Animal Welfare Guidelines – Fish (excluding Zebrafish)

v2 Aug 2022

The University of the Sunshine Coast (UniSC) is committed to the highest ethical standards in the use of animals for scientific purposes. These guidelines have been developed in line with the *Australian code for the care and use of animals for scientific purposes 8th Edition* (the Animal Code), which is the primary guiding document for animal ethics in Australia. Staff and students who submit ethics application must have read and applied the values and principles outlined in the Animal Code, in addition to the UniSC Animal Ethics – Governing Policy, the UniSC Animal Ethics – Procedures and the *Australian Code for the Responsible Conduct of Research*. The use of animals for scientific purposes must not commence until UniSC ethics approval has been granted.

These guidelines are intended to assist those planning and reviewing the use of fish for scientific purposes (excluding Zebrafish). The variety of animals classed as fish is immense and each species may require specific conditions. This document includes general information and methods for the use of fish for scientific purposes.

The structure of the guidelines aligns with the structure of the UniSC Animal Ethics Application Form and the Animal Code. If these guidelines are not suitable for the proposed use of fish for scientific purposes, investigators are welcome to liaise with the Animal Welfare Officer (AWO) and/or justify why alternative methods are being proposed. The Animal Ethics Committee (AEC) will consider the alternatives as part of the ethical review process.

If a team member has not yet been deemed competent at performing a necessary procedure or requires further training, the AWO is available to provide or organise training and assess competency as required.

Table of Contents

1. Overview

Introduction

With the worldwide growth in the aquaculture industry and the move away from wild caught fish to alternative sources of fish for both the food and the pet industry, fish research has seen a rapid rise in investment in areas such as fish growth rates, reproduction success, quality of the fish produced and the variety of fish available for the industry. Fish are also regularly replacing other vertebrate species in specific toxicology, molecular and genetic research studies.
There are 20,000 known species of bony fish (teleosts) and cartilaginous fish (chondrichthyans) in the world, which constitutes about half of all living vertebrates. They range in size from a few centimetres to over fifteen metres in total length (TL) and vary significantly in their morphology, genetics, behaviour, physiology, ecology and taxonomy. Therefore, providing a comprehensive general guideline for all fish species is both difficult and limited in its extent. **Species-specific information on all new fish being considered for research projects at UniSC should be provided by the investigator during the application process with their specific background and welfare requirements regarding housing, husbandry and handling clearly outlined.** The areas and depth of details required for an AEC application can be found within each section of this guideline.

When selecting fish for research projects, the first consideration should be the species. Fish are generally considered as either marine or freshwater species, and there are some that reside across both habitats during their various life stages. The choice of fish for research purposes is often determined by a paucity of data that is required to adequately manage sustainable wild populations. This may be related to fishery pressures and targeting nature, effects of climate change and loss of habitat, or some other critical aspect of species conservation.

Considerations when selecting a fish species for captive studies may include availability of a captive diet, resistance to disease, social compatibility, availability of suitably sized housing facilities, appropriate water supply and temperature control. The frailty of the species should also be assessed, with those requiring multiple daily visits or potentially having higher mortality rates being less desirable for good data collection and time management.

Note: Zebrafish (*Danio rerio*) are a breed of fish that are used extensively within research projects involving molecular and genetic studies, as such, they will be covered in a separate guideline.

Safety information

The aquaculture environment is prone to many potential occupational health and safety risks including:

- skin wounds such as lacerations, stings, and bites
- skin contamination from cleaning chemicals and natural fish toxins
- potential zoonotic infections from water borne bacterial or fungal organisms
- slips and falls on wet floors

- electrocution
- lifting injury
- boating accidents and drowning.

To reduce the potential for harm to investigators and other animal carers, a risk assessment should be carried out for each aquaculture project. As a general basic guide, all persons working or visiting the aquaculture facilities should wear non-slip covered footwear, laboratory coats/overall or aprons, nitrile gloves and safety goggles.

A first aid box (including eye wash equipment) should be available in each housing facility.
In the case of projects involving boats, all persons should be supplied with appropriate standard personal floatation devices (life jackets). Further recommendations made by the formal risk assessment review must be adhered to at all times.

Animal handling of potentially dangerous species

Certain species of fish present a greater risk to animal carers than others due to venomous toxins, spines, or bites. Animal carers should always work in pairs (at least) when handling these types of fish, to ensure appropriate first aid can be carried out and emergency services can be contacted. Investigators should also work closely with the risk assessment team when dealing with fish in this category.

For further occupational health and safety, risk assessment, laboratory procedures and fieldwork considerations contact SafetyTechOps@usc.edu.au prior to writing your AEC application.

Compliance requirements

Ensure that:

- procedures are undertaken as per the approved AEC application
- all required fishing permits are current and available for all persons involved in the project
- all current and relevant routine laboratory and workplace OH&S procedures are followed
- all persons involved in the project wear the designated PPE required for the tasks as detailed in the procedures.

Fish welfare and legislation

Alongside the increased research into the aquaculture industry, there is also an emerging area of research into fish welfare and health, including the occurrence of nociception and pain. Below are some of the welfare codes of practice within the various states of Australia.

- Australian Seafood User's Manual by GK Yearsley et al. (2000). Queensland Department of Primary Industries, Queensland
- Code of conduct for Australian Aquaculture – Australian Aquaculture Forum
- Fish handling (Fact sheet 18) – Department of Primary Industries, Water and Environment, Tasmania
- Guidelines on Fish and Crustacean Welfare – Department of Natural Resources and Environment, Victoria
- Tasmanian Salmonid Farming Industry- Code of Practise (2004), Tasmanian Salmonid Growers Association Ltd.

Each Australian state/territory also has its own legislation regarding fish welfare, which is listed in the following table.

State/Territory	Legislation	Coverage
ACT	*Animal Welfare Act 1992* and Animal Welfare Regulations 2001	Covers fish and crustaceans held for human consumption
	Fisheries Act 2000 Fisheries Regulations 2001	Legislative control of aquaculture
NSW	*Prevention of Cruelty to Animal Act 1979* Prevention of Cruelty to Animals (general) Regulations 1996	Covers fish and crustacea held where food is prepared or for direct retail sale for human consumption
	Fisheries Management Act 1994 Fisheries management (Aquaculture) Regulations 2001	Legislative control of aquaculture
NT	*Animal welfare Act 1999* Animal Welfare Regulations 2000	Covers live fish in captivity and live crustacea held where food is prepared or for direct retail sale for human consumption
	Fisheries Act 1988 Fisheries Regulations 1992	Legislative control for aquaculture
QLD	*Animal care and protection Act 2001* Animal care and Protection Regulations 2002	Covers live fish and some live invertebrates
	Fisheries Act 1994 Fisheries (General) Regulations 2019 Fisheries (Commercial Fisheries) Regulations 2019	Legislative control of aquaculture and fisheries resources
SA	*Prevention of cruelty to Animal Act 1995* Prevention of Cruelty to Animals Regulations 2000	Aquatic animals excluded
	Fisheries Act 1982 Fisheries (Exotic fish, Fish farming and Fish Diseases) Regulations 2000 *Aquaculture Act 2001*; Aquaculture Regulations 2002	Legislative control of aquaculture
TAS	*Animal Welfare Act 1993* Animal Welfare Regulations 1993 (and 1994, 1995, 1997 amendments)	Covers live fish
	Inland Fisheries Act 1995 Marine Farming Planning Act 1995	Legislative control of aquaculture

	Living Marine Resources Management Act 1995 (and 2000 amendment)	
VIC	*Prevention of Cruelty to Animal Act 1986* Prevention of Cruelty to Animals Regulations 1997	Covers live fish and crustacea
	Fisheries Act 1995 Fisheries Regulations 1998	Legislative control of aquaculture
WA	*Animal Welfare Act 2002* Animal Welfare (General) Regulations 2003	Covers live vertebrates or prescribed live vertebrates other than humans of fish
	Fisheries Resources Management Act 1994 Fisheries Resources Management Regulations 1995	Legislative control of aquaculture

General background on the fish species in a project

For the AEC to gain a better insight into the species of fish the investigator has chosen to study, it may be worth supplying some basic facts about the species. The following is an example of the type of information that may be useful to include as part of the AEC application.

Background information on honeycomb grouper (*Epinephelus merra).*

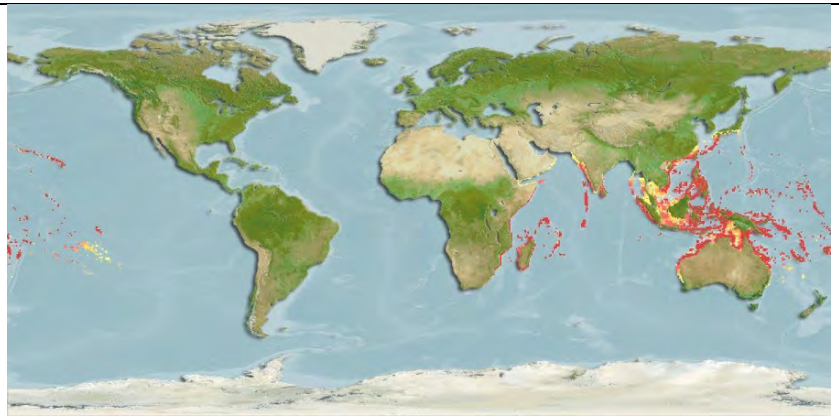
(Image by Undersea, viewed 19 Aug 2019)

(Image by Randall, JE, viewed 19 Aug 2019).

(Image by Jon Hanson, London, UK, viewed 19 Aug 2019)

Classification

Class	Order	Family	Genus	Species	Common Names
Actinopterygii	Perciformes	Serranidae	*Epinephelus*	*merra*	Honeycomb grouper, dwarf-spotted grouper, honeycomb cod, honeycomb rock cod, wire-netted reefcod, wire-netting cod

<table>
<tr>
<td>Natural population distribution</td>
<td>


(Image: Reviewed distribution maps for Epinephelus merra (honeycomb grouper), with modelled year 2100 native range map based on IPCC A2 emissions scenario. www.aquamaps.org, version of Aug. 2016. Web, viewed 19 Aug. 2019.
The honeycomb grouper is found in the tropical waters of the Indo-Pacific from South Africa to French Polynesia. In Australia, it is found from the coast of Western Australia, around the north of the country and down the east coast to the coast of New South Wales.
Note: Not known from the Red Sea, Persian Gulf, nor Asian mainland.
</td>
</tr>
<tr>
<td>General appearance and distinguishing features</td>
<td>
Size: Length from 28-32cm, with a typically fast growth rate.

General appearance: Head, body and fins are pale and are covered by close-set (sometimes coalesced), reddish-brown or dark brown spots, with the interspaces forming an irregular pale reticulum. The pectoral fins are covered with distinct small black spots, largely confined to the rays. (Note: the latter is the best diagnostic colour character of this species.)

Short description
<table>
<tr><th>Spines</th><th>Greatest body depth</th><th>Head length</th><th>Preopercle</th><th>Abdominal Serrae</th></tr>
<tr><td rowspan="2">11 dorsal spines
15-17 dorsal soft rays
Dorsal fin spines third to last subequal, the longest 2.4-3.2 in HL.

3 anal spines
8 anal soft rays
Anal fin spines second and third subequal, 2.1-3.0 in HL and longer than depth of peduncle</td><td>2.8-3.3 in SL.</td><td>2.3-2.6 in SL.</td><td>Rounded or subangular</td><td>At an angle and enlarged</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Operculum</td><td>Jaw/teeth</td><td>Interorbital & head area</td><td>Scales</td></tr>
<tr><td></td><td>Upper edge almost straight</td><td>Mid-lateral part of lower jaw -2-4 rows of teeth
Inner teeth about twice length of outer ones

They do not have many teeth on the edges of</td><td>Interorbital area is flat, and the dorsal head profile is convex</td><td>Lateral line
48-54, in series 98-114
Scales ctenoid on body except cycloid anteriorly above lateral line, on thorax and lower abdomen – body with auxiliary scales</td></tr>
</table>
</td>
</tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><td>Caudal fin rounded, peduncle depth 3.2-4.1 in HL

Pectoral-fin rays 16-18</td><td>Gill rakers
First gill arch
6-9 + 14-17</td><td>their jaws, but they have heavy crushing tooth plates inside the pharynx</td><td></td><td>Nostrils
Subequal anterior and posterior nostrils (latter are larger)</td></tr>
</table>
</td></tr>
<tr><td>Natural diet</td><td>Groupers are not fast swimmers over long distances, and they often lie in wait for their prey or use their mouths and gills as powerful pumps to suck their prey from crevices (ambush predators). They swallow prey rather than biting pieces off it. They are carnivores and mainly eat fish, small sharks, juvenile sea turtles, octopuses and spiny lobsters.
Juveniles consume mainly crustaceans and small fish, increasingly consuming fishes with growth.</td></tr>
<tr><td>Main predators in Australia</td><td>Fishing is not known to be driving global-level declines at this time, and there are no current species-specific conservation measures.</td></tr>
<tr><td>Natural habitat</td><td>Preferred temperature ranges from 24.6 – 29°C with a mean of 27.8°C.
Sea depth range is 1-50 metres but usually occurs shallower than 20 m.
Normally, they are a solitary fish with one male having a group of several females in an area of coral bed within a shallow lagoon or marginal coral reef habitat.
Juveniles shelter in these coral areas (e.g., staghorn Acropora coral thickets) and adults commonly inhabit these shallow lagoons and semi-protected seaward reefs. Spawning occurs at aggregation sites where adults gather to reproduce.
The species is normally site attached with relatively small home ranges where individuals live and feed in the same area throughout their life.</td></tr>
<tr><td>Life expectancy and breeding</td><td>Life expectancy is 5-15 years, with this species having a relatively fast growth rate and a natural mortality ranging from 0.9 to 1.67.
They typically reach reproductive maturity at 30 to 50 per cent of their lifespan (11-19cm length).
This breed is a protogynous hermaphrodite. Most grouper species start out life as females (~) and change sex to males (|) at an age of about half their lifespan (that is, from 3 to 7 years depending on species). Usually, the groupers with a length of 16cm are still females.
Many species move to particular areas at the same time each year to reproduce in spawning aggregations. In these aggregations, females release eggs, and these are fertilised by sperm released by males.
The fertilised eggs hatch to very small forms (larval stages) that drift in ocean currents for 1 to 2 months. Less than one in every thousand of the small floating forms survives to settle as a juvenile in shallow water near reefs.
As they grow, they move onto coral reefs and less than one in every hundred of the young fish (juveniles) survives to become an adult.

Resilience is considered high, with the minimum population doubling time less than 15 months.
Vulnerability is considered low to moderate.

Breeding times

<table>
<tr><td>Australia</td><td>Spawning occurs shortly after the full moon during summer</td></tr>
<tr><td>Micronesia</td><td>March to April</td></tr>
<tr><td>New Caledonia</td><td>September to February with a peak in November/December</td></tr>
</table>
</td></tr>
</table>

	<table><tr><td>Okinawan waters</td><td>May to August with a peak in June</td></tr><tr><td>Society Islands</td><td>January and April for a duration of 3 to 4 days during the full moon</td></tr><tr><td>Tahiti</td><td>January to April</td></tr></table>
Other information	**Threat to humans** There are reports of ciguatera poisoning associated with this species. **Human uses** Fisheries: commercial, in artisanal (traditional/subsistence *fishing)* fisheries aquaculture, gamefish, private and public aquarium industry.

2. Acquisition, transport, admission, and acclimatisation

2.1 Scope

This section relates to live fish acquisition, transportation, admission, and acclimatisation, whether from a breeder/supplier or wild caught for scientific or teaching purposes.

2.2 Background information

The person acquiring the fish must be deemed competent in the acquisition process and in possession of a current animal ethics approval before ordering the animals, either through the licensed supplier/breeder or by catching them from the wild environment, as stated on the animal ethics application.
The number of fish required will be stated on the ethics application and should not be exceeded. If further fish are required during the lifetime of the project, an amendment to the animal ethics approved project will need to be requested and approved before extra animals are ordered.

The Animal Code (clause 2.5.1) defines that the animal carer is responsible for the care of the animals prior to their commencement into a scientific project. An animal carer can be the facility manager, an animal technician or the project investigator. The designated animal carer/s and their responsibilities should be detailed on the ethics application. According to the Animal Code (clause 2.5.3), if multiple persons are responsible for caring for the fish, a person must be identified who has the ultimate responsibility for their care.

2.3 Equipment and resources

Acquisition and Transport

- Delivery invoice, health assessment sheets, animal record form, permits.
- Appropriate fish catching equipment and holding facilities for wild caught species.
- Appropriate transportation tanks/facilities for use during the transportation of the fish to the UniSC campus (air, road, train).
- Fish sedation medication as required.

Admission

- Paperwork: monitoring sheets, tank identity cards, door signage, permits and AEC approval paperwork with amendment attachments.
- Appropriate aquaculture room with drainage and non-slip flooring, separate cleaning facilities and food preparation facilities.
- Appropriate fish tanks for species, aeration and filtration equipment, lighting and heating, water quality monitoring equipment, reliable clean appropriate water supply for species, catch nets, health assessment equipment (e.g., biological sample pots, camera). Extra tanks for separation of fish as required.
- PPE including safety goggles/glasses, laboratory coat/overall/apron, enclosed shoes, nitryl unpowdered gloves, first aid box with eye rinsing products. Hand washing facilities.

2.4 Recommended procedures

Acquisition procedure (general)

Obtaining fish stock

Once ethics approval has been granted, the investigator should order the quantity of fish required according to the current UniSC purchasing policy and AEC application, or manage the catching of wild caught fish. The fish should be ordered to coincide with the commencement of the project to avoid a prolonged period in captivity prior to the project commencing. Consideration regarding the time of capture should be given to the fish's normal life cycle stages, such as spawning, especially if these are a key element of the project.

Investigators must ensure their fish suppliers are reliable and licensed by researching their approval criteria prior to engagement. Accreditation documentation should be included as part of the AEC application and will be assessed as part of the ethical review process.

As a basic guide, the following organisations may be useful to assess the suitability of fish suppliers in Australia:

- The Australian Fisheries Management Authority https://afma.govcms.gov.au/domestic-compliance
- Marine Stewardship Council https://www.msc.org/about-the-msc/what-is-the-msc
- Australian Maritime Safety Authority https://www.amsa.gov.au/vessels-operators/domestic-commercial-vessels
- The World Association of Zoos and Aquariums (WAZA) www.waza.org/
- Ornamental Fish International https://ofish.org/

Booking the animal holding room

The relevant animal holding room/s should be booked in preparation for the fish's arrival and for the total duration of their anticipated stay according to UniSC room booking policy. The investigator must ensure that there is sufficient space to allow for quarantine facilities and extra tanks in case group dynamics in territorial species of fish require re-arrangement.

Obtaining the relevant fish permits

Obtaining details of the relevant state fishing permit requirements is the responsibility of the investigator prior to commencing the project. Information on state/territory permits can be found in the table below.

Queensland	www.qld.gov.au www.daf.qld.gov.au
Tasmania	www.dpipwe.tas.gov.au www.fishing.tas.gov.au www.tasfish.com/licence-info
Victoria	www.vfa.vic.gov.au
South Australia	www.pir.sa.gov.au
Northern Territory	www.nt.gov.au
Western Australia	www.fish.wa.gov.au
New South Wales	www.dpi.nsw.gov.au

The *Fisheries Management Act 1991* section 33 details information on fish collection using a boat. During the capture process, consider information that may be required by the permitting body of each state regarding wild caught fish.

A fish supplier (either the investigator themselves or another person engaged by an investigator to catch fish on their behalf) must keep a copy of the relevant permit and letter of engagement/identification with them at all times during the catching process. Other information that may be required by fishing authorities includes:

- the date and location of sampling
- the capture equipment used
- the number and description of all species caught and their fate
- the number and description of any samples/biopsies collected
- any interactions with protected species and their fate
- any other information regarding size, breeding or anything deemed relevant or of interest that is able to be volunteered.

Additional permits required when using native animals for scientific or teaching purposes

There may be additional permits required when fishing operations are proposed to occur in marine parks. For Queensland permits in state marine park waters, the investigator must apply through the completion of the *Application form Scientific and Educational purposes permit* found at https://environment.des.gov.au.

Before submitting an application, the investigator should discuss the project with the permit assessment officer by emailing them at wildlife@des.qld.gov.au with their specific enquiry. There may be additional permits required for fishing to occur in Australian marine parks or other state regulated waters (Fisheries Queensland). http://www.gbrmpa.gov.au/access-and-use/permits https://onlineservices.environment.gov.au/parks/australian-marine-parks

Considerations prior to capture of fish

The Queensland *Fisheries Act (1994)* and *Fisheries (General) Regulations 2019* set out clear guidelines for recreational fishing that can be undertaken without the need to obtain a licence, except if fishing occurs in specific stocked impoundments (where a stocked impoundment permit is required). Any proposed research on listed noxious fish species will require a Restricted Matter Permit from Biosecurity Queensland, administered under the Queensland *Biosecurity Act 2014*.

If an investigator's project works outside these guidelines with regards to:

- catching techniques
- fish size/development stage
- possession limits
- waterway closure zoning and
- protected species

they will be required to complete and submit a General Fisheries Permit to the Department of Agriculture and Fisheries (DAF) online (prior to having their AEC application approved) at: https://www.publications.qld.gov.au/dataset/general-fisheries-permit/resource/db09e7aa-8227-4acb-b3a8-c4ad495e169d https://www.daf.qld.gov.au/business-priorities/biosecurity/policy-legislation-regulation/biosecurity-act-2014/biosecurity-matter-report/restricted-matter

Capture techniques

There are a variety of capture techniques that have been deemed suitable for catching wild caught fish in Queensland for recreational purposes. Each capture technique has a different welfare implication for both the target species and any by-catch species. A list of capture techniques can be viewed in Appendix 2.12, which highlights techniques allowed by the Department of Agriculture and Fisheries for recreational fishing purposes, with a description of its purpose and limitations. If an investigator wishes to use another form of capture technique for their project, they will require a General Fisheries Permit.

Choice of catching hooks, handling the fish and a humane method of removing hooks

The following information was taken from the Department of Primary Industries NSW: (https://www.dpi.nsw.gov.au/fishing/recreational/fishing-skills/catch-and-release/circle-hooks-benefits-and-tips) and from Bryant, CW 2020, https://adventure.howstuffworks.com/outdoor-activities/fishing/fish-conservation/responsible-fishing/how-to-remove-fish-hook3.htm .

Circle hooks

The use of circle hooks with line fishing has been shown to increase the survival of angler released fish because they typically have a higher incidence of 'mouth-hooking' compared to other hooks. They are produced in barbed and non-barbed forms with the latter being a more humane method of capture. Generally, the survival of fish released by anglers relates to where the fish was hooked, handling time and the dehooking process. Increased morbidity and mortality in fish caught with hooks is associated with 'deep hooking', where the fish are hooked through the abdomen, gills or deep into the throat near internal organs. In comparison, 'shallow hooking', where the hook attaches through the mouth, cheek, or jaw, have better survival rates, as long as the dehooking process is efficient.

Unless justified otherwise, circle hooks as opposed to J hooks are recommended for use by investigators using line fishing to capture fish for their project. They should be the correct size for the type/size of the fish being caught. Consideration should also be given to the strength of the fishing line, ensuring it is sufficient for the species of fish to be caught to make the retrieval process smoother.

Barbed circle hook

Image of a non-barbed circle hook by Angler innovations, 2020, https://www.anglerinnovations.com/products/no-348-maruto-barbless-circle-hook

A circle hook is a fishing hook manufactured so that the point is turned perpendicularly back to the hook shank to form a generally circular or oval shape.

How to use circle hooks

- When loading baits, do not bury the hook (particularly with tough baits). Instead, lightly hook the bait so that the point and barb are exposed ('bridle' the bait).
- Do not strike at the fish (yank on the line), instead allow the fish time to take the bait into its mouth and then apply slow and steady pressure to set the hook in the mouth area. The fish will often hook themselves in this manner.
- Float rigs, short leaders and keeping the fishing line tight may also increase the number of fish that are hooked in the mouth.
- Non-offset circle hooks are recommended for the best mouth-hooking results.
- Use a de-hooker or needle-nosed pliers to help with the unhooking process.

The following images (from Department of Primary Industry NSW Government website) show how to load a circle hook with various types and sizes of bait.


Strip bait with hook point clearly exposed


Peeled prawn mounted on circle hook


Bait rigged for shark fishing using electric cable ties

Handling the fish during capture and hook removal

During the line fishing capture process, fish can exert a great deal of energy due to struggling, which can deplete its tissues of oxygen through the stress of exertion. This physiological change can lead to lactic acid build-up in its muscles and eventually in its bloodstream, which can cause death if the handling and dehooking process is drawn out and oxygenated tanks are not provided for recovery.

Once the fish is hooked and brought in alongside the boat, the investigator should use a quick, efficient, and steady technique to bring the fish inside the boat.

- The fish should never be pulled onto the boat by the hook and line alone.
- The fish should always be lifted either by hand or in a net to support its body.
- The net should be clean, in good order (without rust) and made of knotless cotton mesh or rubber that is less likely to harm the slime/mucous layer on the fish's skin.

Fish caught in shallow water can injure themselves thrashing around on rocks and uneven ground during capture; therefore, investigators should avoid landing the fish in shallow water wherever possible. Prior to capture, the investigator should search for a suitable pool area nearby to the fishing zone where the water is deeper.

Removing the hook

Handling the fish with dry hands or gloves or by using a coarse, dry net can remove the outer mucous membrane coating that helps them fight topical disease. Therefore, this coating must always be kept wet. Fish can only survive for a few minutes out of the water; therefore, handling out of water must be kept to a maximum time of <30 seconds. Where possible, remove the hook while the fish are either still in the water or once they are in an aerated tank or other onboard housing apparatus.

If the investigator must handle the fish out of the water, they should wet their hands with the water from wherever the fish are captured prior to lifting the fish. The fish should be held firmly by the tail whilst supporting the animal gently under the abdomen. Investigators should avoid touching the gills or squeezing the fish. Small fish should quickly be placed into an aerated tank where the de-hooking process can occur underwater. The water used for the aerator tank should be taken from the fish's normal environment to prevent distress from altered water parameters.

The hook should be removed by using needle-nose pliers to grasp the hook by the stem, and while holding the fish in the water twist and pull gently, backing the hook out the way it went in. The hook should not be wriggled or pulled with too much force if it is snagged.

If the fish is hooked into the abdomen, or the hook is too deep into the throat that removal would cause major trauma, the fish should be humanely killed (see section six for humane killing of fish techniques), and an adverse event may need to be completed and submitted to animalethics@usc.edu.au.

Once the hook is removed, the fish should be monitored whilst in the aerated tank prior to performing any other procedures. In general, non-barbed hooks greatly speed up the de-hooking process, as does crimping the barbs with needle nosed pliers prior to removal. To reduce secondary animal welfare problems associated with fishing line entanglement, all equipment including damaged line and hooks must be brought back to shore and disposed of responsibly and safely in the appropriate garbage bins and sharps containers.

Fish size limitations

There are limits on the size (length) of a fish species an investigator can collect and have in their possession. The general ruling on fish size relates to the fish having reached a size to be able to have spawned at least once (in order to add to the species numbers) prior to being captured. A list is provided in Appendix 2.13 detailing the size limitations for various Queensland fish species. If an investigator wishes to take undersized fish for their project, such as fingerlings, they will require a General Fisheries Permit.

Possession limits

There are also limits on the number of fish allowed to be in a person's possession at any one time. This limit does not equate to a 'per day' basis. Therefore, any fish already caught by or on behalf of an investigator and being housed either as live or dead specimens at or away from the capture site will be included in the overall count. Generally, twenty fish of any one species (excluding some bait species) may be kept at any one time without a permit. If an investigator wishes to exceed this number of fish for their project, they will require a General Fisheries Permit.

Waterway closures and restrictions

Certain waterways are regulated or closed for a period of time for reasons including:

- to protect endangered or threatened species
- to protect fish spawning activity
- to prevent overfishing of certain fish populations and to allow successful migratory patterns.

If an investigator wishes to collect fish from restricted waterways for their project, they will require a general fisheries permit. Other areas of waterway, such as designated marine parks, require a further level of permitting for any type of fishing activity. The Great Barrier Reef Marine Park Authority is one example in Queensland where a fishing permit is required prior to **any** fishing activity, regardless of fish type or size. An investigator planning a fish-based project in a marine park should apply online for the specific fishing permit prior to an AEC application (for example http://www.gbrmpa.gov.au/).

Protected and no-take species

Some fish are endangered or particularly vulnerable to exploitation and therefore require stricter regulation or protection. This regulation may cover both males and females of a species or just one gender. For example, one such specimen would be a female egg bearing crustacean of a female blue swimmer crab. Some species are identified as no-take species for similar reasons as above or because they may pose a risk to people eating them.

The following species are protected throughout Queensland and are therefore prohibited from being in anyone's possession without a permit. If accidentally caught, they must be immediately and carefully returned to the water.

Protected sharks and shark-like rays	No-take freshwater fish / elasmobranch / crustacean species	No-take tidal water fish / elasmobranch species
• Great white shark (*Carcharodon carcharias)* • Grey nurse shark (*Carcharias taurus*) • Narrow sawfish (*Anoxypristis cuspidate*) • Dwarf sawfish (*Pristis clavate)* • Green sawfish (also called Long comb sawfish, Narrow snout sawfish) (*Pristis zijsron*) • Spear-tooth shark (*Glyphis glyphis*)	• Australian lungfish *(Neoceratodus forsteri)* • Mary River cod (except in certain stocked impoundments) *(Maccullochella mariensis)* • Bloomfield River cod *(Guyu wujalwujalensis)* • River blackfish *(Gadopsis marmoratus)* • Cling goby (of the subfamily *Sicydiinae*) • Freshwater sawfish *(Pristis microdon)* • Edgbaston hardyhead (*Craterocephalus sp.)* • Myross hardyhead (Thomson River) (*Craterocephalus sp*.) • Spiny crayfish *(Euastacus sp.)*	• Barramundi cod *(Chromileptes altivelis)* • Chinamanfish *(Symphorus nematophorus)* • Humphead Maori wrasse *(Cheilinus undulates)* • Paddletail *(Lutjanus gibbus)* • Potato rock cod *(Epinephelus tukula)* • Queensland groper *(Epinephelus lanceolatus)* • Red bass *(Lutjanus gibbus)* • Manta rays (*Manta birostris* and *Manta alfredi*) • Sawfish (all) *(Pristidae sp.)* • Hammerhead sharks (all) (*Sphyrnidae spp*.) • Sand tiger sharks *(Carcharias taurus)* • Spear-tooth sharks (*Glyphis glyphis*)

Note: The Animal Code does not currently require an investigator to apply for animal ethics approval for Malacostraca species of animals. However, the following animals are classed as no-take species according to the QLD *Fisheries Act 1994* and may be caught as by-catch during the fish capture process:

- Black teat fish *(Holothuria whitmaei)* and white teat fish *(Holothuria fuscogilva)* (sea cucumber species)
- Female mud crabs *(Scylla sp.)*
- Female blue swimmer crabs *(Portunus armatus)*
- Egg-bearing spanner crabs *(Ranina ranina)*
- Egg-bearing three-spot crabs (*Portunus sanguinolentus*)
- Egg-bearing bugs (Balmain bugs and slipper lobsters) (*Thenus orientalis)*
- Egg-bearing and tar-spot (Sperm packets) tropical rock lobsters (all species, including red champagne lobsters and painted crayfish) *(Panulirus ornatus)*
- Helmet, trumpet and clam shells; regulated by species. All of these large and vulnerable shells are totally protected

Further information can be gained from the following Queensland website: https://www.daf.qld.gov.au/fish-identification-information/fish-species-guide/protected-and-no-take-species

Endangered species and the Nature Conservation Act 1992

There are other species of fish and marine animals protected under the *Nature Conservation Act 1992*. These include:

- Edgbaston goby *(Chlamydogobius squamigenus)*

- Elizabeth Springs goby *(Chlamydogobius Micropterus)*
- Grey nurse shark (*Carcharias taurus)*
- Red-finned blue eye *(Scaturiginichthys vermeilipinnis)*
- Honey blue eye (*Pseudomugil mellis)*
- Oxleyan pygmy perch *(Nannoperca oxleyana)*

Note: whales, porpoises, dugongs, turtles, and dolphins are also protected marine species and their welfare should be considered during fish capture to ensure they do not become a by-catch species. For more information on animals protected under the *Nature Conservation Act* contact the Department of Environment and Science at https://www.des.qld.gov.au/.

By-catch process and noxious fish

Each state has its own list of noxious fish species for their area, and information can be found on the relevant government websites where fishing permits are managed. Investigators should be familiar with these noxious fish because penalties can apply if the person undertaking the fish acquisition is found in possession of these species, regardless if they are an accidental by-catch specimen. Noxious fish are invasive fish that have been declared as harmful by each state because they are, or may become, a serious pest to native aquatic communities and require specific actions and restrictions to manage them. Noxious fish have characteristics that are detrimental to other fish, aquatic habitats, or humans. The following website may help with the identification of noxious fish in Queensland https://www.qld.gov.au/environment/plants-animals/animals/pests-diseases/invasive-fish/identifying.

The Queensland *Biosecurity Act 2014* identifies 125 species as prohibited noxious fish (refer to Schedule 1, noxious fish). These species are not in Queensland nor are they able to be brought into Queensland. If sighted:

- they must be reported to Biosecurity Queensland within 24 hours of the sighting
- they must not be given away, sold, or released back (dead or alive) into the environment without a permit
- the investigator has a general biosecurity obligation to take all reasonable and practical steps to minimise the risk of these fish from escaping until they receive advice from an authorised officer.

If caught:

- The investigator or supplier under the direction of the investigator must immediately humanely kill and dispose of the fish away from the water body.
- The noxious fish must not be used as bait–dead or alive.

Further information can be gained from the following Queensland website: https://www.qld.gov.au/environment/plants-animals/animals/pests-diseases/invasive-fish/legal-obligations. If an investigator wishes to include a noxious species of fish in their project, they will require a restricted matter permit from Biosecurity Queensland.

References

Bryant, CW, 2020, *How to remove a hook without injuring the fish*, viewed 11 February 2020, https://adventure.howstuffworks.com/outdoor-activities/fishing/fish-conservation/responsible-fishing/how-to-remove-fish-hook3.htm

NHMRC, 2013, The Australian code for the care and use of animals for scientific purposes, viewed 20 November 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes

Transportation procedure for fish

The transportation process is a very stressful time for fish whether during the wild catch procedure, transportation from supplier to research housing facility, transportation between research facilities or simply when moving fish between housing tanks. The distress is due to factors such as:

- changes in the environment
- different types of noises
- movement
- close confinement.

A fish's ability to cope with this stress depends on many factors including:

- initial state of health
- species
- age
- sex
- stocking density
- period without food
- trip duration
- mode of transport
- water quality (e.g., temperature, oxygen level, pH, carbon dioxide and ammonia in particular).

Careful planning is needed for the transport stage of a scientific fish project to reduce these stressors as much as possible. Details of any species-specific conditions in place to reduce the fish stress should be clearly outlined in the AEC application. These conditions will also vary depending on the fish's age and gender.

Some specific conditions to consider include:

- All types of container should be escape proof.
- Providing a pre-transport 'tempering' to adapt the fish to the new transport conditions.
- Only transporting healthy fish.
- Withholding food from/fasting the fish for one day (smaller fish) to three days (larger fish) prior to transport, to reduce faecal output and water contamination (ammonia) and consequently help to maintain oxygen levels. If the digestive tract of the fish is not cleared prior to transport it will reduce the potential transportation time in half. Note: fish larvae requirements for food intake are greater than other older fish. Herbivorous larvae should not be transported for more than twenty hours and many aquarium species are restricted to transportation times of less than twelve hours.

- Fish that are to be transported from interstate for more than one hour from UniSC must only be undertaken when forecast air temperatures during transport will not exceed 35°C and all stages of transport and housing during transport can maintain temperatures within an acceptable range for the specific species. Fish must be transported **inside** a vehicle with temperature control, so that fish can be maintained within their ideal temperature range when being collected from airports or long-distance transportation depots. The transport containers should never be placed in ute trays or car boots.
- Using insulated containers/tanks to maintain a stable temperature. In general, any water temperature increase of 10^{o}C doubles the oxygen demand of the fish.
- Transporting fish in dark or low light conditions to reduce stress.
- Careful design of the loading systems to reduce handling stress and associated excitement. Excitement of fish seen at the loading stage increases the requirement for oxygen three to five times that of a fish at rest. The excitement phase can last for several hours or even the whole transportation period.
- Provision of continual pure oxygen supply to replace that consumed by respiration during both loading and transportation in order to maintain a D.O. of >5mg/L. However, the ability of fish to use oxygen depends on their tolerance to:

    - stress
    - water temperature (higher temperature uses more oxygen)
    - pH and ammonia levels
    - CO_2 concentration
    - fish size (larger fish require less oxygen per unit weight than smaller fish).

    Closed systems are usually good at maintaining oxygen levels so long as fish density is at the correct level. Closed system bags/tanks should be kept moving slightly to allow mixing of oxygen with water. Dead fish and their associated bacteria are also competitors for the available oxygen, causing potential deaths by hypoxia.

- Provision of water agitators and ventilation units within transportation tanks to 'off-gas' carbon dioxide produced by normal respiration processes.

    Higher CO2 is associated with longer transportation times and overstocking and affects water pH (increases acidity). Although this can combat the effects of ammonia build up to a limited level, it will reduce the oxygen carrying capacity of fish blood, even when tank oxygen levels appear adequate. pH changes rapidly stress fish but water buffer products can be used during transportation. (For example, Trishydroxylmethylaminomethane is organic and can be used in both freshwater and saltwater systems at a level of 1.3-2.6g/litre.)

- Mildly and slowly reducing the water temperature to reduce the metabolic rate of the fish (but keeping within the fish's thermal comfort zone) by the use of natural ice.

    As a guide, 25kg of ice will cool 1000 litres of water by 2^{o}C. If the water contains fish at the time of cooling, there should be no direct contact between the ice and the fish.

    The temperature drop should not be faster than 5^{o}C per hour, nor exceed a total temperature difference in excess of 12-15^{o}C from the original temperature (depending on fish species requirements).

Note: younger fish will not tolerate colder temperatures as well as adult fish.

- Ensuring that the fish density for size and species is not exceeded. Always err on the side of conservative numbers. The stocking density of fish will vary with the size and age of the fish and is also species specific.

  Generally, a lower individual weight of fish means a much lower total weight of fish that can be kept in the transport container. This is due to the higher oxygen consumption and greater demand for space in smaller species.

  Stocking density guidelines can be found online for various farmed fish species.

- Ensuring the fish density allows for an unexpected time delay of up to 1.5 times the original transport time, to allow for events such as cancelled plane schedules or transportation breakdown.

- Ensuring the stocking density of endangered species are kept very low when the primary focus is on 100% survival.
- Inclusion of separate compartments in a transport container for more aggressive fish species.

- Matching fish age and size wherever possible to reduce aggression.

- Use of sedation in overly stress prone fish species. Note, however, some medications such as MS-222 can block water filters quickly due to their powder format, which may be detrimental to the maintenance of water quality during transportation.

- Ensuring a battery powered aerator is taken along when picking up fish, in case of an air leak from a transport bag (see next section for information on transport bags and containers).

Types of transportation and associated animal welfare requirements

The two main methods of transporting fish are either through closed or open systems.

- Closed systems generally comprise of either polyethylene transport bags or specifically designed self-contained transportation units. For most fish research at UniSC, closed systems will be used due to the lower number of fish in the projects.
- Open systems can range from small transport fish cans/containers for transport within the territory of a fish facility up to fish transport trucks and tank wagons.

Appendix 2.14 compares the two most common transport systems most useful for the scale of fish research projects at UniSC and any fish welfare factors to be considered when using them for a research or teaching project.

Monitoring of fish during transportation

The person supervising the transport must provide some form of aeration such as oxygen cylinders for the fish transporter system in case the closed system dissolved oxygen (D.O.) drops below 5mg/L. On longer trips (over 2-3 hours) water quality monitoring equipment should be taken to ensure water can be monitored hourly during the trip (especially oxygen and temperature levels).

Fish waste must be siphoned out (if possible) and water exchanged (max 20%) if ammonia levels exceed safe limits. Safe ammonia levels will be discussed in the husbandry section of these guidelines.

During transport: fish transported in a purpose-built fish transporter system should be inspected every 2-3 hours for physical signs of distress including increased respiration rates/gasping, loss of equilibrium, erratic swimming behaviour and changed skin colour.

For large numbers of fish being transported in a fish transporter, it is recommended a dose of 10mg/L to 20 mg/L of AQUI-S®' sedation/anaesthesia medication be put into the water during the trip. This is particularly important with larger biomasses of fish. **If anaesthetics are being used, then aeration must be provided.**

If fish are showing significant signs of distress (gasping, erratic swimming) during transportation, then an increase in the anaesthetics dose should be administered to sedate them to reduce the stress. Increasing the dose of Aqui-S slowly by 1-2mg/L will allow the level of sedation to be closely controlled. The overall level of Aqui-S anaesthetic administered **should not exceed 20mg/L,** otherwise most fish species will slowly lose equilibrium completely.

References

- American Fisheries Society, 2014, *Guidelines for the Use of Fishes in Research*, viewed 28 November 2019, http://frdc.com.au/Archived-Reports/FRDC%20Projects/1993-184-DLD.pdf

- Berka, R 1986, *The transport of live fish A review*, European inland Fisheries Advisory Commission (EIFAC), viewed 20 November 2019, http://www.fao.org/3/af000e/af000e00.htm
- Fisheries Research and Development Corporation, 2017, *Fisheries growth and new research priorities,* viewed 29 November 2019, https://www.frdc.com.au/media-publications/fish/FISH-Vol-251/Fisheriesgrowth-and-new-research-priorities
- Flinders University, 2019, *Standard Operating Procedure Working with Fish*, viewed 2 December 2019, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-working-with-fish.pdf
- Government, N*A* 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, Primary Industries, NSW Department of Primary Industries: Nelson Bay, NSW, viewed 2 November 2019, https://www.dpi.nsw.gov.au/fishing/aquaculture/publications/general/a-guide-to-acceptable-procedures-and-practices-for-aquaculture-and-fisheries-research
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- NHMRC, 2013, The Australian code for the care and use of animals for scientific purposes, viewed 20 November 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
- Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the welfare of animals during transport, (Question N° EFSA-Q-2003-094) 2004, *The welfare of animals during transport,* European Food Safety Authority journal, vol. 44, pp. 1-36, viewed 2 December 2019, https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2004.44
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, *Guidelines for the Care and Use of Fish in Research,* Institute for Laboratory Animal Research, vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

Further Reading

- Bayne, K & Turner, P (eds), 2013, *Laboratory Animal Welfare,* Academic Press, Cambridge, Massachusetts
- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Southern Region Aquaculture center, *Transportation of Warmwater Fish factsheets*, viewed 29 November 2019, https://srac.tamu.edu
- White, L 2017, *By transforming plastic bags and polystyrene into continuously oxygenated bulk-transport containers Australian engineering is revolutionising the transport of live fish,* viewed 29 November 2019, https://www.fishfiles.com.au/media/fish-magazine/FISH-Vol-23-2/Fish-breathe-easy-en-route

Admission procedure for fish

- Ensure all PPE is worn by the animal carers.
- The admission process should start before the fish arrive at the housing facility.

- Knowledge regarding the quality of water the fish are coming from (such as temperature, pH, salinity levels and hardness) should be duplicated in their future housing tank as closely as possible and any differences changed gradually once they arrive, if required.
- Pre-conditioning of water and biofilter, alongside testing of all tanks, water recirculation equipment and facilities should be undertaken prior to the arrival of aquatic animals.
- Once fish are received, they should be held in quarantine tanks (see below for further details).
- Fish transported in bags should be acclimatised by floating the bags in the new housing tank until the water temperature equilibrates (usually around 30 minutes). The bags should be secured to the sides of the tank with clamps to facilitate the opening and aeration of the water in the bags and to allow for water parameter checks to be undertaken.
- An alternative method of tempering or acclimatisation when using transport bags or larger transport tanks is to slowly transfer water from the quarantine tank to the transport tank whilst providing water aeration. The two sets of water parameters should be assessed initially to ascertain their differences, which will then determine the duration of the water transfer process. Normally water transfer to equilibration should take around 30 minutes. Water testing should take place during this time and acclimatisation will be assessed as complete once the parameters are the same.
- During the transfer of the fish to the quarantine tank, the fish health arrival examination should be conducted and the 'Fish arrival health assessment sheet' completed (Appendix 2.15). Areas to note include:

    - external lesions/trauma
    - eye health
    - colour changes
    - swimming ability
    - amount and type of mucous
    - respiration/opercular movement and rates
    - general buoyancy.

- Mucous samples should be taken from the scales and a gill biopsy taken to assess for parasites. A 'health score' should be assigned to the fish as part of the assessment. Information on health scoring can be found in the monitoring section of these guidelines (see section 5).
- Fish should be handled gently and as quickly as possible by experienced animal carers using a smooth sided container or fine mesh nets. Unnecessary chasing of fish for capture should be avoided.
- Any health issues should be highlighted at this time and fish separated from the main group. Sick or injured fish should be grouped together (if appropriate for species) into the same housing facility for ease of identification, ease of treatment (if appropriate), and to ensure increased monitoring is undertaken.
- The chief investigator and investigator responsible for the project should be notified of the unhealthy animals if other animal carers are assessing the animals and an adverse event report completed according to the approved AEC application. The investigator should communicate with the supplier regarding the adverse event details in case the situation could be prevented in the future. The sick animals should be dealt with according to the criteria set out in the monitoring sheet component of the AEC application for each project, including the engagement of an aquaculture veterinarian.
- The housing facility identity card details should be completed as per the fish housing section of these guidelines, for each housing facility and these should be displayed clearly on or close to the fish housing tank.

- The ‘animal admission form-research and teaching’ (Appendix 2.16) should be completed once all the fish have been health assessed and housed. All arrival paperwork should remain with the monitoring sheets and the copy of the AEC approved application documents and placed in a secure dry position that can be viewed during AEC or AWO inspections.
- The ‘Attention access restricted door signage’ should be completed and placed on the fish housing facility door (Appendix 2.17).

Note: correct handwashing is essential when handling fish for both the safety of the fish and the handler.

- Hands and arms should be washed thoroughly before and after handling any animals, to reduce risk of infection to animals or transfer of zoonoses to animal carers.
- Hands and equipment must also be washed between handling different groups of animals with an unknown disease status.
- Detergents are not recommended for hand washing, as they may be toxic to aquatic animals. Ethanol hand wash gel and a through rinse in water are recommended.

References

- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- NHMRC, 2013, *The Australian code for the care and use of animals for scientific purposes*, viewed 20 November 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, *Guidelines for the Care and Use of Fish in Research,* Institute for Laboratory Animal Research, vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

Acclimatisation procedure

The process of acclimatisation is to allow the animals to recover from the stress of transportation and return to normal physiological and behavioural function. This process will reduce any physiological abnormalities that may affect the research data. Acclimatisation should take approximately 48-72 hours (this may differ for each animal and is given as a guide only). The fish will need to have closer monitoring for the first 3-5 days to ensure they are settling into their new environment. Signs of stress to note during the acclimatisation process include:

- loss of appetite or reduced feed intake or general changes in feeding behaviour
- slower growth, loss of weight or condition
- lower resistance to infection
- reduced fecundity
- abnormal or unusual colour (pale, dark or blotchy), reddening of body or fins, hypo or hyper pigmentation
- abnormal behaviour (flighty, erratic swimming, slow swimming, gasping at the surface)
- lack of response to stimuli, lethargy
- congregation near the surface or edge of the holding facility
- protruding or missing scales
- clamped or frayed fins
- air bubbles in the eyes or in the capillaries of fins or gills
- flared operculum covering the gills
- opacity of the cornea, cataracts or exophthalmos
- coelomic distention.

Minimisation of stress in fish can be achieved by:

- Maintaining good water quality and undertaking a slow change from the fish's original transport water to its new water supply. The new water supply should be cross matched to the transport water quality parameters before introducing it to the tank.
- Appropriate stocking densities for the species and provision of spare tanks in case of aggression between fish.
- Adequate quantities of a nutritionally complete food for the fish receiving an artificial diet. Food should be offered by hand initially to monitor eating patterns before an automated system is used.
- A stable quiet environment with limited disturbance. Fish tanks should allow light to penetrate but be constructed of an opaque material or covered during the acclimatisation process to reduce visual stimulation until the fish settle.
- Careful gentle handling when required using knot free hand nets and sedation drugs such as Aqui-S (see anaesthesia and analgesia section 7).
- Protection from predators such as birds by using tank covers/netting if the fish are to be housed outside within purpose-built tanks.

Quarantine procedure

All new fish arrivals must undergo a quarantine period of at least two weeks (or longer as directed by the housing facility) to facilitate the removal of parasites, bacterial or fungal pathogens. Although many of these pathogens are found normally in healthy populations of fish, the stress and close proximity of animals held in captivity may rapidly facilitate advancement into active disease, which may significantly affect the research data as well as cause serious animal welfare concerns. Quarantine duration will also depend on the successful clearing of any potential infections noted on arrival. Adequate lead time should be built into a project plan to allow for this quarantine period. Fish should not be fed for the first 24 hours in the quarantine tanks. Fish that need to be quarantined include:

- Broodfish from the wild or other facilities
- Fingerlings from other facilities
- Fry/fingerlings after harvest
- any harvested fish that are to be restocked or dispatched
- any wild caught fish
- any fish suspected or known to be diseased.

Quarantine involves:

- The use of separate housing facilities and designated holding tanks where water should not be able to communicate in any way with other fish including cross contamination from:
    - splashing
    - backwashing filtration systems
    - aerosolised water particles.

- Designation of separate syphoning, tank cleaning, feeder and catching equipment to the specific quarantine tank.
- Availability of footbaths, hand washing and hand sanitising facilities outside the quarantine room and the encouragement of use by the investigator to reduce the potential spread of nosocomial infection to other fish holding rooms.

Biosecurity requires that facilities provide treatment for external pathogens and parasites (especially in wild caught marine fish species) at this time (if the supplier has not already provided this service). There are a variety of options available depending on the type of ectoparasite (see section 9 medication appendix 9.12).

For fish held at the Bribie Island Research Centre, the following protocol is performed:

- Once the fish are visibly settled, the flow through water to the tank is turned off and the fish will be treated with a light hydrogen peroxide solution (200mg/L) for 30 minutes. During this operation, aeration will be provided to maintain adequate dissolved oxygen concentration (>5mg/l). After the 30 minutes, the flow of freshwater will be returned to the tank to flush out any residual hydrogen peroxide and to allow the fish to recover before their transfer to the fish laboratory housing facility.

- Other methods of treating external parasites and pathogens are chelated copper sulphate, formalin and Trichlorfon. Each has its own potential associated animal welfare concerns and should be used according to the manufacturer's instructions.

Generally, freshwater fish need less proactive measures during the quarantine period. Samples taken as part of the initial health assessment should form the basis of a treatment plan, if required.

Monitoring records of the quarantine period should be completed daily and stored alongside all other project paperwork for potential inspection by the AEC or AWO.

Effluent from quarantine units should be treated:

- at a minimum with passing the waste through screens (fine enough to retain small, escaped fish from the tank), or
- by using chemicals or other treatments to kill any pathogens that are present in the water.

References

- Department of Primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW.
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption,* F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia.
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, Guidelines for the Care and Use of Fish in Research, *Institute for Laboratory Animal Research*, vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

Further reading

- Barton, BA, Morgan, JD & Vijayan, MM 2002, 'Physiological and condition related indicators of environmental stress in fish', in Adams SM (eds), *Biological Indicators of Aquatic Ecosystem Stress*, pp. 111-148.

2.5 Animal health and welfare considerations

Transportation health and welfare concerns

- Supplier transportation and its associated handling processes during capture are stressful and can cause trauma.
- Death can occur from hypoxia, water fouling, overstocking, inappropriate grading and insufficient temperature control during transportation.
- Delays in transportation cause major animal welfare considerations if the system is not aerated or able to be monitored during the journey.

Admission and acclimatisation health and welfare concerns

- Out of water overhandling of fish during the health assessment can cause death or ongoing general poor health due to a lowered immune system.
- Fish may undergo shock/stress/death if transferred to new tanks where the water has unfamiliar water quality parameters to its previous tank.
- Proximity of humans or other predatory animals may cause fish to stress and lower their general immune system.
- Housing aggressive fish or inappropriate groupings may stress other fish in the tank.
- Fish may be reluctant to feed initially, especially when caught from the wild because they do not recognise the new diet and have not settled into their new environment.
- Increased fish stocking causes stress which may increase the potential for disease outbreaks.

Investigators should provide a detailed description on their AEC application covering acquisition, transportation, admission, and acclimatisation and state how they plan to manage the potential welfare issues associated with these stages of their project.

2.6 Training plan and competency assessment

Investigators and animal carers should complete the Introduction to Animal Ethics training available on the Student Portal and must be fully trained and assessed as competent in the process of fish acquisition, transportation and acclimatisation before undertaking related procedures. Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application. The AWO is available to provide or organise training and to assess competency as required. Investigators and animal carers must be aware of the OH&S and risk considerations surrounding the fish species they plan to use in their project and potential first aid procedures required in case of emergencies.

2.7 References and acknowledgements

- American Fisheries Society, 2014, *Guidelines for the Use of Fishes in Research*, viewed 28 November 2019, http://frdc.com.au/Archived-Reports/FRDC%20Projects/1993-184-DLD.pdf
- Barton, BA, Morgan, JD & Vijayan, MM 2002, 'Physiological and condition related indicators of environmental stress in fish', in Adams SM (eds), *Biological Indicators of Aquatic Ecosystem Stress*, pp. 111-148.
- Berka, R 1986, *The transport of live fish A review*, European inland Fisheries Adbvisory Commission (EIFAC), viewed 20 November 2019, http://www.fao.org/3/af000e/af000e00.htm
- Department of primary Industry NSW Government, Circle hooks - benefits and tips, viewed 11 February 2020, https://www.dpi.nsw.gov.au/fishing/recreational/fishing-skills/catch-and-release/circle-hooks-benefits-and-tips
- Department of primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW.
- Fisheries Research and Development Corporation, 2017, *Fisheries growth and new research priorities,* viewed 29 November 2019, https://www.frdc.com.au/media-publications/fish/FISH-Vol-251/Fisheriesgrowth-and-new-research-priorities
- Flinders University, 2019, *Standard Operating Procedure Working with Fish*, viewed 2 December 2019, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-working-with-fish.pdf
- Government, N*A,* 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, Primary Industries, NSW Department of Primary Industries: Nelson Bay, NSW, viewed 2 November 2019, https://www.dpi.nsw.gov.au/fishing/aquaculture/publications/general/a-guide-to-acceptable-procedures-and-practices-for-aquaculture-and-fisheries-research
- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- NHMRC, 2013, The Australian code for the care and use of animals for scientific purposes, viewed 19 April 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
- Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the welfare of animals during transport, (Question N° EFSA-Q-2003-094) 2004, *The welfare of animals during transport,* European Food Safety Authority journal, vol. 44, pp. 1-36, viewed 2 December 2019, https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2004.44
- Southern Region Aquaculture center, Transportation of Warmwater Fish factsheets, viewed 29 November 2019, https://srac.tamu.edu
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, Guidelines for the Care and Use of Fish in Research*, Institute for Laboratory Animal Research,* vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

2.8 Other information and attachments

Appendix 2.9: Fish capture equipment
Appendix 2.10: Fish size and possession limits
Appendix 2.11: Transportation containers
Appendix 2.12: Fish arrival health assessment sheet UniSC
Appendix 2.13: UniSC Animal record form – Research and Teaching
Appendix 2.14: Attention access restricted door signage

Appendix 2.9: Fish capture equipment

Name of equipment	Tidal / marine water use	Freshwater use	Specifications for use	Other considerations
Breathing devices	**Yes**	**Yes**	o Apparatus (other than a snorkel) is not permitted when taking fish by spear, spear gun, hand or other means o A knife should be carried for quick humane killing in case of mis-directed spears	Efficient system with trained operator. Should cause rapid death. Not suitable for fish studies involving otolith aging of fish.
Box traps or opera house traps	**Yes**	**Yes**	o Opera house or box traps are commonly baited with frozen pilchard or cat food and deployed for a soak time of 24 hours before being retrieved o Ensuring traps are submerged for entire soak time, particularly with tidal movement along the intertidal zone o Minimising capture by restricted entrance hole diameters (<7.5cm) and soak times of 24 hours, and o Minimising handling time of animals captured which are then released immediately to the waterway where they were captured o Experienced handlers must be present upon retrieval of the traps when releasing the fish and other fauna back to the waterway o In Queensland, the entrance of an opera house pot must be rigid and no more than five centimetres if it's being used in fresh water east of the Great Dividing Range, excluding some dams where the hole can be ten centimetres. Further guidelines are available at: https://environment.des.qld.gov.au/__data/assets/pdf_file/0028/90739/biological-assessment-sampling-fish-communities-using-bait-traps.pdf	Used to capture and determine which scavenging species are present within protected waters and include streams, rivers, lakes, and estuaries. Requires General Fisheries Permit.
Canister trap	**No**	**Yes**	o The trap must not exceed 60cm in length or 50cm in width, height or diameter o It must open at one end o Size of the mesh must be no more than 25mm (if not made of rigid material) o When the width, height or diameter is measured anywhere along the length of the trap, it must not exceed the diameter of the trap's opening	Requires General Fisheries Permit.
Cast nets	**Yes**	**No**	o Must not exceed 3.7m from the point of rope attachment to the rest of the net, the net lead line or the bottom of the lowest pocket of the net o Mesh size should not exceed 28mm	
Collapsible trap	**Yes**	**Yes**	o The trap should be made of rigid material and have at least one collapsible side	**(Crabs only)**

Name of equipment	Tidal water use	Freshwater use	Specifications for use	Other considerations
Crab pots and dillies	**Yes**	**No**	o No more than four pots, dillies or a combination of both may be used per person o There must not be more than four pots on a boat per person when on the water o You must have an identification tag on the pot bearing the surname and address of the person **using** the apparatus. When the pot is tied to a fixed object a tag must be attached to a part of the rope (above the high-water mark) that identifies the user's name o All crab pots must have a light-coloured solid float attached when not tied to a fixed object The float must not be less than 15cm in any dimension and also identify the user's name o A dilly for spanner crab fishing should be made of solid steel with a thickness of at least 6mm and not exceeding 1m in each dimension	
Dilly nets	**No**	**Yes**	o The net must not have a diameter of more than 125cm and a mesh size of more than 25mm.	
Electrofishing	**Limited**	**Yes**	o Operator must be trained in animal welfare and electrofishing techniques due to the high risk of fish and human harm using this capture method. Operator needs to detail number of hours experience in electrofishing o Current must be pulsed to prevent injury to the fish o Must be tuned differently for different target fish to reduce impact on non-target species o Target animals must be monitored for electrofishing effects o Net narcotised fish and place in appropriate aerated transport containers (for number and size of fish) for processing. Weigh and measure fish as needed efficiently to reduce handling time o Monitor recovery continually for distress, trauma and abnormal behaviour patterns such as jerky imbalanced movements and rapid opercular movement o Once fully recovered, release the fish back to the same location as the capture site Further information can be found at: https://environment.des.qld.gov.au/__data/assets/pdf_file/0022/90751/biological-assessment-sampling-fish-communities-using-electrofishers.pdf	The larger the fish the more unpleasant the effect is on them. Further procedures (e.g., tagging) will require a suitable anaesthesia and ongoing monitoring.
Fishing lines Rod and reel?	**Yes**	**Yes**	o No more than six fishing lines should be used alone or combined o A set fishing line must not be used o A set fishing line must not be used as a cross-line o Only one hook, artificial fly, bait jig or lure can be attached to a line o You can't be more than 50m away from any of your fishing lines	Barbless hooks/non-offset barbless circle hooks should be used which are biodegradable.
Fishing lines Set lines, drumlines, drop lines?	**Yes**	**No?**	o Up to three fishing lines are permitted consisting of a total of six hooks o An artificial fly, lure, bait jig or gang hook is considered equal to one hook o A cross line, drum line, free-floating line or set line is not included as a fishing line o You must be present with the line at all times	Hooks should be used which are biodegradable. Where possible, barbless hooks should be used where appropriate to the project and species targeted.

Name of equipment	Tidal water use	Freshwater use	Specifications for use	Other considerations
Funnel traps	**?**	**Yes**	o Maximum length of 70cm and width or height of 50cm o A maximum of four entrances, with each measuring no more than 10cm in any dimension o Trap entrance must be made of rigid material o Size of the mesh must be no more than 25mm (if not made of rigid material)	
Fyke nets	**Yes**	**Yes**	o The very last section of the trap should be out of the water to allow for by-catch species such as turtles to be able to access air to prevent drowning o If set in freshwater and it rains, or in tidal zones, the traps should be checked to ensure the final end remains out of the water o If left for too long target fish can be consumed by opportunistic bird predators o Use knotless or woven mesh where possible o Use suitably sized nets to reduce by-catch numbers o Checked regularly or monitor constantly, using sub sampling techniques if possible o Ensure there are enough people to collect the fish efficiently	Fish trap consists of cylindrical or cone-shaped netting bags mounted on rings or other rigid structures. It has wings (leaders) which guide the fish to the entrance of the bags. It is set and then left. Suited to smaller areas with lots of nooks and crannies for fish to hide.
Gill nets	**Yes**	**No**	o Other marine species by catch is common (turtles, birds??, mammals and other non-target fish) and so nets should be constantly monitored when set for routine scanning o Investigators using this type of net must have considerable experience with this equipment prior to the project o Number of nets should be limited to the competency of the handle	Nets are set and left in place as they float horizontally using buoys near the surface of the ocean. Fish become entangled through their gills and spines as they swim into the net.
Hand pumps	**Yes**	**?**	o To be used on foreshores for collecting yabbies only	
Open-top pyramid trap	**?**	**Yes**	o Must have a single, rigid top opening, with a minimum size of 15cm in all its dimensions, parallel to the base of the trap o Must have a mesh size of no more than 25mm o Must have a maximum base size of 60cm in length and width	
Scoop or dip nets	**Yes**	**Yes**	**Freshwater** o Must not exceed 1m in any dimension o Handle should be no longer than 2.5m o Mesh size should not exceed 25mm **Tidal water** o Must not exceed 2m in any dimension o Handle should be no longer than 2.5m o Mesh size should be at least 25mm You can use a gaff or landing net to secure a line-caught fish	

Name of equipment	Tidal water use	Freshwater use	Specifications for use	Other considerations
Marking traps		**Yes**	o A tag must be attached to the trap with the surname and address of the person using the trap o A light-coloured solid float should be attached to the trap if it is not fixed to a stationery object above water level. The float must feature the user's surname and be 15cm in each dimension	
Round traps		**Yes**	o No longer than 70cm in diameter and 50cm in height o A maximum of four entrances, with each measuring no more than 10cm in any dimension o Size of the mesh must be no more than 25mm (if not made of rigid material)	
Seine nets (bait or drag nets)	**Yes**		o Must not exceed 16m in length and 3m in drop and mesh size should not exceed 28mm o The net must not contain a bag, pocket or anything similar and it must not be anchored, staked or fixed o The net should not be out of the water when containing fish other than to remove and release them o All regulated fish not intended to be taken should be released into deep enough water to allow escape o Remove fish around the periphery of the net first o Active trapping technique	The net is anchored at one end on the shore and the free end brought around in a semi- circle where the two ends are joined and the net slowly pulled into the shore, whilst herding the fish into the centre (bunt) of the net Useful for all types of study
Shell dredges	**Yes**		o Mouth of the dredge must not exceed 60cm across o Teeth should be no longer than 7.5cm o Conditions should be checked further in the Fisheries (General) Regulations 2019	
Spearfishing and spear guns	**Yes**	**Yes**	o A power head is not permitted unless in defence against a shark o You are not able to hunt barramundi between 6pm and 6am	
Tropical rock lobster fishing gear	**Yes**		o Free-diving using a mask and snorkel and a rubber-powered spear or spear gun is the only form of breathing and equipment apparatus permitted	
Worm digging	**Yes**		o To be used on foreshores for collecting worms only	

The use of other equipment for fish capture or changes to any of the methods as described above should be arranged through a general fisheries permit.

References

- Gill, H, Ashton, C & Rowland, A, 2017, Working towards the development of best practices in fish and fisheries research or The troubles with fish and fish biologists!, viewed 27 November 2019, https://anzccart.org.nz/app/uploads/2017/06/gill-ashton-workin.pdf
- Queensland Government, 2019, Recreational fishing rules and regulations guide, viewed 20 November 2019, https://www.qld.gov.au/recreation/activities/boating-fishing/rec-fishing/rules/equipment

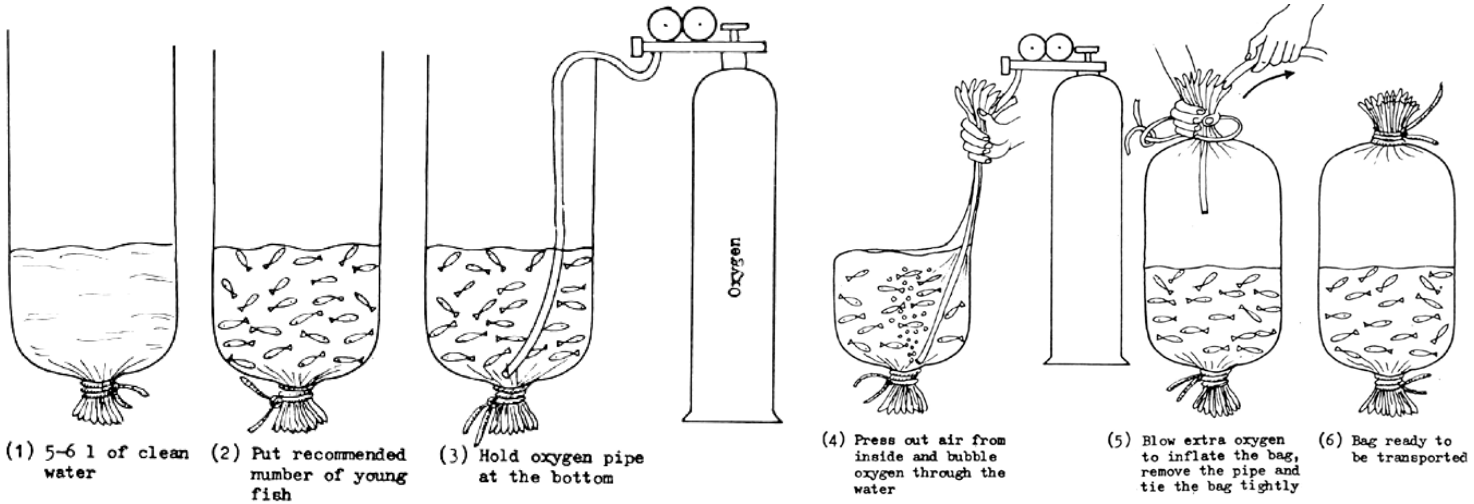
Fish size and possession limits

Please refer to the Department of Agriculture and Fisheries website for current fish size and possession limits.

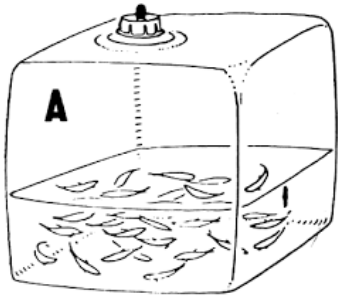
Appendix 2.10: Transportation containers

Closed systems

Type of container	Specifications	Procedure	Animal welfare considerations
Polyethylene bags	Double lined bag with thinner, softer layer internally and harder thicker outer layer.	1. Before putting fish (especially fry) into the bags, the procedure of catching, counting and distributing the fish to the bags should have been thoroughly prepared to allow an efficient transfer operation. 2. If ice is to be used as a cooling mechanism it should be placed inside plastic bags and laid on the bottom of the insulated (Styrofoam) transport box at this time. As a rule, the volume of ice placed under the bag with transport water is usually 10-20% of the transport water. Place a protective cover over the ice such as foam to avoid direct contact with the fish. 3. Place the 'two layered bags' into the insulated transport box and fill the bag with 40-50% original water from fish's housing tank ensuring that no contaminants such as faeces are included. 4. Add the fish carefully by holding around the bag edges to ensure that fish do not spill out. 5. Displace the air from the space above the water, and then hold the neck of the bag fairly tightly, whilst slowly introducing the oxygen tube inside the bag to create a good manual seal during the filling process. Use technical oxygen via a regulator valve to fill the bag (usually 50-60% of the bag's volume), until it feels like a filled balloon, and when pressed with a thumb, the bag returns to its original position rapidly. Note: There is currently research being undertaken in Japan, involving transport bags that can produce their own oxygen supply, which requires a smaller volume of water during transportation whilst maintaining good oxygenation. These may be commercially available soon. 6. Quickly withdraw the oxygen tube and twist the neck of the bag to prevent oxygen from leaking and to produce a slight overpressure (by reducing the bags volume a little). Note: bags transported horizontally should have a pressure of 0.05-0.06 MPa compared to bags transported vertically which have a pressure of 0.02-0.04MPa. Also, slightly less oxygen is added to the bag for air transportation. 7. Secure the bag at the top with a tie such as two rubber bands or string. The ends can also be heat welded closed for security from water loss following the tying process, but this makes monitoring more difficult. 8. Pack the bags closely into the insulated containers for protection from tearing and better temperature control.	Padding (e.g., newspaper) should be provided between the two layers of the bag for spiny fish species to prevent double layer punctures Polyethylene bags are considered a good method of transport especially for smaller fish species Allows easy transportation of individual territorial/aggressive fish. There are some specially designed transportation bags that incorporate an oxygen port This adaption facilitates oxygen top up procedures during transportation, which increases survival rates for young fish

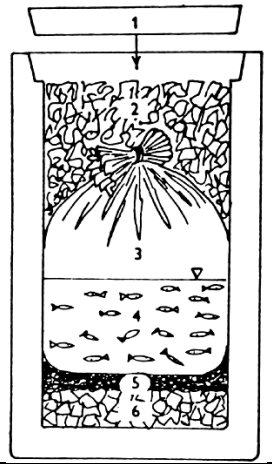


Procedure of filling the bag with water, stocking with the fish, displacing the air, introducing oxygen and closing the upper end (Image by Woynarowich and Horváth, 1980)



Picture of a transport bag with incorporated oxygen supply port

(Image by Woynarowich and Horváth, 1980)



Right: Transport of a bag in a Styrofoam insulated transport box

1 - lid,
2 - insulation filling,
3 - oxygen atmosphere,
4 - water with fish,
5 - insulation lining, e.g., foam rubber,
6 – ice

(Image by Vollmann-Schipper 1975)

Type of container	Specifications	Procedure	Animal welfare considerations
Transport tanks	Vary in complexity from plastic rectangular tanks (such as Pentair holding tanks) to complex pieces of machinery with complete biological, aeration and refrigeration systems (such as Stackpac bins or Austmarine Oceantronic© live transport tanks). The 'Stackpac bin' with its associated 'Fishpac' oxygen regulator system is the only system of its kind available for air freight. A comprehensive operations manual is available for this choice of transport tank due to its clearance for use during air transportation and use of oxygen cylinders whilst flying. Austmarine Oceantronic© live transport tanks are available in various sizes. They are a fibreglass double skinned, vacuum sealed unit with all its equipment located in the sub frame and is powered by a generator and for road or rail transportation.	1. Choose a quality transport tank suitable for the fish species within the project. Consideration should be given to the filtration, aeration and temperature control systems which should ideally be inbuilt. Consider those systems that can support a healthy water quality even during periods without power in case of emergencies. 2. Before putting fish (especially fry) into the tanks, the procedure of catching, counting and distributing the fish to the tanks should have been thoroughly prepared to allow an efficient transfer operation. 3. Tanks need to be preloaded with water to ensure the tank water parameters match those of the fish's normal environment. The water should be monitored for at least 24 hours prior to introducing the fish. This will test out the inbuilt motor system, inbuilt biological filtration system and carbon filters. Tanks such as the Austmarine Oceantronic brand have inbuilt alarms for water parameters such as temperature, oxygen flow and water levels which makes monitoring easier during transport. Ensure these water parameters inside the tanks are communicated to the future fish housing facility prior to transportation. 4. Load the fish gently to the recommended density for the species and developmental age of the fish according to the manufacturer's guidelines. Allow a period of acclimatisation prior to transportation to reduce fish stress. 5. Consider that a fish's body will absorb water through osmosis (approximately 10% of its body mass), so this should be considered during extended transportation where water may need to be topped up. 6. Many tanks have their own designated trailers or loading equipment/support systems in the case of airline transportation and should always be used. 7. Use sedation only if required to reduce stress during transportation but assess the fish prior to closing the tanks for travel. Note: some sedative drugs such as MS-222 are powder based and can clog up filtration systems of these tanks, rapidly reducing water quality during transportation. 8. Apply minimum handling of fish procedures during transfer to permanent housing tanks by using appropriate 'hoppers' and hoses for the fish species in the project. 9. Investigators should consider what actions they would take in case of emergencies during transportation, and how they would alleviate any animal welfare issues if transport tanks had mechanical failures (e.g. back up polyethylene bag system or other). **Reference:** Pers comm, 2020, with Michael Hanrahan from Austmarine Gold Coast, QLD.	The system must have a continuous oxygen flow at 3 litres per minute into an insulated tank. Simpler holding tanks will require a separate aeration system. All tanks should have a thermally insulated double layer construction to allow for better temperature control of the water during transportation. Tanks have smooth surfaces to prevent trauma to the fish. Features of the tanks to consider when assessing animal welfare include: Aeration grates, double bottomed containers, filters and water distributors, separate aerators and drainage valves. Hoppers and associated hoses are built into some units with a removable gutter for releasing the fish into their permanent housing tanks. These can be adjusted to suit the size of the fish, such as: o 20-30cm for fry o 30-40cm for fingerlings o 50-60cm for large fish of 1kg in weight

Examples of live fish transportation tanks available in Australia.

Pentair holding tanks

Austmarine Oceantronic© live transport tanks for road transport.

Stackpac live fish transport for air freight.

References

- Berka, R, 1986, *The transport of live fish A review*, European inland Fisheries Adbvisory Commission (EIFAC), viewed 20 November 2019, http://www.fao.org/3/af000e/af000e00.htm
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia

Appendix 2.11: Fish arrival health assessment sheet UniSC

(Intervention point score……/Humane end point score…….)

AEC application number:		**Chief investigator & contact details:**	/
Housing facility number		**Investigator details & contact details:**	/
Number of animals start		**Animal carer details:**	

Distant visual examination

Animal welfare parameter	Normal expected result	Score	Notes	Is intervention or humane killing required?
Swimming pattern and ability	Swimming throughout the tank with non-erratic movement, exploring all enrichment facilities at all levels in the water table.			
Alertness to stimuli	Fish rush to hiding places when investigator approaches the holding tank or goes to normal feeding location if comfortable with the animal carer.			
Equilibrium in water/balance	Fish able to hold body with dorsum pointing towards the water surface. Fish able to remain underwater without effort.			
Scale condition	Scales are shiny and intact without evidence of trauma or infectious lesions (such as fluffy white patches or bleeding). Scales are a consistent colour according to their natural species appearance. Lateral line scales are evident.			
Colour of body	Normal uniform colour/pattern for the species (devoid of pallor, darkness or blotchy patches). No external indicators of internal haemorrhage/bruising.			
Defaecating	Normal amount and colour for the fish species.			
Breathing rate and pattern	Breathing rate is steady and undertaken under water. (No gasping especially around air inlets or at the surface of the water).			
Body symmetry	Body appears even on both sides when viewed from above. Free from lumps and swellings at any angle.			
Feeding pattern	All fish interested in food and equally eating within 5 minutes of food being offered.			

Direct/close examination during transfer to future housing facility

Animal welfare parameter	Normal expected result	Score	Notes	Is intervention or humane killing required?
Gills/operculum	Normal colour for species, fine gills (devoid of thickening/hyperplasia/fusion, inflammation and excessive mucous).			
Ocular	Eyes bright, non-bulging and no lacerations or lesions. No opacity or 'popeye' noted.			
Skin/scales	No lesions, signs of rubbing, abrasions, or obvious parasites.			
Fins	Fins are in normal position (not bent or misshaped) and all being used for swimming. No lacerations or lesions noted.			
Vent/anus exam	No inflammation, swelling or trauma			
Mucous	Mucous is present and the amount appropriate for species (during both the day and night). No excessive sticky mucous or malodourous mucous especially around the gills or on scales.			
Skeleton	Spine is straight without abnormally shaped deviation.			
Other				

Other notes

The scoring system to identify animal welfare intervention points and humane end points is specific to each fish species, but as a guide the following criteria could be considered.

Monitoring parameters	Assessment method & monitoring level	Clinical signs to consider	Scoring system		
			Normal (0)	Moderate (1)	Severe (2)
Swimming pattern and ability	Visually assess	Erratic rapid swimming, no swimming, hiding near base of tank, uncoordinated swimming, flashing, lethargy.	Swimming throughout the tank with non-, exploring all enrichment facilities at all levels in the water table.	Erratic movement, rubbing on side of tank, uncoordinated, reduced movement.	Floating near water surface, flashing, jumping out of water, minimal movement.
Alertness to stimuli	Alert fish and visually assess	Startled by minor movement or no alertness or reaction to external stimuli.	Fish rush to hiding places when investigator approaches the holding tank or goes to normal feeding location if comfortable with the animal carer.	Fish dart from one hiding place to an another (overreact) or slow to react or get away from stimuli	No reaction to external stimuli, unable to respond
Equilibrium in water/balance	Visually assess	Floating and unable to dive/submerge for normal periods of time for feeding or investigating tank.	Fish able to hold body with dorsum pointing towards the water surface. Fish able to remain underwater without effort.	Fish struggling to remain in a normal upright position. Fish repeatedly dive but always gradually float back to the surface.	Fish unable to die and float at the surface. Body floats on a diagonal or upside down.
Scale condition	Visually assess and close up examination including skin scrape and sampling of mucous	Trauma lesions, bleeding, bite marks, ulceration, 'fluffy white' lesions, rub marks.	Scales are shiny and intact without evidence of trauma or infectious lesions such as fluffy white patches or bleeding. Scale colour is appropriate for species and lateral line scales are evident. No obvious parasites.	Some scales show trauma, rubbing injury, lesions, changes in colour. Increase or loss in mucous in affected areas. Mild bouts of skin rubbing, protruding scales or a few missing.	Excessive abnormal mucous. Large non healing ulcerative multifocal lesions. Fish constantly rubbing.
Colour of body	Visually assess	Body appears very pale, or dark or blotchy which is abnormal for the species and not associated with husbandry changes such as light intensity.	Normal uniform colour/pattern for the species (devoid of pallor, darkness or blotchy patches). No external indicators of internal haemorrhage/bruising.	Fish colour has changed in limited areas of its body but no other signs of ill health-pale, dark or blotchy. Some mild signs of internal bruising noted.	Large portions of body changed in colour and other signs of ill health noted. Signs of bruising under the skin more obvious and greater coverage.
Defaecating	Visually assess	Amount is increased, changed colour (without diet change), contains blood. No faecal production whilst still eating.	Normal amount and colour for the fish species.	Faeces becoming more frequent, changed in consistency or colour for 1-2 days.	Faeces seen to contain blood and be excessive in quantity. No evidence of faecal production for several days.

Monitoring parameters	Assessment method & monitoring level	Clinical signs to consider	Scoring system		
			Normal (0)	Moderate (1)	Severe (2)
Breathing rate and pattern	Visually assess	Rate is increased. Gasping at surface or near air inlet.	Breathing rate is steady and undertaken under water.	Staying around air inlet. Respiration rate mildly increased.	Gasping at surface or near to air inlet. Changes to gill health also noted.
Feeding pattern, weight loss, loss of condition	Visually assess	Fish not feeding in first 5 minutes of food being offered. Weight loss. Losing body condition.	Fish feed immediately, (may even be waiting at feeding point at normal feeding times).	Feeding amount reduced mildly, but there is still some feed intake. Mild weight loss <5%. Mild changes in muscle mass.	Fish stops eating completely and does not show interest in the feeding zone during feeding time. Weight loss >5%. Loss of muscle tone.
Gills/operculum/nares	Close examination, biopsy	Gills are red, inflamed and lamellae are thickened and covered in excessive mucous.	Normal colour for species, fine gill lamellae based on the species.	Gills becoming mildly red and thickened. Mucous amount has increased, and appearance has mildly changed.	Gills are severely inflamed and fused in areas. Lamellae are greatly thickened, and mucous is thick, sticky and voluminous. Air bubbles evident in capillaries of gills. Operculum is flared covering the gills.
Ocular	Close examination,	Eyes are 'popping' out of eye socket or sunken. Lacerations, swelling or redness noted. Cornea is opaque. Air bubbles in eyes.	Eyes bright, non-bulging from eye sockets and normal size for the species.	Mild trauma or inflammation. Eyes mildly bulging from sockets. Cornea becoming slightly discoloured/opaque.	Corneal ulcerations. Lacerations to eyelids. Air bubbles in eyes. Popeye/exophthalmos. Corneas are white in colour/cataracts.
Fins	Palpation and visually assess	Bent fins. Non-functioning fins. Trauma/lacerations to fins including bite wounds. Ulcerative lesions or fluffy growth on the fins surface.	Fins are in normal position and all being used for swimming.	Minor non-infected bite wounds or lesions noted. Fins are mildly misshaped but still being used. Fins are clamped or frayed. Air bubbles evident in capillaries of fins.	Fins are distorted and held limply or severely clamped. Deep lesions or ulcerations noted. Large areas of trauma or sections missing that are affecting swimming ability.
Vent/anus exam	Visually assess	Lacerations, inflammation, gut or urogenital prolapse. Coelomic distention.	No inflammation, swelling or trauma	Mildly inflamed, swollen appearance.	Prolapse of gut or urogenital tract. Severe swelling or lesions noted.
Mucous	Visually assess and sample	Excessive mucous for species. Colour or consistency change. Mucous is congregating around gills, other body parts or close to lacerations in the scales.	Mucous is present and the amount appropriate for species (during both the day and night).	Mild increase in amount of mucous in certain areas of the body.	Mucous has greatly changed in appearance. It is excessive, sticky and tenacious.
Skeleton	Visually assess and palpation	Spine is deviated, bent or generally misshaped. Fish is unable to swim in a straight line.	Spine is straight with fish swimming normally.	Slight deviation in spine, but fish swimming patterns is only mildly changed.	Spine obviously bent or deviated. Fish unable to swim in normal directional movement.

Appendix 2.12: UniSC Animal Record Form – Research and Teaching

ANIMAL RECORD FORM – Research & Teaching

Please complete upon animal's arrival of at UniSC

Animal ethics approval number:	
Chief Investigator name:	
Contact phone numbers:	
Animal carer name:	
Contact phone numbers:	
Scientific name of animal:	
Common name of animal:	
Number of animals approved by Animal Ethics Committee for use:	
Number of animals received:	
Date of arrival:	
Approved length of stay (hrs, days etc): (include dates for housing in faculty)	
Source of animals:	
Transportation to UniSC:	
Condition on arrival: Please see health assessment forms attached	
Physical location of housing:	
Any movement between locations:	

Animals were satisfactorily delivered with no adverse findings

Yes No

Chief Investigator (signature)
_____ Date _____

Animal Carer (signature)
_____ Date _____

Appendix 2.13: Attention access restricted door signage

Attention access restricted

Only authorised AEC approved investigators or animal carers may enter this room.

From:……To:……

Contact person:……

Ph:……

3. Housing

3.1 Scope

This section relates to organising and managing the housing needs of fish within the laboratory environment.

3.2 Background information

When investigators are writing their AEC application, they should provide as much detail as possible regarding housing. Substandard fish housing along with poor husbandry practises are the two most common factors affecting fish health and welfare in any fish facility, and in both cases these are easily avoidable and unacceptable. Section three will help guide the investigator with regards to what is considered essential information to be included within the housing section of the AEC application, because each fish species will have very specific conditions required to ensure the maintenance of best practise animal welfare standards during their time in a scientific project. The use of photographs showing facilities will be useful in this section of the AEC application.

3.3 Equipment and resources

Housing

Suitable housing facilities (tanks, ponds, sea cages) for the fish species, fluorescent lighting equipment for indoor tanks, room and water thermometers, heating and cooling systems, water filtration equipment, water supply (fresh or sea water), dichlorination equipment (if using municipal water), vibration pads for machinery, tank and aquaculture room cleaning equipment and chemicals and storage facility, separate food storage and preparation areas, suitable floor drainage and waste water processing and back-up generators for power cut emergencies.

Paperwork

Monitoring sheets, cage identity cards, ‘Authorised Access only’ sign, AEC approval letter and a copy of the latest version of the ethics approved application including amendments.

PPE

Safety goggles/glasses, laboratory coat/overall/apron, enclosed shoes/waterproof boots, nitryl unpowdered gloves, first aid box with eye rinsing products. Hand washing facilities.

3.4 Recommended procedures

Fish housing facility preparation the day before fish arrive

Knowledge regarding the quality of water from where the fish are coming from (such as temperature, pH, salinity levels and hardness) should be duplicated in the future housing tank initially and changed gradually if required. Pre-conditioning of water and biofilters, alongside testing of all tanks, water recirculation equipment and facilities should have been undertaken prior to the arrival of aquatic animals. Note: Only fish deemed as healthy and having successfully completed the quarantine process should be allowed into the permanent fish housing facility.

Housing tank identification

Attach a laminated cage card (Appendix 3.12) to each housing facility with the following details:

- animal's identification
- number of animals
- tank/pond/sea cage number
- AEC approval number
- investigator responsible for the project and their details including emergency contact numbers.

Note: A permanent marker should be used in aquaculture rooms to ensure the chart details are not erased in the water-based environment.

Aquaculture room/housing facility design and maintenance

- The housing facility should be designed to allow flexibility for the various fish species' requirements of each project.
- There should be numerous floor drains appropriately spaced for each area. The floors should be free from unnecessary equipment to aid in drainage with the cleaning process and in cases of flooding.
- The floor should be constructed from a material impervious to water but also slip resistant. The walls should be designed to be easily cleanable to avoid mildew and mould build up.
- Ground-fault interrupted electrical connections should be fitted for animal and personnel safety.
- Logbooks to schedule general maintenance and calibration of equipment should be available and kept up to date according to the manufacturer's instructions. These logbooks should be ready for viewing by the AEC and AWO during an inspection.
- A separate room or a partitioned specifically designated area for food preparation, food storage (fridge/freezers) and cleaning of food equipment must be available. It should be:

    - appropriate for the food type being stored (dry store, fridge, freezer)

  - easy to clean and maintain
  - free from pests
  - not used for storing any other non-food items.

- A separate room/cupboard/partitioned-specifically-designated area for general room cleaning products and equipment must be available. It should:

  - be away from food or laboratory testing/procedural areas
  - be close to a first aid box in case of accidental exposure to toxic/irritating substances
  - contain the required PPE needed for the chemical handling process
  - have ventilation to allow any chemical fumes to dissipate safely
  - contain safety data sheets for any chemicals stored in the room.

- General room lighting should be adequate to provide a level of luminescence suitable for a safe general working environment. The lights should be non-flickering and quiet.
- There should be good ventilation and a clean maintained air conditioning system that supports 15 air changes per minute to allow drying out of equipment after cleaning and prevent the growth of mildew and mould.
- Room temperature (20-24^{o}C) and humidity (30-50%) should be kept at a comfortable level for the personnel working in the environment, because the fish tanks will have their own inbuilt heating and cooling mechanisms to suit the fish species being housed.
- There should be sufficient room to work safely around and between each fish housing tank to prevent injury to personnel and to ensure access in case of a fish emergency.
- There should be a back-up power system in case of electrical power cuts to ensure aeration, filtration and temperature control systems are always maintained.

Fish housing tanks

Aquaculture/laboratory fish housing tanks

- Fish tanks, valves, delivery lines and drains should be constructed from materials including glass, type 316 stainless steel, nylon, fluorocarbon plastics, concrete, polyethylene sheeting, rigid PVC, Teflon and fibreglass.
- Brass, copper, lead, cadmium, zinc and rubber should be avoided, as should any corrodible substance in any part of the fish holding facility. The latter point is particularly relevant in saltwater fish systems.
- There should be spare clean housing tanks always available in case of emergency spillage from other housing tanks.
- All housing tanks should be made from opaque coloured material or have a coating to make them opaque. This opacity will allow some light to penetrate but will reduce the disturbance from outside factors such as personnel moving around the room.
- The housing tank should be sufficient in its size, shape and depth for the fish species (including any grow out size) to enable them to move around freely and perform as many of their natural behaviours as possible.

- Fish tanks should be designed to enable easy cleaning with the minimum of disturbance to the fish.

Large scale fish cage housing

With the expansion of the aquaculture industry for food production, the use of fish cages either within river systems or at sea may be an increased area of research for UniSC students. These facilities are purpose built and rely on the natural flow of water within the environment to maintain the overall water quality. However, they are associated with potential animal welfare issues and should therefore be described in detail when writing an animal ethics application.

Water exchange systems

Investigators should explain in detail which system they are using for their project, why they have chosen the system, and how it will benefit the welfare of their fish species.

There are three basic systems of water exchange/circulation as follows:

1. Static systems (ponds)
    - Do not receive a continuous water flow.
    - They rely on low stocking densities and biomass.
    - Most rely on natural processes within the system to maintain water quality.
    - Most are aerated with periodic additions of water to replace that lost by evaporation and seepage.
    - Water exchange is achieved by overflow pipes or manual draining and then replacement with clean water.

2. Flow through systems
    - A single pass of water which goes through the tank and is used only once.
    - Water levels are maintained by a balanced input and output of water.
    - Water inflow is designed to concentrate solids near the drainage or outflow area by creating a circular flow pattern.
    - Out flowing water leaves the tank by the overflow and the water is drawn from the bottom of the tank creating a self-cleaning system.
    - These systems rely on a constant water supply (fresh or saltwater) so are normally situated next to a natural water source such as the ocean or a river.
    - Water must go through a ‘continuous treatment plant’ prior to entering the fish tanks. This will ensure the water quality is consistent. The treatment may involve the use of inline filters (e.g. carbon), chillers or heaters, UV disinfection and dichlorinators. Their use will differ depending on the needs of each fish species and the equipment available at the fish housing facility.

3. Recirculating aquaculture systems (RAS)

    - Tank-based recirculating systems can be used for a range of purposes in aquaculture and allow flexibility across fish projects.

- The basis of a RAS is the process of re-conditioning and then re-using the tank water through a means of:
  - mechanical filtration to remove solids, such as micron felt filter bags
  - biological filtration to convert ammonia to nitrite and nitrate
  - foam fractionation where air is bubbled through a column of water to trap and remove organic particles.
- They require a pure oxygen injection to increase the dissolved oxygen concentration.
- The process of ozonation or UV filtration is included in reticulating systems to reduce the pathogen load.

Information on biofilters

The biofilters must be managed as a living breathing organism. They take time (1-3 months) to initially establish depending on the nutrient level supplied, temperature of the water and water flow characteristics of the system. There are commercially available bacterial preparations and associated nutrients available to speed up the biofilm establishment process. Care must be taken not to destroy the biofilter with chemicals used to treat fish such as formalin or to starve it of nutrients. It requires regular monitoring especially when fish stocking densities are altered in the tank.

Seawater systems – the maximum nitrification capacity of a biofilter is lower in saltwater systems compared to freshwater systems and therefore the biofilm takes longer to naturally establish. There are adaptations available for freshwater biofilter systems which can speed up the process in saltwater systems, but consideration should be given to this time delay during the planning of the acquisition process to ensure the system is ready and has been thoroughly checked prior to the delivery of fish for a scientific project.

Water replacement

Static ponds

Water should be topped up as it evaporates and should generally not be siphoned off unless there is a serious toxic event such as an algal bloom (explained in section 9 of these guidelines).

Flow through systems

Water replacement is delivered in a constant flow.

Reticulating aquaculture systems

Water changing in this system is based on three factors:

- Where regular ongoing maintenance of water quality affords a water exchange of between 10-15% per week as a standard procedure.
- When water quality has been monitored and has identified three consecutive days of dropping water quality, on top of the normal ongoing water exchange maintenance procedure.
- Where fish have become sick or died in the tank.

Water of the same volume (to be added) should be siphoned out to waste and clean water slowly added. The water should be of a similar temperature and have similar water parameters to the tank water. If the new water is greatly different, it should be added very slowly over several hours to allow a gradual equilibration of ≤ 2^{o}C/hr. Water parameter testing should be performed once the operation is complete.

Freshwater supplies

All freshwater supplies should have been treated prior to its use to remove the chlorine and chloramine if municipal water has been used, because these are toxic to fish. The best methods available to investigators to clear both chlorine and chloramine are using:

- a UV steriliser system
- a carbon-based filtration system or
- a chemical treatment such as sodium thiosulfate (according to the manufacturer's instructions).

Other methods such as aeration and allowing the water to sit outside for 24 hours are less reliable and will only remove the chlorine component.

Saltwater supplies

Seawater will ideally be supplied onsite either directly through pipes (if based alongside the coast), or if the facility does not allow for this approach, seawater should be supplied in bulk and stored in containers ready for use. The production of artificial seawater requires careful blending of all essential elements, correct mixing, appropriate storage and pre-treatment with algicides and disinfectants prior to use. Incorrect production of artificial seawater can have severe animal welfare issues and affect scientific data and should therefore be avoided in scientific projects wherever possible.

Water turbulence and velocity

Turbulence is generated by the aeration or water inflow system within the tank environment, which is necessary for the oxygenation process and to keep solids suspended in the water column. Fish normally experience water turbulence and velocity in their natural environment but depending on the fish species or the fish's development stage, they may seek shelter as required at different stages of turbulence. For example, fish larvae less than 10 days of age require very low levels of turbulence so that they can still reach the water's surface to inflate their swim bladders.

In an artificial tank environment where water velocity is under the control of the investigator, it is important to ensure that the species of fish being held are generally able to hold their position against the current and to ensure the flow is adjusted as required. This monitoring check should be undertaken when initially setting up the housing facility to prevent exhaustion and stress in the fish.

Water temperature

Fish are poikilothermic animals, relying on the water temperature to set their body temperature. Water temperature affects the fish's bodily functions such as metabolism, digestion, growth, sexual maturity and reproduction. Each species will have its own preferred water temperature range and therefore tank water should remain in this zone **at all times**. Any deviation out of this range will cause undue stress and lead to a lowered immune system, poor health, decreased growth rate and death if allowed to continue. Sudden changes in water temperature as small as 5^{o}C can cause stress responses in fish that are otherwise healthy.

Rapid increases in water temperature are particularly detrimental to the fish because they become more active, consume more food, use more oxygen and grow faster (to a certain degree) even if they are still within their preferred 'temperature range'. Dissolved oxygen saturation decreases in higher temperatures and increased food intake produces greater faecal output and increased water contamination. Cooler temperatures in comparison, tend to send the fish into a torpor state.

Most experienced investigators do not routinely expose fish to temperature changes greater than 2^{o}C per day. If water temperature changes are unavoidable or required for a procedure in a project, the maximum rates of change of ≤ 2^{o}C/hr to a maximum of ≤ 10^{o}C/day should be used as a guide.

Fish tank illumination

Indoors

The photoperiod and light intensity are important housing considerations for fish in an aquaculture/laboratory environment and requirements vary with different fish species.

Period of light

Most species will do well with 12/12 hours light and dark cycle, but 8-10 hours of light per day is generally acceptable for most fish species. The exception to this rule is that of tropical fish which require 12-14 hours of light on average per day. It is important to gradually fade off or on the light in a dusk and dawn set up, with a timer that turns a low light on for 30 minutes prior to making the full change of lighting intensity. Instantaneous changes (flicking a light switch) may cause a panic reaction in the fish leading to distress.

Quality of light

Fluorescent light is the most common type of light used in indoor fish tanks. The specifications for the light quality directly over the fish tank are:

- an intensity of 10-12,000 lux of full spectrum lighting
- a colour temperature of 5000-7000 Kelvin
- a peak wavelength ranging from 475-650 millimetres.

Outdoor lighting

Fish can become sunburnt when they are exposed to bright sunlight especially in clear shallow water. Fish tanks should have an area of shade, suitable hiding places and deeper areas of water into which the fish can retreat if needed.

Grouping and fish density

Overstocking/overcrowding is a key welfare issue within fish facilities. The optimal stocking density varies due to several factors including:

- the type of housing tank
- the species of fish
- the size/age of fish
- the water quality.

The most immediate issue with overstocking is the maintenance of a supply of dissolved oxygen and elevation of water temperature followed closely by the accumulation of waste products, especially ammonia. Therefore, generally stocking levels can be slightly higher in flow through water exchange systems. New advances in the filtration systems of a reticulating aquaculture system have also allowed stocking density levels to increase compared to static pond systems, which allow the lowest level of stocking.

The following table provides a basic guide to stocking density within tanks, cages and ponds for fish species typically used in the food aquaculture industry.

Housing type	Optimal	Upper density (Requires greater control of water quality)
Tanks	10kg/m^3	100kg/m^3
Cages	20kg/m^3	100kg/m^3
Ponds	5t/ha	20t/ha

When planning a fish project, it is important to research the ideal stocking density for that specific fish species. Some fish prefer a higher density and others a lower density, with animal welfare issues associated with each. If the stocking information is not available for a particular fish species, then investigators should

research the natural grouping patterns found in the fish's wild/natural state and use this as a guide to setting up the tanks. This grouping pattern is particularly important for more aggressive species of fish.

The Food and Agricultural Organization of the united nations has some useful information on the best stocking density levels for certain species of fish at http://www.fao.org.

Close monitoring of water quality, fish behaviour and health will help to identify any potential issues with overstocking, which should be addressed immediately by incorporating back up tanks in the laboratory to relocate fish if needed.

Protection from external predators

The presence of external predators will cause undue distress to the fish as well as potential stock losses. Therefore, fish holding facilities should be designed to prevent both fish escape and to exclude predators. Outside tanks should be covered by predator exclusion nets and consideration should be given to acoustic deterrents and fencing on outer perimeters wherever possible.

Housing enrichment

Enrichment strategies should be used to make the captive breeding, growing out or experimental/testing environment, stimulating allowing fish to perform their natural activities and behaviours. The enrichment strategies must therefore be species specific. Examples of enrichment for laboratory held fish include changes to the environment, social interactions and diet.

Environmental based enrichment:

- changing structures within the tanks such as new hiding places with varied shapes
- adding novel items to encourage exploration such as aquatic plants
- moving long term items to new positions
- changing background colours
- making changes in water flow direction
- periodic changes in the photoperiod or intensity of lighting
- addition of suitable substrate to encourage foraging.

Social interaction-based enrichment:

- Ensuring the tank is as natural as possible for socially interacting species of fish, to encourage normal behaviour.

Dietary enrichment:

- use alternating forms of food such as pellets, flaked food, frozen or live foods
- change feeding times
- placing feed in different parts of the tank or holding facility.

Managing waste from 'controlled disease research' holding facilities

- Access must be restricted to all unauthorised personnel by use of signage and security access passes.
- Housing facility units must be isolated and self-sufficient in all aspects of equipment and resources.
- Physical barriers must be in place with sufficient capacity to prevent overflow of any water in the emergency case that all the tanks are emptied in a single event.
- Appropriate cleaning chemicals and techniques must be used depending on the nature of the pathogen within the research project.

3.5 Animal health and welfare considerations

Poor water quality

This is the most important animal welfare consideration for fish, which can rapidly cause stress, ill health and mass death of fish if it is not monitored and managed daily.

Distress from predators

Fish houses outside are particularly vulnerable to predatory animals such as birds and domestic pets.

Distress from noise

Excessive human noise – talking, moving equipment, walking by tanks, machinery noise and general vibrations can cause high levels of stress in fish. All personnel should avoid any unnecessary noise and activity around fish tanks.

Equipment noise – all equipment and machinery should be placed on vibration reducing matting and be maintained and calibrated according to a regular manufacturer's schedule.

Overstocking/overcrowding

Overstocking or inappropriate grouping will lead to fish being stressed, having poor health and poor ability to thrive.

Emergency situations/power outage

Loss of power, especially in tanks with increased stocking density, can be a major animal welfare event. All fish housing tanks typically used in scientific projects require power to operate aeration and filtration systems to maintain good water quality at all times. Any power outages will require back-up generators and increased monitoring of water parameters as required to ensure water quality does not deteriorate to unsafe levels.

3.6 Reference and acknowledgements

References

- Bayne, K & Turner, P, (eds) 2013, *Laboratory Animal Welfare,* Academic Press, Cambridge, Massachusetts
- Department of primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW, viewed 14 January 2020, https://www.dpi.nsw.gov.au/data/assets/pdf_file/0004/638680/ACEC-Guide-2015-FINAL-WITH-AQUI_S-2.pdf
- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- National research council of the national academies, 2011, *Guide for the care and use of laboratory animals Eighth Edition*, viewed 23 December 2019, https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, *Guidelines for the Care and Use of Fish in Research,* Institute for Laboratory Animal Research, vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159
- Victorian Fisheries Authority, 2008, *Best Practice Environmental Management Guidelines for Recirculating Aquaculture Systems,* viewed 17 December 2019, https://vfa.vic.gov.au/aquaculture/publications/best-pratice-environmental-management-guidelines-for-recirculating-aquaculture-systems

Further reading

- Moe, MA, 1992, *The marine aquarium reference: Systems and invertebrates*, Green turtle publications, Plantation, Florida
- Food and agricultural organization of the Nations European inland fisheries advisory commission, 1994, *European inland fisheries advisory commission report of the third session of the working party on stocking*, viewed 23 December 2019, http://www.fao.org/3/T2735E/T2735E05.htm

3.7 Other information

Appendix 3.8: Cage identity card

Do not touch the animals or their holding facility

Chief investigator

Name:_____ _____

Work hours phone number: _____

After hours phone number:_____

Investigator

Name:_____

Work hours phone number: _____

After hours phone number:_____

Animal carer

Name:_____

Work hours phone number: _____

After hours phone number:_____

4. Husbandry

4.1 Scope and background information

This section relates to organising and managing the husbandry needs of fish within scientific projects in aquaculture/laboratory environments.

When investigators are writing their AEC application they should provide as much detail as possible regarding the husbandry factors of their project. This section is critical to both the maintenance of fish welfare and health in scientific projects, because the majority of the husbandry requirements of fish are species specific.

The investigator should take the time to research the husbandry needs of their fish species and use this section of the guidelines to ascertain the level of information required on the AEC application.

Husbandry is a key part of maintaining best practise animal welfare standards.

The use of photographs showing equipment and methods will be useful in this section.

4.2 Equipment and resources

Diet and nutrition

Appropriate varied diet for the fish species, cold room/refrigeration/freezer/dry store for food storage, designated food preparation equipment, containers and scales, automated feeders.

Water parameter monitoring

Monitors to measure carbon dioxide, salinity, dissolved oxygen, alkalinity or carbonate hardness (KH), general hardness, hydrogen sulphide, ammonia, nitrite and nitrate, temperature, suspended solids, turbidity and heavy metals.

PPE

Safety goggles/glasses, laboratory coat/overall/apron, enclosed shoes/waterproof boots, nitryl unpowdered gloves, first aid box with eye rinsing products. Hand washing facilities.

Cleaning

Specific aquaculture cleaning and disinfecting products. Cleaning cupboard. Wet floor signs. Mop buckets and cleaning mops, skimmers.

4.3 Recommended procedures

Diet, nutrition, feeding regime and fasting

Fish are one of the most efficient animals to convert food nutrients into body tissues. Proteins make up 60-70% of fish tissue on a dry weight basis. They rely on proteins and fats to provide their energy requirements and therefore diets high in carbohydrates are not recommended for good growth and good health in fish.

Diet and nutrition

- The food fed to the fish should be specific for the species and should be nutritionally balanced. Essential amino acids, fats, vitamins and minerals must be in the proper ratio to ensure a well-balanced diet.
- Food should be palatable.
- The commercially formulated diets must also be pelleted and processed in such a manner that they are durable, are of the correct size for the fish species, and are water stable with a minimum quantity of fines.
- Most commercially available feed stocks can be adapted for each specific fish species and varied according to their stage of development, therefore manufactured diets may be useful for scientific projects.
- Where whole, natural, or unprocessed fish are to be fed, they should be stored in such a way that all nutrients and quality are maintained. They should also be sourced and treated in such a way as to reduce the risk of disease or contaminants entering the housing facility.
- The fish's natural diet should be researched prior to designing the fish project diet and this should be copied as closely as possible. It is important to consider that supplying a single type of natural diet may not provide a balanced diet for a fish that normally has a varied diet in the wild.

Feeding regime

- The foodstuff should be dispersed in such a way that ensures all fish have access to the feed. The feeding procedure should be closely monitored at the start of a project before using any automated feeding equipment, because chronic failure to address this requirement will likely create social hierarchies where dominant fish will consume or guard food and subservient fish will starve.
- Generally, most fish eat less with a commercially available diet than with a natural diet. A general guide for a commercial diet is between 1-8% of the fish's body weight per day. This amount will vary according to the fish's age, size, and environmental conditions, especially water temperature.
- Food should be fed to satiation point for all fish and no more. This amount should be calculated against body mass and recorded for future use on the monitoring sheet.
- Over feeding will lead to increased levels of waste production if not eaten rapidly when offered (usually within five minutes is ideal). This will negatively affect water quality causing deoxygenation of the water, production of nitrogenous waste products and toxic gases.

- Alternating the time of feeding and the area of the tank to which the dispensers deposit the food can act as a form of enrichment for the fish and should be used once the fish have fully settled into their tanks.
- Any uneaten food should be removed from the tank within a short period after feeding has finished.
- Consider the natural feeding behaviour for the fish species (e.g., bottom feeders fed with sinking pellets) and provide this type of food to encourage normal feeding activity.

Fasting

It is natural for wild caught fish to have periods of fasting (no food intake) due to irregular food supply or limited food resources. Also, at some stage of a fish's life, such as spawning or migration, fish naturally become less interested in feeding.

Within scientific projects, fasting may also be necessary at certain times. For example, before and during transportation fasting may be required for 1-2 days. At other times fasting, for handling with or without anaesthesia/sedation may require only several hours of fasting.

Furthermore, the use of therapeutic agents may necessitate a clear gut to reduce the morbidity rate of the drug, so fasting is necessary before certain treatment regimes. This information should generally be supplied from the veterinarian who is treating the fish and supplying the medications.

Poor nutrition

A poor quality, imbalanced diet for the specific fish species can lead to a slow decline in fish health, stress and susceptibility to disease. However, because these clinical signs are not always obvious or specific, they can go either undiagnosed or incorrectly diagnosed. Appendix 4.12 shows a chart highlighting common nutritional deficiencies and their associated signs of poor health.

Storage of food

The diet may initially be of a good balanced standard, but due to incorrect storage techniques, the diet may lose essential components. Typical examples include the loss of water-soluble vitamin C or the rancidity of essential fats. All foods should be stored in a designated food area (fridge, freezer, dry store) exactly as recommended by the manufacturer. Feed 'use by dates' should be adhered to at all times. Any contaminated food (including cases where high humidity or water have infiltrated dry feeds) should be discarded immediately to prevent bacterial and fungal/mould growth and their associated toxins.

Water testing

Water testing is arguably the most important monitoring practise that investigators need to perform on a daily or regular basis (depending on the parameter). It is important that investigators write as much detail about water parameter monitoring as possible on their AEC application and include factors such as:

- the method or equipment they will use to monitor each parameter

- what the normal or expected value of each parameter should be
- the frequency of the monitoring.

Water testing should be performed immediately at the site of the fish tanks, because water will change its dynamics if collected and time elapses prior to testing. The following list details the water parameters to be tested and the typical equipment used within the aquaculture industry.

Carbon dioxide (CO2)

CO_2 is a product of fish respiration and high levels can lead to fish suffocation, regardless of the oxygen level in the tank. However, it is rarely a problem (low risk) in flow through systems or reticulation systems with efficient aeration equipment. Problems are more commonly noted in closed systems, such as ponds, or during periods where there has been an equipment failure due to malfunction or power cuts.

Monitoring of CO_2 should be undertaken periodically in low-risk housing systems (once weekly) and more routinely (daily) in higher risk housing systems such as ponds. There are a variety of CO_2 monitoring products on the market with many incorporated into the same devices as those used to monitor pH and water temperature. The equipment should be made available for times of emergency/adverse events in any type of fish housing facility.

Always follow the specific manufacturers guidelines for general use, routine maintenance, and safety of the equipment. This will ensure that the results are accurate for quality data collection and any abnormal results can be managed to prevent animal welfare emergencies.

Image shows an example of portable pH CO_2 controller meter - Aquarium 014pH 110v digital tester by Gain express.

pH

Monitoring pH is an essential component of fish husbandry. Incorrect pH has many effects on the level of dissolved gases and metals in the water. It also affects dissolved oxygen uptake by the fish causing distress as well as skin and eye disorders. Each fish species will have a preferred pH range, which should be researched by the investigator prior to setting up the fish housing. Generally, freshwater tanks tend to have a slight lower pH (6.5-7.5), compared to seawater tanks (8.0).

pH should be tested daily in all types of fish housing systems. There are two commonly sed measuring systems: digital monitors; and chemical based kits.

Digital pH monitors

In large facilities a multifeatured digital monitor such as the one shown above is the timeliest and most accurate solution to undertaking pH monitoring. It is also a more expensive option. This type of digital monitor must be calibrated before its initial use, and then re-calibrated periodically (according to the manufacturer's recommendations) to ensure accurate results can be consistently obtained. The manufacturer will normally supply the calibration solution.

Chemical based pH kits

Alternatively, there are a large range of test kits that are cheaper options. There are test kits that cover the whole pH range from 0-14, but the better option are the test kits that are designed to provide specific read outs within a more limited range of pH.

It is important to choose the test kit specific for the fish housing system and fish species in the project. For example, using a 'low range kit' with a pH range of 5.4-7.5 designed for freshwater tanks may show maximum results at the 7.5 level but in fact the actual pH could be higher if it is used in a seawater tank. Typically, 'high range kits' will measure from 7.6-8.8 and are designed for seawater measurements.

Test kits may differ slightly in their method of use and timing of contact, so it is important to read the manufacturer's instructions carefully, especially when trying new kits, to ensure the measurement is accurate. When using the kits, ensure that all equipment is clean and dry prior to use to avoid contamination and ensure the kits are stored correctly according to the manufacturer's instructions.

Salinity

Salinity levels within natural water supplies ranges from 1 in freshwater to 40 parts per thousand (ppt), with oceanic sea water being typically around 35-36ppt. Many species of fish can adapt to minor salinity changes over time due to their natural migration within the different areas of waterways.

However, other fish species are far more sensitive to salinity and therefore investigators must research the preferred level of salinity for the fish species they intend to use in their project. Sudden changes in salinity can be severely detrimental to the health of any type of fish and these emergencies can occur in any type of fish housing facility. Therefore, monitoring of salinity levels should be undertaken daily.
Salinity should be measured as a percentage salt to water rather than as specific gravity because salinity is a true measurement of the concentration of salt in the ocean. It is calculated as the total weight of 'dry' salt dissolved in a total of 1000 weight units of water or parts per thousand (ppt). Salinity is a pure weight per weight measurement, and it is not influenced by temperature, unlike specific gravity.

There are three main methods to monitor salinity, being the use of: a hydrometer; a seawater specific refractometer; or a digital seawater refractometer.

Seawater hydrometer

A seawater hydrometer is a tool that measures the salinity in the fish tank, returning a result in either specific gravity (SG) or salinity PPT (parts per thousand). It works on the principle of floatation where the measuring component floats more in saltier water than in less salty water. They are cheap and generally easy to read. However, strict steps must be followed in their use and complications such as bubbles can give inaccurate results. There are two forms of hydrometer generally available.

Swing arm hydrometers

- Before use, the hydrometer must be soaked in saltwater for 24 hours, known as 'conditioning'.
- The swing arm hydrometer is slowly filled by dipping the bottom corner fill port below the tank water's surface until water flows up and over the inner weir.
- Any air bubbles should be dislodged by gently tapping the hydrometer or pointer. Air bubbles on the pointer may result in inaccurate results.
- The hydrometer should then be placed on a level surface to read the specific gravity (inside scale) and salinity (outside scale).
- The unit should be rinsed thoroughly in freshwater after each use to remove salt and calcium deposits that accumulate on the pointer, which can build up and affect accuracy. If crystallisation does occur inside the unit, it should be soaked in lukewarm water or vinegar for 30 minutes and then rinsed with freshwater and air dried.

Image shows an example of a swing arm hydrometer by Coral sea.

Floating hydrometers

- Floating hydrometers are placed directly into the tank water and allowed to float naturally at a level according to the salinity of the water.
- The reading is taken from where the water's surface aligns to the measuring scale on the hydrometer. Basically, the more salt in the tank water, the more it floats.

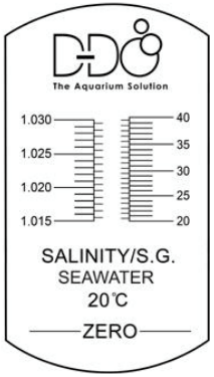
It is generally more accurate than a swing arm hydrometer and easier to read, but being made of glass it is easily broken, more expensive and is less readily available to purchase.

Seawater specific refractometers

There are two main types of seawater specific refractometers, being the manual hand-held instrument or the digital equivalent.

Manual seawater specific refractometers

A seawater specific refractometer is a hand-held instrument used for the measurement of salinity and specific Gravity (S.G.) by refractive index.
A refractometer does not measure salinity directly but measures the refractive index which is then displayed as salinity. The refractive index of a solution **does vary with temperature**; therefore, the reading that is measured with a refractometer is always temperature dependant. Most modern seawater refractometers have the structural ability in the reading scale to compensate for temperature changes.



Images shows an example of a typical seawater reading scale and seawater specific refractometer by Aquarium Solutions.

The reading scale and specific gravity readings in this type of instrument are specifically designed to read natural sea water, which contains mainly sodium chloride (salt) ions, but also has the presence of other significant ions such as magnesium and calcium.
Therefore, a standard salt (brine) refractometer will not give the correct salinity for natural sea water and a conversion factor must be applied. Standard brine refractometers also have a much larger reading scale from 0-100ppt compared to seawater specific models whose range is generally more focused around 20-40ppt; therefore, the latter type is easier and more accurate to read.

The following details are an example of how to calibrate and use a seawater specific refractometer. The investigators should always refer to the manufacturer's guidelines to ensure that their use of the equipment is providing accurate readings to prevent any potential fish welfare emergencies.

Calibrating a saltwater refractometers

Most salt refractometers must be calibrated initially at 20^{o}C, which is close to the average aquaculture room temperature in most cases. It is important to check the preferred temperature on each refractometer, which is normally marked on the scale as example 20^{o}C or 20/20.

During calibration, it is the temperature of the instrument and not the water that is important, because the tiny amount of water used for the test will equilibrate within seconds to the temperature of the refractometer. It is important to let the refractometer stand for 30 minutes to achieve this temperature if needed, prior to testing.

- Clean the glass prism (blue surface) and translucent cover flap of the refractometer with pure or de-ionised water to ensure that there are no salt residues that would affect the reading.
- Lift the translucent cover flap and using the pipette dropper supplied, fill and empty the dropper several times in pure or de-ionised water and then whilst holding the refractometer horizontally place a sample of the pure water onto the glass prism.
- Drop the cover flap onto the sample and wait for 15 seconds for the temperature to equilibrate with the instrument. Then look through the eyepiece with the prism end pointing towards a bright light source (similar to that of natural daylight, if possible). The eyepiece can be adjusted by screwing it in or out to adjust the focus and compensate for individual eyesight.
- Read the value by locating the interface between the upper blue area and lower clear area on the reading scale. During calibration this boundary line should line up exactly on the zero mark of the scale.
- If the boundary is not on zero, remove the protective cap covering the small screw on the cover flap and either tighten or loosen the screw slightly with a small screwdriver until the zero reading is achieved. The refractometer is now calibrated.

Once correctly calibrated, the refractometer can then be used in environments where the ambient temperature, and therefore instrument temperature, would heat up or cool down within the range of the instrument, which is between usually between 10 – 30^{o}C.
It is important to routinely re-check the calibration as recommended by the manufacturer to ensure accuracy of measurements.

Measuring salinity with a seawater specific refractometer

- Rinse the prism area before and after each use with pure water and dry it with the polish cloth provided to avoid scratching the surface. This will eliminate any potential contaminates affecting the reading.
- Using a dropper or pipette, take a representative sample of the sea water from the tank ensuring that the dropper is filled and emptied a few times to rinse out any salt deposits from the previous use. Lift the cover flap and then place the sample of water onto the prism and gently lower the cover flap onto the water. Note: it is better to put more water on the plate and to let the cover flap push the excess away than to use too small a volume.
- Allow the water to equilibrate to the temperature of the instrument for 15 seconds and then look down the eyepiece to take the salinity reading at the bottom of the blue and clear interface.
- One side of the scale will show salinity (normally between 20-40ppt) and the other side the specific gravity (normally between 1.015-10.30) calculated at 20^{o}C pure water density.

Digital seawater refractometers

A digital seawater refractometer is an optical instrument that employs the measurement of the refractive index to determine the salinity of natural and artificial seawater, ocean water or brackish intermediates.

The digital refractometer eliminates any user uncertainty associated with mechanical refractometers and is quick and easy to use. It does require a simple calibration step with either distilled or deionised water before each test. After calibration the prism component is dried off and the seawater sample is added through by a pipette. A rapid readout of both water temperature and salinity are usually given, and in some machines the salinity measure is converted into one of three popular measurement units: Practical Salinity Units (PSU), Salinity in parts per thousand (ppt), or Specific Gravity (S.G. (20/20)).

Image shows an example of a Digital refractometer for seawater by Milwaukee (Refractometer - MA887)

As with all machinery, it is important to follow the manufacturer's instructions to eliminate any contaminants and ensure regular maintenance procedures are carried out to prevent any false readings.

Dissolved oxygen (D.O.)

Oxygen is lost from water through respiration by fish, plankton and other organisms, and by the aerobic decay of organic matter. It is indirectly correlated with temperature and can cause severe animal welfare concerns leading to rapid death when levels of D.O. decrease.

Mechanical aeration (air bubblers) is the main source of oxygen in most tank systems, but some ponds and seawater pens rely on factors such as water surface wind turbulence to increase the general D.O. supply.

As a general rule absolute oxygen saturation should exceed 5mg/L to ensure good fish welfare; however, different fish species, life stages and fish sizes can affect how much oxygen is needed in each housing tank. Investigators should research the fish species they intend to use, and the choice of housing tank available, and base their project's oxygen needs according to this information during the planning stages.

When monitoring oxygen levels, consideration should be given to the temperature of the water because this will affect frequency of testing especially in tanks located outdoors. Higher external air temperatures may require two D.O. monitoring checks per day, with one of those being in the early afternoon when temperatures peak (unless the water supply is able to be kept at a constant temperature in systems for example using a flow through design).

The water sampling location is also a factor to consider in pond systems, where shallow water will heat up more rapidly than deeper water. This may create a variety of oxygen concentrations within one housing system. Therefore, oxygen levels should be tested in a variety of areas in pond housing systems.

Oxygen sensors/meters

There are various types of oxygen sensors available, but generally they all operate in a similar way. The sensor reacts with oxygen and an electrical signal is produced in proportion to the oxygen concentration. That signal is then amplified, translated into concentration units, and displayed by the meter. The meter circuitry also compensates the reading for changes in temperature, altitude or salinity automatically.

Images show examples of a dissolved oxygen meter with probe (Ecoscan and Handy Polaris).

Essential features to consider if an investigator needs to purchase an oxygen meter for a project include:

- rapid response
- ease of calibration
- water resistance
- sturdy and rugged construction
- automatic temperature compensation
- salinity and barometric pressure compensation
- measurement range from 0 to 200 percent saturation
- easily changed cable or probe.

Oxygen meters need to be calibrated prior to use. Most machines need to be standardised to a reference condition that compares an electrical current to a known D.O. concentration. The most common reference condition is the oxygen concentration of air saturated with water vapour (100 % humidity).

To use most oxygen meters, the probe should be held in the water using the handle component and then slowly moved around and up and down to allow oxygen to be consumed across the membrane. Simply holding the probe still in the water will create an oxygen depleted micro zone around the sensor tip and an inaccurate low D.O. reading will be recorded.

The response of dissolved oxygen sensors is not instantaneous, but once it starts reading, the measured values will change rapidly at first and then begin to stabilise within 15 to 20 seconds. In some systems, such as ponds, the stabilisation stage may be difficult to reach due to temperature variables causing constant D.O. variation.

Investigators should routinely refer to the manufacturer's guidelines regarding calibration, use, ongoing care and maintenance methods for each specific machine. This will ensure oxygen readings are always accurate and reliable and help to prevent serious animal welfare emergencies.

Alkalinity or carbonate hardness (KH)

Alkalinity is the measure of the acid neutralising capacity of the water supply in a fish tank. Bicarbonates, carbonates, borates, phosphates and other anions contribute to alkalinity and the level of alkalinity will normally vary between freshwater and seawater tanks.

It is therefore a very important monitoring component in aquaculture-based projects and to ensure good fish welfare, alkalinity should be measured daily, especially in tanks with increased stocking levels.

There are two main methods to monitor alkalinity, being chemical reaction test kits and digital sensors/meters.
Chemical reaction alkalinity test kits

These are simple to use and cheap. Each test kit may require a slightly different technique; therefore, investigators should refer to the manufacturer's instructions to ensure an accurate result each time.

In general, the test requires a clean test tube to be filled with a set amount of tank water, and the KH test solution will be added slowly 1 drop at a time. Each drop should be counted, and the test tube capped after each drop and gentle shaken.

When the liquid turns from a blue colour to a yellow colour the test is complete. Sometimes holding the test tube against a white background will help to identify when the colour change occurs. The number of drops can then be compared to the conversion chart to find the measure.

Digital alkalinity and KH sensors

These machines can be adapted to read multiple water parameters at a time. They are easy to use and in some machines the information can be sent directly to a phone or computer via Bluetooth. Again, each machine will have its own specifications and the manufacturer's recommendations should be followed to ensure accurate results.

As a general guide:

- The machines come with indicator strips which are dipped into the water to be sampled for a designated period of time.
- Excess water is shaken from the indicator strip and the strip is held for a recommended holding period.
- The indicator strip is then slid past the sensor and the results can be read.
- In some machines, these results can then be sent to personal communication devices via an APP.

Image shows an example of a Bluetooth KH sensor.

Hardness - also called total harness or general hardness (GH)

Total hardness is the measurement of divalent cations ($^{+}2$ ions) in the water and, like total alkalinity, is expressed as milligrams per litre (mg/L) or parts per million (ppm) of calcium carbonate ($CaCO_3$), but it doesn't mean that the hardness is in this form.

In the aquarium industry, total hardness may be referred to as "general hardness (GH)" which is often measured in degrees (dGH) rather than mg/L or ppm. One dGH is equal to 17.9 mg/L or 17.9 ppm. The two most common elements that contribute to hardness are calcium and magnesium.

Waters are often categorised according to degrees of hardness as follows:

Water defined as	**mg/L**	**dGH**	**ppm**
Soft		0-3	0-50
Moderately soft	0-50	3 - 6	50 - 100
Slightly hard		6 - 12	100 - 200
Moderately hard	75-150	12-18	200-300
Hard		18 - 25	300-450
Extremely hard	>300	>25	>450

The source of the water for a project will directly impact the hardness of the water. Groundwater tends to be harder and surface water tends to be softer.

Where the alkalinity (KH) is derived from calcium or magnesium carbonate, hardness and alkalinity values are similar. However, if alkalinity is derived from sodium bicarbonate (Na HCO_3), it is possible to have soft water with a high alkalinity.

The welfare level of fish becomes a concern in water hardness values over 400mg/L and less than 20mg/L, with each fish species having varied levels of tolerance in values between these two extreme levels.

Total hardness is a particularly important water parameter to monitor when spawning fish and raising fry, because calcium is critical to egg, bone and tissue development. However, some tropical aquarium species that originate in areas with extremely soft water may require low hardness water to spawn and develop. Therefore, it is important to know the specific requirements for each fish species that will be spawned or maintained.

Hardness ranges from 10mg/l to over 400mg/l depending on the region it comes from, with the most productive waters ranging between 20-250mg/L Ca Co_3.
As a very rough guide the following table can be used to see what is generally considered a normal reading.

Fish group	**Total hardness (dGH)**
Tropical fish tank	4-12 dGH
Discus fish tank	3-8 dGH
Planted Tank	3-8 dGH
Brackish Tank	12-20 dGH
Pond	4-12 dGH

Total hardness should be tested at least once per month in most fish tanks and more frequently in projects involving:

- breeding of fish or raising fry
- setting up of new tanks or
- the use of new water sources.

There are several ways to measure hardness available to investigators. Many are integrated into other water parameter measuring devices and kits. The three main methods are: aquarium test strips; liquid test kits; and scientific meters and probes.

Aquarium test strips for water hardness

These strips generally cover multiple water parameters, are easy to use and are based on the principle of litmus paper. They are also normally slightly more expensive than a liquid test kit. Located along the test strips are small, colour-changing pads. Each one of these pads is designed to detect a certain water parameter.

Depending on the number of pads most of these strips typically test nitrite (NO_2), nitrate (NO_3), pH, general hardness (GH) and carbonate hardness (KH).
To use them, the test strip is dipped into the fish tank water directly or into a tube containing a sample of the tank water and then pulled out. The strip should be left for a designated amount of time (according to the manufacturer's instructions) while the pads change colour. The coloured pads are then compared to a colour chart on the container label. The used test strip is discarded after use.

Images showing an example of a test strip system by Living Reef Aquariums.

Aquarium test strips are generally the least accurate method of testing water parameters (even though they are commonly used) for the following three reasons:

1. Users do not consider the expiry date on the label. Therefore, results are not reliable after this time because the reagents in the colour-changing pads begin to break down.
2. The scale used on the label is not very precise with big jumps between each reading.
3. They can easily be affected by exposure to moisture, touching the pads with fingers and improper storage.

Liquid water hardness test kits

To use the liquid test kits, the manufacturer's instructions should be followed precisely, because each kit may differ in its technique. As a general guide, the designated amount of tank water should be collected with a pipette and added to the test tube. Then the exact amount of testing solution is added.
The water will start to cloud and change colour. The test tube should be capped and shaken gently to mix the contents thoroughly. The mixture inside the test tube should turn a single, uniform colour. The colour of the liquid is then matched to the colour chart supplied and the closest result can be recorded.

Test kits will also have an expire date, so this should be checked to ensure results are always accurate.

Image shows an example of a liquid test kit (Images by Ian Sterling from Fish lab 2019).

Single or multi-task photometers for water hardness

The photometers use specific reagents mixed precisely with the tank water to get an accurate reading of water hardness. They still require some manual input, but the results are far more accurate. They are more expensive to buy initially, but multi-task models can be used for most fish tank water parameter testing with the associated reagent products.

Image shows an example of a multiparameter photometer by Palintest water analysis technologies, 2020.

Hydrogen sulphide (H_2S)

Hydrogen sulphide is produced by the bacterial decomposition of organic matter under anaerobic conditions. Ideally, freshwater fish should not be exposed to more than 2µg/L of hydrogen sulphide for long periods.

Shrimp and other marine species tend to be more tolerant of hydrogen sulphide than freshwater species, but concentrations should not exceed 5 µg/L in aquaculture ponds with brackish water or full-strength seawater.
Total sulphide concentration measurement is a complex task by standard laboratory methods, but investigators can use hydrogen sulphide kits for easier total sulphide analyses. The kits provide relatively reliable data.

The following table by Boyd, CE 2014, shows factors for estimating hydrogen sulphide concentration from measured concentrations of total sulphide in freshwater. For seawater sampling, the factors will need to be multiplied by 0.9 to calculate an accurate reading.

pH	Temp (°C)								
	16	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**
5.0	0.993	0.992	0.992	0.991	0.991	0.990	0.989	0.989	0.989
5.5	0.977	0.976	0.974	0.973	0.971	0.969	0.967	0.965	0.963
6.0	0.932	0.928	0.923	0.920	0.914	0.908	0.903	0.897	0.891
6.5	0.812	0.802	0.792	0.781	0.770	0.758	0.746	0.734	0.721
7.0	0.577	0.562	0.546	0.530	0.514	0.497	0.482	0.466	0.450
7.5	0.301	0.289	0.275	0.263	0.250	0.238	0.227	0.216	0.206
8.0	0.120	0.114	0.107	0.101	0.096	0.090	0.085	0.080	0.076
8.5	0.041	0.039	0.037	0.034	0.032	0.030	0.029	0.027	0.025
9.0	0.013	0.013	0.012	0.011	0.010	0.010	0.009	0.009	0.008

Test strip kits for hydrogen sulphide

These kits are easy to use by investigators. As with other tests, manufacturer instructions should be followed at all times to ensure accurate results.

As a general guide, a test tube is filled with the fish tank water.

A test strip is then added to the tube for the designated amount of time, swirled around and then removed. The test water will change colour in the presence of the hydrogen sulphide. The water colour can then be compared to the colour chart for a reading.

Image showing an example of a hydrogen sulphide test kit and a colour chart by Waterworks.

Digital probe detectors for hydrogen sulphide

Digital probes detect the hydrogen sulphide in the water as well as temperature and pH, to enable the calculation of the total dissolved sulphide concentrations in mg/l as seen in the previous table.

Testing should be undertaken at least once per month, but more frequently in housing systems such as ponds where there is an increased amount of organic matter.

Frequency should also be increased in all housing systems where aeration has been turned off or reduced for a period of time due to malfunction or power cuts.

Nitrogenous waste / metabolites (Ammonia, nitrite and nitrate)

Metabolites are the waste products from respiration and digestion that are released into the water. If these are not diluted or neutralised sufficiently, they can build up to harmful levels.

Ammonia

Ammonia is the key metabolite product continuously excreted from the gills and digestive tract. On mixing with water, an equilibrium mixture forms between the less toxic form (ammonium ion NH_4^+) and a more toxic unionised form (ammonia NH_3). Where pH and water temperatures are higher, the toxic form is more prevalent.

Closed recirculating seawater systems are particularly prone to incidents of ammonia toxicity due to the relationship between salinity and pH and therefore require more frequent monitoring than flow through systems.

Fish species differ in their sensitivity to ammonia and research should be undertaken by the investigator to assess the best system available for the fish species they wish to use in their scientific project, to reduce the risks of toxicity.

As a general rule the following levels are suggested as safe for chronic exposure from an animal welfare perspective.

Fish group	Safe level of ammonia
Sensitive species (e.g. salmonids) in freshwater	<0.002mg/L
Less sensitive species (e.g. non-salmonids) in freshwater	<0.01mg/L
Marine species	<0.05mg/L

Nitrite ($NO2^{-}$)

Nitrite is a product of the natural degradation of ammonia compounds and can also build up to toxic levels causing potential welfare emergencies. Again, this is less likely in an open flow system compared to a recirculating system.
The toxic effect of nitrite is strongly mitigated by chloride ions; therefore, it is less common in seawater systems compared to freshwater systems.
Chronic exposure levels of nitrite in freshwater systems should not exceed 0.1mg/L.

Nitrate (NO3-)

Nitrate is a product created when biofilters typically found in recirculating systems convert ammonia first into nitrite then into nitrate. Nitrate is colourless, odourless and less toxic to fish than ammonia or nitrite unless it reaches very high levels where algal blooms can be seen. Toxic levels of nitrates are rarely an issue in flow through systems unless the water source is already heavily contaminated.

Safe levels of nitrate should not exceed a maximum of 5 to 10 ppm.

Freshwater tanks can be at the higher end of the limit, with seawater fish tanks at the lower end of the limit. Any fish tanks incorporating living reef structures must remain at 0ppm.

Young fish are more sensitive to nitrates than adult fish; therefore, any breeding projects should consider more regular monitoring of this water parameter.

Faecal matter is rarely toxic in its own right but can cause deoxygenation of water during its degradation process, along with production of extra nitrogenous compounds and toxic gases. It will, however, contribute to the organic suspended solid component of water, which is detrimental to gill health. Therefore, faecal levels should never be allowed to build up.

There are three main ways to test the level of these various metabolites in fish tanks being Nessler based test strips, liquid salicylate kits and digital monitors.

Nessler ammonia test strips

These strips measure total ammonia (free ammonia NH_3 and ammonium NH_{4+}).

The results can be affected using water conditioners that convert ammonia into ammonium and other similar non-toxic versions. Tests will come out as positive results, while in fact the ammonia can be at zero.

The test strips consist of a dry felt pad of amber or yellow colour. The pad is dipped into the fish tank water, and after a few minutes, the pad colour is compared to a rough colour chart. As with all test kits the manufacturer's guidelines should be followed at all times.

The strip kits are a cheap method of monitoring but result quality can be affected by:

- extended use past the expiry date
- humidity affecting the pad quality prior to use during storage
- touching the test pads with fingertips
- subjective judgement when comparing the sample to the colour chart.

Images shows an example of a test strip kit for testing metabolites by Aquarium Pharmaceuticals

Salicylate liquid ammonia tests

These liquid tests measure free ammonia (NH_3). They are generally less affected using water conditioners so tend to give less false negative results. The initial cost is higher, but they have a longer expiry date and work out cheaper per test overall.

As with all the tests, the manufacturer's guidelines should be followed at all times, because each kit may vary in its method.
Salicylate kits have a yellow/green or blue colour comparison chart.

Nitrate liquid testing

Most nitrate test kits transform nitrates back into nitrites before measuring the combined values. This is only of importance if nitrites are present.
When nitrites are at zero in established tanks, this "combined reading" is accurate regarding nitrates.

If nitrites are present, the nitrate value is the combined measurement, resulting in a lower nitrate concentration than is indicated.
Although liquid tests kits are more reliable than the strip tests, they can be affected by:

- inaccurate measuring of the water sample
- the water sample being discoloured which will darken or lighten the test water in the vial and result in inaccurate readings when compared to a colour chart
- non-adherence to time guidelines in-between applications of solutions.

Image shows an example of a multi liquid test kits by Aquarium pharmaceuticals.

Digital probes for metabolites

There are a variety of digital devices available for investigators to monitor metabolite levels, with some being incorporated into the previously mentioned multi-task models. Other models are more specific to solely measuring metabolites, but normally always incorporate pH and temperature measures as well, because these water parameters are highly linked to ensure an accurate reading.

Digital monitors are more accurate than chemical kits.

Whichever method of testing is chosen, it is best to:

- Stick to a set time of testing each day, because pH levels will naturally fluctuate throughout the day, with the lowest levels being in the morning.
- Always test the water before performing a water exchange.

Suspended solids, turbidity and clarity

All three parameters (suspended solids, turbidity and clarity) are related to particles in the water column, whether directly or indirectly.

- Turbidity and total suspended solids refer to particles present in the water column.
- Turbidity and clarity are both visual properties of water based on light scattering and attenuation (reduction).

Total suspended solids (TSS) are a total quantity measurement of solid material per volume of water. This means that TSS is a specific measurement of all suspended solids, organic and inorganic, by mass. TSS includes settleable solids and is the direct measurement of the total solids present in a water body. As such, TSS can be used to calculate sedimentation rates, while turbidity cannot.

Turbidity is determined by the amount of light scattered off the particles in the water. Therefore, a turbidity reading can only be used to estimate the total dissolved solids concentration. It will not be exact because it does not include any settled solids. Turbidity measurements can also be affected by coloured dissolved organic matter, which can cause artificially low turbidity readings because it absorbs light instead of scattering it.

A certain level of turbidity is normal in any aquaculture system. It only becomes a problem when poor filtration or contaminated water supplies reach excessive levels, causing interference to the fish's oxygen update through mechanical gill damage. Certain species of fish are more susceptible than others to suspended sediment levels, so setting definitive levels is more difficult. As a general guide:

Fish group	Turbidity levels
Sensitive species (e.g. salmonids) in freshwater	<50mg/L
Less sensitive species (e.g. non-salmonids in freshwater	<200mg/L
Marine species	<100mg/L

There are various ways to measure suspended solids and turbidity including laboratory testing, Secchi disc transparency and digital nephelometers/turbimeters.

Laboratory testing for total suspended solids

- The most accurate method of determining total suspended solids (TSS) is by filtering and weighing a water sample. This process is both time consuming and difficult to measure accurately due to the precision required. In pond systems showing a brown coloured water (mineral turbidity), samples should be sent to the laboratory for testing if there are concerns for fish welfare.
- Total suspended solids, as a measurement of mass are reported in milligrams of solids per litre of water (mg/L).
- When collecting the water samples for the laboratory, always take water from different depths with minimal disturbance to the water column if possible. Note: surface water samples normally have the least suspended solid, so may give a false assessment of overall water quality if used alone for measurement.

The following table shows typical measures of turbidity in pond systems.

Mineral turbidity	TSS (mg/L)
Low	<25
Medium	25-100
High	>100

Secchi disc transparency testing for turbidity

- The Secchi disc is a simple tool that can be used to give an 'estimate' of turbidity. It is particularly useful in green-coloured ponds to estimate plankton turbidity. This measurement is then called the Secchi disc transparency.

- To use this instrument, the disc is slowly lowered into the water, until it disappears. Note on the attached line at what point it breaks the water's surface and mark it as A. Then lower the disc a little further before raising it slowly until the disc just reappears. Mark this on the line as point B.
- Remove the disc and mark the midway point between points A and B as point C and then measure the distance from the top of the disc to point C, counting the knots along the line. This figure is the Secchi disc transparency measurement.

Digital nephelometers/turbimeters

Turbidity is measured in nephelometric turbidity units (NTU). The instrument used for measuring is called nephelometer or turbidimeter, which measures the intensity of light scattered at 90 degrees, when a beam of light passes through the water sample. It is much simpler to measure turbidity than TSS.

Image shows an example of a turbidity meter by LaMotte.

Water clarity is strictly relative to sunlight penetration. While this is usually determined by the amount of suspended solids in water, it can also be affected by chromophoric dissolved organic matter (CDOM) from plants and animals and other dissolved solids. Water clarity is the most subjective measurement of these three parameters, because it is usually determined by human observation.

The most common metals are iron, lead and copper, though others can harm fish, like aluminium, antimony, arsenic, bismuth, cadmium, chromium, cobalt, copper, lead, mercury, silver, zinc and tin, or alloys like brass and bronze.

Heavy metals

The most common metals are iron, lead and copper, though others can harm fish, like aluminium, antimony, arsenic, bismuth, cadmium, chromium, cobalt, copper, lead, mercury, silver, zinc and tin, or alloys like brass and bronze.

Heavy metal contamination can come from:

- Metallic salts introduced into the water.

Heavy metal contamination can come from:

- Metallic salts introduced into the water.
- Metals (especially copper) dissolving from the pipework in the tap water, especially if its acidic (pH <7) or hot.
- Copper in medications. If dosing with copper-based medications, then know that some species of fish will be more sensitive than others. Shrimp and invertebrates usually die in the presence of even low levels of copper.
- Metal ore in rocks added to the fish tank.
- Metal in the frame of the tank or in a piece of equipment.

There are test kits available for heavy metals, such as iron and copper, similar in design to other water parameter kits if investigators suspect they may be causing health issues in the fish.

Image shows an example of a heavy metal testing kit by Aquarium pharmaceuticals

Controlling these heavy metals in fish tanks may need:

- A water change (if the water is not the source of the problem).
- The addition of a commercial water conditioner that de-toxifies (metal chelating) metals, typically using chemicals like Ethylenediaminetetraacetic acid (EDTA).
- Addition of a foam or resin that removes metals when water is passed through it (e.g. Poly-Filter) because activated carbon filters are not good at absorbing metals.

Temperature

Each fish species will have its own preferred water temperature range and therefore tank water should remain in this zone **at all times**. Any deviation out of this range will cause undue stress and lead to a lowered immune system, poor health, decreased growth rate and death if allowed to continue.

Sudden changes in water temperature as small as 5^{o}C can cause stress responses in fish that are otherwise healthy.

As a general rule, the water chemistry in cold water systems tend to be more stable than in warm water systems.

It is better to keep fish at the lower end of their temperature range if possible, to decrease their oxygen and appetite requirements rather than at the top end of their range.

Measuring water temperature

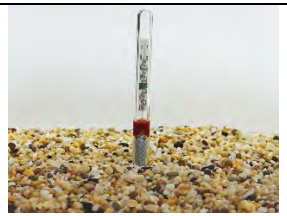
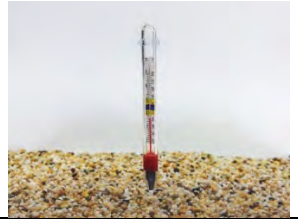
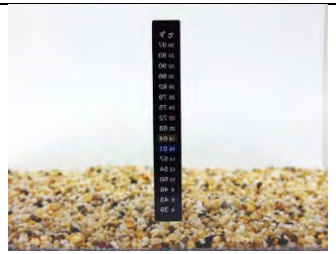
There are many models of thermometer available for investigators to use to measure water temperatures in their projects. Some are simple whilst others have more complex alarm and heating components.

Any thermometer should be checked for calibration on a regular basis to ensure an accurate reading, because poor temperature control can be a serious animal welfare risk in aquaculture-based projects.

The following chart details a brief description of the types of thermometer available. These are based on smaller fish tanks, but commercial thermometers are also available for large scale projects.

Type of thermometer	Image	Information

Digital thermometer and probe		This thermometer is made up of two different parts: A sensor probe which sits underwater within the fish tank and checks the temperature. The probe can be moved anywhere inside the tank. A digital display which remains outside the water and displays the temperature. They are normally battery operated. The probe can be removed from the water once the temperature has been taken.
Fixed probe (instant read thermometer)		The meter attaches directly to the sensor probe. The probe is dipped into the aquarium temporarily and the temperature is shown on the digital display screen.
Wired probe		In this model the probe is separate from the meter and attached with a cord. This allows the thermometer probe to be set up permanently in the fish tank, while the meter sits on the outside, displaying the current temperature. It provides a continuous monitoring and many models have an inbuilt alarm if the temperature goes above or below a pre-set limit. These thermometers are generally very accurate and easy to use but are more expensive to buy. They also require batteries or an electrical supply.
Submersible aquarium thermometer. There are 3 types:	Floating submersible design:  Standing submersible design:	Generally, they are cheap and made either of glass or plastic. They remain in the tank and the readings are on the instrument itself. Floating design: Floats on the water's surface. Can be affected by water flow causing swirling of the instrument making it harder to read. Filtration can push the thermometer into tank corners or behind plants blocking the reading scale. Standing submersible design: It is weighted, allowing it to sink to the bottom of your aquarium where it remains upright and can be easily read.

	 Suction cup submersible design: 	Suction cup submersible design: It is a floating or sinking thermometer with a suction cup which can be attached to any side of the fish tank and remain in position. Note: some models use magnets for the suction component.
Digital submersible thermometer (with or without inbuilt heat pump)		Alarmed Heat adjusting thermometers can activate heating devices if required. They remain in the tank at all times
Thermometer sticker strips		Also referred to as *LCD thermometers*. LCD stands for *liquid crystal display*, which refers to the liquid crystal ink that changes colour according to the temperature of the fish tank. The sticker is peeled and attached to the outside of the tank which reads the water temperature. However, it is also affected by the temperature of the room, leading to inaccuracy. This problem is worse for larger aquariums with thicker glass.

Note: analogue thermometers are those which require the matching of the liquid lines with the temperature reading. The markings are generally very small to read leading to inaccuracy and are made of glass so they can break easily. Hence plastic digital thermometers have surpassed this older style technology and should be considered as the better option for all fish research and teaching projects at UniSC.

In conclusion, there are many types of equipment necessary to monitor fish within scientific projects and the associated costs involved should be considered when planning the budget of a fish based scientific project. More complex digital systems such as photometers can monitor multiple water parameters simultaneously, saving time, but can be cost prohibitive for many investigators.
Appendix 4.12 explains the acceptable levels for each water parameter, the reasons for undertaking the testing and the fish health considerations if the parameters are not within the recommended guidelines.

Wastewater disposal

Wastewater from fish research projects should be disposed of in accordance with the guidelines of the *Environmental Protection Act 1994* (EP Act) and subordinate legislation. Projects using new therapeutic treatments, housing diseased fish or noxious fish species may be required to perform more stringent wastewater procedures prior to water being released into the normal UniSC systems or other waterways.

Investigators should source this information from https://environment.des.qld.gov.au or the department of agriculture and fisheries QLD and include the information in the AEC application.

For basic information on this topic, the following website provides information as a technical guideline to officers when assessing and deciding applications for wastewater releases to Queensland waters against the provisions of the *Environmental Protection Act 1994* (EP Act) and subordinate legislation
https://environment.des.qld.gov.au/__data/assets/pdf_file/0031/88636/pr-gl-wastewater-to-waters.pdf

Housing facility cleaning

Skimmers

Water surface skimmers are essential in fish tanks where larvae are being reared for scientific projects. The aim of the skimmer is to reduce the oily scum build-up on the surface of the water, which would restrict the larvae from reaching the surface to gulp air needed to inflate their swim bladder. After the larvae stage of fish development, the skimmers are no longer necessary, but can aid in the general tank cleaning process by removing excess lipids and proteins.

Cleaning of in-use fish tanks

Static tanks should be cleaned regularly by using a siphon or a vacuum pump to remove the accumulation of organic matter such as uneaten food or faeces and the associated fouling organisms, bacteria and algae.

Sand filters need to be backwashed regularly and cartridge filters cleaned and dried periodically according to a maintenance schedule to prevent build-up of waste material and to ensure efficient operation of the filtration system.

Water parameter measures should also be used to guide the investigator towards the best timetable for cleaning the filters and tanks, because cleaning requirements of the filters are dependent on the fish biomass and feeding regime of the project. Reference to the filter manufacturer recommended guidelines is also useful for setting up a cleaning procedure.

Note: cleaning the tanks is a stressful process for the fish but is an essential requirement for maintaining water quality.

General cleaning of fish tanks, equipment and aquaculture rooms

Dilute pool chlorine or sodium hypochlorite (NaOCl at 20ppm concentration) or caustic soda (NaOH at 1% concentration) have traditionally been used to disinfect drains and floors of aquaculture rooms. However, chlorine is inactivated in its disinfection activity by the presence of organic matter. Therefore, other specialised aquaculture cleaning and disinfecting products are now available with greater efficacy across a broad spectrum of pathogens and generally have a safer handling margin for the users.

Whichever products are chosen, the cleaning process of the aquaculture room, equipment and **empty tanks** requires **a five-stage approach:**

1. Remove large organic matter manually.
2. Use of a specifically designed 'aquaculture general multipurpose heavy-duty alkaline' cleaner (such as 'Antec Biosolve©') for removing general small sized attached organic matter.
3. Removing all traces of the detergent and suspended organic matter by thoroughly rinsing with clean water.
4. Disinfection with a broad-spectrum product (such as 'Antec Virkon© for aquaculture') by following the manufacturers guidelines for use. If this product is not available, then traditional disinfectants such as chlorine (as above) or iodine solution of 1.5% (or according to the manufacturer's instructions) may be used and dilution specifications noted on the AEC application.
5. Final rinse with clean water.

All components of the tank system should be cleaned after use including pipework and valves, according to the recommendations of the cleaning product manufacturers.

All equipment, shelving, benchtops, sinks, food storage facilities, floors, walls, and any other equipment should be cleaned by the five-stage system and then left to dry naturally.

When cleaning or removing water spillages from the floors, a wet floor sign should be placed in the area to alert other personnel to potential slipping hazards. The following table outlines a basic cleaning plan using Biosolve© and Virkon© cleaning products.

Product	Task	Dilution rate	Application process
Biosolve© alkaline detergent	For pre-cleaning of surfaces and equipment Heavily soiled Lightly soiled	 1:100 or 1% 1:200 or 0.5%	Coverage is 500mls per square metre. Leave the product in contact for 15-20 minutes. Rinse off thoroughly with clean water.
Virkon© for aquaculture disinfectant	Disinfection for pre-cleaned surfaces and equipment	1:100 or 1%	300mls per square metre
	Foot dips	1:100 or 1%	Fill footbath with a freshwater solution of Virkon© and replenish every 4 days or when it is heavily soiled.
	Hand nets, weighing equipment.	1:200 or 0.5%	Visibly clean with Virkon© after each use.
Biosolve© alkaline detergent and Virkon© for aquaculture disinfectant	Protective clothing such as aprons and gumboots	1:200 or 0.5%	Pre-clean with Biosolve as above, rinse with clean freshwater then immerse in Virkon© for 10 minutes before hanging up to dry.
	Transport tanks and equipment	1:200 or 0.5%	Preclean with Biosolve© to remove organic matter, rinse thoroughly, visibly clean with Virkon© after each use. Dry tanks before next use.
	Fish tanks	1:200 or 0.5%	When empty, preclean with Biosolve© to remove organic matter, rinse thoroughly, then disinfect with Virkon©.
	Waste disposal area	1:200 or 0.5%	Preclean with Biosolve© to remove organic matter, rinse thoroughly, then disinfect with Virkon©.

The material safety data sheet for Virkon aquatic can be found at https://aquarium.org/wp-content/uploads/2016/11/VIRKON-SDS-11-18-2016.pdf.

Pyroneg

Pyroneg is an alkaline concentrate and a pyrogen free detergent suitable for cleaning of laboratory and surgical instruments and is compatible with manual washing, ultrasonic cleaners and spray washing. It is available in powder and liquid concentrate formats and is currently used in the fish laboratory at UniSC but is not specifically designed for aquaculture facilities. It is recommended that a 2-4mL (mg) /L concentration is used as a working solution. Note: the material safety data sheet can be found at https://www.cleaningshop.com.au/contents/media/msds/liquid-pyroneg-msds.pdf and has the following ecological information:

- Toxicity – if released to waterways, alkaline products may change the pH of the waterway. Fish will die if the pH reaches 10-11 (goldfish 10.9, bluegill 10.5).
- Mobility in soil – May leach to groundwater with toxic effects on aquatic life as above.

Note: General detergent products are highly toxic to fish and aquatic species and should never be used for aquaculture cleaning in any capacity.

Maintenance and cleaning of biofilters on recirculatory fish tank systems

Biofilter maintenance

The biofilter is an essential component of a water recirculating fish tank system and must always be kept in good working order to maintain optimal water quality. The following recommendations are taken from the paper by Jacob Bregnballe, 2015, from The Food and Agricultural Organisation of the United Nations entitled *A Guide to Recirculation Aquaculture, An introduction to the new environmentally friendly and highly productive closed fish farming systems.*

The later recommendations should be used as a guide for the investigator when writing their biofilter maintenance plan, which will also include any manufacturer's instructions. These maintenance plans should be communicated to all persons involved in the project to ensure a consistent approach to monitoring.

Regular monitoring and cleaning schedule

- Every two weeks brush the top plate to avoid blocking of the holes in the perforated top plate with bacteria and algae.
- Every two weeks brush and clean the microbubble diffusers in the process water pipe from last biofilter chamber to microparticle filter.
- Over time, the biofilter will accumulate organic matter, which will impact the distribution of air bubbles and increase the size of the bubbles. The distribution of air bubbles across each of the biofilter chambers should be visually checked.
- Check the height between the water surface level in the biofilter and the polyethylene cylinder wall top edge to identify flow changes through the biofilter and microparticle filter.
- Regularly measure the water quality parameters that have most relevance to the biofilter.
- Closely monitor the remaining volume of base or acid used for dosing.

Cleaning and flushing for sludge removal in biofilter

Accumulations of mixed inorganic materials dislodged biofilms and other organic matter that are difficult to break down by the microorganisms may be found below the biofilter. They should be removed by the sludge removal system placed in the chambers according to the manufacturer's instructions.

Simple cleaning of biofilter using air

Twice a week, it is recommended to apply a simple cleaning protocol. In this procedure, the polyethylene biofilters are cleaned by air according to the manufacturer's instructions.

Microparticle filter cleaning

The regularity of cleaning the microparticle filter depends on the loading on the system. As a guideline, it is recommended to clean the microparticle filter every week according to the manufacturer's instructions.

Deep cleaning of biofilter

If the head difference between biofilter and/or microparticle filter chambers is increasing and the normal head difference cannot be re-established by normal cleaning, then a biofilter deep clean procedure is required. Use regular measurements in each biofilter chamber, between the top of the water level and the polyethylene cylinder top edge to identify flow problems through the biofilter and microparticle filter.

Before completing a deep rinse shut the aeration off in the given chamber for two hours before completing the clean. The given chamber will then act like a microparticle filter for this short period collecting extra waste, which is to be discharged during the cleaning process. As a guideline, it is recommended that all areas of the biofilters are deep cleaned every month. Again, follow the manufacturer's instructions for this procedure.

The following table details some of the problems associated with biofilters, their potential causes and actions required to resolve the problems.

Problem	Reason	Solution
Increased turbidity	Too much aeration	Lower aeration
	Reduced flow rate to biofilter	Open valve between degasser and biofilter, increase flow
Increasing TAN level	Too much aeration leads to reduced nitrification performance due to damage to the biofilm	Lower aeration
Increasing nitrite & TAN levels	Too high organic loading	Make sure feeding does not exceed system specs. Check mechanical filter function.
Decreasing nitrate level	Anaerobic activity	Increase aeration, clean biofilter
Hydrogen sulphide (smell of rotten egg when cleaning)	Anaerobic activity	Increase aeration, clean biofilter
Increasing alkalinity	Anaerobic activity	Increase aeration, clean biofilter
Reduced flow to biofilter	Closed inlet valves partly	Open valve between degasser and biofilter, increase flow
	blocking of biofilter, insufficient cleaning of the biofilter	Clean biofilter according to schedule & production specific demands
Reduced or no aeration	Blower failure	Check blower, intake air filter, fuse and power

4.4 Animal health and welfare considerations

Nutritional deficiencies **–** from poor food storage, an inappropriate or imbalanced diet for the species, or under or overfeeding regimes will have a slow but deleterious effect on fish health leading to reduced immune response and illness.

Water parameters – if not measured correctly and with enough frequency, can lead to both distress and sudden death in certain circumstances. They are a critical component of fish husbandry.

Cleaning product toxicity – can occur when the incorrect cleaning products are used causing severe animal welfare concerns. The incorrect use of cleaning products and procedures can lead to outbreaks of disease in fish populations.

Biofilter failure – leads to a rapid deterioration in water quality that can cause distress and death in the fish population.

4.5 Training plan and competency assessment

Any UniSC staff and students who work with animals should complete the online introduction to animal ethics training found on the Student Portal. Investigators and animal careers must be fully trained and assessed as competent in the process of fish husbandry before undertaking related procedures. Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application. The AWO is available to provide training and assess competency as required.

Investigators and animal carers must be aware of the OH&S and risk considerations surrounding the fish species they plan to use in their project and potential first aid procedures required in case of emergencies. The AEC and AWO will monitor competency during inspections of animal ethics approved projects.

4.6 References and acknowledgements

References

- Bayne, K & Turner, P, (eds) 2013, *Laboratory Animal Welfare,* Academic Press, Cambridge, Massachusetts
- Bregnballe, J 2015, A Guide to Recirculation Aquaculture - *An introduction to the new environmentally friendly and highly productive closed fish farming systems*, viewed 2 December 2019, http://www.fao.org/3/a-i4626e.pdf
- Department of primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW.
- Fondriest environmental learning centre, 2020, Turbidity, Total Suspended Solids & Water Clarity, viewed 14 January 2020, https://www.fondriest.com/environmentalmeasurements/parameters/waterquality/turbidity-total-suspended-solids-water-clarity/
- Halver, JE & Hardy, RW, 2002, *Fish nutrition 3rd ed*., Academic Press, san Diego, California
- Hargreaves, JA & and Tucker, CS 2002, Measuring Dissolved Oxygen Concentration in Aquaculture, viewed 2 December 2019, http://agrilife.org/fisheries/files/2013/09/SRAC-Publication-No.-4601-–-Measuring-Dissolved-Oxygen-Concentration-in-Aquaculture.pdf
- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- National research council of the national academies, 2011, *Guide for the care and use of laboratory animals Eighth Edition*, viewed 23 December 2019, https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, Guidelines for the Care and Use of Fish in Research, *Institute for Laboratory Animal Research,* vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

Further reading

- Milwaukee 2019, *Instruments for fresh & salt water aquariums*, viewed 2 December 2019, http://www.milwaukeeinst.com/site/products/products/digital-refractometers
- The aquarium solution, 2019, Instructions for ATC natural sea water refractometer, viewed 2 December 2019, https://www.theaquariumsolution.com/sites/default/files/downloads/SEAWATER%20REFRACTOMETER%20V2.pdf
- World Organisation for Animal Health, 2019, *Disinfection*, viewed 23 Decembers 2019, https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2009/1.1.3_DISINFECTION.pdf
- Lenntech, 2020, *Turbidity*, viewed 14 January 2020, https://www.lenntech.com/turbidity.htm#ixzz6AyGi2NVu
- OIE Aquatic Animal Health Standards Commission, 2003, *Methods for disinfection of aquaculture establishments*, viewed 20 November 2019, https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2009/1.1.3_DISINFECTION.pdf

4.7 Other information and attachments

Appendix 4.8: Nutrient deficiencies and their associated clinical signs of disease.
Appendix 4.9: Water parameter testing – acceptable levels, general information and reasons for testing, fish health considerations if the parameters are not within the recommended guidelines and methods to control the parameters.

Appendix 4.8: Nutrient deficiencies and their associated clinical signs of disease

Clinical signs of deficiencies in finfish	Nutritional Deficiency
Cardiac system	
Anaemia	Folic acid, inositol, niacin, pyridoxine, riboflavin, rancid, fat, vitamins B12, C, E&K Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, protein, riboflavin
Clotting blood, slow	Vitamin K
Epicarditis	Vitamin E
Fragility of erythrocytes	Biotin, vitamin E
Fragmentation of erythrocytes	Biotin, vitamins B12 and E
Haematocrit (reduced)	Iron, vitamins C and E
Haemoglobin (low)	Iron, vitamins B12 and C
Myopathy (cardiac)	Essential fatty acids
Oedema	Niacin, pyridoxine, thiamine, vitamins A & E
Ocular	
Cataract	Methionine, riboflavin, thiamine, zinc
Cloudy lens	Methionine, riboflavin, zinc
Deformation, lens	Vitamin A
Exophthalmos	Pyridoxine, vitamin A, C and E
Haemorrhage (eye)	Riboflavin, vitamin A
Lesion (eye)	Methionine. riboflavin, vitamins A and C, zinc
Pigmentation, iris	Riboflavin
Photophobia	Niacin, riboflavin
Vascularization (cornea)	Riboflavin
Gastrointestinal system and food absorption	
Anorexia (poor appetite)	Biotin, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine vitamins A, B12 and C
Distended stomach	Inositol
Feed efficiency, poor	Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, protein, riboflavin
Growth, poor	Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, pantothenic acid, protein, pyridoxine, riboflavin, thiamine, vitamins A, B12, C, D, and E
Lesion (colon)	Biotin, niacin
Pinhead	Starvation
Hepatic system	
Ascites	Vitamins A, C &r E

Ceroid liver	Rancid fat, vitamin E
Fatty liver	Biotin, choline, fatty acids, inositol, vitamin E
Haemorrhage (liver)	Vitamin C
Lipoid liver	Fatty acids, rancid fat
Necrosis (liver)	Pantothenic acid
Pale liver (glycogen)	Highly digestible carbohydrate, biotin,
Immune system	
Disease resistance (low)	Protein, vitamin C
Integument	
Coloration, dark (skin)	Biotin, folic acid, pyridoxine, riboflavin
Decolouration (skin)	Fatty acids, thiamine
Dermatitis	Pantothenic Acid
Diathesis (exudative)	Selenium
Erosion (fin)	Fatty acids, riboflavin, vitamin A, zinc
Haemorrhage (skin)	Niacin, pantothenic acid, riboflavin, vitamins A and C
Lesion (skin)	Biotin, inositol, niacin, pantothenic acid
Slime (blue coloured)	Biotin. pyridoxine
Metabolic system	
Goitre	Iodine
Lethargy	Folic acid, niacin, pantothenic acid, thiamine, vitamin C
Musculoskeletal and body condition	
Atrophy (gills)	Pantothenic acid
Atrophy (muscle)	Biotin. thiamine
Cartilage abnormality	Vitamin C, tryptophan
Clubbed gills	Pantothenic acid
Deformation, bone	Phosphorus
Degeneration, gills	Biotin
Dystrophy, muscular	Selenium, vitamin E
Fragility of fin	Folic acid
Haemorrhage (gill)	Vitamin C
Lordosis	Vitamin C.
Rigor mortis (rapid onset)	Pyridoxine
Scoliosis	Phosphorus, tryptophan, vitamins C and D
Spasm (muscle)	Niacin
Tetany & white muscle	Niacin, vitamin D

Neurological	
Ataxia	Pyridoxine, pantothenic acid, riboflavin
Convulsions	Biotin, pyridoxine, thiamine
Equilibrium loss	Pyridoxine, thiamine
Irritability	Fatty acids, pyridoxine, thiamine
Nerve disorder	Pyridoxine, thiamine
Prostration	Pantothenic acid, vitamin C
Shock syndrome	Essential fatty acids
Swimming, erratic	Pyridoxine, pantothenic acid
Renal system	
Calcinosis (kidney)	Magnesium
Haemorrhage (kidney)	Choline, vitamins A and C
Respiratory system	
Exudated gills	Pantothenic acid
Gasping, rapid breathing	Pyridoxine

Reference

- Young, Cho C, 2018, *Nutrition and fish health*, viewed 23 December 2019, https://dokumen.tips/documents/nutrition-and-fish-and-fish-health-c-young-cho-university-of-guelph-department.html

Appendix 4.9: Water parameter testing

(Acceptable levels, general information and reasons for testing, fish health considerations if the parameters are not within the recommended guidelines and methods to control the parameters.)

Water parameter	Acceptable levels	General information and reason for testing	Fish health considerations and methods to control the parameters.
Salinity Measured in parts per thousand (ppt)	Freshwater species: 1ppt or 0-500mg/L Oceanic species: 36ppt or 35,000mg/L or 35g/L Levels vary between species and at different life stages. Some species have a broader tolerance of acceptable salinity levels than other species.	Fish require specific elements to carry out vital biochemical processes. The amount of dissolved salts in the water affects the water's density and temperature requirements of the fish. Natural waterways vary in salinity from 1ppt to 40ppt. Increasing salinity or salinity baths are often used as a method of treatment in freshwater fish to reduce stress, increase mucous production, promote healing of damaged skin and kill some endoparasites. Check daily.	Fish held in water outside their normal salinity levels can suffer from: o Poor maintenance of fluid and electrolyte balance o Stress due to excessive water retention (Haemodilution) o Excessive water loss (Dehydration) **Managing salinity levels:** When changing salinity, fish should be allowed to adjust slowly (e.g. 1-5g/L/day).
Dissolved oxygen (D.O.) Measured in milligrams per litre (mg/L)	The D.O. should be near saturation at any temperature and salinity level. Amounts of oxygen required will depend on life stage, species, size and water temperature. A flow rate of 0.7 x 10-3 assures saturation or >5mg/L for optimal fish health.	Oxygen is lost from water through respiration by fish, plankton and other organisms, and by aerobic decay of organic matter. **Causes of Low D.O.:** o Aerator malfunction or loss of power o Aerator design is insufficient for housing system o Overstocking densities of fish and high feeding rates o "Crash" of phytoplankton/zooplankton booms in outside ponds systems o Excessive turbidity, i.e. no or limited oxygen production through photosynthesis in outdoor systems o Large blooms of phytoplankton, zooplankton and other pond organisms respiring during the night in outdoor systems o Series of cloudy, or windless days in outdoor systems o Check at least once daily or twice in highly stocked tanks or where water temperatures have risen	**The signs fish exhibit with low D.O.:** o Loss of appetite, poor growth and poor feed conversion efficiency o Stress and increased susceptibility to disease o Lethargy o Respiratory difficulty such as gasping near the surface or fish facing into current of inlet or aerator o Death of larger fish followed by smaller fish and other organisms **Managing low D.O. levels:** o Provide generator back up in case of power failure o Monitor D.O. routinely and chart diurnal fluctuations to predict periods of low D.O. o Maintain & provide additional aeration day and night o Spray water across the water surface in outdoor systems o Stop or reduce feeding o Exchange water o Remove dead plant and animal material from the water. o Add plants to the tank o
Water parameter	**Acceptable levels**	**General information and reason for testing**	**Fish health considerations and methods to control the parameters.**

Temperature Measured in degrees centigrade (°C)	Temperature is species specific and life stage specific. Any change in temperature should be very gradual <2oC/day.	Increasing temperatures are more stressful than cooler temperatures. High temperatures reduce available oxygen. Check at least once daily or twice daily if required during heat events.	**High temperatures cause:** o Stress o A lowering of oxygen levels available in the water leading to hypoxaemia o Increased vulnerability to disease o Reduced growth rates o Death o Cooler temperatures cause torpor **Managing temperature change levels:** o Monitor temperature daily and adjust thermostat for heating and cooling system as required, ensuring the change is a gradual process (ideally <2°C/day) o Provide shade in outside tanks o Ensure tanks have good insulation and that tanks are grounded with appropriate ground fault circuit breakers o If temperatures rise increase monitoring of oxygenation levels
Hardness Measured in milligrams per litre (mg/L)	Desirable levels for fish culture generally fall within the range of 20-250 mg/l. Note: As a general rule, the most productive waters for fish culture have a hardness and alkalinity of approximately the same magnitude. For example, a water with an alkalinity of 100 mg/l and hardness of 100 mg/l is good for fish culture.	Measures the mineral content of the water including: Major cations - calcium, magnesium, sodium and potassium and other divalent cations. Major anions – carbon trioxide, chloride, hydrogen bicarbonate and sulphate. Waters are often categorised according to degrees of hardness as follows: 0-75 mg/l - soft 75-150 mg/l - moderately hard 150-300 mg/l - hard over 300 mg/l - very hard. Alkalinity and hardness are not greatly affected by biological activity or aquaculture operations, and the initial concentrations in ponds are determined by their level in the water supply; any changes are largely the result of rainfall and evaporation. Check weekly and / or following water exchanges.	If hardness is too low, lime can be added to outdoor ponds. In fish tanks, either specialised aquarium re-mineralising products can be added to tanks to titrate levels or crushed coral, oyster shell or limestone can be used. Decreasing hardness is more difficult and options are: o Use of Reverse Osmosis/Deionized water (RO/DI) machines mixed with normal water source prior to titration o Commercially made distilled water mixed with normal water supply as above or o Peat is added to the tank in bags to trap the GH minerals, but this is not a precise method Inappropriate hardness may cause osmotic shock and reduced breeding. Appropriate hardness may decrease stress, toxicity of dissolved metals and ammonia.
Water parameter	**Acceptable levels**	**General information and reason for testing**	**Fish health considerations and methods to control the parameters.**

Nitrogen	Nitrogen is not normally toxic to fish except at very high levels.	Nitrogen is present in water as gas, nitrite, nitrate and ammonia. Supersaturation of water with nitrogen can occur when water is pumped under pressure or derived from underground supplies.	High levels of nitrogen can cause 'gas bubble disease' which will kill fish. Bubbles typically form under the skin, behind the eyes or in the blood vessels. **Managing high nitrogen levels:** o Underground water supplies should be passed through 'degassing columns' or vigorously aerated before use
Nitrite **Measured in milligrams per litre (mg/L)**	Freshwater systems <0.1mg/L.	Nitrite is the natural degradation of ammonia compounds and can reach toxic levels. High nitrite levels are less common in open flow systems. The toxic effect of nitrite is mitigated by chloride ions so is rarely a problem in marine systems. Check daily.	High nitrite causes methaemoglobinaemia where gill filaments become brown known as 'brown blood disease'. **Managing high nitrite levels:** Add salt at 5g/L to reduce toxicity.
Carbon dioxide (CO_2) **Measured in parts per million (ppm)**	<30 parts per million (ppm) or 30 milligrams per litre (mg/l).	High levels are typically found in closed systems. All organisms add carbon dioxide to the water via respiration. Phytoplankton and aquarium plants reduce carbon dioxide in daylight hours via photosynthesis. When CO_2 gas dissolves in water it creates carbonic acid. This will acidify the water and pH will decrease. Fish in water with high carbon dioxide concentrations can suffocate even if oxygen levels are high. However, CO_2 toxicity is rare in aerated fish housing facilities and therefore CO_2 only requires regular monitoring in tanks where large amounts of plants are being grown or if aeration systems have failed. Check daily.	**Managing high CO_2 levels:** o Increase aeration o Add aquatic plants to the tank
Alkalinity or carbonate hardness (KH) **It is expressed as milliequivalents per litre (mEq/L).**	Freshwater 0.2-10mEq/L or 50-200mg/l. Seawater 2.5 mEq/L. This ensures buffering of acid metals and proper functioning of bio filters.	Alkalinity is the measure of a tank's acid neutralizing capacity. Bicarbonates, carbonates, borates, phosphates and other anions contribute to alkalinity. Check daily.	**Managing alkalinity levels:** o Levels of alkaline may be raised by the use of lime in outdoor systems or with commercial alkaline buffers for indoor tanks o Levels of alkaline may be reduced by using commercially available pH decreasers or alternatively by undertaking water exchanges o Examples include: 'Proper pH 8.2' which is a bicarbonate and carbonate buffer and contains no phosphate used for seawater systems and 'Proper pH 6.5, 7.0, and 7.5' which are phosphate buffers used for freshwater aquariums.
Water parameter	**Acceptable levels**	**General information and reason for testing**	**Fish health considerations and methods to control the parameters.**

<table>
<tr>
<td>Ammonia Measured in milligrams per litre (mg/L).</td>
<td>Sensitive species (e.g.) salmonids in freshwater = <0.003mg/L.

Less sensitive species in freshwater = <0.01mg/L.

Marine species = <0.05mg/L.</td>
<td>Ammonia is the major waste product of protein or nitrogenous metabolism in fish and other aquatic organisms. Therefore, the rate of ammonia production of fish is proportional to the feeding rate.

In water, the total ammonia-nitrogen (TAN) occurs in two forms, un-ionised ammonia (NH_3) which is toxic to fish, and the ammonium ion (NH_4) which is relatively non-toxic, except at extremely high concentrations. Ammonia can change from one form to the other creating a balance between the two forms. Water pH and temperature influence the proportion of total ammonia occurring as the toxic (NH_3) form.

Therefore, ammonia levels should be measured at the hottest time in the day.

The following table shows: the percentage of total ammonia-nitrogen (TAN) in the toxic un-ionised form NH_3 at different temperature and pH values, taken from Boyd (1982) "Water quality management for pond fish culture"

<table>
<tr><th colspan="8">Temperature (°C)</th></tr>
<tr><th>pH</th><th>8</th><th>12</th><th>16</th><th>20</th><th>24</th><th>28</th><th>32</th></tr>
<tr><td>7.0</td><td>0.2</td><td>0.2</td><td>0.3</td><td>0.4</td><td>0.5</td><td>0.7</td><td>1.0</td></tr>
<tr><td>8.0</td><td>1.6</td><td>2.1</td><td>2.9</td><td>3.8</td><td>5.0</td><td>6.6</td><td>8.8</td></tr>
<tr><td>8.2</td><td>2.5</td><td>3.3</td><td>4.5</td><td>5.9</td><td>7.7</td><td>10.0</td><td>13.2</td></tr>
<tr><td>8.4</td><td>3.9</td><td>5.2</td><td>6.9</td><td>9.1</td><td>11.6</td><td>15.0</td><td>19.5</td></tr>
<tr><td>8.6</td><td>6.0</td><td>7.9</td><td>10.6</td><td>13.7</td><td>17.3</td><td>21.8</td><td>27.7</td></tr>
<tr><td>8.8</td><td>9.2</td><td>12.0</td><td>15.8</td><td>20.1</td><td>24.9</td><td>30.7</td><td>37.8</td></tr>
<tr><td>9.0</td><td>13.8</td><td>17.8</td><td>22.9</td><td>28.5</td><td>34.4</td><td>41.2</td><td>49.0</td></tr>
<tr><td>9.2</td><td>20.4</td><td>25.8</td><td>32.0</td><td>38.7</td><td>45.4</td><td>52.6</td><td>60.4</td></tr>
<tr><td>9.4</td><td>30.0</td><td>35.5</td><td>42.7</td><td>50.0</td><td>56.9</td><td>63.8</td><td>70.7</td></tr>
<tr><td>9.6</td><td>39.2</td><td>46.5</td><td>54.1</td><td>61.3</td><td>67.6</td><td>73.6</td><td>79.3</td></tr>
<tr><td>9.8</td><td>50.5</td><td>58.1</td><td>65.2</td><td>71.5</td><td>76.8</td><td>81.6</td><td>85.8</td></tr>
<tr><td>10.0</td><td>61.7</td><td>68.5</td><td>74.8</td><td>79.9</td><td>84.0</td><td>87.5</td><td>90.6</td></tr>
<tr><td>10.2</td><td>71.9</td><td>77.5</td><td>82.4</td><td>86.3</td><td>89.3</td><td>91.8</td><td>93.8</td></tr>
</table>

Check daily.</td>
<td>Excessive ammonia and nitrite cause ‘new tank syndrome’.

Low levels of ammonia cause:
o Damage to gill tissues
o Abnormal swimming behaviour including lethargy
o Reduced feeding
o Congregation around water inlet or pond edges
o Note: these signs may reduce at nighttime

High levels of ammonia cause:
o Reduced growth rate
o Increased susceptibility to diseases
o Elevated blood pH
o Tissue and internal organ damage
o Poor osmoregulation
o Death

Managing high ammonia levels:
o Reduce or stop feeding
o Water exchange
o Reduce the stocking density
o Increase aeration
o In emergencies - reduce pH of pond</td>
</tr>
<tr>
<th>Water parameter</th>
<th>Acceptable levels</th>
<th>General information and reason for testing</th>
<th>Fish health considerations and methods to control the parameters.</th>
</tr>
<tr>
<td>Suspended solids or turbidity</td>
<td>Sensitive species (e.g. salmonids) in freshwater <50mg/L.</td>
<td>Normal levels of turbulence cause solids to be suspended in the water such as clay, organic material or plankton in water. Excessive levels of solids due to irregular cleaning of tanks and then disturbances during the tank</td>
<td>High levels cause excessive mucous formation and swelling of gill filaments interfering with oxygen uptake. It can also deplete the oxygen levels of the tank.</td>
</tr>
</table>

Measured in milligrams per litre (mg/L)	Less sensitive species (e.g. non-salmonids) in freshwater <200mg/L. Marine species <100mg/L.	cleaning process can cause stress by clogging gills or producing mechanical gill damage if the material is an irritant. Visual daily checks and weekly testing.	**Managing high turbidity levels:** o Ensure filtration system is working well regularly maintained and sufficient for the fish housing system
Hydrogen sulphide Measured in micrograms per litre (µg/L)	Even concentrations <1mg/L can be lethal to fish. Freshwater fish should not be exposed to more than 2µg/L of hydrogen sulphide for long periods. Marine species tend to be more tolerant of hydrogen sulphide than freshwater species. Generally, hydrogen sulphide concentration should not exceed 5µg/L in aquaculture ponds with brackish water of full-strength seawater.	Produced by the bacterial decomposition of organic matter under anaerobic conditions. It smells like rotten egg and is called 'rotten egg gas'. Check weekly to fortnightly or more frequently during high risk episodes such as serration failure or in pond systems.	**Managing high hydrogen sulphide levels:** o Maintain continuous aeration o Add potassium permanganate in toxic events
Nitrate Measured in parts per million (ppm)	<10ppm. Most fish can tolerate brief exposure to levels around of up to 550ppm. However chronic exposure to much lower levels 30ppm can cause long term health issues.	Nitrate is critical for the growth and reproduction of plants in the natural aquaculture environment and is a critical component influencing algal production. In aquaculture systems where the water is recirculated over and over again, even though toxic substances are converted to less toxic substances through the actions of biofilm filtration systems (ammonia to nitrite to nitrate), accumulation of their biological by-products can build up and pose a health risk to the fish. Nitrate is not a common problem in flow through systems because water is not reused. Exceptions are where there have been algal blooms due to high levels of nitrate in the waterways from where the water has been sourced. Test weekly in recirculating systems and monthly or as required in flow through systems following high risk events.	**Signs of high nitrate levels:** o Poor cell development o Lethargy o Poor skin colour, o Reduced immune system o Weakened feeding response **Managing nitrate levels:** o Ensure water supplies are low in nitrate o Manage food levels to reduce overfeeding o Undertake water exchanges if levels become too high o Use a chemical filter media between water changes o Provide plants in the tank to use the nitrate for growth Maintain a healthy biofilm filtration system
Water parameter	**Acceptable levels**	**General information and reason for testing**	**Fish health considerations and methods to control the parameters.**

pH	pH between 6.5 and 9.0. Ideal is 6.9 in most species. Freshwater and seawater tanks will generally have different pH levels, with freshwater being slightly more acidic. A pH of <4 or >10 is lethal for most fish. Fish species vary in their tolerance at different life stages.	pH has many effects on the level of dissolved gases and metals in the water. It also affects dissolved oxygen uptake by the fish. Levels of ammonia, carbon dioxide and organic acids are all important for proper pH maintenance. **Causes of Sub-Optimal pH:** o Acidic water and soils in outdoor systems o Poorly buffered water, i.e. low alkalinity (<20 mg/l) o Waters having high alkalinity and low hardness Check daily.	**Signs of Sub-optimal pH:** o Increase of mucous on gill surfaces o Damage to eye lens and cornea o Abnormal swimming behaviour o Fin fray o Stress o Increased susceptibility to disease o Low production levels o Poor growth o Death **Managing high pH levels:** o Water exchange o Reduce feeding rates to lower nutrient input o Add gypsum ($CaSO_4$) to increase the calcium concentration or add alum ($AlSO_4$) for immediate reduction of pH to avert imminent fish mortality in outdoor ponds systems **Managing low pH levels:** o Water exchange o Add lime to outdoor ponds Add buffering to tanks

Other water parameters that may require periodic measurement

Heavy metals and algae should be measured if there is a known problem in the waterways from where the project's water supply is sourced. Note: larger water volumes are generally more stable than smaller volumes.

References

- American Fisheries Society, 2014, *Guidelines for the Use of Fishes in Research*, viewed 28 November 2019, http://frdc.com.au/Archived-Reports/FRDC%20Projects/1993-184-DLD.pdf
- Boyd, CE 2014*, Hydrogen Sulfide Toxic, But Manageable*, viewed 2 December 2019, https://aquafishcrsp.oregonstate.edu/sites/aquafishcrsp.oregonstate.edu/files/boyd2014hydrogensulfide_gaa.pdf
- Department of primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW, viewed 14 January 2020, https://www.dpi.nsw.gov.au/data/assets/pdf_file/0004/638680/ACEC-Guide-2015-FINAL-WITH-AQUI_S-2.pdf
- Department of primary industries NSW Government, 2019*, Water quality references*, viewed 2 December 2019, https://www.dpi.nsw.gov.au/fishing/aquaculture/publications/water-quality-management/water-quality-references

- Florida Department of Agriculture and Consumer Services, 2019, *Aquarium water quality: Carbon dioxide*, viewed 2 December 2019, https://www.fdacs.gov/Consumer-Resources/Recreation-and-Leisure/Aquarium-Fish/Aquarium-Water-Quality-Carbon-Dioxide
- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- Rowland, SJ 1998, *Water quality in freshwater aquaculture*. *Fishfact 1,* NSW fisheries.

Further reading

- Boyd, CE & Lichtkoppler, F 1979, *Water quality management in pond fish culture,* Auburn AL: Auburn University, International centre for Aquaculture, research development series No. 22.
- Environmental Protection Agency, 1976, *Quality criteria for water*, EPA-440/9-76-023, Washington, DC.

5. Fish monitoring and unexpected adverse event identification

5.1 Summary / Scope

This section relates to the monitoring of live fish to be used for scientific purposes and identification of adverse events.

5.2 Background information

Research involving living animals including fish, must be based on sound experimental design and animal care practices that lead to scientifically valid results. Fish are acutely sensitive to distress and responses may include changes in behaviour, reduced growth, changes in osmotic status, suppressed immune systems leading to illness, and altered reproductive capacity. All of these factors will impact on the quality and reliability of the scientific data being collected. Therefore, it is in the best interest of the investigator to ensure the fish are maintained, handled and tested under conditions that will not create such responses.

To achieve these conditions a fish health scoring system that is communicated between all project team members should be compiled. It acts as a consistent and objective form of fish health and welfare appraisal, which will enable any person involved in the project to recognise an 'intervention point', where the fish requires immediate veterinary attention, possible medications and increased monitoring.

An even more critical stage of welfare that investigators must be able to recognise is the 'humane end point', where the fish are very sick, or deemed to be suffering, and any previous treatment given or changes in husbandry, have been unsuccessful, calling for the immediate humane killing, necropsy and correct disposal of the fish.

This formal method of monitoring fish welfare will ensure no fish are left suffering whilst waiting for a communication from the investigator or chief investigator.

5.3 What details need to be included in a monitoring sheet?

General fish health assessment, including:

- normal expected results for each health parameter
- attached scoring sheet detailing how intervention and humane end points will be identified by assessing abnormal findings
- details of exactly how each health parameter will be assessed
- frequency of assessment varying from high frequency during anaesthesia/sedation (every five minutes) to general daily monitoring between scientific procedures.

Water monitoring, including:

- what water parameters will be assessed
- expected normal values for the system being utilised in the project
- equipment used to assess the parameters including their calibration and maintenance
- actions to be taken in case of abnormal results.

Other machinery monitoring (e.g., biofilters), including:

- Schedule of assessment, maintenance and calibration.

The monitoring sheets can be in either a handwritten or digital form (the latter being more practical in a water-based environment). If written, the handwriting must be legible in order to be read by AEC members and/or the AWO during an inspection and be stored in watertight packaging. All monitoring notes/findings should have a signature for easy identification of the person responsible for the finding, in case follow up information is required (i.e., in the case of adverse events).

It is also essential that results are not altered in any way once they have been recorded, to ensure a quick diagnostic trail can be followed in the case of an adverse event. This practise will help reduce the impact to any other fish in the project.

Storing the monitoring sheets

The completed animal monitoring sheets will be made available for viewing until the animal is released from the project as per the AEC application. The sheets are official documents, and at the conclusion of the project they should be archived according to UniSC policy. The AEC and AWO may request to view the monitoring records for several reasons:

- annual or final report assessment
- after a complaint or adverse event
- during scheduled or unannounced visits.

Monitoring personnel

Animals will be monitored by animal carers deemed to be competent at any stage of the project. In general, the investigators will undertake the procedures relating to the project and other animal carers may undertake the daily routine husbandry requirements under the direction of the investigators. At the start of a project, investigators must give clear instructions regarding who is responsible for what aspect of the animal's care, and this should be clearly communicated to all parties.

An animal carer other than an investigator can be involved in more complex monitoring procedures if:

- they have been given a copy of the AEC protocol
- they have had training in the procedure and are deemed competent
- they have accepted the role
- they are named on the AEC application as co-investigators.

5.4 Equipment and resources

PPE for all procedures: Safety goggles/glasses, laboratory coat, enclosed shoes, nitryl unpowdered gloves, first aid box with eye rinsing products and hand washing facilities.

Monitoring paperwork: Pens, monitoring sheets for various frequency levels or a digital monitoring sheet.

Adverse event paperwork: Recorded details on the electronic adverse event form.

Monitoring equipment: Wristwatch or stopwatch, doppler coloured ultrasound monitor for recording heart rate during surgical procedures, all water monitoring equipment as in section four.

5.5 Recommended procedures

The monitoring process starts at the admission stage with the initial health assessment. Once this is completed the ongoing monitoring program should begin as scheduled in the AEC application. It is essential to make a list with all the routines to be checked each day and also lists for checking at longer intervals.

Monitoring frequency guidelines

Investigators have a responsibility to inform the animal carers if, when, and for how long, the different types of monitoring intensity are required above the baseline daily monitoring.

High risk monitoring (Every five minutes - hourly)

- Ensure all PPE is worn by the animal carers.
- Use the monitoring form for high-risk procedural monitoring called 'In field/in laboratory anaesthesia and post procedure monitoring sheet for fish as detailed in the AEC application process.
- Record the parameters every five minutes during surgery/procedural/general anaesthesia/sedation tasks.
- Maintain this level of high intensity monitoring until the animal is fully conscious/recovered, then revert to medium frequency monitoring (every hour to every four hours) depending on the requirements of the procedure and AEC application.

Record any abnormalities and advise the investigator immediately. If an unexpected adverse event is noted, refer to the guideline 12 of the USC animal ethics guidelines on the Student Portal.

Medium frequency monitoring (hourly-every four hours)

- Medium frequency monitoring would be expected, for example, after drug trials with some known effects, unique procedures, post-surgery (> five hours), during signs of illness or disease, mildly painful procedures, prolonged trapping/handling, or on admission of animals into projects and new holding facilities.
- The level of monitoring (i.e., hourly or four hourly) will be dependent upon the requirements of the animal's specific condition to ensure they are not left unattended in a painful or distressed state.
- A veterinarian should be consulted by the investigator if this level of monitoring is required for sick animals.

Daily monitoring (once or twice in a 24-hour period)

- Visually examine the behaviour and appearance of the fish according to the monitoring sheet parameters.
- Visually examine the water quality (transparency/turbidity) and other daily water parameters as outlined in appendix 4.13.
- Check hydrodynamics (flow) in tanks.
- Check distribution of feed from feeding machines.
- Remove and register dead fish.
- Flush outlet from tanks if fitted with standpipes.
- Wipe off membrane of oxygen probes.
- Registration of actual oxygen concentration in tanks.

- Record all findings on the monitoring sheet and add initials.

Weekly monitoring (once or twice weekly)

- Check water levels in pump sumps.
- Check nozzle sprayers on mechanical filters.
- Registration of volume of new water used.
- Check pressure and volume in oxygen tanks.
- Undertake weekly/fortnightly water parameter checks.
- Check function and timing of UV-lights.
- Check weekly machinery calibrations as required by the manufacturer's instructions.
- Record all findings on the monitoring sheet and add initials.

Weekly or monthly

- Clean the biofilters according to the manual.
- Drain condenser water from compressor as required.
- Check water levels in buffer tanks of automated recirculation systems.
- Check monthly calibration of machinery and equipment according to the manufacturer's instructions including emergency back-up systems.
- Check alarms – make alarm tests.
- Check that the emergency oxygen supply works in all tanks, where applicable.
- Check all pumps and motors for failure or dissonance.
- Check filter sumps.
- Check fish tank lighting and alter scheduling according to seasonal changes.
- Record all findings on the monitoring sheet and add initials.

6-12 monthly monitoring

- Clean UV steriliser and change UV lamps yearly.
- Replace tank lighting as required.
- Undertake maintenance of all fish tank machinery according to the manufacturer's instructions.
- Record all findings on the monitoring sheet and add initials.

Unexpected adverse event management

What is an adverse event and how should an adverse event be managed and reported?

What constitutes an unexpected adverse event?

- When an animal or group of animals die unexpectedly, such as during surgery or anaesthesia, or after a procedure or treatment.
- When a much larger number of animals were adversely affected by a procedure, compared to the number predicted in the ethics application.
- When the level of pain or distress is greater than the level predicted in the ethics application.
- When an animal becomes ill or diseased, which may affect other animals within the project.
- When unexpected external factors occur that may adversely impact the animal's welfare, e.g., human pandemics, flooding, emergency power cuts, inclement weather affecting fieldwork, where access to the animal's husbandry needs and general monitoring assessment are impeded.

What action need to be taken in the case of an adverse event?

Prompt action must be taken in response to unexpected adverse events regarding animals and emergencies including:

1. Alleviate animal pain and distress. If necessary, animals may require humane killing by a competent person without delay.
2. Limit the impact to other animals by isolating the affected animals.
3. Perform treatment, following veterinarian review and advice.
4. Immediately notify the chief investigator.
5. Notify the technician in charge and the ethics team within the Office of Research.
6. Record all the details of the event clearly and comprehensively.
7. If the fish dies, place its body in a plastic bag and into a fridge. Clearly label the plastic bag with the ethics application number, date, identification details ready for a necropsy to be performed, if required.

Complete and submit the Adverse Event Report to animalethics@usc.edu.au no later than the next working day after the unexpected adverse incident.

5.6 Animal health and welfare considerations

All fish should be observed or handled under sedation/anaesthesia for monitoring in a gentle, quiet and calm manner, using welfare parameters to assess humane end points and intervention points. There is an obligation for all investigators using animals for scientific purposes to:

- Ensure the animals are only used for the shortest time necessary for the scientific or teaching outcomes to be reached.
- Prevent or minimise any pain and distress to that which is unavoidable for the purpose of achieving the scientific outcomes.

Therefore, an animal's involvement in a project must end when:

- A scientific endpoint has been reached.
- For some reason, there is no way the scientific outcome can be met.
- The animal develops severe pain or discomfort that does not respond to analgesia.
- The animal develops a morbidity that is unexpected, not part of the study termination point, or that cannot be treated without interfering with the scientific outcomes.

An animal must be removed from a project on a temporary or permanent basis if the pain or distress being suffered is unable to relieved. This could mean ceasing the activity for a period of time, humane killing, releasing the animal back to the wild/abandoning field observation work.

The welfare of the animals must always take precedence over research or teaching outcome.

The end points, intervention points and humane end points are made up of a set of clear and objective criteria/clinical signs that define how these points can be recognised. These criteria should be measurable physiological or behavioural changes. Setting pre-determined criteria allows investigators and animal carers to make decisions independently, leading to prompt treatment and removal of animals from an activity if animal welfare is deemed as being unduly compromised, particularly if the leading investigator cannot be contacted.

The investigator should define these points for each ethics application and indicate them at the top of the monitoring sheet to help guide the animal carers in their daily assessment of the animal's welfare and wellbeing.

Examples of monitoring sheets, scoring sheets and action plans for both animal welfare parameters and water parameters are found in appendices 5.12-5.16. These examples should be used as guidelines only to form the basis of fish species-specific requirements for each project.

5.7 Training plan and competency assessment

Investigators and animal carers should have completed the online Introduction to animal ethics training found on Student Portal. Investigators and animal carers must be fully trained and assessed as competent in the process of fish monitoring before undertaking related procedures. Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application. The AWO is available to provide or organise training and to assess competency as required.

Investigators and animal carers must be aware of the OH&S considerations surrounding this species and the first aid procedures required in case of emergencies. The AEC and AWO will monitor competency during inspections of animal ethics approved projects.

5.8 References and acknowledgements

References

- Bayne, K & Turner, P, (eds) 2013, *Laboratory Animal Welfare,* Academic Press, Cambridge, Massachusetts
- Bregnballe, J 2015, *A Guide to Recirculation Aquaculture - An introduction to the new environmentally friendly and highly productive closed fish farming systems*, viewed 2 December 2019, http://www.fao.org/3/a-i4626e.pdf
- Department of primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW.
- Fondriest environmental learning centre, 2020, *Turbidity, Total Suspended Solids & Water Clarity*, viewed 14 January 2020, https://www.fondriest.com/environmentalmeasurements/parameters/waterquality/turbidity-total-suspended-solids-water-clarity/
- Flinders University, 2019, *Standard Operating Procedure Working with Fish*, viewed 2 December 2019, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-working-with-fish.pdf
- Godfrey, C 2018, *Animal Monitoring and Humane Intervention Points Guideline*, viewed 18 December 2019, https://www.jcu.edu.au/__data/assets/pdf_file/0005/632948/Animal-Monitoring-and-Humane-Intervention-Points-Guideline.pdf
- Hargreaves, JA & and Tucker, CS 2002, *Measuring Dissolved Oxygen Concentration in Aquaculture*, viewed 2 December 2019, http://agrilife.org/fisheries/files/2013/09/SRAC-Publication-No.-4601-–-Measuring-Dissolved-Oxygen-Concentration-in-Aquaculture.pdf
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- National research council of the national academies, 2011, *Guide for the care and use of laboratory animals Eighth Edition*, viewed 23 December 2019, https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf
- NHMRC, 2013, The Australian code for the care and use of animals for scientific purposes, viewed 19 April 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, Guidelines for the Care and Use of Fish in Research, *Institute for Laboratory Animal Research*, vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

Further reading

- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Ostrander, G, Bullock, G & Bunton, T 2000, *The Laboratory Fish*, Academic Press, Cambridge, Massachusetts

5.9 Other information and attachments

Appendix 5.10: In field/in laboratory anaesthesia and post procedure monitoring sheet for fish.
Appendix 5.11: Medium frequency monitoring sheet (Post anaesthesia/procedure visual examination.
Appendix 5.12: Daily monitoring sheet for fish projects and scoring sheet for all animal welfare parameter monitoring forms.
Appendix 5.13: An example of proposed investigator nominated actions and interventions based on the scoring sheet results.
Appendix 5.14: Water parameter monitoring form - daily and once weekly including normal/expected values, monitoring equipment and actions taken. Scoring sheet and instructions for using the monitoring form.

Appendix 5.10: In field/in laboratory anaesthesia and post procedure monitoring sheet for fish

(Note: a new sheet should be completed for each animal undergoing a procedure or anaesthesia) UniSC AEC application number:……

Animal identification	**Procedure notes:**	**Investigator:** **Contact details:**	**Chief investigator:** **Contact details:**
Holding facility details:	**Intervention score =** **Humane killing score=**		

Fish health parameters to be assessed	**Assessment time during anaesthesia and procedure (Monitoring every 5 - 15 minutes) depending on type of procedure and level of animal welfare risk** **Scoring system: 0-same as "normal" 1-very slightly different from "normal"** **2-noticeably different from "normal" causing serious welfare concerns. (See chart below for explanation)**																		**Notes** **Detail how this parameter's values differ from the normal level and action to be taken based on scoring.**
Time	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	**IN EACH BOX INCLUDE TIME (e.g. 08:30, 15:25)**
Gills/operculum score Normal = Normal colour for species, fine gills (devoid of thickening/hyperplasia/fusion, inflammation and excessive mucous).																			
Heart rate																			
Ocular score Normal = Eyes bright, non-bulging and no lacerations or lesions.																			
Skin/scales score Normal = No lesions, signs of rubbing, abrasions, or obvious parasites.																			
Fish health parameters to be assessed	**Assessment time during anaesthesia and procedure (Monitoring every 5 - 15 minutes) depending on type of procedure and level of animal welfare risk** **Scoring system: 0-same as "normal" 1-very slightly different from "normal"** **2-noticeably different from "normal" causing serious welfare concerns. (See chart below for explanation)**																		**Notes** **Detail how this parameter's values differ from the normal level and action to be taken based on scoring.**
Time	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	**IN EACH BOX INCLUDE TIME (e.g. 08:30, 15:25)**

Fins score Normal = Fins are in normal shape & position. All used for swimming. No lacerations or lesions noted.																			
Vent/anus score Normal = No inflammation, swelling/trauma																			
Mucous score Normal = Mucous is present and the amount appropriate for species No excessive sticky mucous or malodourous mucous especially around the gills or on scales.																			
Skeleton score Normal = Spine is straight without abnormally shaped deviation.																			
Total score																			

Appendix 5.11: Medium frequency monitoring sheet

(Post anaesthesia/procedure visual examination. Hourly to 4 hourly, as required until normal welfare parameters achieved).

Animal identification	Procedure notes:	Investigator: Contact details:	Chief investigator: Contact details:
Holding facility details:	Intervention score = Humane killing score=		

Fish health parameters to be assessed	Normal expected result	Time allocations and score assessment (e.g. 08:30, 09:30)								Notes Detail how this parameter's values differ from the normal level and action to be taken based on scoring.
		:	:	:	:	:	:	:	:	
Swimming pattern and ability score	Swimming throughout the tank with non-erratic movement, exploring all enrichment facilities at all levels in the water table.									
Alertness to stimuli score	Fish rush to hiding places when investigator approaches the holding tank or goes to normal feeding location if comfortable with the animal carer.									
Equilibrium in water/balance score	Fish able to hold body with dorsum pointing towards the water surface. Fish able to remain underwater without effort.									
Scale condition score	Scales are shiny & intact (no evidence of trauma or infectious lesions (such as fluffy white patches or bleeding). Scales- consistent in colour as per natural species appearance. Lateral line scales are evident.									
Fish health parameters to be assessed	**Normal expected result**	**Time allocations and score assessment (e.g. 08:30, 09:30)**								**Notes** **Detail how this parameter's values differ from the normal level and action to be taken based on scoring.**
		:	:	:	:	:	:	:	:	

Colour of body score	Normal uniform colour/pattern for the species (devoid of pallor, darkness or blotchy patches). No external indicators of internal haemorrhage/bruising.									
Defaecating score	Normal amount and colour for the fish species.									
Breathing rate and pattern score	Breathing rate is steady and undertaken under water. (No gasping especially around air inlets or at the surface of the water).									
Body symmetry score	Body appears even on both sides when viewed from above. Free from lumps and swellings at any angle.									
Feeding pattern score	All fish interested in food and equally eating within 5 minutes of food being offered.									
Total score										

Appendix 5.12: Daily monitoring sheet for fish projects

UniSC AEC application number:……

Animal identification:	Procedure notes:	Investigator: Contact details:	Chief investigator: Contact details:
Holding facility details:	Intervention score = Humane killing score=		

Fish health parameters to be assessed	Normal expected result	Dates							Notes Detail how this parameter's values differ from the normal level and action to be taken based on scoring.
		/ /	/ /	/ /	/ /	/ /	/ /	/ /	
Swimming pattern and ability score	Swimming throughout the tank with non-erratic movement, exploring all enrichment facilities at all levels in the water table.								
Alertness to stimuli score	Fish rush to hiding places when investigator approaches the holding tank or goes to normal feeding location if comfortable with the animal carer.								
Equilibrium in water/balance score	Fish able to hold body with dorsum pointing towards the water surface. Fish able to remain underwater without effort.								
Scale condition score	Scales are shiny & intact (no evidence of trauma or infectious lesions (such as fluffy white patches or bleeding). Scales-consistent in colour as per natural species appearance. Lateral line scales are evident.								

Fish health parameters to be assessed	Normal expected result	Dates							Notes Detail how this parameter's values differ from the normal level and action to be taken based on scoring.
		/ /	/ /	/ /	/ /	/ /	/ /	/ /	
Colour of body score	Normal uniform colour/pattern for the species (devoid of pallor, darkness or blotchy patches). No external indicators of internal haemorrhage/bruising.								
Defaecating score	Normal amount and colour for the fish species.								
Breathing rate and pattern Score	Breathing rate is steady and undertaken under water. (No gasping especially around air inlets or at the surface of the water).								
Body symmetry score	Body appears even on both sides when viewed from above. Free from lumps and swellings at any angle.								
Feeding pattern Score	All fish interested in food and equally eating within 5 minutes of food being offered.								
Total score									

Appendix 5.13: An example of a fish scoring system and associated fish scoring sheet

The scoring system to identify animal welfare intervention points and humane end points is specific to each fish species, but as a guide the following criteria could be considered.

Monitoring parameters	Assessment method & monitoring level	Clinical signs to consider	Scoring system		
			Normal (0)	Moderate (1)	Severe (2)

Swimming pattern and ability	Visually assess	Erratic rapid swimming, no swimming, hiding near base of tank, uncoordinated swimming, flashing	Swimming throughout the tank with non-, exploring all enrichment facilities at all levels in the water table.	Erratic movement, rubbing on side of tank, uncoordinated	Floating near water surface, flashing, jumping out of water
Alertness to stimuli	Alert fish and visually assess	Startled by minor movement or no alertness or reaction to external stimuli.	Fish rush to hiding places when investigator approaches the holding tank or goes to normal feeding location if comfortable with the animal carer.	Fish dart from one hiding place to an another (overreact) or slow to react or get away from stimuli	No reaction to external stimuli, unable to respond
Equilibrium in water/balance	Visually assess	Floating and unable to dive/submerge for normal periods of time for feeding or investigating tank.	Fish able to hold body with dorsum pointing towards the water surface. Fish able to remain underwater without effort.	Fish struggling to remain in a normal upright position. Fish repeatedly dive but always gradually float back to the surface.	Fish unable to die and float at the surface. Body floats on a diagonal or upside down.
Scale condition	Visually assess and close up examination including skin scrape and sampling of mucous	Trauma lesions, bleeding, bite marks, ulceration, 'fluffy white' lesions, rub marks.	Scales are shiny and intact without evidence of trauma or infectious lesions such as fluffy white patches or bleeding. Scale colour is appropriate for species and lateral line scales are evident. No obvious parasites.	Some scales show trauma, rubbing injury, lesions, changes in colour. Increase or loss in mucous in affected areas. Mild bouts of skin rubbing.	Excessive abnormal mucous. Large non healing ulcerative multifocal lesions. Fish constantly rubbing.
Colour of body	Visually assess	Body appears very pale, or dark or blotchy which is abnormal for the species and not associated with husbandry changes such as light intensity.	Normal uniform colour/pattern for the species (devoid of pallor, darkness or blotchy patches). No external indicators of internal haemorrhage/bruising.	Fish colour has changed in limited areas of its body but no other signs of ill health- pale, dark or blotchy. Some mild signs of internal bruising noted.	Large portions of body changed in colour and other signs of ill health noted. Signs of bruising under the skin more obvious and greater coverage.
Monitoring parameters	**Assessment method & monitoring level**	**Clinical signs to consider**	**Scoring system**		
			Normal (0)	**Moderate (1)**	**Severe (2)**
Defaecating	Visually assess	Amount is increased, changed colour (without diet change), contains blood. No faecal production whilst still eating.	Normal amount and colour for the fish species.	Faeces becoming more frequent, changed in consistency or colour for 1-2 days.	Faeces seen to contain blood and be excessive in quantity. No evidence of faecal production for several days.

Heart rate	Assess with doppler or ECG monitor on pectoral or anal fins	Erratic racing pace with irregular pattern. Very slow rate with intermittent missed beats.	Regular patterned strong heartbeat.	Pattern starts to become less regular and intermittent loss of beats. Heart is racing 1.5 faster than normal rate.	Heart slows greatly with multiple events of missed beats. Heart is racing 2 times faster than normal rate.
Breathing rate and pattern	Visually assess	Rate is increased. Gasping at surface or near air inlet.	Breathing rate is steady and undertaken under water.	Staying around air inlet. Respiration rate mildly increased.	Gasping at surface or near to air inlet and changes to gill health.
Feeding pattern	Visually assess	Fish not feeding in first 5 minutes of food being offered (even after new feed choices have been offered to stimulate appetite)	Fish feed immediately, (may even be waiting at feeding point at normal feeding times).	Feeding amount reduced mildly, but there is still some feed intake.	Fish stops eating completely and does not show interest in the feeding zone during feeding time.
Gills/operculum/nares	Close examination, biopsy	Gills are red, inflamed and lamellae are thickened and covered in excessive mucous. Operculum movement is abnormal.	Normal colour for species (pink to light red), fine gill lamellae based on the species. Operculum movement is steady and rhythmic.	Gills becoming mildly reddened and thickened or becoming pale. Mucous amount has increased, and appearance has mild changes. Operculum movement is becoming arrhythmic.	Gills are severely inflamed and fused in areas. Lamellae are greatly thickened, and mucous is thick, sticky and voluminous. Gills very pale. Operculum movement is rapid and erratic.
Ocular	Close examination,	Eyes are 'popping' out of eye socket or sunken. Lacerations, swelling or redness noted. Cornea is opaque.	Eyes bright, non-bulging from eye sockets and normal size for the species.	Mild trauma or inflammation. Eyes mildly bulging from sockets. Cornea becoming slightly discoloured/opaque.	Corneal ulcerations, lacerations to eyelids. Popeye evident. Corneas white in colour.
Fins	Palpation and visually assess	Bent fins. Non-functioning fins. Trauma/lacerations to fins including bite wounds. Ulcerative lesions or fluffy growth on the fins surface.	Fins are in normal position and all being used for swimming.	Mild bite wounds or lesions noted. Fins are mildly misshaped but still being used.	Fins are distorted in shape and held limply. Deep lesions or ulcerations noted. Large areas of trauma or sections missing that are affecting swimming ability.
Vent/anus exam	Visually assess	Lacerations, inflammation, gut or urogenital prolapse.	No inflammation, swelling or trauma	Mildly inflamed, swollen appearance.	Prolapse of gut or urogenital tract. Severe swelling or lesions noted.

Skeleton	Visually assess and palpation	Spine is deviated/bent/ misshaped. Fish is unable to swim in a straight line.	Spine is straight with fish swimming normally.	Slight deviation in spine, but fish swimming patterns is only mildly changed.	Spine obvious bent/deviated. Fish unable to swim in normal directional movement.
Mucous	Visually assess and sample	Excessive amount of mucous for species. Mucous has changed colour/consistency & is congregating around gills, other body parts or close to lacerations in the scales.	Mucous is present and the amount appropriate for species (during both the day and night).	Mild increase in amount of mucous in certain areas of the body.	Mucous has greatly changed in appearance. It is excessive, sticky and tenacious.

This scoring system for both the animal welfare parameters and the water parameters are used to create a consistent objective approach across all persons involved in a scientific or teaching project. The aim is to identify intervention points and humane end points rapidly, and then identify what action should be taken based on the scoring system. The table outlined below shows a simple action plan based on a scoring system as a guideline only. Each scoring system should be designed to be specific to each animal project, to encompass individual fish species variations.

Score	Score Assessment	Actions/Interventions
1	Animal demonstrates slight or moderate deviation from normal	Notify chief investigator or laboratory team leader and/or AWO – commence treatment if recommended by AWO or nominated veterinarian.
2	Animal demonstrates significant or sustained deviation from normal	Immediate consultation with the AWO or nominated veterinarian or immediate humane killing. Notify animal ethics, office of research. Complete a necropsy. If animal welfare compromise or mortality rates fall outside of approved AEC application conditions, an unexpected adverse event report must be submitted.
Accumulative score of 3 or greater	Animal demonstrates significant or sustained deviation from normal	Immediate consultation with the AWO or nominated veterinarian or immediate humane killing. Notify animal ethics, office of research. Complete a necropsy. If animal welfare compromise or mortality rates fall outside of approved AEC application conditions, an unexpected adverse event report must be submitted.

Appendix 5.14: Water parameter monitoring form

(Daily and once weekly including normal/expected values, monitoring equipment and actions taken)

Water parameter monitoring device expected value	**Week starting……**							**Notes and actions taken in case of abnormal results**
	Dates							
Oxygen (D.O.) (mg/L) am/pm Oxygen meter >5mg/L / >80%								
Temperature (^{0}C) Thermometer= (species specific enter details)								
Salinity (ppt) or (mg/L) Tester= Freshwater sp. 1ppt/0-500mg/L Oceanic sp.: 36ppt /35,000mg/L								
Ammonia (mg/L) Tester= Sensitive freshwater sp. <0.003mg/L. Less sensitive freshwater sp. <0.01mg/L. Marine sp. = <0.05mg/L.								
Nitrite (mg/L) Tester= Freshwater systems <0.1mg/L.								
Nitrate (ppm) Tester= <10ppm.								

pH **Tester=** **pH between 6.5 and 9.0.** **Ideal is 6.9 in most species.**								
Alkalinity or carbonate hardness (KH) (mEq/L). **Tester=** **Freshwater 0.2-10mEq/L or 50-200mg/l.** **Seawater 2.5 mEq/L.**								
Hardness (mg/L) **Tester=** **20-250 mg/l.**								
Suspended solids or turbidity (mg/L) **Tester=** **Sensitive freshwater sp. <50mg/L.** **Less sensitive freshwater sp. <200mg/L.** **Marine sp. <100mg/L.**								
Hydrogen sulphide (mg/L) **Tester=** **Freshwater sp. < 2µg/ periods. Marine sp. < 5µg/L**								
Carbon dioxide (ppm) or (mg/l). **CO_2 meter.** **<30ppm or 30 mg/l**								
Total daily score								

Note: Prior to using the monitoring sheet, the method of measuring each parameter should be completed for all forms. If measuring equipment changes, the details should be recorded on the sheet, to identify potential equipment variation.

Instructions for completing the water parameter monitoring sheet

1. Each parameter/animal/tank/enclosure is examined at each nominated monitoring time point.
2. Each criterion is scored, and the score marked on the monitoring sheet. Training by the AWO and/or chief investigator may be required to ensure all personnel are consistent in terms of scoring.
3. Scores are then added together, and a total score is recorded on the monitoring sheet.
4. Appropriate to the score, specific actions/interventions are undertaken.
5. Comments concerning abnormalities are recorded in the 'notes' section.
6. Any other abnormalities are recorded in the 'other' section.
7. Any abnormality that is observed to be of greater severity than the normal levels or any major deviation requires immediate consultation with the chief investigator and AWO or immediate humane killing and recorded as an unexpected adverse event.
8. All unexpected adverse events must be reported immediately to animal ethics and an 'unexpected adverse event' report completed.

Example of a scoring system for water parameter monitoring

This chart will need to be **adapted for each fish species** when writing the AEC application. A few examples of ranges are given as a guide only.

Water parameter	**No obvious deviation from normal** **Score 0**	**Slight or moderate deviation from normal** **Score 1**	**Sustained deviation from normal** **Score 2**	**Recommended actions to be taken in case of adverse results**
Oxygen (D.O.) (mg/L) (%) am/pm	>80%	70 – 80%	<70%	E.g. Aeration to be increased to tank
Temperature (^{0}C) (*Species specific*)	+/- 5^{o}C from optimum	+/- 10 from optimum	> 10 from optimum	
Salinity (ppt) or (mg/L)	+/- 5ppt from optimum	+/- 10 ppt from optimum	> 10ppt from optimum	
Ammonia (mg/L)	<1	1-5	>5	E.g. 50% of tank water to be replaced
Nitrite (mg/L)	<1	1-5	>5	
Nitrate (ppm)	<50	50 - 100	>100	

pH	< 1 unit from optimum	1 – 1.5 units from optimum	> 1.5 units from optimum	E.g. Bicarbonate or acid to be added
Alkalinity or carbonate hardness (KH) (mEq/L).				
Hardness (mg/L)				
Suspended solids or turbidity (mg/L)				
Hydrogen sulphide (mg/L)				
Carbon dioxide (ppm) or (mg/l).				

6. Techniques for the humane killing of fish

6.1 Summary / Scope

This section covers the humane killing of fish in the laboratory and field setting for scientific purposes.

6.2 Background information

Reasons for humane killing in scientific projects

Within a university environment, there may be several reasons why fish need to be humanely killed including:

- completion of a teaching or research project (end points)
- for tissue collection
- adverse events where intervention points/humane end points have been identified.

Criteria to evaluate the various methods of humane killing include:

- the method chosen must cause a rapid loss of consciousness followed by death
- the method must be reliable and irreversible
- any drugs must be readily available to the investigators
- there should be no major risk to the handlers during the humane killing process.

The process of killing any fish should always follow humane procedures

There are two general groups of humane killing suitable for fish: chemical immersion/bath (anaesthesia overdose); and physical. Chemical baths containing anaesthetic agents will allow the fish to become sedated prior to humane killing and this method goes through various stages as follows.

Level number	Level of anaesthesia	Signs associated with this level
Level 0	Normal	Normal swimming behaviour and reaction to external stimuli

Level 1	Light sedation	Still swimming but reduced reaction to external stimuli; equilibrium normal' normal opercular rate
Level 2	Deep sedation/light anaesthesia	No swimming; loss of equilibrium (rolls over – belly up) but still may try to right itself; normal to slightly decreased opercular rate; still maintains a tail reaction following slight pinch
Level 3	Surgical anaesthesia	Complete loss of equilibrium; complete loss of reactivity (negative tail reaction); very slow opercular rate; slow heart rate
Level 4	Medullary collapse	Total loss of opercular movement followed by cardiac arrest. Humane killing stage.

6.3 Equipment and resources

Field and laboratory humane killing

Immersion procedures
Container to house animal during procedure, aeration supply for container, fresh or saltwater supply at temperature appropriate for species and the same parameters as the fish's tank water, measuring pipette/syringe, anaesthetic agent, clean container to mix stock solution in, oxygen/temperature meter, stirring rod/paddle, Nitrile gloves, apron or laboratory coat and eye protection.

Physical procedures
Pithing/spear gun, sharp knife, chainmail gloves, solid chopping board/surface.

Necropsy procedures
Necropsy kit, laboratory OH&S, PPE, laboratory sampling pots, slides, transport packaging and paperwork. Fish monitoring sheets.

6.4 Recommended procedures

Humane killing of fish - Immersion methods (Aqui-S, Benzocaine)

Chemical immersion methods with Aqui-S

Aqui S immersion procedure is the preferred method of humane killing at UniSC.
Aqui -S is a water-soluble liquid anaesthetic and is suitable for both freshwater and saltwater aquaculture systems. There is potential for irritation at higher concentrations depending on fish species; therefore, it is preferred to gradually add the product, until the suitable dose has been achieved.

Method of humane killing with Aqui-S:

1. Mix the bottle of Aqui-S by inverting several times.
2. Prior to dispensing Aqui-S into the tank containing the fish, mix it 1:9 with tank water.
3. Expose the fish to an anaesthetic concentration of Aqui-S (10-25mg/L), and once signs of sedation are observed, add sufficient Aqui-S to reach a concentration of 40 mL per 1000 litres. This will usually require adding 15 to 25 mL of additional Aqui-S per 1000 litres.
4. Once the fish are narcotised the concentration can then be gradually increased to 150mg/L and the fish left in the solution for 30 minutes to achieve medullary collapse and death in most species.
5. Fish should be left in the solution for at least 10 minutes after showing no signs of life (i.e. no response to touch, no gill and mouth movement, and no swimming).

If fish are to be removed from treated water within 30 minutes of immersion with Aqui-S, a secondary form of humane killing should be used to ensure death has occurred, once an anaesthetic level three has been reached (e.g. brain spiking). Note: brain spiking should only be undertaken by individuals assessed as competent and approved on the AEC application.

Further information on calculating dosage rates can be found at **www.aqui-s.com**.

Where fish in research projects are not bred for human consumption, there is no withholding period with Aqui S.
This drug is currently only registered for use in commercial salmonid species; however, 'off label' use for other fish species in research projects is allowed with caution, because there are no known recommended doses for many fish species. Therefore, for fish safety, the investigator should start at the lower end of the dose rate and slowly increase the dose as required for the plane of anaesthesia (level 3) prior to increasing the concentration to achieve humane killing (level 4 anaesthesia).

Chemical immersion methods with Benzocaine (Ethyl-p-amino benzoate)

Use this chemical as a backup method if Aqui-S is unavailable or impractical:

- Benzocaine requires some pre-preparation. It must be dissolved in 100% ethanol at a recommended concentration of 1 gram per 10 millilitres (1g/10ml) to form a stock solution prior to use.
- The stock solution must be kept in the refrigerator in brown glass bottles and not exposed to sunlight. One millilitre of the stock solution contains 100 milligrams (mg) of benzocaine i.e. 1mL stock – 100mg benzocaine.
- It is used as an immersion bath for anaesthesia or humane killing.

- To make an immersion bath of concentration of one milligram per litre (1mg/L) stock solution is added at the rate of 0.01 millilitre per litre of bath water 0.01ml/L or 1ml/100 L of immersion bath.

Dilution rates for benzocaine stock solution.

Dose of benzocaine required for immersion bath (mg/L)	Dilution rate of stock solution (mL/100litres of immersion bath)
25mg/L	25ml/100 litres
50mg/L	505ml/100 litres
75mg/L	75ml/100 litres
100mg/L	100ml/100 litres

Immersion bath dose rates for benzocaine. (This is a guide only because doses will vary between fish species).

Handling /sedation	20-35mg/L immersion bath
Surgery/anaesthesia	50-75mg/L immersion bath
Humane killing	100 or >100mg/L immersion bath

Method of humane killing with benzocaine

1. Fish should initially be placed in immersion baths of lower concentrations (35mg/L) and the concentration slowly increased to reach the humane killing level.
2. Fish must be left in the solution for 10 minutes after opercular movements and other signs of life have ceased. Note: respiratory paralysis can precede cardiac arrest by some fish species tolerant of hypoxia. For example, 'ram ventilator species' such as tuna, generally show very little respiratory movement under anaesthetic conditions during normal procedures.
3. If the investigator is unsure if death has been achieved, a secondary humane physical killing technique should be performed for assurance (such as decapitation or brain spiking). Brain spiking should only be undertaken by individuals assessed as competent and approved on the AEC application.
4. Benzocaine has a long withholding period in fish to be used for human consumption, but there is no withholding period for fish used in research projects, and off label use is allowed.

Chemical immersion methods with Tricaine methanesulfonate (MS-222)

This anaesthesia drug has been used in the past as an immersion bath for humane killing. However, a more recent study on goldfish (which are an example of hypoxia tolerant fish) has shown that recommended concentration levels of this drug are ineffective. Therefore, its use may be limited and more favourable options such as Aqui-S should be used in its place. Drug dosing concentrations have been included in the medications for fish section as a reference guide only.

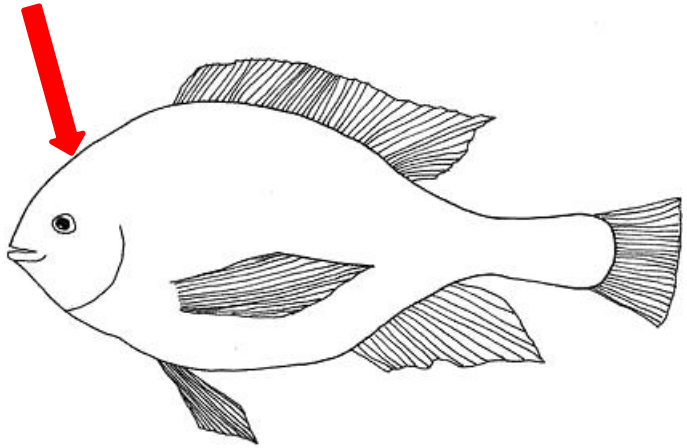
Humane killing of fish - Physical methods

Any physical method of humane killing should be approached as a two-stage process where an anaesthetic agent such as Aqui-S is used in the first stage to render the fish unconscious, followed by the physical killing procedure, wherever possible. Examples of where this may not be possible would be in a field situation, where the fish is too large to move onboard and chemical measures cannot be employed. In this situation, only the physical methods can be applied.

Percussion stunning/cranial concussion/blunt force trauma or clubbing

1. The fish should be placed in lateral recumbency on a solid non-slip board.
2. Stunning involves a rapid blow to the head of the fish with sufficient force to render it immediately insensible or unconscious. The blow should be delivered by a hammer, mallet or a solid piece of wood.
3. It is not advisable in a small fish due to inaccuracy of the contact.
4. Ensure the animal carer is both confident and skilled in this procedure to ensure the process is humane.
5. This technique should be followed by brain pithing to ensure death has occurred.
6. Confirm that death has been successfully established. Death will occur very quickly with this method with minimal distress when performed by an experienced animal carer. On the other hand, if inexpertly carried out, for example with insufficient force, stunning can result in wounding that may cause considerable pain and suffering.

Operator protection should be taken when handling sharp instruments. See picture below for site of the stunning.



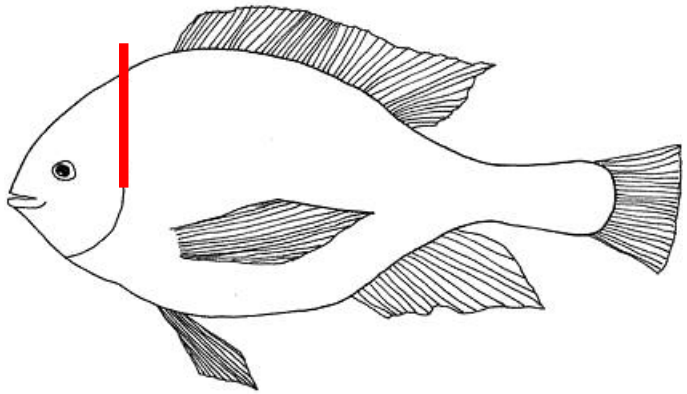
The site for percussion stunning (arrow and cross mark the actual site)
(Image by the Aquarium vet)

Decapitation

This involves the separation of the brain from the spinal cord by severing the spinal cord with a sharp knife (larger fish) or scalpel blade (smaller fish) to remove or partly remove the head.

1. The fish should be placed in lateral recumbency on a solid non-slip board.
2. Knife insertion occurs at the back of the head/operculum opening and the operator uses a rotation of the wrist once the knife is inserted to assist in the decapitation process.
3. Used alone decapitation is generally not an acceptable method of humane killing and should be followed by brain pithing. Experienced operators may be able to use complete spinal transection as a sole procedure without the need for pithing if enough brainstem (high spinal) injury has resulted in rapid painless death. This may only be appropriate for fish of a certain size and species.
4. Confirm that death has been successfully established.

Operator protection should be taken when handling sharp instruments. See picture below for area to cut.



Red line marks the site for decapitation
(Image by the Aquarium vet)

(Brain Spike/pithing/Iki-jime)

- This process involves killing the fish with a sharp tool (such as a sharp knife, a specifically designed Ikigun® or a carbon tipped speargun (the latter for larger fish species), where the skull is pierced, and the brain destroyed.
- The site of insertion is dorsal (above) and slightly caudal (behind) to the eye of the fish.
- **There is some variation in the exact site of the brain in some fish species and this should be investigated prior to undertaking humane killing in this manner.**
- Smaller fish able to be lifted easily must be placed in lateral recumbency on a solid non-slip board and secured by placing light pressure with the opposing hand.
- Larger fish may need to be cradled prior to pithing to ensure the fish is restrained enough to make an accurate pithing technique.

Operator protection should be taken when handling sharp instruments.

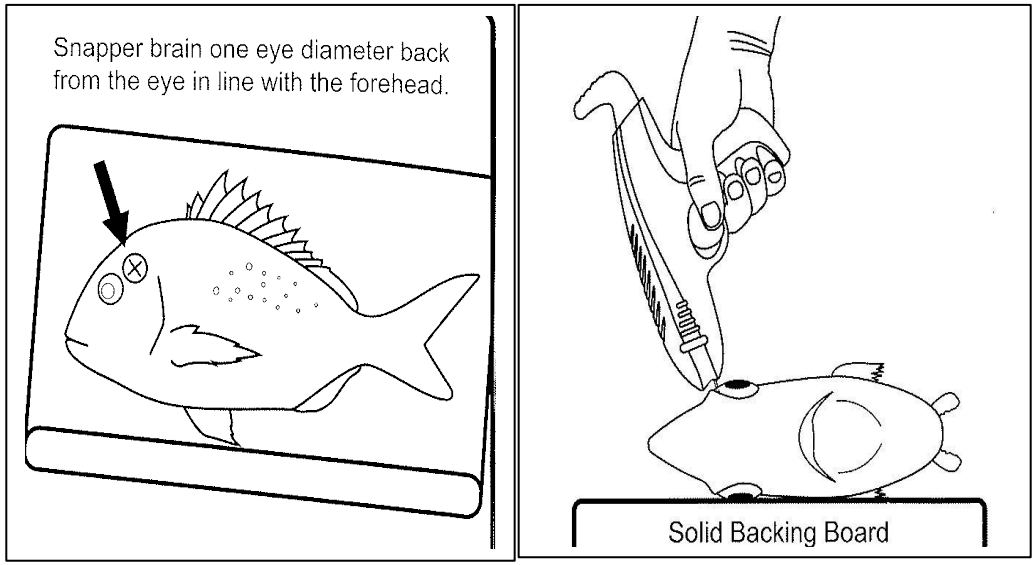
Notes on the Ikigun® for pithing fish

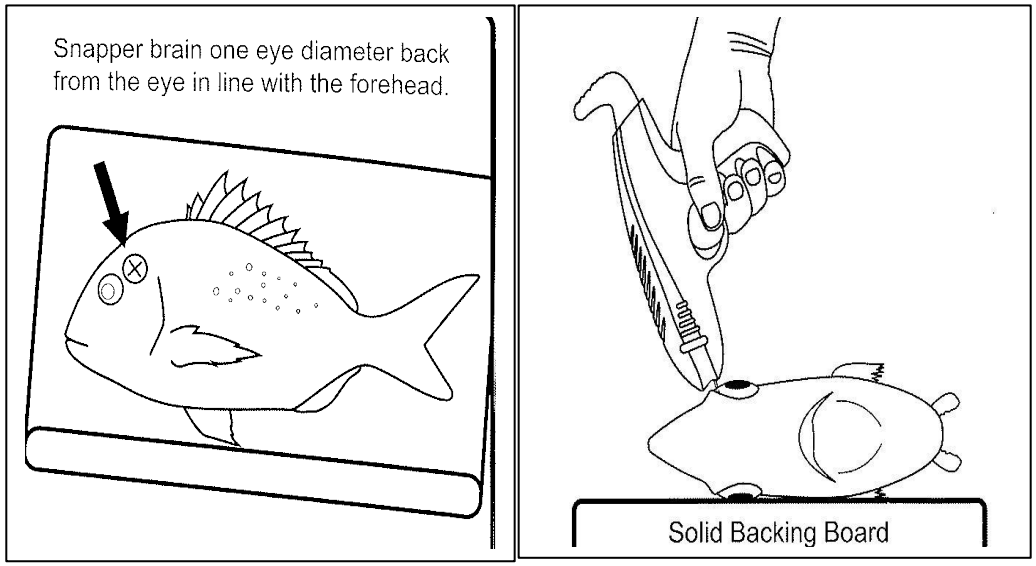
The Ikigun® is a specifically designed instrument for pithing fish and is recommended when brain spiking is used for the humane killing of fish from 200 to 350 mm in length.

The Ikigun® is also a potentially dangerous instrument and should be used only once fish are restrained on a flat surface using the Ikiboard restraint (see image below) to stabilise the fish for the killing process, which keeps the opposing hand away from the sharp instrument.

Procedure for using the Ikigun®

1. The fish are placed in lateral recumbency on the Ikiboard and secured by placing light pressure using the clasp.
2. The bolt is cocked by pulling back on the ram (up to three clicks).
3. The gun muzzle is pressed onto the side of the head, one eye diameter caudodorsal from the eye, in line with the forehead.
4. The trigger is then pulled.
5. After the killing, the gun should be cleaned with cold water.





(Image from: Ikigun, Adept Ltd, Auckland, NZ)

Further information can be found at: http://www.ikijime.com/ for photos of where to administer the brain spike for different fish species.

Confirmation of death in the fish

Reliable indicators of death may not be available for some fish species. However, there are some standard approaches that can be useful for many of the more commonly encountered species when assessing death in a fish.

Test	Expected confirmation signs of death
Touching the tail	Loss of body movement and loss of reactivity to any stimulus.
Holding the fish	Initial flaccidity prior to rigor mortis.
Visualise respiration	Respiratory arrest / cessation of rhythmic opercular activity for 30 minutes.
Rock fish from side to side to assess eye movement	Loss of eyeroll (no vestibulo-ocular reflex or movement of the eye when the fish is rocked from side to side). However, this is also lost in anaesthetised fish, so should not be used as a sole indicator of death.
Cardiac arrest	The heart can continue to contract even after brain death or removal from the bodies of fish, so the presence of a heartbeat is not a reliable indicator of life. However, sustained absence of heartbeat is a strong indicator of death.

For more sessile, less active organisms, or those with specific anatomical or physiological adaptations that prevent the use of these indicators, it may be more difficult to assess loss of consciousness and death and consultation with species experts is recommended if animal carers or investigators are unsure.

Necropsy techniques for fish

The necropsy can help determine causes of morbidity and mortality in fish. The relevance of recent history and the fish's environment play a key role in the necropsy process, more so than in most other species of animal because the vast majority of disease problems in fish relate to water quality and poor management practises. Therefore, biopsies alone may not be enough to determine an accurate diagnosis.

Complete information on life support systems, water quality, husbandry/general management, the fish species and other fish in the same facility, bird and predator contact, and any recent procedures or changes to the fish's environment must be included in a necropsy report and sent to a pathology laboratory. A list of any previous fish health issues should also be included.

Performing a necropsy

1. In accordance with the Animal Code, when an animal dies unexpectedly, or is humanely killed due to unforeseen complications, a necropsy should be performed by a competent person.
2. In the face of a disease outbreak, fish at varying stages of illness should be culled and samples collected from each. This will prevent secondary diseases often found in late disease stage fish samples, masking the underlying cause. Fish should be collected ideally before they die in the water, because in as little as 30-60 minutes their bodies will be contaminated and start to undergo autolysis.
3. The dead fish should be wrapped in plastic and placed in the fridge (4^{o}C) or on ice as soon as possible if necropsy is to be delayed a few hours. However, any delay in the necropsy process will affect tissue quality and pathological results. Therefore, if a necropsy is needed, it is best it's performed as a priority when fish die of unknown causes. Fish for necropsy **should not** be frozen.
4. A full set of samples should be collected in every case whether the plan is to send them off to a laboratory immediately in the face of a disease outbreak or to have histopathology samples ready to send off in case a potential disease outbreak is imminent.
5. Wet preparation samples should be made for both inhouse viewing and for laboratory analysis and tissues collected and placed in 10% formalin for histopathology analysis at the laboratory. In a case where PCR sampling is required, tissues should be placed in 90% ethanol solutions.
6. Pathology reports should be made available in the case of adverse events, annual/final reporting and AEC/AWO inspection visits.

Appendix 6.12 outlines a plan for investigators to follow, ensuring a systematic approach to each fish necropsy. A copy of this report should be sent with any samples to the chosen pathology laboratory to enhance the chances of an accurate diagnosis and treatment/management plan. Information regarding necropsy samples sent to Biosecurity Queensland can be found at the following web address: https://www.publications.qld.gov.au/dataset/veterinary-laboratory/resource/56026801-3046-4182-b645-46965924f35d

References

- Chong, RSM 2009, Aquaculture production, VETS 4021 lecture notes, University of Queensland
- European Association of fish pathologists, 2015, Fish necropsy manual, viewed 2 December 2019, http://www.necropsymanual.net/en/
- Meyers, TR 2004, Standard necropsy procedures for Finfish, NWFHS laboratory procedures manual, 2nd ed. Alaska Fish and game C.F. Division Juneau & Anchorage
- Yanong, RPE 2003, Necropsy Techniques for Fish, seminars in avian and exotic pet medicine, vol. 12, no. 2, pp. 89-105

Disposal of bodies

Once fish have been confirmed as dead, dispose of them according to the UniSC laboratory body waste procedures.

Cleaning process after necropsy

At the end of the necropsy, all equipment including necropsy room benches and floors must be cleaned with hospital grade detergent, thoroughly rinsed and then disinfected with either 70% or 99% ethanol, bleach, or sodium hypochlorite.
Note: if necropsy is performed within the fish housing area, the detergent must be suitable for aquaculture (see general cleaning notes in husbandry section 4).

6.5 Animal health and welfare considerations

Each of the procedures outlined above will cause some level of distress to the fish, simply by the direct handling of the animal. It is important to humanely kill fish away from other fish or animals. Ideally the room will be quiet, and the lights slightly dimmed to reduce levels of stress. Aqui S anaesthesia immersion bath method has been shown to cause no increase in cortisol levels during the humane killing process.

Any of the physical methods of humane killing will cause distress. However, undertaking an anaesthesia immersion bath process beforehand, will provide a more humane and accurate procedure. Poor technique does, however, have the ability to cause severe suffering and pain.

Disposing of live animals- is preventable by thorough assessment of the fish for multiple signs of death prior to disposal.

6.6 Training plan and competency assessment

Investigators and animal carers should have completed the online introduction to animal ethics training found on the Student Portal.

Investigators and animal carers must be fully trained and assessed as competent in the process of fish humane killing techniques before undertaking the related procedures. Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application. The AWO is available to provide training and assess competency as required.

Investigators and animal carers must be aware of the OH&S considerations surrounding this species and the first aid procedures required in case of emergencies. The AEC and AWO will monitor competency during inspections of animal ethics approved projects.

Training should include:

- Safety issues and risk factors surrounding the handling of noxious fish species and the use of knives and chemicals.
- Appreciation for the mechanism by which the humane killing procedure produces loss of consciousness and humane death.
- Recognition of signs of pain and distress in fish.
- Necropsy techniques.

6.7 References and acknowledgements

References

- American Veterinary Medicine Association (AVMA), 2020, *The American Veterinary Medicine Association Guidelines on euthanasia,* viewed 14 January 2020, https://www.avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf
- Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART), 2016, *Humane killing of Animals Used for Scientific Purposes*, viewed 1 May 2019, https://www.adelaide.edu.au/ANZCCART/docs/an29022016.pdf
- European Association of fish pathologists, 2015, *Fish necropsy manual*, viewed 2 December 2019, http://www.necropsymanual.net/en/
- Fenn, CM, Glover, DC & Small, BC, 2013, Efficacy of AQUI-S 20E as a Sedative for Handling and Cortisol Suppression in Pallid Sturgeon, *North American Journal of Fisheries Management*, vol. 33, no. 6, pp. 1172-1178, https://doi.org/10.1080/02755947.2013.831000, viewed 30 January 2020, https://www.tandfonline.com/doi/abs/10.1080/02755947.2013.831000
- Flinders University, 2019, *Standard Operating Procedure for working with fish 18/06/19*, viewed 2 January 2020, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-use-of-marine-aquaculture-facilities.pdf
- Jones, R & Daly, J, *Humane euthanasia techniques for ornamental fish,* viewed 12 January 2020, https://www.google.com.au/search?q=humane+euthanasia+for+ornamental+fish&ie=&oe=
- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, Guidelines for the Care and Use of Fish in Research, *Institute for Laboratory Animal Research,* vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

Further reading

- AVMA, 2000, Report of the AVMA Panel on Euthanasia, Journal of the American Veterinary Medical Association vol. 218, no. 5, pp. 669-696
- Barker, D, Allan, GL, Rowland, SJ & Pickles, JM, 2002, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research. Port Stephens, NSW: NSW Fisheries Animal Care and Ethics Committee,* Port Stephens Fisheries Centre, New South Wales
- Blessing, JJ, Marshall, JC & Balcombe, SR, 2010, Humane killing of fish for scientific research: a comparison of two methods. *Journal of Fish Biology* vol. 76, pp. 2571-2577
- Callahan, HA & Noga, EJ, 2002, Tricaine dramatically reduces the ability to diagnose protozoan ectoparasite (*Ichthyo- bodonecator*) infections. Journal of Fish Disease, vol. 25, no. 7, pp. 433-438
- Kestin, SC & Van De Vis, JW, 2002, Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. *Veterinary record*, vol. 150, pp. 302-307
- NHMRC, 2013, The Australian code for the care and use of animals for scientific purposes, viewed 20 November 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
- Ostrander, G, Bullock, G & Bunton, T, 2000, *The Laboratory Fish,* Academic Press, Oxford, UK

- Van De Vis, H, Kestin, S, Robb, D, Oehlenschläger, J, Lambooij, B, Münkner, W, Kuhlmann, H, Kloosterboer, K, Tejada, M, Huidobro, A, Otterå, H, Roth B, Sørensen, NK, Akse, L, Byrne, H & Nesvadba P, 2003, Is humane slaughter of fish possible for industry? *Aquaculture Research,* vol. 34, pp. 211-220
- Wildgoose, WH (ed), 2001, British Animal Small Veterinary Association, Manual of ornamental fish, (ed 2), Gloucester, England

6.8 Other information and attachments

Appendix 6.9: Quick reference guide to humane killing of fish
Appendix 6.10: Necropsy information for fish

Appendix 6.9: Quick reference guide to humane killing of fish

The following quick reference chart shows the recommended humane killing techniques for fish, starting with the most preferred method for UniSC investigators.

Method of humane killing	Acceptability of technique with regards to humaneness	Efficacy	Cost	Dose/concentration	Comments
Chemical immersion methods - fish are placed in a separate tank of known anaesthetic agent at euthanasia dose rates. The water parameters of the euthanasia tank must be as close to the fish's normal tank water as possible in all aspects. Fish must remain in the solution for at least 10 minutes after cessation of opercular movement.					
Aqui S Immersion	Most acceptable method	Very good	Cheap	40ml/1000L	Suitable for all fish species and life stages.
Benzocaine Immersion	Acceptable method	Good	Cheap	>250mg/L	Suitable for all fish species. Solution must be buffered.
Tricaine ms-222 Immersion	Acceptable method	Very good	Cheap	250-500mg/L (or 5-10 times the anaesthetic dose).	Suitable for all fish species. Solution must be buffered. Dose depends on species, life stage and water chemistry parameters.
Physical methods of humane killing must be performed by a trained and experienced operator. Preference for using one of these methods instead of the more humane immersion methods above, must be justified by the investigator. Unless unable to undertake chemical euthanasia (i.e. at sea, on large *in situ* fish), all other fish must be anaesthetised prior to physical humane killing procedures. Confirmation of death must be assessed.					
Stunning and brain destruction Physical	Conditionally acceptable	Acceptable if accurately undertaken.	Cheap	Not applicable	Must be followed by pithing to confirm death. Not suitable for all fish species.
Decapitation or spinal section Physical	Conditionally acceptable	Acceptable if accurately undertaken.		Not applicable	Must be followed by pithing to confirm death. Not suitable for all fish species.

Methods not deemed to be acceptable according to humaneness are: Skin absorption of chemicals, death by anoxia and immersion in carbon dioxide. Note: ice slurry has been suggested as a humane method to kill small tropical fish in the past. However, the very specific requirements, potential welfare issues associated with the direct contact with ice and complications associated with saltwater fish species and osmotic complications following dilution, has rendered this a less favourable killing procedure compared to Aqui-S, and is therefore not recommended in these guidelines. When considering any of the humane killing options noted above, the investigator should seek advice from other colleagues who are experienced in the particular requirements of the fish species in the project.

Appendix 6.10: Necropsy information for fish

General overview guidelines

Operators should wear appropriate OH&S eye protection, apron/overall/ covered footwear and nitrile gloves according to UniSC laboratory guidelines. The necropsy process should take place in a clean location to prevent contamination of samples.

Step 1 - General observation of the fish in the tank prior to death if possible

If the fish is to be culled to collect samples for general disease surveillance or disease outbreak investigation, it is important to make notes on their appearance prior to the cull. (Some of this information may already have been noted on the fish daily monitoring sheet.) Notes should include:

- general behaviour, swimming pattern and movement
- signs of deformity or external lesions such as ulcers and trauma
- any changes in scale colour, thickening or thinning/loss or external parasites
- signs of bleeding or haemorrhage
- changes in mucous colour, quantity, consistency or distribution
- eye health
- gill health, operculum and respiratory patterns
- changes in body condition, shape and weight loss/gain and food intake.

Step 2 – Close up external assessment of carcass and sample collection

Once the fish is dead (see humane killing techniques), a close-up examination of all external structures should be made and notes completed on the necropsy report. Samples of external tissues, tissue swabs/scrapings (gill and skin) can be collected and wet mounted on slides for microscopic analysis and staining as required.

Step 3 - Internal organ assessment and sample collection

Once the internal body of the fish is exposed, the organs should be visually assessed noting changes in location, size and general appearance whilst in situ. Following this, each internal organ should be dissected in a systematic fashion to ensure contamination of tissues is prevented. Tissue samples should then be collected and stored according to the requirements of the laboratory. Each sample/pot should then be clearly labelled and notes made on the necropsy report.

Step 4 – Package samples, collate supporting documents prior to transport to pathology laboratory

Once all samples have been collected, the laboratory report is completed and the samples placed in the specific laboratory transport packaging. Include all supporting paperwork, such as water parameter sheets for four weeks, fish health monitoring sheets, any recent changes or procedures (e.g. Stress testing, changes in diet or lighting, use of drugs, transportation), diet information and any other recent history that may be relevant to facilitate an accurate diagnosis and treatment or management plan. Remember, that formalin fumes may affect the quality of fresh/wet mounted slide samples and should therefore be packaged separately to avoid contamination. Caution: Do not inhale formalin vapours or allow it to come into contact with any part of the investigators body. If accidental contact is made follow the laboratory safety guidelines for first aid measures.

Necropsy equipment and supplies

General supplies	Microbiological supplies	Histopathology supplies	PCR supplies
Compound microscope, dissecting microscope or magnifying lamp	Cultural swabs for pathology laboratory testing	10% buffered formalin for light histopathology	90% alcohol
Pathology report form, permanent marker pen, laboratory transport packaging	Microbiological media plates including: BHIA (Brain heart infusion agar) with or without 1% saline for saltwater species is BHIA medium halophilic bacteria are suspected as disease agents. TSA (Tryptic soy agar) with 5% sheep blood Sabouraud's dextrose agar	Trump's solution for electron microscopy	
Latex/nitrile gloves, apron, enclosed shoes, eye protection as required by laboratory guidelines for PPE	Sterile loops		
Scissors (small ophthalmic, medium and large with blunt and pointed tips	Alcohol burner		
Scalpel and handle	Sterile gauze		
Forceps (rat tooth, fine/microdressing)	Glass jar/container for instruments		
Necropsy tray or table	Alcohol pads		
Slides and cover slips, sample pots	Matches/lighter		
Paper towels			
Needles and syringes for blood collection			
Dechlorinated water or saltwater for wet mounts			
Aqui-S anaesthetic for humane killing			
Drill, Dremel, saw for larger fish			
Camera for picture collection			

Cleaning equipment including brushes, cleaning chemicals as per UniSC laboratory guidelines, drying racks, biological waste disposal garbage bags/bins			

Necropsy report for AEC application number:……

Date/time of fish death:……/…..:….. How did the fish die: Humanely killed with …… Died by ……

Date /time of necropsy:……/……:…….. How was the fish stored if the necropsy was delayed?.....

Identification details:

Fish tank ID: **Tank/life support system:**	**Animal identification (breed, life stage):**	**Investigator:** **Contact details:**	**Chief investigator:** **Contact details:**

Step 1 – General observation of the fish in the tank prior to death if possible
(If parameters are considered to be normal findings, investigator should write 'NAB'-no abnormalities noted in appropriate box)

Health parameter	Description (What can be seen in detail)	Comments (Possible causes)
General behaviour, swimming pattern and movement		
Signs of deformity or external lesions such as ulcers and trauma		
Any changes in scale colour, thickening or thinning/loss or external parasites		
Signs of bleeding or haemorrhage		
Changes in mucous colour, quantity, consistency or distribution		
Eye health		
Gill health, operculum and respiratory patterns		
Changes in body condition, shape and weight loss/gain and food intake		

Step 2 – Close up external assessment of fish carcass and sample collection recommendations

Anatomical location	Description/comments and potential cause of abnormality (if known)	Procedure /samples to be collected
Blood collection (Check for blood parasites, viral inclusion bodies, bacteria and other cell morphology changes)		Small fish specimens: Excise the caudal peduncle vein and allow a drop of blood to be deposited onto the slide for a blood smear and use a diff quick stain. Larger fish specimens: collect blood from the caudal vein puncture into a heparinised syringe and prepare a blood smear.
Eyes (Check for cloudiness, haemorrhages, increased mucous, exophthalmia, buphthalmia)		Collect eye for PCR sampling Collect samples of lesions for fixing or microbiology testing.
Mouth (Check internal and external surfaces for lesions or parasites)		Visual check and collect samples of lesions is noted for fixing or microbiology testing.
Skin and scales (Record size, location, distribution and number of skin lesions/erosions. Use a drawing to mark location of lesions). List sites of haemorrhages, parasites and fungi.		Perform a skin scrapping. Collect samples of external lesions such as ulcers/areas of discolouration/increased mucous/abrasions/haemorrhage/masses/parasites. Inoculate ulcers onto appropriate medium.
Gills/opercular List sites of haemorrhages, parasites, fungi, and microbubbles (indication of supersaturation) , telangiectasia (ballooning of capillaries in gills suggestive of ammonia or toxins), hyperplasia, discolouration of gill (brownish colouring suggestive of nitrite toxicity, white/pale colour suggestive of anaemia). Examine the inner side of the opercular.		Perform a gill scrapping and wet mount Collect and fix gill samples for histopathology Collect gill sample for PCR sampling If bacteria are noted, mince the gill tissue, allow it to air dry and gram stain slide sample.
Fins (Record deformities, trauma and lesions)		Collect and fix fin samples for histopathology
Spine (Record deformities)		Visual check and collect samples of abnormal spine during internal investigation
Vent/anus/genital pore (Check for swelling, redness, protrusions and parasites).		Visual check and collect samples of lesions for fixing or microbiology testing.
Muscle (Check for colour, haemorrhage, masses, wasting).		See notes below.
Body score (Designate body score out of 5)		See below for an example of a fish scoring system used for zebrafish species.

Step 3 - Internal organ assessment and sample collection

Internal necropsy procedure

1. Place the fish on their right-hand side on the clean necropsy tray or table.
2. Disinfect the outside of the fish's body by flooding it with 70% ethanol.
3. Disinfect the equipment with 100% ethanol and by passing the metal instruments through a Bunsen flame enabling the alcohol to burn off and repeat the process.
4. Incise into the abdominal cavity (see supporting information below for technique).

Visual assessment

Visually examine the viscera in situ for abnormalities such as displacement, discolouration or mottled appearance, enlargement (hypertrophy), haemorrhage or erythema, abscesses or cysts, excessive fluid in the abdominal cavity (ascites, causing pot belly), foreign bodies such as fungus, metazoan parasites or tissue growth. Organs to include are: heart; liver and gall bladder; kidney; pancreas; adipose tissue; spleen, air bladder; pyloric caecae and entire GI tract.

Techniques for internal and skin/muscle microbiology testing for laboratory assessment

1. Dips scissors and forceps into 100% alcohol and then flood the kidney surface with 70% alcohol. Cut a small portion of kidney and place in a labelled transport tube.
2. Flood the spleen surface with 70% alcohol and flame the tips of the forceps and scissors with a Bunsen burner to aseptically collect the spleen sample and place it in a labelled transport tube.
3. Heat a clean scalpel blade with a Bunsen burner and sear the area of skin to be sampled. Make the sample deep enough to also collect the muscle below and place the sample in a labelled transport tube.
4. If lesions are noted on other visceral organs collect samples in a similar sterile manner to the kidneys.

Inhouse microbiology assessment

1. Perform a squash smear of the spleen with a drop of PBS (Phosphate-buffered saline) and gram stain for bacterial rod and fungal hyphae. Place a cover slip and examine.
2. Perform a squash smear of the rectum with a drop of PBS (Phosphate-buffered saline), place a cover slip and examine for evidence of protozoa, fungal hyphae or food content. Abundant bacteria are expected in this area.
3. Perform a squash smear of the other visceral organs as above if required and stain with Diff Quick.
4. Sample the spinal column by severing it along the dorsum. Blood will be evident. Use a sterile loop, collect a sample and streak it onto a TSA plate or use a culture swab.

Internal viral testing

1. Collect tissues for viral assay of larger fish including kidney, spleen and place in sterile tissue culture fluid for refrigeration. In the case of fry place the whole dead fish in the container.
2. Collect kidney smears for Immunofluorescent assay testing (FAT) and Enzyme-linked immunosorbent assay (ELISA) testing if required.

Histopathology testing

1. Taking histopathology samples from fish that have been dead for several hours or longer are generally not suitable for histological examination due to rapid autolysis.
2. In the case of small fry, the whole dead body can be placed into the labelled 10% formalin solution sample pot.
3. Deceased fingerlings should have their abdominal cavity opened to achieve better fixative penetration.
4. 1cm cubed pieces of liver, spleen, intestine, heart and kidney can be placed into a labelled 10% formalin solution sample pot.
5. Open the skull of the fish and remove the whole brain prior to slicing it in half in a longitudinal direction. Remove an eye and place both half the brain and eye in a separate labelled formalin solution sample pot.
6. Collect 1cm cubed piece of muscle including the lateral line and also skeletal muscle if obvious lesions are noted, and place in 10% formalin solution.

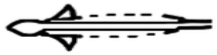
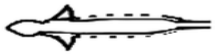
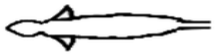
Supporting information for necropsy techniques

General points

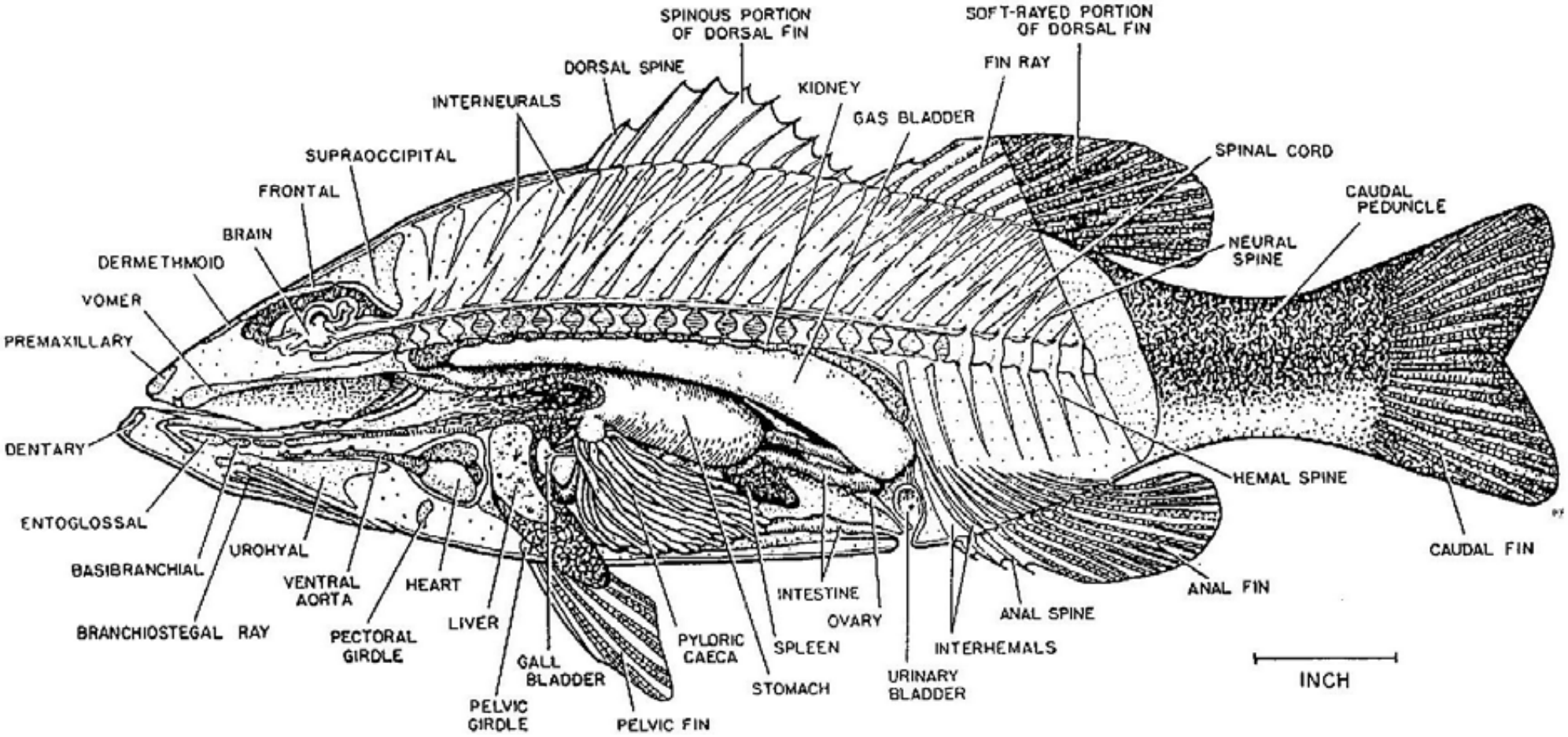
- Three wet mounted samples can be placed on the same slide, with a cover slip over each sample site.
- Collection of thin 2-3mm sections of sampled organs is sufficient for squash preparation with addition of a water droplet, especially in larger fish sampling that have more fibrous tissue making the squash preparation more difficult.
- Dechlorinated freshwater can be used for both marine and freshwater species for internal organ wet mounts. However external sources of tissues for wet mount assessment will require drops of saline for marine fish species.

Skin scraping procedure: Using the blunt edge of a clean scalpel blade, scrape in direction from the head to the tail, collecting mucous and some scales from the skins surface. Place the sample on a clean slide and add two drops of dechlorinated water (freshwater fish) or saline (Marine fish). Cover with a cover slip and label the slide.
Gill scraping procedure: Lift the operculum with forceps and cut a small section from the gill tips and other areas with obvious lesions. Also sample the gill arches (base of gill where filaments connect) and place the sample on a slide. Add a drop of dechlorinated water (freshwater fish) or saline (Marine fish) to the sample. Cover the sample with a cover slip and label the slide.
Entering the abdominal cavity procedure: Gently pull the pectoral fin with sterile forceps while cutting into the abdominal wall at the base of the pectoral fin with a pair of scissors and continue the cut dorsally to just below the lateral line where resistance is encountered. Start again at the same place (base of the pectoral fin) but this time, continue the cut along the ventral abdominal wall toward the vent whilst staying slightly above the abdominal tract to prevent puncture. At the vent continue the cut dorsally to just below the lateral line, then extend the cut cranially to meet up with the end of the first cut. Remove the flap of abdominal tissue to expose the viscera and cavity. The swim bladder and viscera should remain intact. Remove organic matter from the scissors and re-flame them prior to the next procedure.
Fin biopsy procedure: Cut a small section from each fin. Darker fins should be flattened to improve viewing with the microscope. Place the sample on a clean slide and add two drops of dechlorinated water (freshwater fish) or saline (marine fish). Cover with a cover slip and label the slide.

Example of a fish body scoring system

Adult Zebrafish BCS		
	Lateral View	Dorsal View
BCS 1: • Head larger than body (big head) • Lateral- concave ventral surface between head and abdomen (narrow abdomen) • Dorsal- body is more narrow than head and linear • **Fish is thin (emaciated)**		
BCS 2: • Head and body equal size • Lateral- flat ventral surface between head and abdomen • Dorsal- head and width of abdomen are equal • **Fish is underconditioned**		
BCS 3: • Body larger than head • Lateral- slight convex ventral surface • Dorsal- head is slight smaller to a fusiform body • **Fish is well-conditioned**		
BCS 4: • Body significantly larger than head • Lateral- body moderately convex ventral surface • Lateral- Symmetrical ventral surface • Dorsal- head visually smaller to a moderately distended abdomen • **Fish is over-conditioned**		
BCS 5: • Body significantly larger than head • Lateral- body significantly convex ventral surface • Lateral- Symmetrical or asymmetrical ventral surface • Dorsal- head visually smaller to a significantly distended abdomen • **Fish is obese (large)**		

(Image by Joseph Schech, 2018)



(Image of teleost fish anatomy by Tes, https://www.tes.com/lessons/Xw5GYZVw8wsNRw/fish)

Further in-depth necropsy information can be found at:
European Association of fish pathologists, 2015, *Fish necropsy manual*, viewed 2 December 2019, http://www.necropsymanual.net/en/

7. Fish anaesthesia and analgesia

7.1 Summary / Scope

This section relates to anaesthesia and analgesia options for fish used for scientific purposes. They cover chemical based general anaesthesia, local anaesthesia and analgesia drugs.

7.2 Background information

Defining and assessing pain in fish

Fish pain has been an area of scientific debate for the past two decades in part because of the potential implications associated with the fish food production industry. However, in the past five years, strong scientific evidence from key investigators (see reference list at end of section) into the definition and assessment of pain have concluded that fish along with lower order animals, such as invertebrates, feel pain and can suffer as a consequence.
Pain in animals is described by Zimmerman, 1986 as "an aversive sensory experience caused by actual or potential injury that elicits protective and vegetative reactions, resulting in learned behaviour and may modify specific behaviour".

The inability to communicate verbally does not negate the possibility that an individual is experiencing pain.

To assess whether animals can experience pain, scientists proposed that they must meet a set of criteria (references?). The animals must:

- Possess nociceptors (receptors that detect damaging stimuli on or in the body).
- Possess pathways from the nociceptors to the brain (brain structures analogous to the human cerebral cortex that process pain).
- Possess opioid receptors and endogenous opioid substances in a nociceptive neural system.
- Show a reduction in adverse behavioural and physiological effects after administration of analgesics or painkillers.
- Learn to avoid potentially painful stimuli, and that this learning is rapid and inelastic.
- Show that they suspend their normal behaviour for a prolonged period, rather than show a reflex response, with adverse changes in behaviour reflective of signs of discomfort.

The following table adapted from Sneddon et al. (2014) shows the various criteria for pain perception across animal vertebrate groups. 'Yes' indicates selected taxa that at **least one species** within the animal class fulfils the criterion.

Criteria for pain	Mammals	Birds	Amphibians & reptiles	Fish	Elasmobranchs	Molluscs	Crustaceans	Insects
Nociceptors	Yes	Yes	Yes	? C-fiber = No	C-fiber = No	Yes	Yes	Yes

				A delta = yes	A delta = unknown			
Pathways to the CNS (via fMRI analysis)	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Central processing in the brain (via fMRI analysis)	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Receptors for analgesic drugs	Yes	Yes	Yes	Yes		Yes	Yes	?
Physiological responses	Yes	Yes	Yes	Yes		Yes	Yes	?
Movement away from noxious stimuli	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Behavioural changes from norm	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Protective behaviour	Yes	Yes	Yes	Yes		Yes	Yes	No
Responses reduced by analgesic drugs	Yes	Yes	Yes	Yes		Yes	Yes	Yes A
Self-administration of analgesia	Yes	Yes	?	Yes		?	?	?
Responses with high priority over other stimuli	Yes	Yes/?B	?	Yes		Yes	Yes	No
Pay cost to access analgesia	Yes	?	?	Yes		?	?	?
Altered behaviour choices/preferences	Yes	Yes	?	? Some species will feed as normal after procedures		Yes	Yes	Yes
Relief learning	Yes	?	?	?		?	?	Yes
Rubbing, limping or guarding behaviour following pain initiation	Yes	Yes	?	Yes		Yes	Yes	?
Paying a cost to avoid stimulus	Yes	Yes	?	Yes		?	Yes	?
Trade-offs with other requirements	Yes	Yes	?	Yes		?	Yes	?

Index

Yes (Indicates at least one species fulfils the criteria).

? (Indicates that more evidence is needed or that it is inconclusive).

A (Indicates that opioids work as analgesics in cockroaches).

B (Indicates pain is imperative whereas others demonstrate reduced pain behaviour when birds are starved or placed in novel circumstances).

The Scientific research projects (noted above) in live fish injected with potentially painful stimuli resulted in prolonged complicated responses including:

- Increased opercular beat rates (ventilation of the gills).
- Guarding behaviour and general changes in normal behaviour patterns.
- Increases in plasma cortisol.
- Reduced swimming activity in some species and increased activity in other species depending on the type of noxious stimuli.
- Rocking to and fro.
- Rubbing at the site of noxious injections.
- Suspension of feeding activity.

The pain system and nociception have been shown to vary between fish species based on varied life history and ecology. For example, some temperate fish species such as the European trout have been shown to be able to tolerate more extreme cold temperatures, which would cause a potentially life-threatening noxious stimuli in tropical fish species.

Therefore, when planning a fish based scientific project, investigators must seek to minimise and alleviate pain in fish wherever possible and consider the specific needs of the fish species being used.

If painful stimuli are necessary for a project, there should be a clear justification for this part of the project and clearly outlined methods of how the pain will be relieved in the form of anaesthesia and/or analgesia.

Indications for anaesthesia in fish

Anaesthetics play a key role in the health and welfare of fish in scientific projects by reducing distress and physical damage. In some situations, they can be used to facilitate:

- fish handling
- transportation
- diagnostic procedures
- surgery and other scientific procedures
- artificial breeding for gamete sampling, hormone injections and egg/milt stripping
- humane killing.

A good general anaesthetic should provide predictable results including effective analgesia, good immobilisation and rapid induction and recovery, whilst allowing for a wide margin of safety. By minimising the distress experienced by fish during handling, the physiological and biochemical cascades that are associated with the fight and flight situation can be minimised, preventing the disruption of osmoregulation, loss of immune function and decreased reproduction.

In some cases, general anaesthesia is preferred over local or topical anaesthesia. The latter two types of anaesthesia can however be used as adjunct/additional treatments to general anaesthesia during surgery or other procedure.

7.3 Equipment and resources

PPE for all techniques
Safety goggles/glasses, laboratory coat, enclosed shoes, nitryl unpowdered gloves, first aid box with eye rinsing products and hand washing facilities.

General anaesthesia maintenance
Aqui S, small weight measuring scales and measuring jug, syringes, distilled water, separate bottle for stock solution, separate tank, oxygen supply for aeration, oft cradle structure, ET tubes for surgical procedures, tank water for hydration, recovery tank.

Local anaesthesia
Lidocaine or bupivacaine, appropriately sized sterile needles (25-27 gauge) and syringes.

Monitoring fish during anaesthesia (See monitoring sheet appendix 5.12)
Doppler heart monitor, stopwatch/wristwatch, pen and monitoring chart.

Recovery from general drug induced anaesthesia
Separate recovery tank, oxygen supply.

Analgesia
Appropriately sized needles and syringes for injections.

7.4 Recommended procedures

General anaesthesia in fish

AQUI-S general sedation and anaesthesia

AQUI-S (iso-eugenol) is the recommended general anaesthesia drug of choice for investigators at UniSC because of its ease of use and effectiveness. This recommendation for its preferred use is based on:

- Its wide therapeutic margin.
- Its freshwater or saltwater solubility.
- Its smooth dose related induction and rapid recovery.

General notes on AQUI-S

Stock solutions can be prepared by diluting concentrated AQUI-S to 100mg/L in distilled water. This stock solution can then be added directly to the anaesthesia bath. There is potential for irritation at higher concentrations depending on fish species, so it is best to gradually add the product until the suitable dose has been achieved by closely monitoring the fish to assess the depth of anaesthesia.

This drug is approved for use in fish intended for human consumption in salmonid species. Veterinary directions are required for off label use in other fish species, but the same food residue limitations apply. Although this factor will generally not affect most scientific projects it should be considered by an investigator working with food production partners.

The dose of AQUI-S depends on the fish species, metabolic ability and overall health of the fish. However, a general guideline for AQUI-S dosing is found in the following table:

Handling	25mg/L
Surgery/procedures	60mg/L

Method for using AQUI-S

A clean separate induction container/tank should be used and filled with 'a measured volume of water'. The water quality parameters must match those in the fish's normal fish tank especially salinity, hardness, pH, dissolved oxygen and temperature. The easiest way to achieve this is to take the water from the fish's housing tank wherever possible.

The correct dose of AQUI-S stock solution should then be calculated based on the water volume. (A useful concentration or dosage calculator along with a download on the preparation of anaesthetic baths can be found at: www.aqui-s.com). Fish should then be moved directly from their housing tank into the new induction tank where an appropriate level of aeration/oxygenation is supplied to ensure a dissolved oxygen rate. (A flow rate of 0.7 x 10^{-3} assures saturation or >5mg/L D.O. levels for optimal fish health). The correct dose of stock solution should be added in small conservative increments on 1-2 representative fish initially, adding anaesthetic as needed to move quickly through the excitement phase where injury is most likely to occur.

During this induction phase, fish should be closely monitoring to prevent overdosing. The following table details the levels of anaesthesia common to most animals.

Level number	Level of anaesthesia	Signs associated with this level
Level 0	Normal	Normal swimming behaviour and reaction to external stimuli
Level 1	Light sedation	Still swimming but reduced reaction to external stimuli; equilibrium normal' normal opercular rate
Level 2	Deep sedation/light anaesthesia	No swimming; loss of equilibrium (rolls over – belly up) but still may try to right itself; normal to slightly decreased opercular rate; still maintains a tail reaction following slight pinch
Level 3	Surgical anaesthesia	Complete loss of equilibrium; complete loss of reactivity (negative tail reaction); very slow opercular rate; slow heart rate
Level 4	Medullary collapse	Total loss of opercular movement followed by cardiac arrest. Humane killing stage.

Monitoring the depth of anaesthesia becomes increasingly difficult as the fish loses its equilibrium, stops swimming, fails to respond to deep pressure and subsequently ceases any opercular activity. Investigators should aim for level 3 anaesthesia for surgery or other potentially painful or stressful procedures. Fish should be maintained at level 3 (surgical anaesthesia) throughout the procedure. Maintenance of anaesthesia is achieved by continued exposure to varying concentrations of AQUI-S either:

- By using an intermittent administration of AQUI-S solution over the gills. Using a 60ml syringe or an alternative drip system, created by using 2 fluid bags connected by an IV tubing line and a 3-way stopcock for mixing the solution, or
- By using an advanced fish anaesthesia machine that allows a continuous flow of oxygen and a titrated dose of anaesthesia to the fish.

Fish anaesthesia with doppler heart flow monitor.

(Image by 'The telegraph' 2020).

Note: levels of oxygenation should be supplied during any anaesthesia procedure by the placement of an oxygen tube through the oral cavity during surgical techniques.

1. High frequency monitoring sheets (see section 5, appendix 5.1 and appendix 5.2) should be completed for the whole anaesthesia period including the recovery stage until the fish are back to normal activity. Respiratory rates should be monitored by watching the operculum movement and the gills should be pink to light red, with pale gills indicating hypoxaemia, hypotension or anaemia. The heart rate should be monitored either by a doppler heart flow monitor or/and by ECG. Doppler probes should be placed in the opercular slit (for large and medium-sized fish) or directly over the heart (for smaller specimens and small scaled/scaleless species) and ECG probes should be placed on the pectoral or anal fins.
2. To help in the recovery stage, the fish should be placed in a well oxygenated, anaesthetic free fish tank, with the water quality parameters matching those of its housing tank (as for the induction stage). To help speed up recovery, the fish's head can be 'gently' propelled through the water such that water is forced through the mouth and over the gills to encourage the removal of the drug whilst also oxygenating the fish. Note: jaw tone will generally return before opercular activity.

Section 9 shows a list of 'medications for fish' detailing several other types of general anaesthesia drugs that are available for use by consulting veterinarians attending sick fish in UniSC scientific projects. However, most sedation and anaesthetic requirements at UniSC will be covered by the use of AQUI-S.

Local anaesthesia in fish

- Scientific literature on the use of local anaesthesia in fish is very limited and should be used with caution.
- Dosing is therefore based on dosing rates for other animal species, because no specific fish dosing is available at this time. Recommendations suggest not exceeding 1-2mg/kg total dose given as a subcutaneous injection.
- Overdosing in small fish species can be a problem and therefore lidocaine should be diluted 1:10 or 1:100 with 0.9% sodium chloride injection or sterile water for injection to ensure doses are sufficiently small enough for these animals.

- Lidocaine may be useful as an adjunct analgesia alongside AQUI-S for surgical procedures to reduce the potential incidence of pain causing lightening of the general anaesthesia and to extend the pain free period following surgery. However, at this stage the duration of local anaesthesia in many fish species is still unknown in many species.
- The drug should be administered in a 'fanning pattern' at the subcutaneous level in the area of the body expected to experience the pain (for example, the surgical incision site).
- Blood vessels should be avoided.

Analgesia in fish

Fish have opioid receptors like other vertebrates, which are the proteins present on neurons that bind to morphine and other similar compounds, including endogenous opioids involved in the body's natural analgesia. Studies have shown that:

- Morphine supplied in the fish's water at normal animal rates is very slow to be absorbed and very slow to be eliminated and is therefore not a suitable route of administration.
- There is a large species difference regarding the elimination half-life of the drug and therefore each species will require its own dosing strategy. The disposition rate is also generally much slower than other animals of the same size, and therefore caution should be applied in repeated dosing situations.
- Known side effects from using morphine in fish include a slow increase in heart rate and cardiac output, therefore it is not suitable to administer to animals at sea due to uncertain recovery times.

The following table lists some of the known analgesia useful to alleviate potential fish pain. It is important to remember that the efficacy of analgesia may vary between fish species.

Analgesic	Dose	Species/route	Side effects	Comments
Lidocaine	0.1-2mg/kg	Trout IM Zebrafish IM	None	1mg/kg has good efficacy. Do not exceed 1-2mg/kg.
Morphine	5-50mg/kg	Trout IM Flounder IP Goldfish IM	None	5-10mg/kg has good efficacy.
Buprenorphine	0.01-0.01mg/kg	Trout IM	Reduced activity. No impact on feeding or ventilation	Effectiveness in some species is poor.
Carprofen	1-5mg/kg	Trout IM	Depressed activity	Reduced time to feed using 2.5mg/kg
Butorphanol	0.25-5mg/kg	Koi Carp (0.5mg/kg) IM Dogfish IM		Improved behaviour in Koi carp but ineffective in Dogfish.

Ketoprofen	1-4mg/kg	Koi carp (2mg/kg) IM Dogfish IM	No impact on behaviour in Koi carp	Not effective.

Index: IM - intramuscular injection, IP - intraperitoneal injection

As at 2018, morphine appears to have the best efficacy for freshwater fish analgesia, but the dose is dependent upon the fish species. At this time, the pharmacodynamics of morphine use in fish is relatively unknown and dosing intervals are unclear. Therefore, until further scientific research has been undertaken, re-dosing of morphine should be based upon the individual fish's behaviour, clinical signs of pain and the pain associated with the method of surgery being performed.

7.5 Animal health and welfare considerations

Overdose of anaesthesia causing death

It is very easy to overdose smaller fish with anaesthetic, therefore close monitoring at the induction stage and careful measuring of anaesthetic drugs is essential.

Poor maintenance of anaesthesia

Titration of drug dosing to maintain anaesthesia in fish requires intensive monitoring of clinical parameters to prevent a fish becoming conscious during a procedure.

Hypoxaemia during the procedure

An aerated/oxygenated water supply must be provided to the fish's respiratory system during the whole anaesthesia process and the method used should be suitable for the fish's normal method of breathing. Oxygenation levels should be closely monitored throughout the anaesthesia process.

Pain associated with procedure

Where appropriate to use, ensuring a steady surgical anaesthesia plane, use of local anaesthesia and appropriate analgesia before and after every medical procedure will help eliminate any risk of ongoing pain.

7.6 Training plan and competency assessment

Investigators and animal carers should have completed the online introduction to animal ethics training found on the Student Portal.

Investigators and animal careers must be fully trained and assessed as competent in the process of fish anaesthesia before undertaking related procedures. Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application. Alternatively, they should be directly supervised by a QLD registered aquaculture veterinarian.

The AWO is available to provide or organise training and assess competency as required.
Investigators and animal carers must be aware of the OH&S considerations surrounding this species and the first aid procedures required in case of emergencies.
The AEC and AWO will monitor competency during inspections of animal ethics approved projects.

7.7 References and acknowledgements

References

- Baker, TR, Baker, BB, Johnson, SM & Sladky, KK, 2013, Comparative analgesic efficacy of morphine sulfate and butorphanol tartrate in koi (*Cyprinus carpio*) undergoing unilateral gonadectomy, *Journal of the American Veterinary Medical Association*, vol. 243, no. 6, pp. 882-890
- Chatigny, F, Creighton, CM, & Stevens, ED, 2018, Updated Review of Fish Analgesia, *Journal of the American Association of Animal Science*, vol. 57, no. 1, pp. 5-12
- Fenn, CM, Glover DC & Small, BC, 2013, *Efficacy of AQUI-S 20E as a Sedative for Handling and Cortisol Suppression in Pallid Sturgeon,* viewed 30 January 2020, https://www.tandfonline.com/doi/abs/10.1080/02755947.2013.831000
- Neiffer, DL, & Stamper, MA, 2009, Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, Methods, and Types of Drugs, *Institute for Laboratory Animal Research*, viewed 20 January 2020, https://vpresearch.louisiana.edu/sites/research/files/NeifferFishSedation.pdf
- Owens, C & Bowker, J, 2017, *U.S. Fish & Wildlife Service DRUG RESEARCH INFORMATION BULLETIN, Use of AQUI-S®20E, Tricaine-STM, and AQUACALMTM to Sedate Rainbow Trout to Handleable*, viewed 16 January 2020, https://www.fws.gov/fisheries/aadap/dribs/DRIB_53.pdf
- Ross, LG & Ross, B 2009, Anaesthetic and Sedative Techniques for Aquatic Animals, 3rd edn, Blackwell Publishing, Oxford
- Sneddon, LU, 2012, Clinical Anesthesia and Analgesia in Fish, *Journal of Exotic Pet Medicine,* Vol 21, no. 1, pp. 32-43
- Sneddon, LU, Elwood, RW, Adamo, SA & Leach, MC, 2014, Defining and assessing animal pain, *Animal behaviour*, vol. 97, pp. 201-212
- Sneddon, LU, 2019, Evolution of nociception and pain: evidence from fish models, *Philosophical Transactions of the Royal Society B:,* vol. 374, 20190290, viewed 11 February 2020, https://royalsocietypublishing.org/doi/10.1098/rstb.2019.0290
- Sneddon LU, 2015, Pain in aquatic animals, *The Journal of Experimental Biology,* vol. 218, pp. 967-976
- Stevens, ED, 2008, *"Pain" and analgesia in fish: what we know, what we do not know, and what we need to know, before using analgesics in fish*, ANZCCART Conference Paper, viewed 12 December 2019, https://anzccart.org.nz/app/uploads/2017/06/stevens-pain-and.pdf
- Walters, ET & Williams, ACdeC, 2019, Evolution of mechanisms and behaviour important for pain, *Philosophical Transactions of the Royal Society B:, 374:20190275*, viewed 11 February 2020, http://dx.doi.org/10.1098/rstb.2019.0275
- Zimmerman, M, 1986, Physiological mechanisms of pain and its treatment, *Klinische Anasthesiologie Intensivtherapie*, vol 32, pp. 1-19

Further reading

- Ashley, PJ, Ringrose, S, & Edwards, KL, Wallington, E, & McCrohan, CR, *2009,* Effect of noxious stimulation upon antipredator responses and dominance status in rainbow trout, *Animal Behaviour*, vol. 77, pp. 403-410
- Chatigny, F, 2019, The Controversy on Fish Pain: A Veterinarian's Perspective, *Journal of Applied Animal welfare Science,* vol. 22, no. 4, pp. 400-410, doi:10.1080/10888705.2018.1530596
- Chervova, LS & Lapshin, LN, 2000, Opioid modulation of pain threshold in fish, *Doklady Biological Sciences*, vol. 375, pp. 590-591
- Davis, MR, Mylniczenko, N & Storms, T, Raymond, F & Dunn, JL, 2006, Evaluation of intramuscular ketoprofen and butorphanol as analgesics in chain dogfish (*Scyliorhinus retifer*), *Zoo Biology*, vol. 25, pp. 491-500
- Fowler, ME & Miller, RE, 2003, *Zoo and Wild Animal Medicine*, W.B Saunders Co, Philadelphia, PA
- Hill, JV, Davison, V & Forster, ME, 2002, The effects of fish anaesthetics (MS222, Metomidate and AQUI-S) on heart ventricle, the cardiac vagus and branchial vessels from Chinook salmon (*Oncorhynchus tshawytscha*), *Fish Physiology and Biochemistry*, vol. 27, pp. 19-28
- Hill, JV & Forster, ME, 2004, Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery, *Comparative Biochemistry and Physiology - Part C*, vol. 137, pp. 167-177
- Martins, T, Valentim, A & Pereira, N, 2018, *Anaesthetics and analgesics used in adult fish for research: A review,* viewed 20 January 2020, doi.org/10.1177/0023677218815199
- National Research Council (US) Committee, 2009, Recognition and Alleviation of Pain in Laboratory Animals, National Academies Press (US), Washington (DC) https://www.ncbi.nlm.nih.gov/books/NBK32658/
- Neiffer, DL & Stamper, MA, 2009, Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs, *Institute for Laboratory Animal Research*, vol. 50, pp. 343-360
- Newby, NC, Mendonca, PC, Gamperl, K & Stevens, ED, 2006, Pharmacokinetics of morphine in fish: winter flounder (*Pseudopleuronectes americanus*) and seawater-acclimated rainbow trout (*Oncorhynchus mykiss*), Comparative Biochemistry and Physiology - Part C, vol. 143, pp. 275-283
- Newby, NC, Wilkie, MC & Stevens, ED, 2009, Morphine uptake, disposition, and analgesic efficacy in the common goldfish (*Carassius auratus*), *Canadian Journal of Zoology*, vol. 87, pp. 388-399
- NHMRC, 2013, *The Australian code for the care and use of animals for scientific purposes*, viewed 19 April 2019, https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
- Queensland Government, 2019, *Veterinary laboratory users guide,* viewed 20 January 2020, https://www.publications.qld.gov.au/dataset/veterinary-laboratory/resource/56026801-3046-4182-b645-46965924f35d
- Roques, JAC, Abbink, W, Geurds, WF, van de Vis, H & Flik, G, 2010, Tailfin clipping, a painful procedure: studies on Nile tilapia and common carp, *Physiology and Behaviour*, vol. 101, pp. 533-540
- Ross, LG & Ross, B, 2008, *Anaesthetic and Sedative Techniques for Aquatic Animals*, 3rd edn, viewed 20 January, DOI:10.1002/9781444302264
- Sloman, KA, Bouyoucos, IA, Brooks, EJ & Sneddon, LU, 2019, Ethical considerations in fish research*, Journal of Fish Biology*, vol. 94, no. 4, pp.556-577

- Small, BC & Chatakondi, N, 2005, Routine measures of stress are reduced in mature channel catfish during and after AQUI-S anesthesia and recovery, *North America Journal of Aquaculture*, vol. 67, pp. 72-78
- Sneddon, LU, 2002, Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss*, *Neuroscience Letters,* vol. 319, pp. 167-171
- Sneddon, LU, Braithwaite VA & Gentle MJ, 2003, Do fishes have nociceptors?: Evidence for the evolution of a vertebrate sensory system, *Proceedings of the Royal society B of Biological Sciences*, vol. 270, pp. 1115-1121
- Sneddon, LU, 2006, Ethics and welfare: pain perception in fish, *Bulletin of the European Association of Fish Pathologists*, vol.26, pp. 6-10
- Sneddon, LU, 2009, Pain perception in fish: indicators and endpoints, *Institute for Laboratory Animal Research*, vol. 50, pp. 338-342
- Sneddon, LU, 2003, Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception, *Brain Research,* vol. 972, pp. 44-52
- Ueta, K, Suzuki, T, Sugimoto, M, Uchida, I & Mashimo, T, 2007, Local anesthetics have different mechanisms and sites of action at recombinant 5-HT3 receptors, *Regional Anesthesia and Pain Medicine*, vol. 32, pp. 462-470
- Velišek, J, Stejskal, V, Kouril, J, Svobodová, A, 2009*,* Comparison of the effects of four anaesthetics on biochemical blood profiles of perch, *Aquaculture Research,* vol. 40, pp. 354-361
- Weber, ES, 2011, Fish analgesia: pain, stress, fear aversion, or nociception?, *Veterinary Clinics of North America: Exotic Animal Practice,* vol. 14, no. 1, pp. 21-32, doi: 10.1016/j.cvex.2010.09.002
- Young, T, Walker, SP, Alfaro, AC, Fletcher, LM, Murray, JS, Lulijwa, R & Symonds, J, 2019, Impact of acute handling stress, anaesthesia, and euthanasia on fish plasma biochemistry: implications for veterinary screening and metabolomic sampling, *Fish Physiology and Biochemistry*, vol. 45, no. 4, pp. 1485-1494, doi: 10.1007/s10695-019-00669-8
- Zahl, IH, Kiessling, A, Samuelsen, OB, Hansen, OB & Kjerulf, M, 2009, Anaesthesia of Atlantic cod (*Gadus morhua*)—effect of preanesthetic sedation, and importance of body weight, temperature and stress, *Aquaculture*, vol. 295, pp. 52-59

8. Fish techniques and procedures

8.1 Summary / Scope

These guidelines relate to techniques and procedures undertaken on fish for scientific purposes.
If a team member has not yet been deemed competent at performing a necessary procedure or requires further training, the AWO is available to provide or organise training and assess competency as required.

8.2 Background information

There are many procedures undertaken on fish for teaching and research purposes within scientific projects. Each has its own level of welfare risk to the fish. The following have been highlighted as being some of the more common procedures undertaken by fish scientists:

- handling techniques
- blood collection
- fin clipping
- fish identification/tracking techniques
- egg, spawn and larvae collection and stocking
- surgical procedures
- weighing fish
- producing triploid fish
- cleaning protocol following procedures.

All procedures undertaken on fish should be performed by fully trained/competent investigators.

8.3 Equipment and resources

Handling techniques

Haul nets, fish cradles, aerated tanks/aerated water flow, PPE, light cloth.

Blood collection

Appropriately sized (single use) sterile needles and syringes, vials/sample tubes, sharps containers/needle removing equipment, sample vials, glass slides, plastic bags for holding samples, waterproof marker pens, labels and pencils, paper towel, anaesthetic, tanks for anaesthetising fish and for recovery, dip nets, ice and esky for storing samples (if remote from a fridge or freezer), sharp knife, aquaculture detergent and disinfectant.

Fin clipping

Surgical (nitrile) gloves, surgical scissors, ethanol or antiseptic preserving solution such as diluted iodine, sterile sample pots, holding tank or polyurethane double layered fish transport bag with aerated water.

Fish identification/tracking techniques

Appropriate tags for the fish species/size, anaesthesia, analgesia, mesh cradles, monitoring paperwork, PIT tag monitors if appropriate, sterile scalpel, drill, tagging pole, cleaning and disinfecting products for equipment, scalpel and suturing equipment and supplies, safety equipment.

Egg, spawn and larvae collection and stocking

Aerating stones, graduated cup and bucket, monitoring sheets, plankton nets, sample tubes, hatching tanks with a banjo screen/filter and flow through water system, an egg collection tank and net, hoses, larvae counting chamber, Sedgwick rafter counter.

Surgical procedures

Anaesthesia drug, oxygen supply, analgesia, antibiotics, surgical equipment suitable to the procedure, surgical supplies such as suture material, high frequency monitoring sheets.

Weighing fish

Weighing scales, monitoring paperwork, dip net, clear ruler, clean detergent free bucket containing water from the fish's housing tank, Aqui-S anaesthetic agent, a suitably sized dish (e.g. a large petri dish) containing a sponge or paper towel dampened by a small amount of tank water for holding the fish on the scales during weighing, battery or mains powered aerator.

Producing triploid fish

Pressurised stainless-steel cylindrical vessel, microscope/slides, clean buckets for animal use, lysing agent, Aqui S anaesthesia, thermometer.

Cleaning protocol following animal procedures

Aquaculture detergent, 'Pyroneg' or 'Virkon' disinfectant, cleaning only buckets, scrubbing brushes, clean drying area.

8.4 Recommended procedures

Handling fish during procedures

Handling during sampling

The principal aim of handling techniques is to minimise distress experienced by fish as much as possible and to prevent any further damage following capture. There must be sufficient staff to restrain animals in a quiet environment and prevent injury to animals and handlers. To achieve this:

- The time for which the fish is held should be minimal and consistent with the aims of the study.
- Fish must be held in such a way as to minimize stress and/or injury. Never carry a fish by the caudal peduncle (the region between the trunk and tail) because carrying a fish upside down may result in paralysis and death. When carrying an animal, always carry it the 'right way up'.
- Fish should be protected from UV damage, especially their eyes by covering them with a light cloth where feasible.
- When sorting the catches of haul nets, traps, etc, the net or trap should be kept in the water for as long as possible to reduce the trauma to all aquatic animals captured, but any air-breathing wildlife species should be released as soon as the fishing gear has been lifted.
- Knowledge of available information on the normal behaviour of the species and its likely response to captivity is essential and must form the basis for management practices.
- Wherever possible, fish must be sampled whilst still in the water. This is particularly relevant when using any trapping or netting sampling methods. Some fish (e.g. sharks) require oxygenated seawater to be passed through the buccal cavity and over the gills during handling.
- Holding areas must be safe, quiet and hygienic.
- If handling or restraint is likely to cause harm, including pain and distress, to the animal, the use of chemical restraint (e.g. sedatives) must be considered.
- Fish must be assessed regularly if prolonged restraint or confinement is required.
- If any adverse impact is detected, the animal must be released, or the method of restraint must be modified to minimise that impact.

- Close confinement devices must:
    - allow fish to rest comfortably
    - minimise the risk of escape or injury
    - be adequately aerated
    - maintain constant temperature
    - minimise the risk of disease transmission.
- To minimise the distress to animals being kept for analysis or preservation, animals must be humanely killed (see section 6) as soon as possible after capture.

Handling during release

- Release should be at the site of capture or in suitable places (safe from advantaged predators or unsuitable water conditions), which may be found close to sorting areas, unless an alternative site is justified in the project proposal.
- The time of the release should be consistent with the species usual time of movement.
- Individuals must be released safely, particularly if the time of day for release is less than optimal.
- When releasing fish from holding tanks, fish must be supported by both hands and gently lowered into the water:
    - Never throw a fish back into the water.
    - Bass species should be held by the lower jaw and eased back into the water.
    - Non-bass teleost fish species should be lowered headfirst with both hands whilst supporting the body under the abdomen.
    - Fish in river systems should be pointed with their head upstream into a slow current. These fish may need to be moved gently back and forth to allow water to flow into the gills. This technique is similar for lake fish.
- Once the fish begin to respond and try to swim away, investigators should release their grasp.
- At the time of release all reasonable steps must be taken to protect animals from injury and predation.

Note: Larger fish may take a little longer to revive than smaller fish.

Blood collection

General background information for blood sampling in fish

- Capture and handling of fish can have a marked effect on the haemogram due to the release of catecholamines (e.g. adrenaline) leading to haemoconcentration and swelling of the erythrocytes.
- Cannulation techniques have been developed in fish research to help alleviate some of these stress related factors, but the fish cannot be held out of water for more than 30 seconds before respiratory distress leads to electrolyte imbalances. Therefore, the use of general anaesthesia (see section 7 for suitable anaesthesia) enables the researcher to be able to collect a blood sample from the fish whilst its head and gills are held in the aerated water.
- Anticoagulant use is important when collecting blood from fish and can be used in three ways:

    1. To coat the syringe and needle with heparin prior to collection–a technique particularly useful for small fish sampling where the collection will be slow and chance of clotting increased. Heparin dissolved in saline is the most commonly used substance to prevent clotting in this manner.
    2. To use a collection tube containing anticoagulant such as ethylenediaminetetraacetic acid (EDTA).
    3. Using a combination of EDTA and heparin or of activated citrate dextrose solution when collecting blood from elasmobranch fish (i.e. sharks and rays).
    The use of anticoagulants does have disadvantges regarding fish blood collection:

        - EDTA can cause the haemolysis (rupture) of erthrocytes in some fish species.
        - Heparin can cause the blood sample to clot if there is already a small clot in the sample creating a blue tinge to blood films stained with Romanowsky stains.
        - If the anticoagulants and diluents used for obtaining cell counts have a lower osmolarity than the plasma osmolarity of the fish (especially sharks and rays) and these are not balanced specifically for the osmolarity of the fish species, haemolysis can occur.

Note: Vacutainer syringes are not recommended for blood collection in small fish, because the amount of suction cannot be controlled, and the vein may collapse.

Unique fish anatomy affecting blood sampling

- Teleost fish have low blood arterial pressure and their capillaries are highly permeable so that osmotic pressure across the capillary walls is low. The intersistial fluid has a high protein concentration and therefore plasma runs freely through the capillary walls. Therefore fish are able to tolerate some changes in plasma concentrations.
- Fish have a secondary circulatory system that is a conduit for almost cell free blood. It has a lower haematocrit and its circulatory time takes hours rather than minutes.
- Fish have an extensive lymphatic drainage system due to the increased capillary permeability and hence the lymphatic fluid composition is the same as the plasma fluid.
- The majority of blood cells are directed to the primary circulatory system at the bifurcation of the two systems located at the major vessels of the gills and various surface areas such as skin and gut.
- Blood sampling from the major vessels represents blood in the primary circulation; however, incorrect sampling can allow contamination from the lymphatic system and affect sample colour (clear fluid mixing with red blood).

- Blood volume of fish is relatively small approximately 5% of body weight, but due to greater fluid balance acute blood loss is generally better tolerted than other animals (within limits).

Techniques for collection of blood from living finfish

Blood collection should only be performed by individuals who have been trained in this method.

1. All equipment for blood collection needs to be prepared in advance of collection to minimise the time fish are restrained. The equipment list includes:

    - appropriately sized needles and syringes
    - sample vials/tubes for blood. Choose the correct tube according to the intended end use (EDTA, plain tube etc)
    - plastic bags for holding samples and laboratory paperwork
    - waterproof marker pens
    - labels and pencils
    - paper towel
    - anaesthetic drug (Aqui-S)
    - smaller fish tanks for anaesthetising fish and for recovery
    - dip nets
    - ice and esky for storing samples (if remote from a fridge).

2. Once fish are anaesthetised, fish should be weighed prior to sampling. No more than **1% of the fish's total body weight should be taken in a blood sample, with a gap of 14 days between samples** (For example, fish weight 50g = blood sample volume not exceeding 0.5ml).

3. They should be restrained ventral side up on a stable surface (e.g. a plastic/metal dissecting tray lined with a wet, soft matting such as a foam cradle covered in plastic).

4. A suitably sized syringe and needle should be heparinised and blood collected from the caudal vein or artery by a ventral or lateral approach. Needle gauge will vary depending on the size of the fish. For example, a 22-gauge needle would be appropriate for a fish in excess of 1kg.

5. The ventral approach is the preferred technique because the internal anatomy of the muscle blocks in the ventral region reduces post sample bleeding. This approach involves:

    a) Gentle insertion of the needle perpendicular to the body under a scale (in teleost fish) along the ventral midline approximately 5mm caudal/posterior to the anal fin/near the base of the caudal peduncle, with the bevel facing the fish's caudal/tail end.
    b) Directing the needle towards the vertebral body whilst applying a slight negative pressure on the syringe plunger and once reached, the needle should be withdrawn slightly ventrally and laterally into the caudal vein that lies just below the vertebral bodies.

c) Identifying the blood within needle hub once in the vessel and starting the blood collection. It is important not to put too much negative pressure/suction on the syringe because the vein may collapse which will impede blood flow. Slow slight adjustments may be required to maintain the blood flow without severely damaging the blood vessel wall. The caudal puncture vein will yield between 0.2ml and 10ml from most fish over 25g in weight but a safe volume should be calculated prior to collection depending on their size (see point 2 above).

The lateral approach to the caudal vertebral vessels is performed by inserting the needle a few millimetres below the lateral line near the base of the caudal peduncle. The needle is then slowly advanced towards the midline under the vertebral bodies, and blood is collected in the same manner as above. This approach is generally used less commonly than the ventral technique.

Blood collection from living elasmobranch fish (i.e. cartilaginous fish such as sharks, rays and skates)

1. Blood can be collected in the same manner as finfish or by using a vascular sinus (meeting point of blood vessels) which is an easier approach.
2. The vascular sinus approach involves:

    - Restraining the anaesthetised fish in ventral recumbency in a sling with its dorsum exposed. The dorsal fin should be lifted dorsally/upwards.
    - Inserting the anticoagulant coated needle into a plane parallel to the back and slightly off the midline through the soft skin just under the caudal aspect of the dorsal fin.

Preparing the blood collected for laboratory analysis

1. Transfer the blood sample to the tube, by removing the needle and gently squirting the blood from the syringe into the collection vial/tube containing the anticoagulant. Apply the lid to the tube firmly, and then gently agitate the tube in a slow rocking motion to mix the contents. Overzealous agitation of the sample will result in poor cytological outcomes during examination.
2. Label all samples clearly as required by the pathology laboratory and to ensure the needs of the projects data requirements are fulfilled.
3. Place all samples in a plastic sealable bag and complete all laboratory paperwork as required. Information required usually includes.
4. Blood samples will need to be handled according to the desired end use and the experimental protocol to be employed. Store the blood in a cool place (refrigeration of 4^{o}C) for most blood samples being sent for laboratory assessment. If there is a delay in getting the blood to a refrigerator, an esky with ice should be used as a temporary holding facility, but the blood samples should not come into direct contact with the ice at any stage. For procedures where clotted blood is required, blood should be stored in a cool, shaded area until clotted and then placed in the refrigerator.

Other factors to consider during blood collection in fish

- A new needle and syringe should be used for each fish.
- The foam cradle/plastic cover should be rinsed with clean water and wiped with a new sheet of paper towel between each fish.

- Following blood sampling, fish should either be humanely killed if this is part of the AEC application specifications or immediately placed in a recovery tank with well aerated/oxygenated clean water as per the anaesthesia recovery notes in section 7.
- Both the anaesthesia stage and recovery stage of the fish should be closely monitored. Full recovery of normal behaviour and function must be carefully confirmed according to the criteria on the monitoring sheet before fish are released back to their tanks or their natural habitat, to reduce the risk of easy predation. Any fish experiencing difficulties with swimming or orientation after the usual recovery period should be humanely killed. A necropsy must be performed to ensure that the blood collection did not cause the demise of the fish, and if so, measures must be taken to ensure that the deleterious technique is not repeated on other fish.

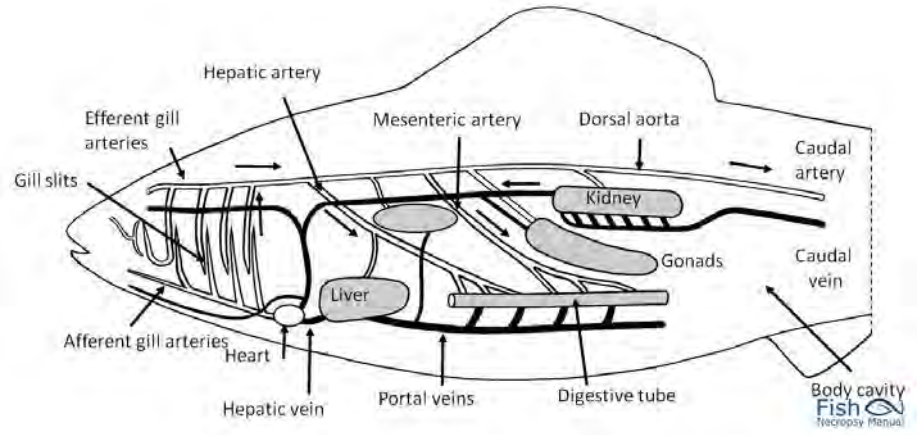
Blood collection from recently humanly killed fish

1. Ensure all equipment for blood collection is prepared prior to the humane killing including:

    - anaesthetic agent (AQUI-S) at humane killing concentration in smaller individual treatment bath
    - doppler monitor to assess heart and confirm death
    - sample vials/tubes for blood
    - plastic bags for holding samples and laboratory paperwork
    - waterproof marker pens
    - labels and pencils
    - paper towel
    - scalpel or sharp knife.

2. Once the fish has been confirmed as dead, use a sharp knife or scalpel and cut off the tail behind the anal fin (the caudal vessels are located directly beneath the spine) and allow the blood to drain from the caudal blood vessels into the blood collection vials/tubes. Fill the sample vials to no more than 1 cm from the top.
3. The knife or scalpel used for blood collection needs to be carefully cleaned and disinfected then rinsed with clean water and wiped with a new sheet of paper towel between each fish to minimise any cross contamination between samples.

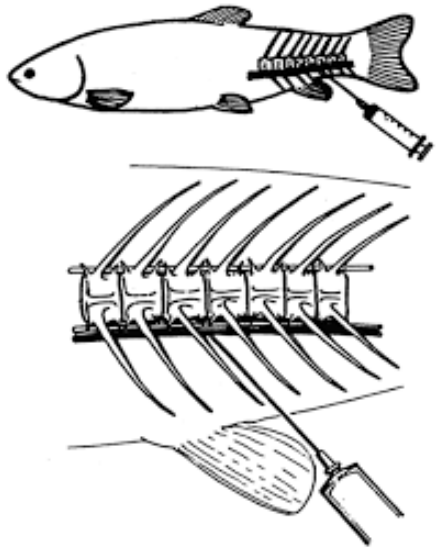
See images below of suitable blood collection sites.



Teleost fish circulatory system. The dotted line shows cutting area for blood collection following humane killing.

(Image from Fish Necropsy Manual, 2015)

Caudal vein blood collection technique. Image from Radboud University, Netherlands, 2013)



Caudal vein location and approach for blood collection (Image from http://www.fao.org/tempref/FI/CDrom/aquaculture/a0845t/volume2/docrep/field/003/ac160e/AC160E09.htm)

(Blood collection in a stingray. (Image from the Cape Eleuthera Institute http://ian-overton-n4wy.squarespace.com/news?offset=1393264546000&category=Research+Programs)

References

- Department of primary Industries, 2015, *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research*, NSW Department of Primary Industries: Nelson Bay, NSW
- European Association of fish pathologists, 2015, *Fish necropsy manual*, viewed 2 December 2019, http://www.necropsymanual.net/en/

- Flinders University, 2019, *Standard Operating Procedure Working with Fish*, viewed 2 December 2019, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-working-with-fish.pdf
- Jenkins, JA, Bart Jr, HL, Bowker, JD, Boswer, PR, MacMillan, JR, Nickum, JG, Rachlin, JW, Rose, JD, Sorensen, PW, Warkentine, BE & Whitledge, BE, 2014, *Guidelines for the Use of Fishes in Research- Revised and Expanded*, viewed 20 November 2019, https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/03632415.2014.924408
- Radbound university, 2013, *Blood sampling in Tromsø* Viewed 24 February 2020, https://www.ru.nl/animal/news-archive/blood-sampling/
- Research institute of fish culture and hydrobiology Vodňany Czechoslovakia, 1991, Diagnostics, prevention and therapy of fish diseases and intoxications, viewed 24 February 2020, http://www.fao.org/tempref/FI/CDrom/aquaculture/a0845t/volume2/docrep/field/003/ac160e/AC160E09.htm

Fin clipping for tissue sampling

General background information on fin clipping in fish

Fin clipping is generally undertaken for genetic, biochemical or other analyses using fish tissue sampling.
This procedure should be conducted by two investigators/animal handlers regarding their personal safety and to ensure minimal disturbance of wild fish whilst minimising the risk of cross contamination of disease.

Techniques for fin clipping from living finfish

1. Ensure all equipment is ready prior to staring the procedure including:
   - surgical (nitrile) gloves
   - surgical scissors
   - ethanol or antiseptic preserving solution such as diluted iodine
   - sterile sample pots (depending on the type of tissue assessment to be performed according to the AEC application).
2. Investigator/animal handler 1 wears gloves to handle and hold fish securely.
3. Investigator/animal handler 2 wears gloves to sample fish.
4. Scissors should be immersed in ethanol or antiseptic solution such as diluted iodine prior to cutting a small wedge (3mm triangle) from a fin. The sample should be placed into a sterile labelled sample pot.
5. The sample site should be monitored for bleeding prior to release.
6. Fish that have been handled are in shock/distressed and similar to fish that are still bleeding after this procedure, they are far more susceptible to being attacked by predators. Therefore, they should be held in an aerated holding tank/polyurethane double layered transport bag for monitoring until they are fully recovered as designated by the monitoring form.
7. Return the fish to the wild habitat at point of capture and release safely.

References

- Flinders University, 2019, *Standard Operating Procedure Working with Fish*, viewed 2 December 2019, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-working-with-fish.pdf

Fish identification/tracking techniques

Using microchips and tagging on animals may have implications under the *Animal Care and Protection Act 2001 (ACPA)* and other acts such as the *Nature Conservation Act 1992*. Under the ACPA, any person who uses identification equipment on animals has the responsibility to ensure that:

- Activities are performed in an appropriate manner.
- The microchip and/or collar is appropriate for the animal.
- The process does not cause the animal unjustifiable, unnecessary or unreasonable pain.

Failure to meet your responsibilities may constitute offences under the duty of care or cruelty provisions of the ACPA.

General background information for identification/tracking techniques in fish

- Tags and markers are used to obtain information on the biology of tagged organisms and to develop rational management strategies.
- The identification of fish over time is required for studies focusing on ecology, fish behaviour, age and growth, mortality rates, abundance, population dynamics, migrations, stock identification and stocking success.
- Researchers can use both intrinsic and extrinsic identification systems, where the nature of the study dictates the type of tag or mark employed.
- Prior to marking fish, investigators must consider the amount of tissue affected, and whether the effects of handling will be momentary or prolonged.
- Investigators should also determine if the animal will:
    - be at greater than normal risk to predation
    - be less desirable as a mate
    - be at risk of substantially increased infection.

Marking techniques for fish have been extensively reviewed and are constantly evolving; therefore, investigators should review recent literature alongside these guidelines.

OH&S and risk considerations should be noted because many of the tagging systems use needles and scalpels (particularly in large fish species) and most operations will be carried out in various forms of waterways. Therefore, a full risk assessment must be completed prior to the project commencing and the recommended safety and personal protection equipment should be worn at all times.

General tagging considerations

It is important that any tagging program has a well-defined set of research questions that are designed to answer a specific objective, because tagging is an invasive procedure and investigators should avoid the need to tag fish unless objectives cannot be achieved in another manner.
Tagging components of research programs are highly public and also time consuming, so it is important that all fish are tagged correctly and released in the best possible health. This maximises benefits to the scientific research project and also ensures the welfare of the fish beyond the tagging exercise.

Correct tagging of fish is a learned skill and it is important to ensure investigators have adequate training to ensure techniques are correct, which will minimise stress on fish and also ensure a high probability of tag retention.

Multiple artificial tagging methods are available and external tags and marks are constantly evolving; therefore, selecting the correct one for the project should be based on:

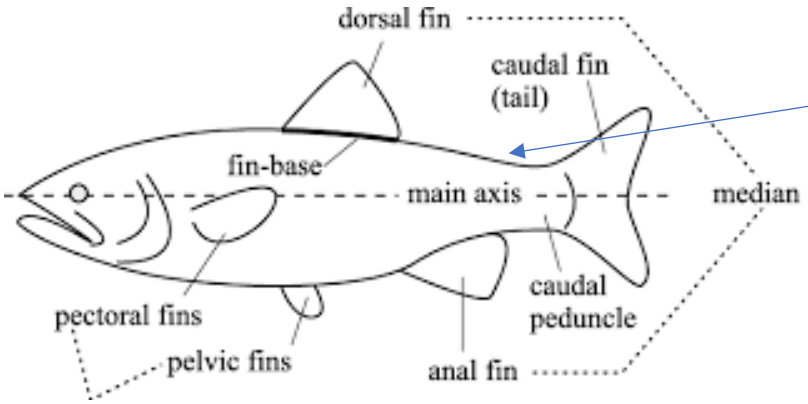
- the objectives of the study and type of data being collected
- fish species
- size/age of the fish
- budget
- effect on survival, behaviour, and growth
- permanency and recognition of the mark
- number of fish to be marked
- stress of capture, handling, and marking
- cost
- recovery of tagged fish
- operator skills
- coordination required among agencies, states or countries.

Natural tags (Natural marks)

Using natural biological marks for identification includes the use of meristic (countable traits), pigmentation, morphometrics (shape and form) and scale characteristics that have minimal animal welfare risks, but their use is limited, because they are subjected to environmental and genetic influences. The shape, size, and circulus patterns of scales are the most frequently used natural marks. The use of biological marks requires extended knowledge about the life history of the organism.

Fin clipping for marking/identification

Hole punching or clipping of fins or other body parts as a form of identification has been used for many decades. The fins selected for clipping or removal will depend upon the species. The fins most commonly subjected to clipping are the adipose fin and pelvis fins, but clipping of the tail fin, pectoral fin, dorsal fin and anal fin has also been tested. The method consists essentially of clipping a fin in a standard pattern with scissors, trying to make the same cut/shape in all fish of the same family group.



Adipose fin: is a small fleshy fin found posterior to the dorsal fin and anterior of the caudal fin. It is only found on a few fish species.

Fish fins.

(Image from https://www.researchgate.net/publication/3231079_Review_of_Fish_Swimming_Modes_for_Aquatic_Locomotion)

Fin clipping has been used to recognise individuals within a group and is commonly used in breeding work and animal tracking in the field. In breeding programs, fish are often tagged as soon as they reach sufficient size (5-10 grams), using a characteristic clip for each family.

This method is suitable for situations where there are only members of a few different families that need to be identified afterwards.
It is also cheap and quick to perform; multiple clip patterns can provide many combinations.

However, there can be associated welfare concerns involving pain, distress during handling, bleeding, infection of clipping sites and changed swimming performance leading to increased mortality through increased predator attack.

Fin clipping is also not always reliable as a marker in young growing fish, where the clip site shape can become distorted. Equally the shape may not be the same due to operator error, use of different clipping techniques between projects or natural regrowth of the fins.

Hence, fin clipping should **not be considered** the first choice of identification marking for most scientific projects using fish at UniSC.

Otolith microstructural features

All teleost fish have otoliths (ear bones) located in the inner ear cavity. Manipulations of environmental temperature, feeding rates, photoperiod, or external chemical baths can induce specific marks in fish otoliths. Otolith microstructural features are permanent and can be viewed and analysed in fish of any age. Elasmobranch ageing is studied by sectioning and counting growth bands deposited on the vertebrae.

The tetracycline drug and other fluorescent compounds are used as markers for calcified structures in fish, and the vertebral cartilage in sharks.

A strength of this system is the ease of application to otoliths at any time during the growth period of the fish. Fish that are bred under controlled conditions are readily available for such manipulations.

Fisheries that require stock definitions and assessment of early life stage breeding success benefit from the otolith and vertebral marking systems.

Fish parasites used for identification of fish

Several taxonomic groups of fish parasites have been used as biological tags, and this method is best suited to the separation of relatively self-contained stocks of fish. Recovery of internal parasites used as biological tags is enhanced if parasites are associated with a specific anatomical site of the fish. The decision to use a parasite as a natural mark on fish is determined by calculating the ratio of incidence of that parasite on one fish population to its incidence on another.

Genetic Markers

Genetic markers are techniques employing markers based on chromosome and nuclear DNA polymorphisms (different DNA sequences among individuals, groups, or populations). The potential uses for such markers in selective breeding programs, evaluating the contribution and effects of stocked species, or delineating specific habitat requirements have emerged quickly.

The fact that adequate samples of the tissues needed for analyses of such markers can be obtained non-lethally (e.g. fin clipping) and with minimal handling, provides additional incentives for their use in a wide array of studies.

Polymorphisms and random amplified polymorphic DNA allow for screening of genetic variation to obtain different molecular fingerprint patterns.
In the face of questions concerning stocking programs and native species, the use of molecular techniques can provide additional information to address these issues. Genetic markers are valuable for managing performance traits such as long-term reproductive success and assessing habitat restoration.

With the rapid development of genetic markers, an investigator will need to update their knowledge of the most current, scientifically accepted genetic identification systems and their potential applications prior to designing their scientific project.

Isotopes

What are stable isotopes?

The nucleus of each atom contains protons and neutrons with the number of protons defining the element (e.g. carbon) and the addition of both the protons and neutrons giving the atomic mass. The isotope of the element is defined by the number of neutrons. In the case of carbon elements, approximately 99 % have 6 protons and 6 neutrons and is written as ^{12}C to reflect its atomic mass.
However, about 1% of, for example, the carbon in the earth's biosphere has 6 protons and 7 neutrons (^{13}C) forming the heavy stable isotope of this important element. Stable isotopes do not decay into other elements, in contrast to radioactive isotopes such as ^{14}C) which are unstable and will decay.

Stable isotopes occur naturally, behave identically to the "typical" isotope and can be identified with a high degree of accuracy and reliability. Variation in the ratios of the stable isotope to the more common form can be used to identify sources of materials and to trace them within individual animals, populations or ecosystems.

The use of stable isotopes, such as ^{13}C, ^{15}N, or ^{34}S, as marks to identify places of origin, nutrient pathways, feed efficiencies and an array of physiological or ecological processes is becoming relatively common.

In contrast to radioisotopes, the uses of which are regulated very tightly, the use of stable isotopes does not require special facilities and permits.
Depending upon the objectives of the research, non-lethal fish sampling is possible by using scales or fin clips for stable isotope analysis (see fin clipping for tissue sampling technique above). However, elasmobranch stable isotope analyses often require muscle, liver or vertebral tissues, as the rate of ^{13}C or ^{15}N turnover varies among these tissues.

Different tissues have different elemental turnover rates; therefore, an investigator should determine and explain on their AEC application which tissues will provide the materials needed to satisfy the requirements of their project.

External tags

The biological tagging system's disadvantages led to the development of physical tags for identification. These tags are conspicuous by their colour, shape, size or attachment location and are made from a variety of materials.

General considerations regarding the use of any external physical tagging methods

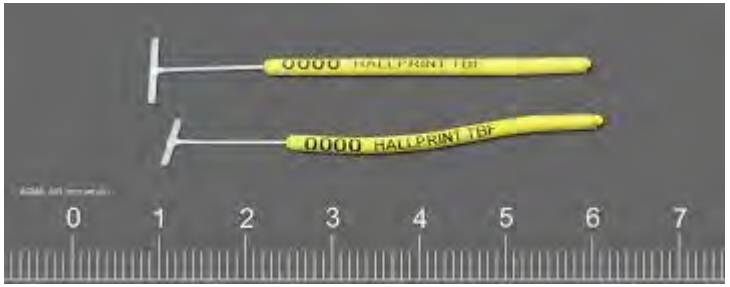
- The fish should always be assessed as healthy without injury or illness prior to placing any type of tag. Fish should never be tagged if they are showing signs of distress.

- The technology around external tags continues to evolve in sophistication, use and data collectable; however, these tags can be lost or fail to be reported in the future. Data logging tags may also cause welfare concerns, due to their larger size and the potential effects on the ability of fish to swim and feed normally.
- The most common identification tags are the T-bar anchor tags, dart tags, streamer tags or spaghetti tags.
- Tagging large fish will require some force, but extra care should be taken with smaller fish to ensure the tag does not pass right through the fish.
- Application effectiveness should be checked by gently tugging on the tag to ensure it does not pull out. When tags are correctly inserted, they will lie in a semi-streamlined position (anchor end towards the head and coloured tail end towards the tail fin).
- Tags should not interfere with an animal's behaviour or cause extra drag when swimming.
- Fish should be tagged within 20 seconds using simple T bar tags. More complex tagging procedures should be completed within three minutes using oxygenated water supplies or a specialised fish anaesthetic machine? Maximum time out of water is six minutes for any procedure with an oxygen supply, but fish may become hypoxic during this time.

General notes on the placement of external T bar anchor tags or dart tags

- Regardless of which type of tag is chosen, the dart-like tip or the bar at the end are inserted into the dorsal musculature such that the anchor locks in between the pterygiophores of the dorsal fin of the fish to secure the tag.
- The first (or primary) tag is the easiest to insert and is placed on the side of the fish facing the tagger when it is lying in the tagging position (right side of the body down, left hand side facing the operator). Care must be taken when inserting the second (or companion) tag to ensure the applicator does not cut through the primary tag. For this reason, the companion tag is inserted slightly further caudal.
- The tag should not be implanted further than 5mm from the dorsum of the fish to avoid interfering with the lateral line (sensory organ).
- The tag should not protrude from the fish at an angle greater than 45 degrees. Angles greater than this can create drag and inhibit swimming ability.

T bar anchor tags



T bar fish tags (image from 'Hallprint fish tags' 2019).

- T-bar anchor tags are used for fish where large numbers may need to be tagged in a short space of time and/or where holding time is critical for fish survival.

- Tags can be varied in length and the minimum length should be determined by the size of the fish and the amount of print required on the tag.
- The main types used in finfish include ‘fine anchor tags-type TBF’ and standard anchor tags (type TBA).

Procedure for placing T bar tags

1. Fish should be anaesthetised using Aqui-S wherever possible (see section 7) and laid in a stable position with the left-hand side of their body upwards and facing the animal carer.
2. T-bar tags are implanted using an applicator gun. The gun contains a magazine which holds multiple tags and a needle for inserting into the fish.
3. Tags are usually administered between the pterygiophores of either the dorsal or anal fin.
4. Prior to inserting the needle, it is good practice to remove scales around the insertion location so that the needle is piercing the skin-only.
5. The needle should be inserted, at a 45-degree angle, between two pterygiophores, preferably the 3rd and 4th dorsal spine.
6. The gun is then deployed and then rotated clockwise before removing the needle to ensure the tag lodges correctly.
7. The needle is then withdrawn and the tag checked for correct placement by gently tugging on the tag.
8. The fish should then be placed into aerated water for recovery, checked to be fully functional and non-bleeding before release.

Dart Tags

- Dart tags are larger than T-bar tags and have been developed for use in studies on larger bodied fish species.
- Small dart tags are applied with needles with an approx. 2.4 mm outside diameter. These are suited to finfish species between 20 cm to 30 cm in size and are popular for small fish likely to undergo fast growth rates.
- Medium dart tags are also available and are applied with needles of an approximately 3.3 mm outside diameter. These are suitable for fish from about 35 cm up to about 55 cm.
- Large dart tags are applied with an approximately 4.0 mm outside diameter needle and are most suitable for finfish and sharks from around 60 cm size including tuna, barramundi, tarpon, adult Murray cod and Spanish mackerel.

Procedure for placing dart tags

1. Fish should be anaesthetised using Aqui-S wherever possible (see section 7) to ensure control during handling.
2. Dart tags are applied using a hand-held needle. The needle should be used to firstly remove scales in the tagging area.
3. Insertion should occur at a 45-degree angle with the barb orientated toward the fish. The needle should be inserted to a depth just beyond the fin spine and no more than 5mm from the top of the fish. The needle should then be rotated to lock the barb between the fin spines and the needle can be withdrawn.
4. The tag can then be tugged slightly to ensure it is set.
5. Fish should be allowed to recover in an aerated tank prior to release, checked to be fully functional and non-bleeding, before release.

Biotelemetry and biologging tagging devices (acoustic and archival)

The conventional tags detailed above only tell an investigator where a fish was released and where it was caught. To get more detailed information on the movement patterns of a tagged fish, investigators must turn to biotelemetry and biologging devices such as acoustic transponders and archival tags, which are increasingly being used to study spatial ecology of a variety of taxa in the wild. These tags are usually more expensive to buy.
Acoustic and archival tags can be surgically implanted internally or attached externally.

Acoustic tags

They send out unique radio signals on a pre-programmed frequency. Investigators set up an array of receivers in the area where the fish are tagged. These listening stations record the time and signal they receive. When the researchers retrieve the data, they can plot out where the fish went and even how fast it was travelling.

Internal deployment of acoustic tags

Procedures involving the implantation of acoustic tags are advanced and complex. Training is a critical step in delivering compliance with animal care guidelines and ensuring that the quality of the data is of the highest level possible. Individuals who perform these procedures must have an excellent understanding of and experience with anaesthesia, anatomy and the recognition of pain and distress in fish.

Staff undertaking the surgical implantation of acoustic tags must receive training from an aquaculture veterinarian or highly competent/trained and experienced chief investigator who is experienced in fish surgery. Training should focus on:

- wound healing in fish
- behavioural and physiological consequences of tagging
- surgical procedures relating to antiseptic technique
- incision and suturing
- best practice in anaesthetic/analgesic techniques
- high frequency monitoring during fish recovery following surgery.

Procedure for placing internal acoustic tags

Coded acoustic tags for internal deployment come in a range of sizes but the surgical technique is the same for most fish species, with only slight adjustments made to accommodate the fish's size.

1. Fish should be anaesthetised using Aqui-S at all times (see section 7) to achieve total loss of equilibrium and stage 3 of anaesthesia. It should then receive an oxygenated air supply during the procedure.
2. The surgery site in fish is on the ventral surface approximately midway between the pelvic and anal fins. For sharks, the surgery is conducted more towards the anal fin where the skin and musculature are thinner and more pliable.
3. The fish must be placed on its dorsum in a cradle or similar stable surface with ventrum upwards.
4. Once the location of the surgery has been decided, local anaesthetic can be injected subcutaneously in a fanning pattern along the incision line (1-2mg/kg total) prior to cutting. A scalpel blade is used to cut a small slit of about 2- 4 cm length through the skin, fat and muscle, taking care to leave the peritoneum intact. This may require several passes with the knife depending on the condition of the animal.
5. Fingers are used to gently break through the peritoneum to access the abdominal/body cavity.
6. The tag is inserted so that it lies lengthwise inside the body cavity.
7. The incision site should be sutured closed using cruciate stitches and surgical knots, with the thread cut about 5 mm above the knot.
8. One or two external conventional orange coloured T bar or dart tags should be inserted into the pterygiophores/dorsal musculature to indicate that this animal has a surgically implanted tag. This helps to differentiate the animal and avoid a second capture. It also communicates this information to fishers and the public should the animal be caught.
9. If brought on board and anaesthetised, place the fish into an aerated tank until fully recovered, checked to be fully functional and non-bleeding, before release.

Complications associated with implantation of transmitters include compromised buoyancy, infection or ineffective suturing of the incision, which can result in high levels of mortality, or longer-term alteration in movement and behaviour.

External acoustic tags

- Externally applied coded acoustic tags are cylindrical (approximately 94 mm in length and 16 mm in diameter).
- Coded acoustic tags are attached using a nylon or 316 stainless steel tethers attached to a dart or anchor. To date, the tether design has limited the lifespan of the tags to about two years, after which the coded acoustic tag detaches from the animal.
- The tags are embedded in a small high-density float that is coated in antifouling paint. The float minimises the chances of the coded acoustic tag contacting the animal's body and causing irritation. The antifouling greatly reduces the amount of marine growth which may also cause abrasions. The float has the added function of improving the hydrodynamic shape of the tag and also the opportunity for recovery of the tag when it releases.
- External acoustic tags are particularly useful for large species of fish, where anaesthesia is difficult to perform and recovery tanks are impractical.

Procedure for placing internal external acoustic tags

1. If the fish can be caught, they are drawn into the side of the boat and cradled into a sling or tethered (in the case of sharks) with the dorsal fin pointing dorsally/upwards for ease of operation. However, both continuous and coded acoustic tags can be attached to the animal using a hand pole to dart the tag into the dorsal musculature of free-swimming animals.
2. Care must be taken in the orientation of the tag anchor. The point of the anchor must be towards the head of the animal with the tail angle pointing to the opposite side of the animal. This will ensure that the anchor will lay flat and present the greatest surface area to the angle of highest stress. External tags are attached close to the base of the dorsal fin.

3. Some hand pole applicator heads collect a tissue sample at the same time as applying the tag. If this type of applicator is used:

    a) The tissue sample should be removed immediately after the tagging event.
    b) The sample should be placed into a suitable vial with a suitable preservative that does not degrade the DNA (e.g. ethanol or DMSO (Dimethyl sulfoxide or frozen immediately at -80^{o}C) and labelled with the acoustic tag number, date and species. Note: ethanol is a restricted substance on aircraft and consideration should be given to this in projects based away from the processing site.

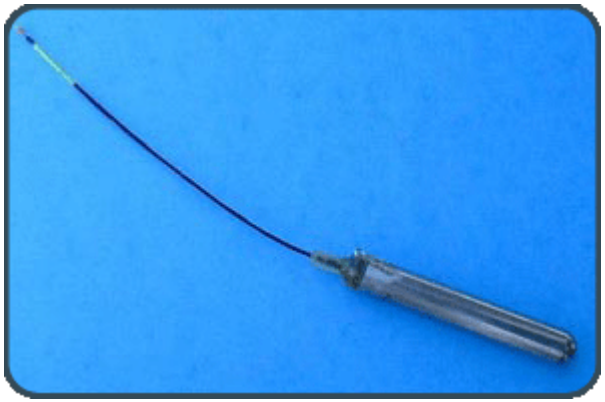
An example of an acoustic tag. (Image from Two Oceans Aquarium https://www.aquarium.co.za/blog/entry/whats-in-a-tag-leervis-garrick-acoustic-telemetry-study-update)

Archival tags

When investigators want to tag large pelagic fish that travel long distances (e.g. tuna, billfish, and sharks), they can use an archival tag, which stores data on a computer chip inside the tag. Although archival tags may be deployed internally or externally in fish, the vast majority are surgically implanted.

Procedure for placing internal archival tags

1. Internally deployed archival tags have a trailing light stalk that is designed to protrude outside of the body cavity once the tag is in place. In order to ensure comfortable placement of the light sensor stalk, the archival tag should be prepared by gently heating the light stalk in warm water and then bending it such that the trailing end lies in a streamlined position when in place.
2. To surgically implant an archival tag, initially follow the same procedure outlined above for internal acoustic tags.
3. After inserting the archival tag into the body cavity, push the tag forward so that the trailing (external) light stalk is near the cranial end of the incision.
4. The wound should be sutured immediately caudal/posterior to the light stalk to prevent it from slipping back and extending the incision and/or irritating the anal fin.



(An example of an archival tag. Image from Marine CSI https://www.marinecsi.org/2010/05/29/archival-tagging/)

When the fish is re-caught and the tag returned, researches can download the data to reconstruct where the fish has been; however, in many cases the fish are rarely caught again once they've been tagged. Therefore, in projects where the likelihood of re-catching fish is unfavourable, a better tagging option involves the use of a pop-up satellite archival tag.

Pop-up Satellite Archival (PSAT) tags.

Pop-up Satellite Archival tags are deployed externally on a variety of larger fish including southern bluefin tuna, yellowfin tuna, broadbill swordfish and various shark species. The tags take readings of environmental conditions like temperature, depth and light level. They are recommended for shorter term projects (up to one year). After a specified amount of time (days to months), the tag pops off and floats to the surface. At the surface, it broadcasts a sub-set of the information it has collected during its deployment to the ARGOS satellite system until the tag battery is spent. The data is then collected by the investigator.

These kinds of tags provide a lot of data about the movement and diving behaviour of these rarely seen animals; however, a single pop-off archival tag and the necessary satellite time cost thousands of dollars. Often, these pop-off tags are only used once, as many are lost at sea once they have transmitted their information. However, tag recoveries do occur too, and the entire data collected during the deployment period can be retrieved.

Procedure for placing external pop-up satellite archival (PSAT) tags

Placement of these type of tags require considerable coordination of crew members on the vessel, so it is important that all people involved know the details of the operation and any role they may have in handling the fish and attaching the tag(s).

Small fish and elasmobranchs can be brought aboard the vessel for this procedure. However, this tag can also be placed into free swimming (in water) fish or those restrained in the water alongside the vessel.
In water attachment method

1. Attaching the PSAT tag to fish, which are not brought on board, is undertaken using a customised tagging pole and performed by one person. If a second person is available, they can provide a support role by passing tagging equipment to the tagger as required and recording tagging details.
2. Assemble the tagging pole as per the manufacturer's instructions in readiness for the tagging. Lock the anchor into the tagging pole tip and stabilise the tag using rubber bands. The rubber bands prevent the tag from being knocked about and/or falling off the tagging pole prior to attachment.
3. Place the tagging pole in an area with the rest of the tagging equipment where they are readily accessible, out of the way of the crew and protected from potential damage or buffeting.
4. PSAT tags are applied by anchoring the tag either through the pterygiophores of the dorsal fin or into the dorsal musculature at the posterior base of the dorsal fin. This procedure will require considerable force when punching through the skin. Often for elasmobranchs, a small incision in the denticles using a scalpel facilitates the tag head insertion and minimises the need for aggressive punching at the skin.
5. Once the tag is attached the fishing line can either be cut or, if possible, the hook removed. Wherever possible the catching hook should be removed, but if this is not possible the line should be cut as close to the hook as possible.

On board boat attachment method

Transferring the fish to the boat

1. In order to bring the fish on board a tagging cradle or sling should be used and operated by two people (one at each end).
2. The tagging cradle/sling should be assembled at the start of the trip before any fishing operations take place. Once fishing operations begin, the tagger should ensure all the equipment required to apply the tag is set-up and ready. Once a fish suitable for tagging has been identified, the tagging cradle or sling is deployed into the water to a submerged position, which will allow the fish to swim into to it easily.

3. The aim is to swim the fish into the cradle so that it is clear of the mesh (thereby avoiding scraping of the scales and skin on the mesh) and then bring the mesh up from underneath the fish, providing even support along the length of the fish.
4. Once at the surface, the fish is quickly, but gently, guided headfirst into the submerged tagging cradle and once completely supported by the cradle, lifted onboard the vessel. Guiding the fish into the cradle in general will require two people to ensure that the fish is orientated correctly and any buffeting against the sides of the cradle is minimised.
5. As soon as the cradle is lowered to the deck a moist cloth is placed over the eye of the fish to aid in calming the animal. A new cloth is used on each animal to reduce the chance of transfer of infections between individuals.
6. The catching hook is removed and the fish is measured.

Placing the Pop-up Satellite Archival (PSAT) tags

1. The PSAT tag is attached using a hand applicator and is done by one person. If a second person is available they can provide a support role passing tagging equipment to the tagger as required and recording tagging details.
2. PSAT tags are applied by anchoring the tag either through the pterygiophores of the dorsal fin or into the dorsal musculature at the posterior base of the dorsal fin. This procedure will require considerable force when punching through the skin, and a skin nick using s small sharp knife/scalpel at the entry point will facilitate the process and ensure correct alignment to the body.
3. If the tag has a secondary anchor, insert the secondary anchor into the dorsal musculature posterior to and in line with the primary anchor so that the tag sits in a line along the dorsal surface of the fish. Try to leave a little bit of slack in the anchor leads (i.e. do not stretch them taut) as this will prevent the leads from chafing and cutting into the animal.
4. After attachment of the PSAT tag the fish and cradle are lowered back into the water.
5. Never tip the fish out of the cradle, instead swim the fish along in the cradle as the vessel slowly steams forwards. This allows the gills to be flushed with water, re-oxygenating the fish and allowing it to revive in its own time.
6. Once the fish is capable of swimming on its own it will swim out of the cradle. The fish is then monitored until it swims out of view and its behaviour noted on the monitoring form.

Shark satellite tagging (SAT tags)

SAT tags are either attached directly to the first dorsal fin or tethered to the dorsal fin using a pin and plate system.

Procedure for placing external shark satellite tags (SAT tags)

1. Sharks should be tagged alongside the vessel after securing with a tail rope and a supporting soft rope or snatch strap like sling posterior to the dorsal fins in a. Capture and handling of a shark involve ropes and wire trace; therefore, gloves should be worn at all times.
2. In general, sharks are caught through the use baited set lines, drop lines, drumlines or by longlining. Sharks caught by the use of a baited line can be coaxed close to the vessel using a teaser line (baited line without a hook) to assess the suitability of the shark for capture. If suitable, a baited line with a hook is put out.
3. Once hooked, guide the shark to the side of the vessel and attach a tail rope immediately – this reduces the amount of force applied at the hooked area of the mouth/jaw. Point the sharks head to bow. Once hooked, depending on the species the shark may be allowed to tire against the pull of a Styrofoam float attached to the rope just behind the trace (additional floats may be required depending on the size of the shark), taking care not to excessively tire the shark. As soon as the shark is restrained, oxygen is bubbled through a seawater stream and used to irrigate the mouth of the shark. Providing oxygen in this way helps to ensure adequate oxygen irrigation of the gills as well as providing a mild sedative effect (reference for all species?).
4. The shark must be restrained while the SAT tag is attached to the dorsal fin (which will generally require 3-4people for larger sharks). Two people are tasked with attaching the SAT tag, one (the tagger) to put the tag on, the second to be a support person to pass tag components as required.. Another team member ensures the oxygen is maintained and generally monitors the procedure. The entire tagging procedure should not exceed six minutes from the time the shark is restrained to the time it is released.

- With the tagger positioned beside the dorsal fin, the support person passes over a template used to locate the position of the bolt holes. The wet/dry sensor and the aerial of the SAT tag must be exposed when the dorsal fin breaks the water's surface; therefore, the template should be positioned in the upper 1/3rd of the dorsal fin to achieve this. Supporting the back of the fin with a cutting board, the tagger drills through the fin using the template as a guide.
- Next, the backing plate for the SAT tag and a bolt is fitted to the fin prior to the SAT tag being secured using the bolt. A second bolt is then fitted to secure the SAT tag firmly and prevent it from rotating.
- If time permits, other tags may be attached to the shark once the SAT tag has been attached.
- The shark is then immediately guided out of the sling headfirst or if brought onboard for tagging, immediately returned to the water headfirst. The tail rope should be released last, only when the shark is deemed of good condition following the procedure.
- The release person must make a mental note of the shark's condition on release and this information should be transferred to the release documentation and monitoring sheet as soon as possible.

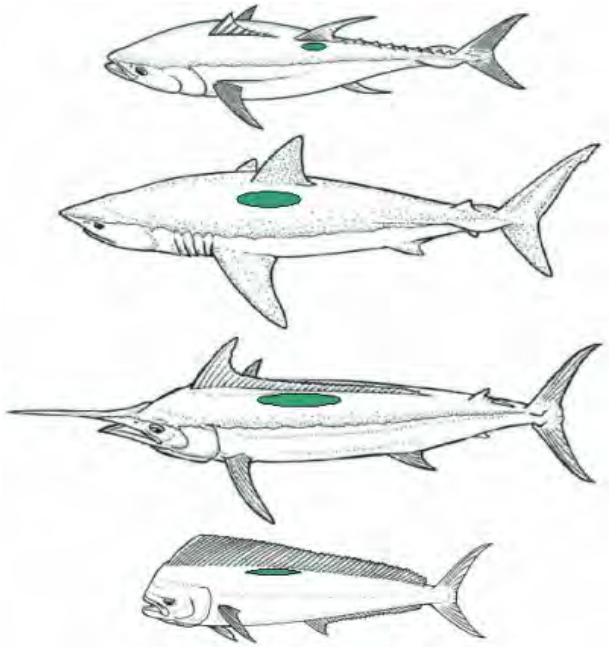


Image of tagging locations for game fish species by New South Wales Government https://www.dpi.nsw.gov.au/fishing/recreational/resources/fish-tagging/game-fish-tagging/how-to-tag-game-fish

Internal Tags and Marks

Implanted wire tags, passive integrated transponder (PIT) tags, otolith marks and natural parasites are internal marking systems used to identify fish.

Coded wire tags

Coded wire tags are tiny pieces of metal with microscopic unique codes that are inserted into fish, usually in the snout. They are often used to tag hatchery-reared fish that are released as juveniles into the wild because the fish are very small themselves at this stage and couldn't handle an external tag. Since coded wire tags aren't visible from the outside of the fish, they may be overlooked by anglers who recapture the fish, so some fish may also have fins clipped to distinguish them from untagged fish.

The coded wire tag identification system has been tested for management and research applications with multiple fish species and does not cause adverse tissue reactions. The coded wire tag is normally injected into cartilage, connective tissue or muscle and is later detected electronically with a handheld device. The use of transparent tissues as injection sites can decrease the necessity for external indicators. Shallow implantation of tags facilitates benign surgical recovery of the tags.

Visual implant tags

Visual implant tags are also implanted internally but are placed very close to the surface of the skin, so they are visible. Visual implant tags are made of a brightly coloured plastic-like substance that is injected underneath the skin, usually near the eye of the fish. Some tags may have a number on them, but many are simply colour-coded to represent the year or body of water they were released in. When they are recaptured, scientists know which "batch" of released fish they came from, but not which individual fish are returned.

Electronic Passive Integrated Transponder (PIT) tag (also called Coded-wire tag (CWT))

A PIT tag is a small radio transponder that contains a specific code, which allows individual fish to be assigned a unique 10 or 15-digit alphanumeric identification number. This small computer chip is injected into the abdomen of the fish for permanent identification.

Rather than the tag transmitting a signal, the tag scanner (or reader) sends out a radio frequency and when a tag is within range, it will relay the identification code back to the receiver. The lack of a battery is the greatest advantage of the PIT tag since it allows for the production of much smaller tags that can be used on smaller organisms, which will last the life of the fish.

These tags can be read easily through soft and hard tissue, seawater, freshwater, glass, plastic, metal and even when tags are moving at some velocity. The reading device may be powered by alternating current or battery for convenient use in the field as well as the laboratory. Information may be downloaded directly to a computer.
PIT tags are commonly used for long term fish migration projects or for identifying broodfish in hatchery situations. The tags do not contain batteries, so once tagged a fish can theoretically provide information for life. A number of different PIT tags are available, but most Australian applications will use either a full duplex (minimum size 8 mm in length) or half duplex (minimum size 12 mm in length) tag.
It is important that PIT tag suppliers are registered with the International Centre for Animal Registration (ICAR) and that tags are ISO 11784 and 11785 compliant (thus reducing the possibility of obtaining duplicate numbers). Food safe plastic-coated tags and glass encapsulated tags are both available and their application will vary among projects.

Procedure for placing a PIT tag

1. PIT tags should be checked with the scanner to ensure the paperwork matches with the code on the tag, prior to insertion.

2. Fish should be at stage 3 of anaesthesia using Aqui S prior to tagging.
3. PIT tags are inserted by using a specialised applicator needle and applicators are usually specific to the type and size of tag being used (i.e. full or half duplex). The manufacturers generally provide applicators for their tags.
4. Tags should be inserted into the coelomic (peritoneal) cavity between the body wall and internal organs (note: a needle angle close to parallel with the fish will reduce the risk of piercing organs). This position has been found to result in the highest rates of tag retention and lowest mortality. Tagging in the shoulder, cheek or pelvic region is not recommended.
5. The needle should then be removed, and the tagged fish placed into an aerated tank for recovery prior to release. A hand-held PIT reader should be used to detect the chip, to verify that the tagging was successfully inserted.

Note: PIT tagging should always be conducted outside of predictable reproductive windows for the fish species in the project, and additional care should be taken when tagging gravid females as rupturing ovaries can result in additional shedding during spawning.

Advantages of PIT tagging	**Disadvantages of PIT tagging**
The tag does not appear to affect the survival and growth in fish	The fish must be captured and anaesthetised to insert the chip
The method does not result in a skin wound that may act as an entry site for bacteria, viruses and fungi	Special equipment (scanner and software) is needed to register the fish and identify individuals
There is no mutilation of fins	Fish must be recaptured again to read PITs
There are online databases where fish breeders/scientists can publish their tagging lists, so that individuals from other areas can be identified	

PIT tag implantation. (Image from Fishbio, 2009, https://fishbio.com/field-notes/fish-monitoring/surgically-implanting-of-pit-tags)

Post tagging procedures

- At the end of each tagging period, regardless of the technique used, all tag applicators, knives and tags should be thoroughly cleaned under running water and then dipped into an antiseptic (e.g. Betadine) or alcohol bath, to help avoid transfer of infections between animals. Most manufacturers will have a preferred method of equipment maintenance on their websites and these should be followed by the investigators.
- Knives if used should be sharpened and any other single use scalpels or other sharp instruments should be placed in the appropriate sharp's container as per routine laboratory practise. Any moving parts should be sprayed with WD40 or similar to prevent rusting from the marine environment.
- Once dry, all equipment should be placed back into the tagging kit toolbox ready for the next deployment.
- All samples taken must be stored, labelled and used as per the AEC application.

References

- Booth, G, 2010, Tagging for results – making it better for you and better for science, viewed 27 February 2020, https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/448509/BW82-Tagging.pdf
- Bradford, RW, Hobday, AJ, Evans, K & Lansdell, M, 2009, *CSIRO Marine and Atmospheric Research Code of Practice for Tagging Marine Animals,* viewed 20 January 2020, http://www.cmar.csiro.au/e-print/open/2009/bradfordrw_a.pdf
- Gill, H, Ashton, C & Rowland C, 2008, *Working towards the development of best practices in fish and fisheries research or The troubles with fish and fish biologists!,* Viewed 20 November 2019, https://anzccart.org.nz/app/uploads/2017/06/gill-ashton-workin.pdf
- New south Wales Government, Department of Primary Industries, How to tag game fish, viewed 27 February 2020, https://www.dpi.nsw.gov.au/fishing/recreational/resources/fish-tagging/game-fish-tagging/how-to-tag-game-fish
- Roques, JAC, Abbink, W, Geurds, WF, van de Vis, H & Flik, G, 2010, Tailfin clipping, a painful procedure: studies on Nile tilapia and common carp, *Physiology and Behaviour*, vol. 101, pp. 533-540
- Sfakiotakis, M, Lane, DM & Davies, JB, 1999, Review of Fish Swimming Modes for Aquatic locomotion*, Journal of Oceanic Engineering*, vol. 24, no. 2, PP. 237-252
- University of Wyoming, *What are stable isotopes?* viewed 20 November 2019, http://www.uwyo.edu/sif/stable-isotopes/what-are-stable-isotopes.html)

Further reading

- Bumgarner, JD, Schuck, ML & Blankenship, HL 2009, Returns of Hatchery Steelhead with Different Fin Clips and Coded Wire Tag Lengths, *North American Journal of Fisheries Management*, vol. 29, no. 4, pp. 903-913
- Champagne, CE, Austin, JD, Jelks, HL & Jordan, F 2008, Effects of Fin Clipping on Survival and Position-Holding Behavior of Brown Darters, (Etheostoma edwini), *Copeia,* vol. 2008, no. 4, pp. 916-919

- Chaprales, W, Lutcavage, ME, Brill, RW, Chase, BC & Skomal, 1998, Harpoon method for attaching ultrasonic and "popup" satellite tags to giant bluefin tuna and large pelagic fishes. *Journal of the Marine Technology Society, vol.* 32, pp. 104-105
- Conover, GA & Sheehan, RJ, 1999, Survival, Growth, and Mark Persistence in Juvenile Black Crappies Marked with Fin Clips, Freeze Brands, or Oxytetracycline, *North American Journal of Fisheries Management*, vol. 19, no. 3, pp. 824-827
- Dietrich, JP & Cunjak, RA, 2006, Evaluation of the Impacts of Carlin Tags, Fin Clips, and Panjet Tattoos on Juvenile Atlantic Salmon, *North American Journal of Fisheries Management,* vol. 26, no. 1, pp. 163-169
- Department of Natural Resources, Michigan 2011, *Coded-wire tag program,* viewed 20 November 2019, http://www.michigan.gov/dnr/0,1607,7-153-10364_52259_10951_11301-97831--,00.html
- Elliot, DG & Pascho, RJ, 2001, Evidence that coded wire-tagging procedures can enhance transmission of Renibacterium salmoninarium in Chinook salmon, *Journal of Aquatic Animal Health*, vol. 13, no. 3, pp. 181-193
- Monamy, V & Gott, M, 2001, Practical and ethical considerations for students conducting ecological research involving wildlife, *Austral Ecology* vol. 26, pp. 293-300
- Oakley, KL, Thomas, L P & Fancy, SG, 2003, Guidelines for long-term monitoring protocols. *Wildlife Society Bulletin* vol. 31, pp. 1000-1003
- Thompson, JM, Hiredotha, PS & Eggold, BT, 2005, A Comparison of Elastomer Marks and Fin Clips as Marking Techniques for Walleye, *North American Journal of Fisheries Management,* vol. 25, no. 1, pp. 308-315
- Vander Haegen, GE, Blankenship, HL, Hoffmann, A & Thompson, DA, 2005, The Effects of Adipose Fin Clipping and Coded Wire Tagging on the Survival and Growth of Spring Chinook Salmon, *North American Journal of Fisheries Management*, vol. 25, no. 3, pp. 1161-1170
- Wagner, CP, Einfalt, LM, Scimone, AB & Wahl, DH, 2009, Effects of Fin-Clipping on the Foraging Behavior and Growth of Age-0 Muskellunge, *North American Journal of Fisheries Management,* vol. 29, no. 6, pp. 1644-1652
- Ward, DL, 2003, Effects of Marking Techniques and Handling on Swimming Ability of Bonytail Chub, *Journal of the Arizona-Nevada Academy of Science,* vol. 36, no. 1, pp. 34-36
- Young, R, 2005, *Sea Safety Strategy*, viewed 19 June 2019, www.marine.csiro.au/intranet/ohse/needtoknow/seasafetystrategy.html
- Zerrenner, A, Josephson, DC & Krueger, CC, 2007, Growth, Mortality, and Mark Retention of Hatchery Brook Trout Marked with Visible Implant Tags, Jaw Tags, and Adipose Fin Clips, *The Progressive Fish-Culturist*, vol. 59, no.3, pp. 241-245

Egg, spawn and larvae collection and stocking

When a spawn occurs, being ready and prepared is the difference between being able to collect a maximum amount of eggs to have a maximum number of larvae to stock and having an unpredictable and variable amount of egg and larvae to stock. Furthermore, the better prepared, the easier it is to collect the eggs/larvae and the less dead larvae resulting from distress due to mechanical (e.g. nets) or physiochemical shock (e.g. temperature, salinity, pH etc…). Equipment needed may include:

- an egg collection tank and net
- a graduated cup and bucket
- hatching tanks with a banjo screen/filter and flow through water system
- hoses

- larvae counting chamber
- sedgwick rafter counter
- sample tubes.

Preparing for the spawning process

- Monitoring should start as soon as the tanks and equipment are set up prior to the eggs being laid.
- Each fish species will have its own spawning time which may be based on factors such as water temperature and full moon timing and the investigator should have this knowledge prior to starting a project.
- The larvae pond/tank will need to be pre-prepared to stock the larvae on day two or when each species of fish hatch.
- Regarding the spawn the following steps need to be followed:
    1. Install the egg collection tank and new egg collection net (300µm) for each broodstock tank at least 2 days before the expected spawn.
    2. Ensure the egg incubators are ready, with easy water access and the appropriate level of aeration (note: eggs are strong compared to larvae so can withstand more turbulence).

Spawning and egg collection and count

To recognise if the spawn occurred, the investigator should check the egg collector. Once eggs are observed, monitoring of the eggs and larvae should begin and include the time the eggs were first observed with photos of the eggs clearly showing the developmental stage. This information is important in order to calculate when the spawn occurred. This will be also an important step to be able to publish the data.

The water flow on the broodstock tank should be checked to ensure it **does not exceed** the flow out of the egg collector tank. If the flow is too high, the flow can make the egg collector overflow and thus eggs will be lost.

Note: It is important for the investigator to know the time scale for spawning in their species of fish and the normal timing of the egg hatching stage to be able to catch as many eggs as possible. The length of this stage can be affected by the water temperature (i.e. warmer temperatures typically speed up hatching and visa versa).

Procedure to collect the fish eggs

1. Turn off the flow on the broodstock tank.
2. Slowly lift the egg collector out of the water and at the same time gently rinse any eggs that have accumulated on the mesh with the tank water (fresh or marine) whilst gently shaking the egg collector.

3. Once the eggs are concentrated in the egg collector in approximately 10 litres of water, the investigator can gently scoop them out using a 1 litre cup or beaker and transfer them into a graduated bucket/measuring bucket.
4. Once all the eggs are in the bucket, an air stone or other gentle aerator should be placed into the bucket, so that the eggs are aerated and kept in suspension.
5. The animal carer's hand should be used to gently mix the water in the bucket, before collecting 3 x 40ml samples in 50ml sample tubes.

Procedure to count the fish eggs

In the laboratory

1. Count 1 ml from each sample using a Sedgwick rafter counter.
2. Take the average of the 3 counts and multiply it by 1000 x number of litres in the bucket. This will calculate the total number of eggs in the bucket that a broodstock tank contributed to.
3. Record the data.

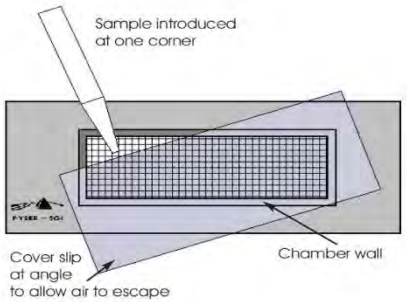


Image of a Sedgewick rafter egg counter example by Adelab Scientific, http://adelab.com.au/haemocytometers-and-microbe-counting-chambers/sedgewick-rafter-counting-cell-plastic-includes-1-cover-glass

Procedure to transfer the eggs to the hatching tank

1. Transfer the eggs to a hatching tank with flow through and "strong" aeration. (It is very important to reduce the aeration once the larvae start to hatch, because the eggs are more robust compared to the larvae.)
2. Aeration should therefore be adjusted to avoid a stressful turbulence to which post-larvae are most sensitive, in particular at two critical stages–during the first feeding and during the formation of the swim bladder. Excessive water movement:
   a) May prevent predatory activity of fish post larvae.

b) May make the action of gulping of an air bubble at the water surface (that is necessary to activate the inflation of their swim bladder) more difficult.

Note: Consider, however, that smaller fish/larvae require higher levels of dissolved oxygen per amount of fish mass than larger fish but prefer less turbulence.

Procedure for larval count and transfer

According to the species, when the larvae are ready to harvest, they should be transferred to the permanent housing.

1. Harvest the larvae by siphoning the water from the hatchling tank into a plankton net suspended in a smaller container of the same water parameters. (Scooping larvae up in nets causes trauma and increased mortality).

An example of a plankton net. Image from Natural History Book Service, 2020 https://www.nhbs.com/plankton-net-300mm-frame)

Once the hatch tank is empty:

1. Slowly lift the plankton net out of the water and at the same time wash with water (fresh or marine as applicable) any larvae accumulated on the mesh and by gently shaking the net.
2. Once the larvae are concentrated in the net in approximately 5 litres of water, they can be gently scooped out using a 1 litre cup or beaker and transferred to a graduated bucket.

3. Once all the larvae are in the bucket place an air stone or other gentle aeration so that the larvae are aerated and kept in suspension.
4. Use a hand to gently mix the water in the bucket, collect 3 x 40ml (approximately) samples in 50ml sample tubes.
5. Note the volume of the sample and then add lugol (aqueous iodine) to make the larvae more visible.
6. Pour its content into the larvae counting chamber.
7. Count all the larvae and then divide that number by the volume of the sample in litres.
8. Multiply this volume by 1000 x by the number of litres in the larvae bucket.
9. Once the number of larvae has been confirmed, they can be transferred into their permanent housing tank.

IMPORTANT NOTE

The water quality in the larvae bucket and the new housing tank will not be the same, so the fish need to be slowly acclimated to their new environment. This is achieved by letting the bucket get to the temperature of the new system (usually 30 minutes minimum). Titration is achieved by adding a small amount of water from the new system every 5-10 minutes, with the number of repetitions depending on the temperature difference between the bucket and the new housing tank. The greater the temperature difference the less amount of volume to add to the larval bucket and the less frequently. After 30 minutes, the water temperature in the bucket and the new housing tank/facility should be checked and if it is the same, the larvae can be released slowly. If it is still different, maintain the process of acclimation until the temperatures match.

References

- Research Institute for Coastal Aquaculture, 2017, Egg and larvae collection, counting and stocking SOP, viewed 11 February 2020, http://www.cari.org.in

Further reading

- Food and Agricultural Organization of the United Nations, *Seed production- an overview*, viewed 11 February 2020, http://www.fao.org/3/AC182E/AC182E01.htm
- Jakobsen, T, Fogarty, MI, Megrey, BA & Moksness, E, (eds), 2009, *Fish Reproductive Biology: Implications for Assessment and Management*, Blackwell Publishing, Oxford United Kingdom

Surgical procedures on fish

There are numerous forms of surgery carried out on fish within scientific projects including implanting of tags and transmitters and examining gonads. All types of fish surgery carry a very high animal welfare risk. Therefore, surgery should only be performed by very experienced and deemed competent investigators.

The investigators must prove that they have had the relevant training, including surgical principles and guidance in performing the specific procedure. This training can be undertaken by an aquatic veterinarian or the chief investigator with extensive experience in the particular procedure being performed. The trainee must demonstrate competency to the trainer or AWO and the training must be documented prior to performing the procedure. All training records should be included as part of the AEC application.

The investigator will be required to describe in detail their approach to any procedure requiring surgery including:

- the location of the surgery and method of ensuring a clean working area
- provision of sterile equipment
- provision of oxygen supply
- method of analgesia (initial and ongoing) and anaesthesia (induction and maintenance)
- surgical site preparation
- surgical procedure including approach, techniques and closure of surgical site
- post-operative care including antibiotics and other medications
- monitoring before, during and post-surgery.

It is not within the scope of these guidelines to document every type of fish surgery possible, but the following points highlight some of the considerations and requirements to ensure the best welfare outcomes for the fish during all surgical procedures.

- A suitably clean environment should be found whether in the laboratory setting or in the field setting for the surgery to take place. It should be quiet and free from potential external contamination during the procedure. The table should be well set up with all the equipment required ready to use prior to starting the procedure.
- All equipment should be sterilised using routine techniques, such as autoclaving within the laboratory setting. In the field, the instruments should be washing in detergent to remove excessive contamination, rinsed and then soaked in a suitable disinfectant such as 'Pyroneg' according to the manufacturer's guidelines (see husbandry section 4) prior to use.
- Given the aquatic environment in which fish live, it is impossible to conduct surgical procedures under aseptic conditions, even within laboratory settings. However, care should be exercised to prevent the introduction of additional infective agents and to minimize the physiological stress of such procedures.
- Fish should be anaesthetised to plane 3 in preparation for a pain free surgery prior to intubating. The intubation process will maintain a good oxygen flow through the gills whilst the fish are out of water. The fish should be checked regularly for the depth of anaesthesia and the heart monitored with a doppler ultrasound heart monitor. Anaesthesia should be maintained with titrated dosing throughout the procedure (see section 7). Fish should be managed in a 'cradle' or similar structure to stabilise the animal during the procedure.
- Analgesia in the form of morphine, or local anaesthesia injection or topical application at the site of the incision, should be used to alleviate pain both during and after the surgery (see section 7).

- Skin should be prepared for surgery by removing the mucous from the surgical site and cleaning the area with sterile saline or/and diluted betadine solution. In some species the scales will need to be removed to facilitate the incision. A plastic drape should be used to maintain skin moisture and also prevent external contaminants from entering the wound site during surgery.
- The incision site should be closed with a monofilament suture material of 5-0 size using either a curved reverse cutting or a tapered needle of the smallest size practical to prevent tearing of the fish skin and minimising the hole left by the needle. Depending on how the fish will be housed during healing (especially the temperature of the water), the choice of absorbable vs non-absorbable suture material should be made. Warmer water increases metabolic rate and hence the breakdown of the suture material). Simple interrupted sutures should be placed 4-6 mm apart with a 2mm tissue bite either side of the incision. A surgeon's knot should be used to ensure the tissues are apposed, but the knot should not be over tightened, to prevent tissue necrosis
- Antibiotic therapy should be considered for surgery where the coelomic cavity or deep muscle has been incised. **However, antibiotic use should not be used as a replacement to clean surgical technique**. Suitable antibiotics for fish can be found in section 9.
- Animals subjected to surgical procedures in the laboratory should be observed carefully for at least 72 hours following the surgery (see high frequency and medium frequency monitoring section 5).

The following references describe various fish surgery techniques that may offer investigators guidance when describing the specifications of the surgery in their project.

- Harms, CA, 2000, Surgery in fish, *Veterinary Clinics of North America: Exotic animal practise*, vol 3, no. 3, pp. 759-774, (https://doi.org/10.1016/S1094-9194(17)30074-9)
- Harms, CA, 2005, Surgery in fish research: Common procedures and postoperative care, *Lab Animal,* vol. 34, no. 1, pp. 28 -34 https://www.researchgate.net/publication/26862057_Surgery_in_Fish_Research_Common_Procedures_and_Postoperative_Care
- Murray, JM, 2002, Fish Surgery, *Seminars in Avian and Exotic Pet Medicine*, vol. 11, no. 4, pp. 246-257, https://www.sciencedirect.com/science/article/pii/S1055937X02800226
- Wagner, GN, Cooke, SJ, Brown, RS & Deters, KA, 2011, Surgical implantation techniques for electronic tags in fish, Reviews in Fish Biology and Fisheries, vol. 21, no. 1, pp. 71-81 https://www.researchgate.net/publication/226061128_Surgical_implantation_techniques_for_electronic_tags
- Wildgoose, WH, 2000, Fish surgery: an overview, *Fish veterinary journal,* vol. 5, pp. 22-26, https://www.researchgate.net/publication/310842827_Fish_surgery_An_overview
- Weber, ES, Weisse C, Schwarz T, Innis C & Klide, A, 2009, Anesthesia, Diagnostic Imaging, and Surgery of Fish, *Compendium*, vol. 31, no. 2, pp. 1-9 http://vetfoliovetstreet.s3.amazonaws.com/mmah/0a/4d8d2e237945408013bcff89a564f7/filePV0209_WEB_Weber_Fish_0.pdf

References

- American Fisheries Society, 2014, *Guidelines for the Use of Fishes in Research*, viewed 28 November 2019, http://frdc.com.au/Archived-Reports/FRDC%20Projects/1993-184-DLD.pdf

Weighing and measuring fish

Equipment

- Scales suitable for the size of fish to be weighed.
- Data recording supplies (E.g. notebook, pen, laptop).
- Dip net.
- Clear ruler.
- Clean detergent free bucket containing water from the fish's housing tank.
- Aqui-S anaesthetic agent.
- A suitably sized dish (e.g. a large petri dish) containing a sponge or paper towel dampened by a small amount of tank water for holding the fish on the scales during weighing.
- Battery or mains powered aerator.

Procedure for weighing fish

Ideally, have a second person present to record data and assist in capture and monitoring fish post sedation. Also, plan the day's workload so that all fish are returned to their tanks and can be monitored intensely to confirm recovery initially and then at least every four hours before the fish are left overnight.

1. Set up the weighing and measuring area as close to or within the fish housing area. All measurements must be completed and the fish returned to their housing tank for recovery within three minutes.
2. Fill the bucket with water from the fish's tank and add the appropriate amount of Aqui-S to sedate the fish for handling (see sedation section 7).
3. Ensure the weighing dish covered with moist paper towel or cloth is tared/zeroed on the scales.
4. Capture the fish with the net as gently as possible, with minimum distress, and place it in the bucket containing the Aqui-S. Carry the bucket to the weighing and measuring area.
5. Place the air stone in the bucket and provide aeration with minimal turbulence to prevent foaming of the water.
6. When the fish appears sedated (loss of equilibrium, uncoordinated swimming movements, etc) quickly transfer it to the dish on the scales.
7. Record the fish's weight in grams.
8. Place the fish on its side with the jaw closed.
9. Measure a straight line from the tip of the snout to the extreme tip of the tail fin.

10. For soft-tailed fish, squeeze the tail fin together to obtain the maximum overall length.
11. For hard-tailed fish, turn the tail to obtain maximum length.
12. Record the length.
13. Return the fish to the bucket and then return the fish to the housing tank.
14. Monitor the fish until it is fully recovered. If high frequency monitoring cannot be undertaken in the fish's normal tank due to distance/lack of observation, smaller recovery tanks can be set up (filled with water from the fish's housing tank) where fish can be placed temporarily, close to the investigator until they are ready to be taken back to their normal housing tank.

Note: if weight is the only measurement needed, this can be performed in a small container of water (water to be taken from the main housing tank) by:

1. Taring the container of water.
2. Adding the fish and recording the weight.
3. Returning the fish to the recovery tank and re-taring the container of water for the next fish.

Reference

- Flinders University, 2019, *Standard Operating Procedure Working with Fish*, viewed 2 December 2019, https://staff.flinders.edu.au/content/dam/staff/research/ebi/animal/sops/sop-working-with-fish.pdf

Producing triploid fish

Example of a triploid fish. (Image from by Katie Burgert, 2019).

What is a triploid fish?

Normal fish have two sets of chromosomes (diploid) compared to triploid fish have three sets rendering them infertile.
Triploid fish appear the same as diploid fish, with the male fish still producing gonads and exhibiting spawning behaviour even though they are sterile. Females, however, do not produce gonads, but as female gonads cannot be visualised even in diploid fish, they still appear the same externally. Fish size may differ slightly, but that can also depend on other factors.

Why are triploid fish useful to fish biologists in scientific projects?

There are several reasons for creating sterile fish including:

- As infertile predators for controlling unwanted, usually invasive, species which cannot reproduce themselves and become their own problem.
- To maintain the genetic purity of wild stocks where wild and hatchery grown stocks of fish are mixed in fisheries. This ensure the genetics of the wild stock are not tainted, but overall productions levels can be increased.
- To improve the growth rate of aquaculture species because less energy is diverted for reproduction.

How triploid trout are created

Creating a triploid fish requires the egg to retain a chromosome it would normally expel. In normal fertilisation, after an egg and sperm combine, one chromosome is expelled, leaving either XX (female) or XY (male) chromosomes behind. If the extra chromosome is retained, the offspring will either be XXX (triploid female) or XXY (triploid male).

There are several ways to cause a chromosome to be retained, but the most common ways are by:

- hydrostatic pressure treatment
- heat shocking
- modifying female fish to produce sperm instead of eggs.

Hydrostatic pressure treatment

The fertilised egg is given enough time to produce the polar body at the stage where the third chromosome is ready to be expelled. Before it can separate out, the egg is subjected to high pressure, causing the polar body to remain. This is presently the most consistent method in the aquaculture industry.

Hydrostatic pressure procedure

1. Pressure shocks are typically administered using a stainless-steel cylindrical vessel closed by a brass piston fitted with an O-ring, pressure gauge and relief valve. An external hydraulic press is used to apply pressure to the piston.
2. Different pressure parameters are required for different fish species. For example, Grass carp require 7,000 to 8,000 pounds per square inch (psi) for a duration of up to 90 seconds, starting 4 to 5 minutes after water is added to the eggs and sperm at 26°C. Decompression is instantaneous at the end of the treatment and the eggs are then transferred to the incubation apparatus.
3. A control group (i.e. a small quantity of the same fertilized eggs) should always be incubated separately, because initial egg quality is a major factor in the success of the triploid fish production. There is a strong relationship between egg viability of the control group and the ability to successfully produce triploid fish. Egg quality is generally influenced by the brood female condition, water temperature and the timing of hand stripping with regards to ovulation.
4. Egg viability of the control group at 20 hours after fertilisation, provides a good indicator of successful triploid induction by hydrostatic pressure shocks. For example, in the case of Grass carp, when the egg viability of the control group is greater than 60 %, hydrostatic pressure shocks usually result in 80 to 100 % triploid fish with a hatch relative to controls of nearly 70%. In comparison, minimal triploids are produced when the viability of control eggs from the same spawn is below 40%.

Heat shocking treatment

This procedure is also fish species specific with regards to the waiting periods prior to the process starting and different temperatures required to form the triploid fish.

Heat shocking procedure

1. To produce the triploid fish, the eggs must be fertilised with normal fish sperm.
2. Depending on fish species a time lapse is applicable whilst the egg starts it development process prior to the eggs being heated. This heating will induce polar/barr body retention within the egg (i.e. it prevents the body from being expelled by disrupting the small fibres that pull the chromosomes apart) and hence retain an extra chromosome. For example, trout are heat shocked at either 29 °C for 10 minutes or at 26 °C for 20 minutes.
3. The heat-shock method of producing triploids can result in a low number of fish that are diploid and hence is not considered as reliable as the other two methods of triploid production.

Modifying female fish to produce sperm instead of eggs

Female fry, which have an XX chromosome complement, are changed into morphological males (referred to as sex-reversed fish/XX males), by feeding them male hormones.

Even though these fish are genetically female they produce sperm instead of eggs. These sperm contain only X chromosomes and no Y chromosomes (the "male" chromosome"). When sperm from an XX male is used to fertilize eggs, the offspring are all female.

Modifying female fish procedure

1. These sperm producing female fish (XX male) are then humanely killed with Aqui-S and the testes are crushed to release the sperm. This sperm is then used to fertilise eggs.
2. Eggs are then gently stripped/squeezed from other normal female fish, with these eggs containing two sets of chromosomes at this time.
3. The eggs are fertilised with the sperm collected earlier. After a brief moment, the eggs are gently rinsed to remove excess sperm and bacteria.
4. The eggs then undergo the heat shocking treatment specific to each species to become triploid, and because these fish have only X sex chromosomes, they are all females.

5. After a warm bath, the egg trays are incubated as normal and eventually develop into fry.

Stripping female fish for egg collection. (Image from Krejszeff s et al, 2011).

Assessing Triploidy success

Triploidy is verified by anaesthetising the fish once they are large enough to sample and collecting approximately 1 microliter of blood. The cell membrane of the red blood cells are lysed/broken with a lysing agent (e.g. Hematall LA-Hgb Reagent™), leaving the nuclei ready to be scanned for ploidy determination.
For example, diploid Grass carp have a red blood cell nuclei volume of 10.06 cubic micrometres (µm3), while the mean volume of triploid red blood cell nuclei is 14.82 µm3 (i.e. triploids have a larger red blood cell nuclei).

Managing the growth of triploid fish

Once triploid fish have hatched, they can be raised in the same way as their diploid counterparts and released as stocker fish in various aquaculture facilities including research establishments.

Information for investigators when preparing AEC applications using triploid fish

Currently under the *Animal Care and Protection Act 2001 (Qld)*, fish eggs and larvae are not considered as animals until they have absorbed their egg yolk sac and started feeding; therefore, they are not covered by the Animal Code. This means that the pressure and heat shock treatment of eggs does not require AEC applications per se, if the eggs have been bred elsewhere and brought into a research project.

However, the modifying of female fish to produce sperm does require an AEC application.
Similarly, inappropriate management techniques during the production of triploid fish leading to egg and larvae death, will indirectly affect the welfare of the broodstock fish used to produce the eggs (held within UniSC projects), by unnecessarily extending the duration required to be housed within the research facility in order to wait for follow up spawning events. Therefore, this would also require an AEC application.

Considering all the general information on producing triploid fish as above, and the complexity involved in each of the processes, the investigator should clearly document the details of their triploid production techniques with the anticipated success factor based on other scientific peer reviewed papers for the species of fish being researched. Details pertaining to general housing, husbandry, use of drugs and hormones, triploid processing and humane killing of the brood fish, eggs and larvae should be included in the AEC application.

References

- Hu, F, 2019, The Sterility of Allotriploid Fish and Fertility of Female Autotriploid Fish, *Frontiers in Genetics*, viewed 11 February 2020, https://doi.org/10.3389/fgene.2019.00377
- Krejszeff, S, Kucharczyk, D, Targońska, K & Żarski, D, 2011, *Optimization of the reproduction of chub (Leuciscus cephalus L.) under controlled conditions, Fish management in a variable water environment*, viewed 11 February 2020, https://www.researchgate.net/publication/309789456_Optimization_of_the_reproduction_of_chub_Leuciscus_cephalus_L_under_controlled_conditions
- Rottmann, RW, Shireman, JV & Chapman, FA, 1991, *Induction and Verification of Triploidy in Fish*, viewed 11 February 2020, http://agrilife.org/fisheries/files/2013/09/SRAC-Publication-No.-427-Induction-and-Verification-of-Triploidy-in-Fish.pdf

Cleaning protocol following fish procedures

When cleaning equipment in aquaculture facilities, a separate sink should be designated as part of a utility room. This separation will prevent chemical contamination of food supplies for the fish, ensure hand washing facilities remain clean and prevent potential intoxication of fish in tanks due to accidental splashing or spillages of toxic substances.

1. The equipment should be cleaned as soon as it has finished being used by the investigators, to facilitate an efficient cleaning process and maintain the quality of the equipment.
2. Obvious large pieces of contamination should be removed and disposed of in the appropriate garbage facility.
3. All equipment must be washed in hot soapy (detergent) water according to the manufacturers recommended dilution rates. Ensure the detergent is appropriate for aquaculture facilities because normal detergent is highly toxic to fish.
4. All equipment must be rinsed thoroughly with clean hot water.

5. Instruments should then be soaked in a solution of Pyroneg or Virkon products (see husbandry section 4 for dilution rates). The specimen tray should be sprayed with the same solution and left for 10 minutes.
6. The disinfectant solution should be rinsed off thoroughly and the equipment left to air dry in a clean area.
7. The clean equipment should be stored in a clean dry area where it will not be contaminated.

8.5 Animal health and welfare considerations

All procedures in this section have the capability of causing severe pain and distress if performed incorrectly and by inexperienced investigators and animal carers.
Anaesthesia and analgesia must be considered for every procedure and included in the AEC application process.
It is essential that high frequency, followed by medium frequency monitoring sheets are completed for all scientific procedures and during recovery, to enable the identification of intervention points and humane killing points as soon as possible.

8.6 Training plan and competency assessment

Investigators and animal carers should have completed the online introduction to animal ethics training found on the Student Portal.

Investigators and animal careers must be fully trained and assessed as competent in the process of all scientific procedures in this section, especially those requiring anaesthesia and surgery. They must also be experienced in high and medium frequency monitoring practises.
Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application.
The AWO is available to provide or organise training and to assess competency as required.
Investigators and animal carers must be aware of the OH&S considerations surrounding this species and the first aid procedures required in case of emergencies.
The AEC and AWO will monitor competency during inspections of animal ethics approved projects.

8.7 References and acknowledgements

References

- Campbell, TW, 2015, *Exotic Animal Hematology and Cytology*, Wiley Blackwell, John Wiley and Sons Inc., UK
- Grace, M, Jørgensen, P, Van deurs M, Anthony I, Butts, E, Jørgensen[c], K & Behrens, JW, 2017, PIT-tagging method for small fishes: A case study using sandeel (*Ammodytes tobianus*), *Fisheries Research,*vol. 193, pp. 95-103, https://www.sciencedirect.com/science/article/pii/S0165783617300930
- Queensland Government, 2016, *Microchipping, pit tagging or tracking injured wildlife using collars for release back into the wild,* viewed 24 February 2020, https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/livestock/animal-welfare/animals-science/activities/microchip-tag-release

9. Guidelines for consulting veterinarians managing the medical and surgical requirements of sick and injured fish species

9.1 Summary / Scope

This section relates to administration routes for medications in fish species and common medications useful for treating fish species. It also covers health considerations including non-infectious and infectious forms of ill health, methods of diagnosis and management controls to prevent these illnesses and diseases.

9.2 Background information

Introduction

The health and welfare of animals within scientifically based projects (research or teaching) are governed by the Animal Code in Queensland. The Animal Code requires that all the animal carers (investigators, chief investigators and animal handlers) monitor the animals sufficiently to be able to identify distress, pain and ill-health at all times throughout the life of the project.

This guideline aims to provide information to investigators on:

- Regulations surrounding the administration of medications to fish.
- How to administer medications to fish.
- The types of diseases and illnesses found in fish species (non-infectious and infectious).
- The diagnostic pathways available and how investigators can help the pathologist to make an accurate diagnosis.
- The management controls available to help prevent further outbreaks of a diagnosed illness.

As with any sick animal, there are usually several factors that lead up to an illness occurring. All of these factors need to be assessed when designing the animal's treatment plan, to ensure that the therapeutic drugs prescribed will have the best opportunity to heal the animal.

Some of the more common areas of animal management associated with preventing illness in fish include:

- An effective quarantine protocol to prevent diseases being introduced into a project.
- Effective housing facility cleaning and disinfecting procedures and the use of fallow tanks between fish stocks.

- A designated treatment facility to allow the rapid isolation of fish showing clinical signs of illness during the monitoring process in order to rapidly reduce the spread of disease.
- Removal of dead fish to reduce disease transmission.
- Reducing fish stocking densities to decrease pathogen densities, reduce distress levels and incidence of immunosuppression.
- Manipulating water temperatures to manage some forms of parasite (as long as the temperatures remain within the thermal tolerance zone of the fish).
- Ensuring water quality is at the optimum level, by close monitoring daily.

9.3 Equipment and resources

Administering medications

Measuring jugs, separate fish housing tanks, appropriately sized needles and syringes, nets, medicated feed, monitoring equipment and paperwork, PPE., sharps bins, alcohol swabs.

Diagnosing diseases

Necropsy equipment and paperwork, PPE, monitoring equipment and paperwork, laboratory paperwork and sample containers.

9.4 Recommended procedures

Regulations surrounding the administration of medications to fish

Prior to administering any type of medication to fish showing signs of illness, a thorough assessment of the fish's environment and clinicals signs must have been given to either an aquaculture veterinarian or a veterinary aquaculture pathologist (if fish have died or are humanely killed) to obtain an accurate diagnosis. The unregulated use of medications such as antibiotics can have severe wide-ranging, long-term effects on:

- The success of certain medical conditions if used incorrectly (e.g. antibiotics resistance).
- The welfare associated issues for fish that are not recovering as well as they should due to incorrect diagnosis.
- An investigators scientific data.

The use of any medications including drugs, vaccines and hormones within scientific projects, are generally limited to those previously tested as safe for fish. Routine and extra label use of medications must be regulated by a qualified veterinarian and similarly some medications can only be administered only by a state licensed veterinarian. Some factors that need to be considered by investigators include:

- Strict guidelines are in place for use of many drugs in any fish intended for human consumption.
- The Australian Pesticides and Veterinary Medicines Authority (APVMA) regulates the use of drugs in animals in Australia. They should be the first point of contact for an investigator wishing to trial veterinary drugs for scientific projects.
- Investigators must keep detailed records of any drugs used including times of administration, dosing intervals, dose in milligrams, route of administration and storage.

Appendix 9.12 outlines some of the drugs used to medicate fish showing signs of illness, with their associated dosing rates.
Note: most drugs have only been tested on commercially farmed fish and therefore dosing regimens are arbitrary for many other species. The investigator should follow the directions of the prescribing veterinarian in all cases.

Administration routes for medications in fish species

Methods of medication administration

- Drugs may be administered by many routes: topically (by dip, short-term bath, indefinite bath, direct topical); parenteral (by injection); or orally.
- Administering medication to fish may require handling and will cause distress.
- All medications and how they are administered must be detailed on the AEC application or on an adverse event form.
- Medications must only be administered by veterinarians, trained animal carers.
- Prior to administration of medication, the investigator should ensure that a quiet location is established, and all equipment required (e.g. appropriate gauge needles, alcohol swabs, sharps bin and protective clothing) is available.

Topical treatments (TOP)

- For any topical medication in the form of baths, dips or tank treatments, it is essential to know the volume of water to which the drug is to be added to calculate the exact drug concentration being used. This calculation will help to reduce the chance of drug overdose.
- Doses are often listed as parts per million (ppm), or in the case of salt, parts per thousand (ppt), which are equivalent to mg/L and g/L, respectively.
- Some liquid therapeutants may be provided at different concentrations, requiring an additional step in the calculations.
- Thorough mixing of the drug into the water is essential to ensure the water has a homogenous state, to prevent toxic concentration zones. Ideally, the drug should be pre-mixed into a measured volume of water prior to applying it to the whole tank, to help with dispersal.
- The water used in any of the topical treatments must have the same water parameters as the fish's normal housing tank to prevent distress and shock during the treatment.

Tank treatment

- When treating fish within a tank, aquarium or pond, the activated carbon filtration should be stopped during the treatment phase to ensure the drug is not removed before its uptake period.
- The water quality should be monitored very closely following tank treatment because the normal nitrifying bacteria (biological filters) may also be adversely affected by the drug, leading to higher than normal ammonia and nitrate levels which may cause toxicity.
- Tanks should be well aerated and the fish monitored closely during the treatment.
- Following treatment, the water changes should occur as directed in the charts, and the filtration systems (carbon and biological) turned back on to remove any residual drugs in order to prevent an overdose.
- The replacement of carbon filters is recommended following drug therapy within tanks.

Bath treatment

- When using the bath system, the fish should be removed from their main housing tank and placed in an aquarium with a known volume of water and concentration of the therapeutic drug, rather than the fish biomass.
- The drug is dissolved into the water and treatment lasts from 15 minutes up to 24 hours, normally with at least a 50% water change between treatments.
- The water parameters of the bath must match those of the fish's normal housing tank.

Indefinite bath treatment

- The fish are placed in the bath as above; however, there is no water exchange or chemical filtration to remove the drug level.
- The process relies on the drug's natural breakdown process to remove it from the system.

Dip treatment

- The fish are removed from their normal tank and submerged into a known volume of water and concentration of the therapeutic drug (rather than the fish biomass) as in the bath scenario. However, both the volume of water and the contact period is usually less than a bath, being just a few seconds up to 15 minutes.
- The water parameters of the dip must match those of the fish's normal housing tank.

Topical medication

Although this method would at first appear impractical due to the reduction in contact time within a water environment, it is useful in certain skin and corneal conditions.

- The normal treatment method is to partially hold the fish out of the water ensuring that its gills remain submerged, apply the topical medication and allow a few minutes for contact prior to releasing it back to its tank.
- Commonly products include: 'Silver-sulfadiazine' and Nystatin (Panalog) used in this manner for active skin lesions; and diluted Betadine ointment, which may also be used prophylactically on surgical incisions.
- Human recombinant platelet-derived growth factor, or bercaplamin (Regranex), is now being used to treat head and lateral line erosion (HLLE) disease in marine tropical fish in this manner.

On initial assessment, tank, bath and dip treatments appear to be a more convenient option for treating fish due to the reduced handling and distress, easy administration of the drug and no requirement of the fish to be feeding. However, dosing of expensive drugs into large volumes of water may be cost prohibitive and the effects of the therapeutic drugs on the filtration system (both biological and carbon based) may eventually be more time consuming.

Parenteral injections

Parenteral injections can be administered into either muscular tissue (IM), subcutaneous tissue (SC), the coelomic cavity (ICe) or a vein (IV). This method of medicating fish is useful when dealing with individual fish or small batches of sick fish that have been isolated. It ensures an exact dose of the drug is given and is less wasteful than topical methods. It does, however, require handling of the fish which will cause them distress, is a time-consuming process and requires a certain level of skill to perform the task.

Intramuscular injections (IM)

The most useful site for an intramuscular injection is in the soft spot of muscle directly behind the insertion of the dorsal fin. This potential space between the epaxial muscle bundles will accommodate any potential leakage from the injection site, which may be encountered in other areas of tissue where the muscles are tighter. However, this method can cause scale depigmentation or needle point abscessation that may affect the fish's appearance or cause damage to the tissue in the area.
A further site used for intramuscular injections is the pectoral muscle mass, just caudomedial to the insertion of the pectoral fin on the ventral surface of the body.

Intracoelomic injections (ICe)

These injections are given craniodorsally into the ventrum and the fish must be sedated/anaesthetised for this procedure. The drug has a rapid uptake systemically.

Intravascular injections (IV)

These injections are normally administered into either:

- the caudal vein (Note: this injection may end up as perivascular due to this blind technique)

- the dorsal aorta at the roof of the mouth
- the sinus venosus accessed through the translucent membrane which is caudal to the gill chamber.

The fish must be sedated/anaesthetised for this injection.

Oral medication

It is important to ensure the correct dosing for oral medications because some doses are provided based on the weight of the fish and others on the weight of the feed. Therefore, the daily feeding regime/feed intake must be considered to work out the correct dosing (which is usually in the region of 1-8% depending on growth stage, metabolic activity and temperature).

Gel food feeding regimes can be useful for this type of drug administration and recipes for various species are available, as are commercially produced products. The difficulty with oral dosing of drugs is that sick fish will often be anorexic and the drug infused food can, in itself, cause inappetence; therefore, ensuring the correct dose for each fish can be difficult.

Regardless of the drug administration method chosen, it is important to consider that the toxicity and pharmacokinetics of many drugs used to treat fish are unknown. Therefore, best practise suggests the isolation of sick fish, and the treatment of smaller numbers of fish initially with the chosen drug, before treating a whole tank.

Reference

- Department of agriculture, water and the environment, 2014, Australia's National Strategic Plan for Aquatic Animal Health, viewed 20 November 2019, https://www.agriculture.gov.au/animal/aquatic/aquaplan

Recognising illness in fish

Many diseases in fish present with very similar clinical signs and therefore it is not ethically acceptable for a guess to be made about what the underlying cause may be and to treat it on the off chance that the diagnosis is correct.

An incorrect diagnosis and corresponding treatment plan will unnecessarily extend the period of illness causing prolonged welfare concerns and may cause the unwanted build-up of antibiotic resistant organisms.

There are ten charts (Appendix 9.13-9.22) attached to this section which explain the various causes of illness and highlight the need for a thorough diagnostic pathway:

- Disease recognition in finfish.

- Quick reference guide to common clinical signs and possible causes.
- Non-infectious disorders of finfish.
- Heavy metal contamination and therapeutic and agricultural chemical contamination charts.
- Pesticides, herbicides, algicides, fungicides, de-mosser products and organochlorine pesticides.
- Fertilisers, detergents and petrochemical charts.
- List of antimetabolites causing malnutrition.
- List of minerals, use in the fish's body and signs of deficiency.
- List of chemical carcinogens, sources and typical clinical signs.
- Infectious disorders of finfish.

Diagnostic approaches

An investigator should always liaise with an aquatic veterinarian/veterinary pathologist in any illness outbreak or incidence of unknown death, and they will guide the investigator to the best diagnostic pathway to take. This will not only alleviate suffering in the fish, but it will ensure as many fish are treated and saved as possible to maintain the quality of the researcher's data. It will also save the investigator time and research funds if the diagnostic path can be more focused.
To ensure the best chance of an accurate diagnosis, the investigator should:

- Take objective notes as part of the daily AEC approved monitoring plan regarding fish behaviour, appearance, changes in husbandry, housing or recent scientific procedures.
- Take water parameter readings (as part of the overall monitoring process).
- Undertake a thorough and prompt necropsy for pathology sampling.

All of this information along with any animal tissue samples and water samples should be sent to the aquatic veterinary pathology without delay.
The chart in appendix 9.23 will help to guide the investigator as to what may be asked of them for diagnostic purposes in each case of infectious disease.

Management and control options

Housing and husbandry within fish projects can have severe negative impacts on fish health if not monitored and managed correctly. It not only causes a direct effect on fish welfare and health through factors such as lack of quarantine, rough handling, overstocking and poor water quality, but it can also indirectly affect fish by causing distress leading to a lowered immune system and increasing the chance of infectious organisms affecting the fish stock.
Appendix 9.24 outlines some of the management controls available to prevent the infectious diseases listed above and how an investigator can help to prevent a disease outbreak in the future, particularly if a current adverse event has been accurately diagnosed.

9.5 Animal health and welfare considerations

If fish illness is not diagnosed correctly and promptly, it can lead to significant distress, pain and mortality.
Regular, efficient and objective monitoring of fish health and water parameters can rapidly alert the investigator to a problem allowing them to isolate sick fish and reduce the spread of the illness to other fish.

The efficient and thorough collection of samples (fish tissues and water samples) with a complete monitoring history, will help the aquatic veterinarian/veterinary pathologist make an accurate diagnosis, recommend a treatment plan and reduce the rate of both morbidity and mortality.

9.6 Training plan and competency assessment

Investigators and animal carers should have completed the online introduction to animal ethics training found on the Student Portal.

Investigators and animal carers must be fully trained and assessed as competent in the process of administering medications to fish before undertaking related procedures.
Decisions regarding who is authorised to provide training and assess competency should be clearly outlined in the animal ethics application.
The AWO is available to provide or organise training and to assess competency as required.
Investigators and animal carers must be aware of the OH&S and risk considerations surrounding the fish species they plan to use in their project and potential first aid procedures required in case of emergencies.
The AEC and AWO will monitor competency during inspections of animal ethics approved projects.

9.7 References and acknowledgements

References

- Carpenter, JW, 2005, *Exotic Animal Formulary*, 3rd edn, Saunders Elsevier, Missouri
- Gill H, Ashton, C & Rowland, A, 2000, *Working towards the development of best practices in fish and fisheries research or the troubles with fish and fish biologists*! viewed 12 September 2019, https://anzccart.org.nz/app/uploads/2017/06/gill-ashton-workin.pdf
- Johnston, C & Jungalwalla, P, *Guidelines on welfare of fish and crustaceans in aquaculture and/or in live holding systems for human consumption*, F.a.F. Aquatic Animal Health Unit of the Department of Agriculture, Editor, National Aquatic Council of Australia
- Penney, A, Bromhead, D, Begg, G, Stobutzki, I, Little, R & Saunders, T, 2016, *Development of guidelines for quality assurance of Australian fisheries research and science information,* viewed 15 October 2019, file:///G:/Office%20of%20Research/Research-Ethics/AWO/Specific%20animal%20guidelines/Fish/Development%20of%20guidelines%20for%20quality%20assurance%20of%20Australian%20fisheries%20research%20and%20science%20information.pdf

- Tolla, LD, Srinivas, S, Whitaker, BR, Andrews, C, Hecker, B, Kane, AS & Reimschuessel, R, 1995, Guidelines for the Care and Use of Fish in Research, *Institute for Laboratory Animal Research,* vol. 37, no. 4, https://doi.org/10.1093/ilar.37.4.159

9.8 Other information and attachments

Appendix 9.9: Common medications useful for treating fish species
Appendix 9.10: Disease recognition in finfish
Appendix 9.11: Quick reference guide to common clinical signs and possible causes
Appendix 9.12: Non-infectious disorders of finfish
Appendix 9.13: Heavy metal contamination and therapeutic and agricultural chemical contamination charts
Appendix 9.14: Pesticides, herbicides, algicides, fungicides, de-mosser products and organochlorine pesticides
Appendix 9.15: Fertilisers, detergents and petrochemical charts
Appendix 9.16: List of antimetabolites causing malnutrition
Appendix 9.17: List of minerals, use in the fish's body and signs of deficiency
Appendix 9.18: List of Chemical carcinogens, sources and typical clinical signs
Appendix 9.19: Infectious disorders of finfish
Appendix 9.20: Diseases and their common diagnostic pathways
Appendix 9.21: Diseases and their management/control options

Appendix 9.9: Common medications useful for treating fish species

Drug name	Dose	Frequency (q = every)	Route	Notes
Antibiotics				
Amikacin	5mg/kg loading dose then 2.5mg/kg q72hrs x 5 treatments. 5mg/kg 5mg/kg	q24-48hrs q72h x 3 treatments q24hr x 3 then q48hrs x 2 treatments	SC, IM, Ice IM ICe	 In Koi carp
Amoxicillin	25mg/kg 40-80mg/kg feed /day	q12hrs 10 days	PO PO	For treating furunculosis, Pasteurellosis, Edwardsiellosis and Streptococcosis. Easily degraded by light, heat & heavy metals.
Ampicillin	10mg/kg 50-80mg/kg feed/day	q24hrs 10 days	IM PO	For treating furunculosis, Pasteurellosis, Edwardsiellosis and Streptococcosis. Easily degraded by light, heat & heavy metals.
Aztreonam	100mg/kg	q48hrs x 7 treatments	IM, Ice	For Aeromonas salmonicida infections. Common in koi carp.
Benalkonium chloride	0.5mg/L 10mg/L for 10 mins	Long term		Quaternary amine with broad disinfection properties.
Ceftazidine	20-22mg/kg	q72-96hrs x 3-5 treatments	IM, Ice, SC	Cephalosporin with good activity against gram -ve bacteria (e.g. Pseudomonas) due to its long half- life.
Chloramine-T	2.5-20mg/L		TOP Flush treatment	Disinfectant used to control bacterial gill disease and some ectoparasites. Wide dosage duration range across species and water quality.
Chloramphenicol	50mg/kg loading then 25mg/kg ongoing 20-40mg/kg 20-50mg/kg 20mg/L bath	q12-24hrs initial then q24hrs q48hrs x 7 treatments q7 days x 2 treatments Change daily	SC, IM, ICe IM, ICe ICe TOP Bath	Florfenicol may be a better option for fish grown for human consumption. For A. salmonicida in goldfish.
Enrofloxacin (Baytril)	5-10mg/kg 1ml/L 10mg/kg 19mg/kg 2.5-5.0mg/L	q24-48hrs for 7-21 days q24hrs q5days q24hrs q24hrs x 5-7 days	PO, SC, IM, ICe TOP Bath 15 mins ICe PO in feed TOP Bath for 5 hrs	May be diluted with sterile saline since the concentrated form (2.27%) may be irritating. For koi at 20°C Atlantic salmon Change 50-75% of water between treatments.

	0.1% feed	10-14 days	PO, In feed	Oral or injectable form can be given orally.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antibiotics				
Erythromycin	50-100mg/kg 100mg/kg 100-200mg/kg	q24hrsx 10 days q24hrs x7-21 days q24hrs x 21 days	PO PO PO	Commonly sold as a tank treatment for aquarium fish but it is toxic to nitrifying bacteria, so it is not recommended for this use. For treating bacterial kidney disease, Streptococcosis, Mycobacteriosis, Chlamydiosis and Piscirickettiosis. To control Renibacterium salmoninarum in salmonid species.
Florfenicol (Nuflor)	5-20mg/kg 40-50mg/kg	q24hrs q12-24hrs	PO PO, IM, ICe	Atlantic salmon Red pacu sp.
Flumequine	50-100mg/L 10mg/kg 10mg/kg 30mg/kg	 q24hrs x 10 days q48hrs	TOP Bath 3 hrs PO, In feed IM, ICe	Quinolone for gram -ve bacteria. Freshwater fish use at pH 6.8-7.2. There is a decreased uptake in hardwater. Increase the dose for marine fish. Cod, wrasse sp. High antibiotic levels persist for several days when given IM
Gentamicin	2.5mg/kg	q72hrs	IM	Nephrotoxic issues in species without determined dose rates.
Iodine potentiated (Betadine)	20-100mg/L		TOP Bath for 10 minutes Topical directly onto wounds	For disinfecting eggs Rinse immediately. Do not use solutions combined with detergent such as betadine scrub.
Kanamycin sulphate (Kantrex)	50-100mg/L 50mg/kg 20mg/kg	q72hrs x 3 treatments q24hrs q72hrs x 5 treatments	TOP Bath 5 hrs PO, In feed ICe	Change 50-75% of water between treatments. Absorbed from the water. Toxic to some fish.
Methylene blue	2mg/L tank water	q48hrs up to 3 treatments		Prevents infection of freshwater eggs. Toxic to nitrifying bacteria. Will stain equipment in the tank. Toxic to plants.
Nalidixic acid	5mg/kg 5mg/kg 20mg/kg 13mg/L	q24hrs q24hrs q24hrs Repeat as required	PO, IM PO, IV PO TOP Bath 1-4hrs	For quinolone gram -ve bacteria such as furunculosis, aeromoniasis, columnaris, vibriosis, bacterial kidney disease. Rainbow trout Note: drug less useful in marine fish species and drug resistance is an issue.
Neomycin	66mg/L tank water	q3days up to 3 treatments		Commonly sold as a tank treatment for aquarium fish. Toxic to nitrifying bacteria. Keep fish densities low.
Nifurpirinol	0.1mg/L tank water 0.45-0.9mg/kg	q24hrs for 3-5 days q24hrs for 5 days	PO TOP Bath 5mins – 6 hrs	CAUTION nitrofuran is carcinogenic. It is toxic to scaleless fish. Absorbed from the water. Drug is inactivated in bright light.

	1-2mg/L 4-10mg/kg	 q12hrs x 5 days	 PO, in feed	
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antibiotics				
Nitrofurazone	2-5mg/L tank water 100mg/L 20 mg/L	q24hrs for 5-10 days Q24hrs for 5-7 days	 TOP Bath 30 mins TOP Bath 5 hrs	CAUTION nitrofuran is carcinogenic. It is toxic to scaleless fish. Absorbed from the water. Drug is inactivated in bright light. Water soluble formulations are preferred. Change 50-75% of water between treatments
Oxolinic acid	25mg/L 1mg/L tank water 5-25mg/kg 10mg/kg 25-50mg/kg	q12hrs x 3 days q24hr q24hrs q24hrs x 10 days q24hrs	TOP Bath 15 mins PO PO, in feed PO	Quinolone for gram -ve bacteria. Has decreased uptake in hard water, but better uptake at pH <6.9. Up to 30mg/kg/day in seawater Freshwater species Marine species
Oxytetracycline	10-50mg/kg 10-100mg/L tank water 20-50mg/L 7mg/g feed 55-83mg/kg/day 20mg/kg 50mg/kg 70mg/kg 25-50mg/kg 10mg/kg 25mg/kg 7mg/kg 3mg/kg	Once off q24hrs x 5 treatments q24hrs for 10 days 10 days q8hrs q24hrs for 10 days q24hrs for 10-14 days q24hrs q24hrs for 5-7 days q24hrs q24hrs	TOP Bath 1 hr TOP Bath 24 hrs PO in feed PO in feed PO PO PO IM, ICe IM IM, ICe IM IV	Note: surface bacterial infections (E.g. Columnaris disease in freshwater fish, vibriosis and streptococcosis). Note: yellow brown foam may develop in the treatment water. Higher doses are needed in hard water. If fish remain sick, re-treat them on day 3 after a 50% water change. The drug is light sensitive so keep the tank covered to prevent photo-inactivation. The drug turns brown when decomposing and 50% of water should be changed immediately. Change 50-75% of water between treatments. Note: Bacterial resistance is common due to overuse. Produces high levels for several days when given IM Red pacu sp. Red pacu sp. Some salmonids. Carp sp.

	20mg/kg 60mg/kg 75mg/kg in feed	 Once weekly q24hrs for 10 days	ICe IM PO in feed	
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antibiotics				
Potassium permanganate (KMnO)	2mg/L 5mg/L 1000mg/L		TOP Bath indefinite TOP Bath 30-60 mins TOP Bath 10-40 seconds	Heavily organic system may require a higher dose. First test efficacy by adding appropriate amount of (KMnO) to a small amount of system water (without the fish). The red colour should remain for at least 4 hrs. Retest until the (KMnO) remains for 4 hrs and then use as treatment. For freshwater fish/skin and gill bacterial infections. Can be toxic in water with a high pH. Do not mix this with formalin. Can be toxic to goldfish.
Sarafloxacin (Saraflox)	10-14 mg/kg 10mg/kg	q24hrs x 10 days q24hrs	PO PO	Fluoroquinolone drug For marine Atlantic salmon
Silver sulfadiazine cream	Cream	q12hrs	TOP	For external bacterial infections. Keep the lesion out of the water for 30-60 seconds post application, but keep gills submerged.
Sulfadimethoxine ormetoprim	50mg/kg/day in feed	For 5 days	PO in feed	Used as powder to add to feed and available as a medicated feed. For treating furunculosis, edwardsiellosis, aeromoniasis and yersiniosis. Process: place brine shrimp (larvae) in 3mg/L seawater for 4 hrs, then rinse them in seawater with a brine shrimp net. Feed the shrimp larvae immediately to the fish to be treated. Works with adult shrimps and other live food.
Trimethoprim & sulfadiazine	30 mg/kg 20 mg/L	q24hrs for 7 to 10 days q24hrs	IM, PO, or Ice TOP Bath for 5 hrs	A good broad-spectrum antibiotic. For treating furunculosis, edwardsiellosis, aeromoniasis and yersiniosis.
Trimethoprim & sulfamethoxazole	20mg/L 30mg/kg 0.2% feed	q24hrs x 5-7 days q24hrs for 10-14 days 10-14 days	TOP Bath 5-12hrs PO PO in feed	Change 50-75% of water between treatments.
Triple antibiotic ointment (polymyxin B sulphate/bacitracin/neomycin sulphate)	Ointment	q12hrs	TOP directly onto wounds	For external bacterial infections. Keep the lesion out of the water for 30-60 seconds post application, but keep gills submerged.

Drug name	Dose	Frequency (q = every)	Route	Notes
Antiparasitic				
Acetic acid	2ml/L		TOP Bath 30-45 seconds	For treating monogenean trematodes, crustacean ectoparasites. It is safe for goldfish but may be toxic to smaller tropical fish.
Chloramine -T	2.5-20mg/L		TOP Flush treatment	Disinfectant used to control bacterial gill disease and some ectoparasites. Wide dosage duration range across species and water quality.
Chloroquine diphosphate	10mg/L in tank water	Once, then as needed.	TOP Tank treatment.	For treating Amyloodinium ocellatum. Monitor for 21 days. Use carbon to remove drug if no relapse.
Closantel (50mg/ml)/mebendazole (75mg/ml)	1ml/400L	Once off but can be repeated if needed in 3-7 days.	TOP Tank treatment. Change water between treatments.	Safe and effective. Useful for external monogeneans in koi. Toxic to goldfish and medaka.
Copper sulphate				For treating marine fish with ectoparasitic protozoan and monogenean trematodes flukes and snail control. Must assess copper levels with commercial kit and adjust regularly as needed. Copper can be toxic to gill tissue and cause immunosuppression. Very toxic to invertebrates and plants. Copper is removed with carbon filtration.
	100mg/L		TOP Bath 1-5 minutes	• Prepare stock solution of 1mg/ml (1g $CuSO_4$ 5 H_2O in 250ml distilled water).
	0.1-0.2mg/L	Maintain in tank water until therapeutic effect.	TOP Tank treatment	• Use higher dose in hard water.
	Maintain free ion levels at 0.15-0.2mg/L of tank water.		TOP Tank treatment	

	0.2mg/L tank water Maintain free ion levels at 0.25-1mg/L.	14-21 days	TOP Bath for 24 -48 hrs	• Citrated copper sulphate. Prepare stock solution of 1mg/ml. (3g $CuSO_4$ 5 H_2O add 2g citric acid monohydrate in 750ml distilled water).
Diflubenzuron	0.01 mg/L	Once weekly x 3 treatments	TOP Bath for 48 hours	Very effective but may kill desirable environmental invertebrates. For treating crustacean ectoparasites by inhibiting chitin synthesis. Drug persists in water long term. Must have an EPA license to administer.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antiparasitic				
Dimetridazole	28mg/kg	q24hrs x 10 days	PO, in feed	For treating Ichthyophthirius multifiliis in Rainbow trout.
Fenbendazole type of benzimidazole	2mg/L tank water 0.2% or (200 mg/100 grams food) 2.5mg/g feed 40mg/kg 50mg/kg Medicated brine shrimp	q7days for 3 treatments For 3 days and repeat in 14-21 days For 2-3 days, repeat in 14 days q4 days for 2 treatments q24hrs for 2 days then repeat in 14 days q24hrs for 2 days and then repeat in 14 days	TOP Tank treatment PO in feed or PO in a gel food PO in feed PO, in feed PO PO Place live brine shrimp in 400mg fenbendazole per 100ml water for 15-20 minutes immediately before feeding to fish.	For non-encysted gastrointestinal nematodes, cestodes & monogenean trematodes (such as skin/gill flukes). For treating Bothriocephalus acheilognathi in carp. A good parasiticide for intestinal nematodes.
Formalin **All doses based on volumes of 100% formalin (=37% formaldehyde).** **A conventional dose which is commonly used is 1.0 mL of**	15–25 mg/L (0.015-0.025ml/L tank water. 0.125-0.25ml/L 0.4ml/L 0.5ml/L	q48hrs for 3 treatments q24hrs for 2-3 days q3days for 3 treatments q3days for 3 treatments	TOP Bath 12 to 24 hours followed by a 50% water change on alternative days. TOP Dip 60 minutes TOP Bath 1 hour TOP Bath 1 hour	Effective for some ectoparasites in koi (Use 0.025ml/L dose for treating Ich) When using maximum dose treat q3days. For soft water tanks. For hard water tanks.

100% formalin (37% formaldehyde)/10 gallons (38 L) of water. **Toxic to plants.**				For treatment of: cryptocaryoniasis, chilodonellosis, brooklynellosis, costiasis, trichodiniasis, oodiniasis, amyloodniosis, tetrahymenosis, cryptobiosis, amoebiasis, ectocommensal cilitaes, skin/gill flukes & saprolegniasis. Can be highly toxic & carcinogenic (if white precipitates of paraformaldehyde are present). Some fish are very sensitive, so test small batch first, monitor fish for distress, piping and pallor. Soft, acid water at high temps increases toxicity level. Treat with vigorous aeration. Always change water between treatments. Ichthyophthirius cystic forms & Cryptocaryon sp. require higher doses and multiple treatments.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antiparasitic				
Formalin (F)/malachite green (M)	(F) 0.025ml/L + (M)0.1mg/L tank water	q48hrs for 3 treatments	TOP Tank treatment	Good for Ichthyophthirius. Change 50% of tank water on alternative days. Commercial products available.
Freshwater		q7days as needed	TOP Bath 3-15 minutes TOP Bath 4-5 minutes	For treating marine fish ectoparasites. Ensure water is well aerated and monitor fish closely. Some small fish are very sensitive.
Fumagillin	5-10mg/kg fish/day	q24hrs up to 3-6 weeks		For treating microsporidial and myxosporean parasites and proliferative kidney disease. Controls the spore forming protozoa but cannot eliminate the infection. Can be toxic at higher or prolonged dosing levels. Narrower safety margin in salmonid sp.
Hydrogen peroxide	1.0-1.5mg/L 17.5ml/L	Once off	TOP Bath 20 minutes TOP Bath 4-10 minutes	For treating: Atlantic salmon, sea lice, external bacteria, protozoa and egg fungal infections, acute environmental hypoxia. Toxic to operator. Corrosive. Toxic to fish in water >14oC. General ectoparasite dose. Monitor fish closely. May be harmful to smaller fish.
Ivermectin	DO NOT USE. CAUSES NEUROLOGICAL SIGNS AND DEATH AT NORMAL THERAPEUTIC DOSES. TOXIC TO MANY ENVIRONMENTAL INVERTEBRATES.			
Levamisole	0.5mg/kg 1-2mg/L 10mg/kg	q7days for 3 treatments	ICe TOP Bath 24 hours PO	Can cause immunostimulation in Rainbow trout. For treating internal nematodes especially larval stages.

	11mg/kg 50mg/L 4g/kg feed	q7days for 2 treatments q7days for 3 treatments	IM TOP Bath 2 hours PO in feed	For treating external trematodes.
Luferuron	0.13mg/L	As required		For treating crustacean parasites
Malachite green **Prepare a stock solution of 3.7mg/ml (1.4g malachite green in 380ml water).**	100mg/L 0.1mg/L tank water 5-60mg/L 1mg/L	 q3days for 3 treatments	TOP TOP Tank treatment TOP Bath 10-30 seconds TOP Bath 30-60 minutes	Caution: mutagenic, teratogenic, toxic to some fish species and fry. Toxic levels increase in acidic high temp water. Toxic to plants. Stains objects such as plastic. Residual chemical is removed by activated carbon after the last tank treatment. Use zinc free formulations. For treating skin lesions on freshwater fish with protozoan ectoparasites.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antiparasitic				
Mebendazole	1mg/L 1mg/L 20mg/kg 100mg/L	 Q7days for 3 treatments	TOP Bath 24 hours TOP Bath for 72 hours PO TOP Bath 10 minutes to 2 hours	For treating monogenean trematodes. For treating branchial monogeneans such as Pseudodactylogyrus bini and P. anguillae sp. For treating gastrointestinal nematodes. Do not administer to brood fish as it is embryotoxic and teratogenic. For treating monogenean trematodes
Methylene blue	1-3mg/L tank water		TOP Tank treatment	Not effective for freshwater fish with ectoparasites. Toxic to nitrifying bacteria. Stains objects. Toxic to plants.
Metronidazole or dimetridazole (DMZ) both nitroimidazoles	5mg/L tank water 25mg/L tank water 25mg/kg feed 100mg/kg feed 6.25-18mg/g feed 50mg/kg	q24hrs for 3 days q48hrs for 3 treatments q24hrs for 5-10 days q24hrs q24hrs for 5 days q24hrs for 5 days	TOP Tank treatment TOP Tank treatment PO, in feed PO, in feed PO, in feed PO, in feed	For treating Spironucleus, (Hexamita) and other internal flagellates, some external flagellates including hole in the head disease. It is poorly soluble in water so dissolve before adding to water or feed. Change the water between tanks treatments. Not for food production fish. Equivalent to 0.25% in feed (250mg/100g food) at 1% Bwt per day. For treating Ich. Equivalent to 1% in feed (1g/100g food) at 1% Bwt per day. 5mg/L for treating Hexamita and 10mg/L for oodinium.

	5-10 mg/L Medicated brine shrimp	q24hrs for 3 consecutive days with 25–50% For 5 days	TOP Bath PO	 Place live brine shrimp in 625 mg metronidazole per 100ml water for 15-20 minutes immediately before feeding to fish.
Piperazine	10mg/kg in feed	q24hrs for 3 days	PO, in feed	For treating non-encysted gastrointestinal nematodes. Equivalent to 0.1% in feed at 1% Bwt per day.
Potassium permanganate	5mg/L 100mg/L 1g/L		TOP Bath 30-60 minutes TOP Bath 5-10 minutes TOP Bath 10-40 seconds	For treating freshwater fish protozoans and crustacean ectoparasites. Can be toxic in acidic water. Never mix with formalin. Toxic to goldfish.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Antiparasitic				
Praziquantel	5-10mg/L 2-10mg/L 5mg/kg 5-12mg/kg feed 5—8 mg/kg 50mg/kg	Repeat in 7days Q7days for 3 treatments q24hrs for 3 days q24hrs repeat dose in 14-21 days Once off	TOP Bath 3-6 hours TOP Bath 2-4 hrs PO IM, ICe, or PO, in feed PO	For treating monogenean trematode ectoparasites, cestodes, eye flukes, skin/gill flukes. Always aerate the water during treatment. Marine fish are more sensitive. Toxic to Corydoras sp. Toxic in water with high pH. Effective at detoxifying hydrogen sulphide. Handle with care and use PPE. For metacercaria sp. Monitor closely for lethargy, incoordination and loss of equilibrium. For treating cestodes, some internal digenean trematodes. Given to fish via oral gavage to treat adult cestodes or as 0.5% of feed at 1% Bwt per day.
Pyrantel pamoate	10mg/kg in feed	Once off	PO in feed	For treating gastric nematodes
Sodium chloride **Use sea water (conc. 30-35g/L) or artificial sea salts. If not use**				For treating freshwater fish ectoparasites: Ich, cryptocaryoniasis, chilodonellosis, brooklynellosis, costiasis, trichodiniasis, oodiniasis, amyloodniosis, skin/gill/eye flukes, anchor worms, leeches, fish lice, isopods, turbellarians, uronemiasis,

non-iodised table/rock salts (some anticaking agents in solar salts are toxic to fish).	1-5 g/L tank water 3g/L 10-30g/L 30g/L 30-35g/L	As needed q24hrs	TOP Indefinite bath. TOP Bath 30 minutes TOP Bath 10 minutes TOP Bath 4-5 minutes	tetrahymenosis, cryptobiosis, amoebiasis, ectocommensal cilitaes, hexamitosis. Endo parasites: coccidiosis, digenean trematodes, nematodes, cestodes, myxozoans, microsporidians. Saprolegniasis, epizootic ulcerative syndrome. Catfish are sensitive to seawater treatment. Toxic to plants at concentrations over 0.5 grams/L. generally safe and cheap. (2 grams/L will usually kill circulating protozoal parasites). Use as a prophylaxis or as a treatment for ectoparasites. Safe for most species of ornamental fishes at this dose. Use for supportive care or with salt sensitive fish or weak fish. Use in fish greater than 100g only Goldfish and koi will tolerate these concentrations used as a general tonic for stress, and control of external parasites.
Thiabendazole	10-25mg/kg in feed 66mg/kg	Repeat in 10 days Once off	PO, in feed PO	For treating gastric nematodes. Note: anorexia may be seen at higher doses lasting from 2-4 days.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Anti-parasitic				
Trichlorfon (dimethylphosphonate)	0.5 mg/L tank water 0.5mg/L 0.25mg/L 0.25mg/L 0.5-1mg/L tank water 0.5-1mg/L tank water 1mg/L tank water	q10days for 3 treatments q3days for 2 treatments q7days for 4 treatments Once off q3days for 2 treatments Once off q48hrs for 3 treatments	TOP Tank treatment with water changes of 20-30% 24-48 hours following each treatment. TOP Tank treatment TOP Tank treatment TOP Tank treatment TOP Tank treatment TOP Tank treatment TOP Tank treatment	This is an organophosphate so is effective in controlling crustacean ectoparasites: copepods, Argulus, sea lice & isopods, and skin/gill flukes, leeches & anchor worms. Ensure water is well aerated. A BIOTEST IS STRONGLY RECOMMENED WHEN USING THESE COMPOUNDS. It is very toxic to larval fish and tetras. Liquid form is marketed for cattle and is convenient to dispense. OH&S factors include avoiding inhalation and skin contact. Use for freshwater fish in water >27oC for Dactylogyrus sp. and other oviparous monogenean sp. For treating anchor worms For treating copepods, other monogeneans sp. Argulus and leeches For treating marine fish for oviparous monogeneans. For treating copepods (except sea lice), other monogenean sp. Argulus and leeches. For treating marine fish for turbellarians.

Anti-fungal				
Bronopol	15-50mg/L	As needed	TOP Bath 30-60 minutes	For mycotic infections of fish and fish eggs. Note: eggs may require a higher dose.
Formalin/formaldehyde	0.23ml/L 1ml/38L 1-2ml/L	 Repeat as required.	TOP Bath up to 60 minutes TOP Bath 12-24 hour followed by 30-70% water change. TOP Bath 15 minutes (for eggs only)	For treatment of mycotic infections on eggs. All doses based on volumes of 100% formalin (=37% formaldehyde). Do not use to treat eggs within 24 hours of hatching. Can be highly toxic especially if white precipitates of paraformaldehyde are present. Can be carcinogenic. Test on a small number of fish initially, as it can be toxic to some species. Monitor fish for distress and signs such as piping and pale colour. Soft, acid water at high temps increases toxicity level. Toxic to plants. Treat with vigorous aeration. Always change water between treatments.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Anti-fungal				
Furazolidone	1-10mg/L tank water 25-35mg/kg in feed 50-100mg/kg in feed	 q24hrs for 20 days q24hrsfor 10-15 days	TOP Tank treatment for 24 hrs PO, in feed	This is a nitrofuran so can be carcinogenic. It is toxic to scaleless fish. Drug is absorbed from the water and is inactivated by bright light. For treating salmonids. Not to be used in fish for human consumption.
Itraconazole (Sporonox)	1-5mg/kg in feed	q24hrs for 1- 7 days	PO, in feed	For treating systemic mycoses
Kanamycin sulphate	50-100mg/L 50mg/kg in feed 20mg/kg	q72hrs for 3 treatments q3days for 5 treatments	TOP Bath 5 hrs	Change 50-75% of water between treatments. Absorbed from the water. Toxic to some fish.
Ketaconazole	2.5-10mg/kg		PO, IM, ICe	For treating systemic mycoses
Malachite green (zinc free)				For treating mycotic infections in freshwater fish. Toxic to plants.

	0.1mg/L tank water 0.25mg/L 0.5mg/L 1mg/L 100mg/L tank water	q3days for 3 treatments q24hrs	 TOP Bath 15 minutes TOP Bath 1 hr TOP Bath 30-60 minutes TOP to skin lesions	Caution: mutagenic, teratogenic, toxic to some fish species and fry. Toxic levels increase in acidic high temp water. Stains objects such as plastic. Wear PPE. Not for use in fish used for human consumption. Residual chemical should be removed by activated carbon after the last tank treatment. For fungal control in fish eggs For fungal control in freshwater fish eggs Better to use 2mg/L if pH is high.
Methylene blue				Prevents infections of freshwater eggs. Toxic to nitrifying bacteria. Stains many objects. Toxic to plants.
Miconazole	10-20mg/kg		PO, IM, ICe	For treating systemic mycoses
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Anaesthesia/restraint/analgesia				
Aqui-S (compound mixture of eugenol and polysorbate 80) (Can take 5-8 minutes in well rested fish until they start to show signs of loss of equilibrium & uncoordinated swimming). Agitated fish will show a faster response time).	10-25mg/L 50-100mg/L 150mg/L	As required for light sedation. For heavy sedation/anaesthesia For humane killing	TOP Bath to effect. TOP Bath to effect. (Gill ventilation slows down).	Sedation of freshwater or marine fish. Useful for harvest, transportation and humane killing. Most husbandry procedures can be carried out after 12-15 minutes later. Ensure the tank water is well aerated for all procedures using this product. This product has a wide safety margin and can be used for extended procedures. In case of overdose, remove the fish from the bath and irrigate gills with clean well aerated water. For general anaesthesia of freshwater and marine fish. Dilute waste anaesthesia solution prior to disposal. Can also be used as a humane killing method at conc. The fish should be narcotised at 40mg/L prior to being placed in the stronger concentration.
Atipamezole (Antisedan)	0.2mg/kg		IM	Reversal agent for medetomidine

Benzocaine	10-40mg/L 50-500mg/L 1g/L spray	As required As required As required	TOP Bath to effect TOP Bath to effect	Do not use products marketed for mammals. Prepare the stock solution in ethanol and store in a dark bottle at room temperature. For transport sedation For anaesthesia (humane killing in prolonged bath) Spray product onto gill of large fish species as an anaesthesia.
Clove oil (eugenol)	40-120mg/L	As required	TOP Bath to effect	Make a stock solution (100mg/ml of eugenol by diluting 1 part clove oil with 9 parts 95% ethanol). There is usually a long recovery time for the fish following anaesthesia. Most fish are anaesthetised with dose of 25-50mg/L.
Etomidate	1-4mg/L	As required		Anaesthesia. Use lower doses in some fish species such as bass.
Ketamine	66-88mg/kg	As required	IM	Immobilisation for short procedures. Complete recovery can take >1 hr.
Ketamine (K) / medetomidine (M)	(k) 1-2mg/kg + (M) 0.05-0.10mg/kg	As required	IM	For immobilisation. Reverse the medetomidine with atipamezole at 0.2mg/kg IM.
Lidocaine	Total max dose 1-2mg/kg	As required	SC	Local anaesthesia. Use cautiously in small fish. Inject SC into surgical areas in a fanning pattern.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Anaesthesia/restraint/analgesia				
Metomidate **Make a stock solution of 10g/L and store in a dark container.**	0.06-2.0mg/L water 0.5-1.0mg/L water 2.5-5.0mg/L 5-10mg/L 2.5-5mg/L 0.2-0.3mg/L 1-10mg/L 0.1-1.0mg/L	As required	TOP Bath TOP Bath TOP Bath TOP Bath TOP Bath TOP Bath TOP Bath TOP Bath	Fish may turn dark coloured temporarily. Gouramis sp. are sensitive to this drug. Do not use in cichlids in water of pH<5. For transportation sedation. For light sedation. For heavy sedation. Anaesthesia. Note some species may require 10-30mg/L. For marine fish anaesthesia- induction. For marine fish anaesthesia maintenance. For freshwater fish anaesthesia- induction. For freshwater fish anaesthesia maintenance.
Pentobarbital	60mg/kg	As required	ICe	For humane killing.
Phenoxyethanol	0.1-0.5ml/L 0.6ml/L	As required	TOP Bath TOP Bath	Anaesthesia of fish. For surgery on Carp sp.

Quinaldine sulphate **Make a stock solution 10g/L and buffer the acidity by adding sodium bicarbonate to saturation. Store in dark container in fringe or freezer.**	 50-100mg/L 15-60mg/L 25mg/L	As required	 TOP Bath TOP Bath TOP Bath	Aerate the water during anaesthesia to prevent hypoxia. Drug is excreted unchanged from the body. Anaesthesia induction Anaesthesia maintenance For anaesthesia of catfish, salmon. Do not use for bass sp. Do not use for long procedures. Note: Can be used for humane killing by keeping fish in solution for >10 minutes after respiration ceases.
Tricaine methanesulfonate (MS-222) **Make a stock solution of 10g/l and buffer the acidity with sodium bicarbonate at 10g/L or to saturation. Store in dark container and keep in fridge or freezer. Discard solutions developing an oily film.**	 10-40ppm 50-100mg/L 50-60mg/L 100-200mg/L 50-100mg/L 15-50mg/L 1g/L spray 8-30mg/L	As required	 TOP bath TOP Bath TOP Bath TOP Bath TOP Bath TOP Spray water TOP Spray water TOP Bath	Sedation to anaesthesia. Aerate water during procedure to prevent hypoxaemia. Safety margin narrower in younger fish, soft and warm water. Sedation Anaesthesia induction Anaesthesia maintenance Anaesthesia induction Anaesthesia maintenance For sedation. For anaesthesia of large fish species. Spray onto gills. For a general sedation of many fish species. Can be used for humane killing by keeping fish in the solution for >10 minutes after respiration ceases.
Drug name	**Dose**	**Frequency (q = every)**	**Route**	**Notes**
Hormones				
Carp pituitary extract	5mg/kg 0.75mg/kg 1.0-1.5mg/kg 1.5mg/kg 2.5-3.omg/kg	Repeat in 6-24 hrs	IM IM IM IM IM	Dose when combined with human chorionic gonadotrophin (20IU/kg) Hormone to stimulate release of eggs. Can be given in 2 doses 24 hrs apart. Give first preparatory dose ≤10% of the total dose. Does not cause eggs to mature. Do not give unless eggs are mature. Female fish <2kg Male fish Female fish 2-5kg Female fish >5kg

Human chorionic gonadotrophin (hCG)	30IU/kg 20IU/kg 5mg/kg 800-1000IU/kg	Repeat in 6 hrs Repeat in 6 hrs Repeat in 6 hrs q8hrs	IM IM IM IM	Hormone to stimulate release of eggs. Does not cause eggs to mature. Do not give unless eggs are mature. Dose when combined with carp pituitary extract. For carp sp.
Human recombinant platelet-derived growth factor, or bercaplamin (Regranex)			TOP directly onto wounds	Breakthrough treatment for Head and lateral line erosion (HLLE) disease in marine tropical fish. The growth factor binds rapidly to receptors in the damaged epithelium, so long contact time is not necessary. Although expensive, it can be diluted 25% without loss of efficacy, and only very small quantities are required
Luteinizing hormone	5-100µg/kg	Repeat in 6-18hrs	IM	Mammalian gonadotropin that has been used to induce the reproductive cascade in fish.
LRH-A	2µg/kg then 8µg 6 hrs later		IM	Synthetic luteinising releasing hormone analogue. Stimulate the release of eggs. It does not cause the eggs to mature and do not give to eggs that are not mature. Used with haloperidol or reserpine for the first injection in some species.
Salmon gonadotrophin releasing hormone analogue & domperiodone (Ovaprim)	0.5ml/kg fish	Split the dose 3 days apart. In salmonids. Single dose in carp. Split dose 25%/75% a few hours apart in catfish.	IM	Only use for broodstock. Not for human consumption fish. Wear PPE and avoid contact with the product.

Drug name	Dose	Frequency (q = every)	Route	Notes
Miscellaneous medications/agents				
Atropine	0.1mg/kg	As required	IM, Ice, IV	Use for organophosphate or chlorinated hydrocarbon toxicity.
Carbon, activated	75g/40L tank water			Removal of medication and other organics from the water. Normally within the filtering system. Discard after two weeks.75g is equivalent to 250cc dry volume.
Dexamethasone	1-2mg/kg 2mg/kg	 Q12hrs	IM, ICe ICe, IV	Adjunct to treatment of shock, trauma, chronic stress syndromes. For treatment of chlorine toxicity.
Doxapram	5mg/kg		ICe, IV	For treatment of respiratory depression.
Ephinephrine (1:1000)	0.2-0.5ml		IM, ICe, IV, IC	For treatment of cardiac arrest.
Furosemide	2-5mg/kg	q12-72hrs	IM	To induce diuresis, for treatment of ascites, generalised oedema. Note: efficacy is limited due to lack of loop of Henle in fish kidneys.
Glucans	2-10mg/kg 2g/kg fed	 For 7 days	ICe PO, in feed	Polysaccharides act as an immunostimulant. Used in rainbow trout.
Haloperidol	0.5mg/kg		IM	Dopamine blocking agent. Use with LRH-A to stimulate release of eggs.
Hydrocortisone	1-4mg/kg		IM, ICe	Adjunct to treatment of shock, trauma and chronic stress syndromes.
Hydrogen-peroxide	0.25ml/L water		TOP Bath	For treatment of acute environmental hypoxia.
Nitrifying bacteria	Use as directed for commercial based products. Adding material from a tank with an active biological filter system and healthy fish to a new tank			Used as a seed product to improve the development of the biological filtration system to detoxify ammonia, nitrite, nitrate and many commercial preparations. Do not expose the bacteria to extreme heat and use before expiry date. Must evaluate disease transmission by this method before use.

Drug name	Dose	Frequency (q = every)	Route	Notes
Miscellaneous medications/agents				
Oxygen			Fill plastic bag with oxygen containing 1/3rd volume water	Used to treat acute environmental hypoxia common during transportation. Close the bag tightly with rubber band and keep fish in the bag until they are swimming and breathing normally.
Reserpine	50mg/kg		IM	Dopamine blocking agent. Use with LHR-A to stimulate the release of eggs.
Salt (Sodium chloride)	1-3g/L tank water 3-5g/L tank water Add chlorine to produce at least 6:1 ratio (w/w) of Cl:NO_2 ions.			Best to use sea water (conc. 30-35g/L) or artificial sea salts. If not, use non-iodised table/rock salts as some anticaking agents in solar salts are toxic to fish. Some species e.g. catfish are sensitive to seawater treatment. Toxic to plants at concentrations over 0.5 grams/L. For prevention of stress induced mortality in freshwater fish. For treatment of nitrite toxicity.
Sodium thiosulphate	10mg/L tank water 10g neutralises chlorine (up to 2mg/L) from 1000L of water 100mg/L tank water		TOP TOP TOP	Use as directed for chlorine/chloramine neutralisers. Often added to commercial chlorine/chloramine neutralisers when using municipal water for fish tanks which is toxic to fish unless pre-treated. Used to treat chlorine exposure.
Zeolite	Use as directed. 20g/L tank water			Used as an ion exchange resin that exchanges ammonia for sodium ions in ammonia toxicity cases.
Vaccines				
For bacterial disease			IM	To prevent: Vibriosis, Aeromonas Salmonicida, Streptococcosis.
For viral disease			IM	To prevent: Viral haemorrhagic septicaemia, Infectious pancreatic necrosis, Spring viraemia of carp, Channel catfish viral disease

References

- Carpenter, JW, 2005, *Exotic Animal Formulary*, 3rd edn, Saunders Elsevier, Missouri
- Chong, RSM, 2009, Aquaculture production, VETS 4021 lecture notes, University of Queensland
- Harms, CA, 2019 *Therapeutics for Fish, Aquatic medicine*, NAVC Conference 2012 Small Animal, viewed 10 September 2019, https://www.vetfolio.com/learn/article/therapeutics-for-fish
- Rottmann, RW, Shireman, JV & Chapman, FA, 1991, *Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish*, viewed 23 September 2019, https://pdfs.semanticscholar.org/a4a2/0c215f05d7c35e50385ffa58aecdf2105514.pdf?_ga=2.255034060.532958373.1569210578-1857874335.1569210578
- University of Minnesota, 2013, *Induced Reproduction in Fish*, viewed 23 September 2019, http://www.seagrant.umn.edu/aquaculture/induced_fish_reproduction

Appendix 9.10: Disease recognition in finfish

To be able to recognise ill health in finfish, it is essential to recognise what is a normal appearance and a normal behaviour pattern in each finfish species.
Often very subtle signs such as changes in feeding patterns can be the first signs of ill health in a fish.

Poor water quality (cloudy or discoloured) that obscures the tank vision may impair the observer's ability to see sick or dying fish that do not feed or are staying on the bottom of the tank. In such cases such, a light source is a useful tool to be able to monitor the fish appropriately whilst the water quality is managed.
Common fish diseases can cause rapid mass fish deaths within minutes to hours. The higher the stocking intensity the more critical is the need for early detection of disease processes.

Daily observations involving water quality checks and fish observational checks combined with carefully prepared monitoring reports and efficient diagnostic pathways during unexpected adverse events are the basis of overall effective fish management within scientific projects and general aquaculture food production.
Regular observations of the fish should be made on a once or twice daily basis, and include:

- swimming patterns
- feeding patterns
- breathing patterns
- location in the water (e.g. crowding around an air supply)
- skin colour.

Appendix 9.11: Quick reference guide to common clinical signs and possible causes

Clinical sign	Possible cause
Sudden death of many fish with few clinical signs	Very low dissolved oxygen (D.O.), acute poisoning, Very acute infection.
Abnormal swimming pattern and imbalance	Viral infections such as Nodavirus.
Breathing distress (rapid mouth and gill movement)	Gill infection (parasitic, bacterial, viral or fungal), anaemia (low RBC count), decreasing D.O., toxins such as carbon dioxide (CO_2) or nitrite.
Irritation (jumping, rubbing or flashing movements)	Skin or gill parasites, chemical irritants.
Bleeding of scales	Bacterial or viral infection, trauma.
Skin ulceration and damage	Fungal infection such as Epizootic ulcerative syndrome (EUS), bacterial infections such as Flexibacteriosis, trauma from skin parasites or other aggressive fish.
Popeye	Bacterial infections such as Streptococcosis, viral infection.
White spots	Parasites such as *Icthyopthirius multlifiliis.*
Corneal cloudiness	Gas bubble disease, trauma, poor water quality, bacterial infection.
Cataract	Nutritional deficiency such as zinc, eye flukes.
Skeletal deformity	Nutritional imbalance such as vitamin C deficiency, phosphorous and calcium imbalance. Genetic abnormalities such as inbreeding, toxins such as pesticides.
Internal organ haemorrhage	Bacterial or viral infections.
Fin and tail rot	Trauma, secondary bacterial infections such as Flexibacteriosis, poor water quality.
'Cotton-wool' growth on skin	Fungal infections such as Saprolegniasis.
Body swellings	Tumours, abdominal free fluid/blood, parasitic cysts.
Excessive mucous	Parasitic infections such as Chilodonella protozoa, skin/gill flukes.

Appendix 9.12: Non-infectious disorders of finfish

Disease	Clinical signs and Impact	Cause	Epidemiology	Diagnosis and Management
Water quality disorders				
Low dissolved oxygen (D.O.)	Gasping, grouping around water and air inlets, open gaping mouths and potential rapid mass (100%) death. Fish die from hypoxia <5ppm D.O. or anoxia <2ppm D.O. Any surviving fish often die later from infections due low D.O. distress.	Fish require at least 5ppm of D.O. for maintenance & growth depending on species.	When fish feed or are stressed due to handling or for transport or have gill disease they require more oxygen. Factors reducing oxygen include: High stocking density, increased water temp, increased organic waste due to overfeeding, no power supply to aeration equipment, inadequate water exchange, normal diurnal oxygen fluctuation (i.e. low D.O. in early dawn), dead fish/decomposing matter, some chemical treatments e.g. formalin baths.	<u>Ensure adequate oxygen supply to tanks (>5ppm at all times) by:</u> Emergency and power supply backups. Maintenance of aerators. Extra aeration when fish are handled or sick. Manage stocking densities appropriately for size of tanks and equipment available. Ensure correct feeding regime for number of fish. Increase flow and flushing rates of water. Monitor oxygen levels closely and adjust as needed. Remove sick fish. Minimise handling of fish that have been through a hypoxic event and reduce feed intake until recovered. Monitor fish health and avoid exposure to chemicals by using separate treatment tanks.
Nitrite (NO_2) poisoning	Breathing issues as above. Light tan to brown gills on postmortem with tan to brown blood and acute mortality. Acute mortality in fish even with minor handling or stress. Also, hypoxia in fish.	Elevated NO_2 >0.6ppm-2.0ppm). NO_2 binds to haemoglobin forming methaemoglobin which cannot bind oxygen. At 40-80% conversion fish begin to suffocate.	High nitrite levels are common in new sets ups after an ammonia peak due to the immaturity of the *Nitrobacter* sp. bacterial population (biofilm) which needs time to stabilise. Any inhibition of these bacteria due to antibiotics methylene blue, formalin or ammonia can cause toxicity, as can overfeeding.	Diagnosis via water test and necropsy. Ensure NO_2 is <0.1ppm at all times, by daily monitoring. Use salt therapy to inhibit the uptake of nitrite through the gills (ratio 6:1 chloride: nitrite). Increase water exchange rate. Increase aeration and filtration. Cease/reduce feeding until stabilised. Stage stocking density increases until biofilm has had time to build up, or reduce stocking density.
Gas bubble disease	Gas bubbles in gills, eyes, skin and internal organs. Popeye (exophthalmia where the eye of the fish is swollen and protrudes abnormally from its socket), abdominal air causing bloating. Fish killed in minutes.	D.O. >20ppm or >125% saturation, or excessive nitrogen or carbon dioxide with total gas saturation of >50-200mm Hg. Excess gas comes out of solution in fish blood and forms gas bubbles that block up the circulation in tissues.	Rapid water temp rise from water source to tank, air being sucked in at water intake pipe, water pump defect which pumps air into solution under pressure, a rapid decrease in pressure from water source to tank, bore water source, excessive direct oxygenation of tank, air transportation of fish, water falling over a deep spillway.	Remove excess gas from the water (oxygen, nitrogen and carbon dioxide) by: by aerating bore water to the atmosphere before use. Packed column degasser for large water volumes. Monitor total gas via a saturometer.

Disease	Clinical signs and Impact	Cause	Epidemiology	Management
Water quality disorders				
Carbon dioxide (CO_2) poisoning/low pH	Breathing problems grouping around water inlet and aerators, severe lethargy and sedation, rolling over in the water after a few hours of CO_2 exposure regardless of well oxygenated water, development of calcium deposits in the kidneys (nephrocalcinosis). Fish die from hypoxia due to high CO_2 in the water.	Increased CO_2 in the water inhibits diffusion of CO_2 out of the blood of fish causing acidosis. This reduces the amount of oxygen able to be carried to the fish tissues.	CO_2 is very soluble in water and can far exceed levels found in the air. Elevated CO_2 can develop when using liquid oxygen to allow for a higher stocking density. High CO_2 water concentrations cause a narcotic effect and hypoxaemia. CO_2 above 10ppm and near 60ppm are very stressful for fish. An algal bloom die off can also increase water CO_2 levels.	Buffer the water to increase pH. At >8.34 free CO_2 is not present. Vigorous aeration. Use a packed volume degasser to remove CO_2 from bore water. Increase venting in live fish transporter tanks by opening the lid to allow CO_2 to disperse. Daily check of water pH to indicate elevated CO_2 levels in the water.
Ammonia (NH_3) poisoning	Gill hyperplasia/fusion, fish at surface showing signs of irritation in acute toxicity, signs of respiratory distress. Acute deaths.	Un-ionised ammonia is lethal at >1ppm and stressful at >0.02ppm. When pH is higher (>8.5) a great % of total ammonia nitrogen exists as the more toxic form NH_3 and less as the ionised less toxic form of NH_4^+. Ammonia is a by-product of fish excretion and bacterial decomposition of organic matter.	Failure of the biofilm to convert the NH_3 to nitrate NH_4 causes a build-up in the system which becomes toxic. Excessive feed, high stocking density or build-up of organic matter. Inadequate water exchange or plant uptake of nitrate. The biofilm relies on a good source of both *Nitrosomonas* and *Nitrobacter sp*. of bacteria which take time to build up and themselves require sufficient surface area, oxygenation and water flow. Antibiotics, disinfectants and other chemical products can trigger an ammonia toxicity adverse event.	Good water exchange. Maintain a healthy biofiltration capacity for the stocking density of the tank allowing 6-8 weeks for its maturation. Daily testing of ammonia levels and adjustment of feeding and water exchange as needed. Adequate oxygenation of the water. Protect the biofiltration from chemicals by using other tanks for treating sick fish. Avoid rapid pH increases above pH 8.5.
Biotic toxins				
Algal toxins	Respiratory distress. Toxic hepatopathy and gill lamellar necrosis. Acute fish mortalities are common.	Blue green algae produce toxins. Prymnesiophytes algae produce mucilage clogging gills of fish. Dinoflagellates form red tides whose toxins disrupt gill epithelia.	Specific environmental requirement are needed for many of the algae to bloom and cause toxicity such as: nutrient rich water, lack of water exchange and favourable climatic conditions. Some can produce potent neurotoxins which can bioaccumulate in fish (e.g. Ciguatera toxin).	Identification of algal species in tanks. Avoid and protect fish from exposure. Maintain good water quality and adequate water exchange. Provide additional aeration during algal bloom crashes.

Disease	Clinical signs and Impact	Cause	Epidemiology	Management
Biotic toxins				
Fungal toxins	Emaciation, liver tumours, cystic swellings, haemorrhage, spleen and kidney metastasis. Outbreaks of fish neoplasia and mortality have been associated with the use of grain seeds with poor feed storage conditions.	Exposure to fungal Aflatoxins produced by mould *Aspergillus flavus.* It is a potent carcinogen inducing hepatic tumours with limited metastasis but causes death by infarctive necrosis and haemorrhage of the fish's liver.	Animal feed containing grains (peanuts, groundnuts, cottonseed) are at risk when stored in humid moist conditions.	Prevent exposure to the mouldy feeds by improving feed storage in low humidity facilities such as by refrigeration. Use food within expiry date and reduce grain consumption levels in fish feed. Aflatoxins can be bound by using mineral clays in the diet.
Botulism	Fish become dark in colour. Slightly swollen and float listlessly with erratic swimming and sinking episodes with gradually increasing mortalities. Outbreaks are rare but can occur.	*Clostridium botulinum* neurotoxin type E produced in the gut of fish, soil, faeces and decaying organic matter associated with anaerobic conditions. Blocks the acetylcholine from synapses.	Most commonly occurs in earthen ponds where dead fish are not removed, fish are underfed or fed contaminated stale trash diets with poor husbandry. Other species such as birds and mammals can also be affected if they eat the contaminated fish.	The tanks need to be fully destocked and disinfected and fish buried in quick lime 1.5kg/m^2. Treated ponds can be reused after a month but at lower stocking levels with good water exchange systems for the first 6 months until stocking can be returned to normal levels.
Plant toxins	Clinical signs are varied depending on the toxin and include, respiratory distress, loss of balance, liver tumours and skin ulceration due to photosensitisation, CNS and altered nerve mechanisms of the heart, immobilisation and stunned appearance in the water column and horizontal gaze nystagmus (HGN).	The plants produce various toxins each having a detrimental effect on fish health.	Exposure routes include heavy rainfall with wash down of plant material, toxic plant material in feed sources and deliberate poisoning. At least 70 species of plant can produce toxins capable of killing fish.	Avoid exposure to unknown plants within the tanks.

Disease	Clinical signs and Impact	Cause	Epidemiology	Management
Other toxins	Acute distress and thrash around in the water surface. Jellyfish stings in marine cages and tanks during summer. Ant feeding on trout after drought.	Jellyfish toxins sting the skin and gills cause severe inflammation and oedema. Formic acid from ants erode gastric mucosa and cause renal degeneration in fish.	Fish undergo severe self-trauma after stings or eating ants.	Avoid jellyfish habitat or infested water supplies. Harvest wild caught fish for projects out of the months when jellyfish are in their greatest numbers. More common in marine cage environments than in tanks or during drought conditions in freshwater ponds. Humane killing of affected fish.
Pollutant toxins				
Heavy metals	Clinical signs are varied and relate to the organs damaged but include: Gill degeneration & hyperplasia, liver /renal necrosis, vertebral deformities and fractures, excessive mucous production to non-specific response to epithelial surface irritation from metals and death from organ failure are common. Associated with fish kills. More problematic in certain areas where leaching into waterways causes higher than normal metal concentrations.	Copper, aluminium, zinc, iron, lead, mercury, tin, cadmium and silver are the main metals associate with toxicity.	Acidic pH and low hardness water enables metals to dissolve into the water as more toxic forms. Chelated forms of metals in organic matter are less harmful. Bore water and rainfall run off water may already contain high levels of dissolved metals.	Test water supply regularly for heavy metal contamination and move projects away from contaminated water supplies. Be aware of iatrogenic sources of metal in fish systems such as copper plumbing and galvanised zinc tanks. See appendix 9.15 for chart displaying acceptable levels of heavy metals in fish aquaculture facilities.
Chlorine	Peracute death with little signs from chemical burns of the gills, skin and fins. Chronic sub-lethal exposure causes gill hyperplasia and respiratory compromise.	Chlorine and chloramine are potent toxins to fish, commonly a problem with using municipal water supplies when filling fish tanks. Levels of >0.003ppm are stressful and fish are killed at >0.1ppm.	Chlorine combines with ammonia to form chloramine, a more stable form of chlorine which is highly toxic to fish. Aeration alone will not neutralise chloramine.	Neutralisation of chlorinated and chloramine water with sodium thiosulphate at 2.85 times the dose of chlorine. Vigorous aeration or exposure to direct sunlight over 2-3 days will affect a slower dichlorination effect. The ammonia will also need to be detoxified prior to usage by water exchange.

Disease	Clinical signs and impact	Cause	Epidemiology	Management
Therapeutic chemicals	Excess mucous production, irritative swimming patterns or splashing when exposed to high concentrations of a chemical. Respiratory distress ad Peracute death may occur if hypoxia occurs during the handling process. Neurotoxic signs may be associated with organophosphate over-exposure.	All drugs/chemicals have a different therapeutic index and overdoses will cause a toxic effect. Factors such as fish species sensitivity, concurrent disease, environmental factors, or advanced physiological stress may limit drug use in fish.	Fish kills due to chemical application usually occur 24-72 hours after exposure. Biofiltration based fish kills due to chemicals can take longer to affect the fish. Stress due to handling of sick fish can also cause mortality. Out of date chemicals can degrade and their metabolites can be more toxic than the parent components. Water factors such as temp, pH, salinity, D.O., and hardness affect chemical bioavailability and toxicity levels.	Ensure a definitive diagnosis through a sound scientifically based pathway prior to administering chemicals. Assess recovery prognosis as the use of drugs can cause more mortality if used incorrectly than the disease itself. Accurately assess biomass and water volume for dosage calculations. Perform a test batch prior to treating the whole tank. Have emergency clean water to remove chemicals quickly. Protect biofiltration from the chemicals. Avoid treatment during adverse weather or poor water quality periods and monitor fish closely during exposure to the drugs.
Pollutant toxins				
Pesticides, herbicides, algicides	Hypoxia, lots of dead fish and other aquatic animals downstream from a spill. Exposure to pollutants due to accidental spillage, malicious discharge, drift sprays/applications or as rainfall runoff.	Acute exposure to levels >LD50 or chronic morbidity with impaired growth, reproduction or protracted mortality at sublethal but stress inducing and cumulative levels of pollutants.	Pollutants exposure should be included in any fish kill investigation. Obtaining sediment, water feed and fish for sampling is often problematic to achieve. It will often affect several species at the same time. Surveying the current local land use will help reveal the sources of pollutant. See appendix 9.16 for the list of common pesticides, algicides and herbicides associated with fish mortality.	Obtain useful samples for laboratory analysis for pollutant levels wherever possible. Commonly associated with prosecution of offenders causing the spill.
Others- fertilisers, detergents and petrochemicals	They exhibit escape behaviours, erratic swimming and surface splashing. The impact is similar to pesticides and herbicides chemicals due to run off pollutants upstream to water supply.	Fertilisers cause a rapid increase in oxygen demand in the water. Detergents damage gill epithelium by the lipid surfactant action. Petrochemical smother the respiratory epithelium. Organochlorines are neurotoxic leading to muscle spasms and deformities.	Fish trapped in contaminated tanks/ponds are unable to escape the pollutant. Multispecies involvement implicates a pollutant toxicity. See appendix 9.17 for the list of common fertilisers, detergents and petrochemicals associated with fish mortality.	The smell may lead to identification of the pollutant. Once exposure has occurred there is no treatment available. Protecting unaffected fish is the priority by diluting the pollutant or removing them to clean water tanks/ponds ASAP.

Disease	Clinical signs and impact	Cause	Epidemiology	Management
Nutritional disorders				
Antimetabolites	Vary with the substance but generally cause poor growth, nutritional deficiency, chronic mortality and deformity. Aquaculture feeds may contain organic and inorganic substances that may be toxic or reduce the nutritional value of the feed, leading to malnutrition and toxicosis.	There are many types of antimetabolites (e.g. vitamin antagonists, toxic amino acids), plus feed toxins (e.g. fungal or bacterial toxins) and also chemical contaminants (e.g. pesticides) which can affect various components of the feed leading to malnutrition.	Cyclopropenoid acids, linamarin and many others can cause malnutrition if not stored or treated correctly prior to feeding to the fish. See Appendix 9.18 for full list of common antimetabolite toxins, their sources and effects on the fish.	Locate the suspect batch of food. Maintain accurate feed monitoring records. Look for specific changes on a daily basis to narrow down the diagnostic pathway. Test the feed. Identify suspect ingredients in the feed. Assess storage facilities and make changes as needed. Change diet, supplement feed with vitamins. Rule out other possible causes such as infections and toxins.
Vitamin deficiencies	Abnormalities such as immunosuppression, skeletal deformities, anaemia, haemorrhages, cataracts, myopathies, gill hyperplasia, neurological derangements peculiar to the specific deficient vitamin. Young growing fish are affected more than adults.	Lead to insufficiency of co-enzyme systems in the animal leading to poor growth, clinical and developmental abnormities. Occur in commercial feeds due to inadequate levels or breakdown due to poor storage.	Takes several weeks for clinical signs to show. Gradual increase in mortality and physical abnormalities.	Identify the deficiency by feed analysis and associated pathology. Supplementation may reverse the clinical signs if caught early enough in the malnutrition event.
Rancid oils	Renal haematopoietic necrosis leading to severe anaemia, hepatic ceroidosis and degenerative myopathy (like Vit E deficiency). Ascites, splenomegaly, pale yellow livers and anaemic extreme pallor in gills and organs are noted.	Feeding too many marine fish oils which have high polyunsaturated fatty acid (PUFAs). Autooxidation of PUFAs produces free radicals and peroxide compounds that react with other feed ingredients such as Vit E. They damage cell membranes.	Poor food storage with high humidity and temperatures. Using expired food with a high fat content. (>15-18% in salmonids) (>5-12% in tilapia) (5-10% in silver perch) (15-16% in snapper) Mortalities in fish fed high level of polyunsaturated fats leading to liver lipoid disease.	Use only properly stored food when it is fresh. Fortify diets with antioxidants such as Vit E or synthetic antioxidants such as 'ethoxyquin'. Note: bloodmeal (Haemoglobin) acts as catalyst for autooxidation, therefore limit their content.

Disease	Clinical signs and impact	Cause	Epidemiology	Management
Nutritional disorders				
Mineral deficiencies	Clinical signs are specific to each mineral deficiency. See appendix 5 for chart detailing minerals, purpose and clinical signs of deficiency. Production loss from poor growth rates, mortalities and developmental deformities.	Major minerals required for good health by fish are: calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur. Trace minerals are: iron, iodine, manganese, copper, cobalt, zinc, selenium. Deficiencies will cause clinical signs with continued deficiencies.	Potentially a problem with fish held in waters deficient in minerals (reduced skin absorption), deficient diets or diets high in phytates restricting uptake. Appendix 9.19 shows a list of mineral deficiencies	Find the suspect feed stock by maintaining good monitoring records. Look at the specific pathology in the fish to help define a diagnostic pathway. Test the feed. Supplement with minerals as required. Change feed brands and change as needed for the fish species. Rule out other causes such as infections and toxins.
Head and lateral line erosion (HLLE)	Progressive lesions of the head that sometimes extend down the lateral line, often in a symmetrical pattern. The depigmented erosions and ulcerations coalesce to produce large crateriform lesions and pits on the head, flanks and lateral line. Fish may feed and behave normally, even when severely affected. Some fish may become anorexic, lethargic and thin and wounds are open to secondary infections which lead to mortality.	Exact aetiology is unknown, but proposed agents include nutrient deficiencies in vitamin A or C, parasites, carbon dust, heavy metals, electrical voltage, UV radiation,	Common chronic disease that affects marine fish, especially tangs and angelfish. Fish can recover from HLLE but often have permanent scarring. The disease is rarely fatal There may not be one particular inciting cause therefore: Improve feed quality. Reduce overcrowding and other stressors. Ensure a grounding device removes stray voltage from the tanks. Replace activated carbon in filtration system. >90% water changes may need to be done to reduce the effects of activated carbon and improve water quality controls. Move the fish to a new aquarium that has never had fish develop HLLE in it.	Treat secondary infections with appropriate antimicrobial/antifungal medications. New treatment: 0.01% human recombinant platelet-derived growth factor) bercaplamin (Regranex), has been successfully used to treat HLLE in marine tropical fish by applying the product to the debrided skin lesions. The growth factor binds rapidly to receptors in the damaged epithelium, so long contact time is not necessary. Although expensive, it can be diluted 25% without loss of efficacy, and only very small quantities are required. Improvements in HLLE lesions can be dramatic; however, in tanks conducive to the condition, lesions can quickly recur. Diagnosis based on history and clinical signs. Wet mount preparations from skin samples, faecal examination for parasites and histopathology samples from all body parts.

Disease	Clinical signs and impact	Cause	Epidemiology	Management
Nutritional disorders				
Nutrient excesses (fats, vitamins and minerals)	Nephrocalcinosis where calcium is deposited into the kidney and soft tissues leads to ureter obstruction and distension of the kidneys. Fatty liver is seen as paleness and enlargement leading to degeneration of the liver tissues. Mortality from toxicosis, growth depression and on-going mortalities.	Vit A, D and E occur because they are stored in lipid tissue and cause metabolic/clinical derangements. High fat diets cause fatty liver degeneration and other conditions.	Over fortification of vitamins and minerals in the diet during processing. Inclusion of dietary fats may exceed nutritional requirement for specific species. For signs of nephrocalcinosis, exposure to elevated carbon dioxide, deficiency in magnesium and increased levels of selenium can predispose to the condition.	Analyse feed and rule out water borne toxins. Correct water quality parameters such as high carbon dioxide. Change to more appropriate diet for the species.
Physical disorders				
Trauma	Damaged fins, body injury such as spinal fractures creating 'bent' fish, jaw and teeth marks in scales. Ova shocks (electric) produce twins,' pugheads' and spinal fractures/curvature. Blackening of the body skin distal to the spinal trauma site due to blockage of the autonomic innervation. Trauma due to poor handling techniques causes morbidity and poor-quality stock.	Predator and competitor attacks, poor handling techniques, mechanical electrical shocks (ova) and electrocution, damaged handling equipment and holding tanks.	Naturally aggressive fish species towards smaller cohort members such as Barramundi and Murray cod. Electrofishing may cause fish with spinal fractures due to excessive current employed.	Identify and correct the causes such as regular grading of fish, monitoring for aggression and protecting vulnerable fish with seal net or by providing more hiding places in the tanks. Improve husbandry and handling techniques. Check electrical wiring for safety levels.

Disease	Clinical signs and impact	Cause	Epidemiology	Management
Genetic disorders				
Inbreeding	Occurrence of twins, pugheads, crossbite, spinal curvature, double fins and opercular deformities. Increased genetic linked abnormities are expressed in new generations of fish.	Using a very small genetic pool-inbreeding.	Increasing rates of abnormalities from one generation to the next, when new genetic stock is not added to the breeding programme.	Increase genetic diversity in the broodstock. Avoid breeding from blood lines that show an increased incidence of deformity in the young fish.
Neoplasia disorders				
Hereditary & radiation	Depends on tumour type. Generally lethal with melanomas. Fish are susceptible to irradiation damage and can transfer damaged genes to their progeny.	UV damage to genetic material and passage of genes to subsequent progeny.	<u>Hereditary neoplasia includes:</u> Melanomas of hybrid Xiphophorus. Irradiated zebra fish led to activation of the melanoma gene leading to melanoma formation. Gonadal tumours in hybrids goldfish crossed with common carp. Thyroid tumours in Amazon mullies after thyroid cells were irradiated in vitro.	Minimise exposure of fish to UV radiation and avoid breeding from affected lines.
Chemical carcinogens	Swelling or distortion of the abdominal organs (liver) or external lesions with cauliflower shaped appearance.	See appendix 9.20 for list of chemicals, source and typical clinical signs.	Routes of exposure include contaminated water, sediment or foodstuff. Chemicals can be oncogenic or anticarcinogenic and their role may involve the activation of oncogenic viruses or as co-carcinogens.	Ensure placement of tanks, ponds etc are as far away from industry and population centres as possible.
Oncogenic viruses	Clinical signs depend on the type of tumour. Occur naturally in wild populations of fish and transmission to cultured populations is problematic.	Herpesvirus (*Onchorynchus masou virus*), Retroviridae (*Walleye dermal sarcoma)*, Plasmacytoid leukaemia of chinook salmon, Atlantic salmon swim bladder sarcoma, Atlantic salmon papilloma, Esox sarcoma, Esocid lymphosarcoma, Pike epidermal proliferation, Damselfish neurofibromatosis, *Xiphophorus sp.,* Hybrid neuroblastoma, Viral erythrocytic infection of sea bass.	(Onchorynchus masou virus- vertical and horizontal transmission occurs with annual epizootics in the young fish. Walleye dermal sarcoma- has a seasonal occurrence in spring but declines into summer. Plasmacytoid leukaemia of chinook salmon- affects both wild and pen reared fish causing high mortalities.	Exclusion of infected fish from cultured stocks (difficult where there is contact with wild stock such as marine pens).

Appendix 9.13: Heavy metal contamination and therapeutic and agricultural chemical contamination charts

Heavy metal contamination chart

Parameters (metals)	Levels in water associated with fish kills (ppm) or as specified.	Acceptable continuous exposure levels in water for culture (ppm) or as specified.
Aluminium	>0.1-5.0 (Low pH)	No information
Cadmium	>1.0-3.7 (Soft water) >5.2 (hard water) >0.1 (Cadmium salts)	<0.0005 (Soft water) <0.003 (hard water)
Chromates	>3.3-133	No information
Cobalt	>30	No information
Copper	>0.03000.007 (Soft water) >0.6-6.4 (Hard water)	<0.006
Copper nitrate	>0.02	No information
Iron	>0.5	<0.1
Lead	>1.0-31.5	<0.02
Lead salts	>0.5	No information
Manganese	>75	<0.01
Manganese chloride	>0.5	No information
Mercury	>0.17	<0.0002
Mercury chloride	>0.008	No information
Methyl mercury	>0.07	No information
Nickel	>4.5-9.8	<0.01
Nickel salts	>0.1	No information
Selenium	>8.1-72	<0.05
Silver	>0.006-0.07	No information
Tin	>55	No information
Tri-n-butyl tin (TBT)	>0.0015-0.02	<0.02ppb
Zinc	>0.4-1.76	<0.005

Therapeutic and agricultural chemical contamination chart

Parameters Therapeutic and agricultural chemicals	Levels in water associated with fish kills (ppm) or as specified.	Acceptable continuous exposure levels in water for culture (ppm) or as specified.
Copper sulphate	>0.14	<0.002
Detergents	>4	<0.1
Formalin	>50	Therapeutic dose for brief period
Hydrogen peroxide	>25	Therapeutic dose for brief period
Malachite green	>0.1	Therapeutic dose for brief period
Phenolic disinfectants	>0.1	No information
Potassium permanganate	>1.0	Therapeutic dose for brief period
Simazine	>10	<0.1
Sodium hypochlorite	>0.1 free chlorine	<0.003
Trichlorphon	>0.8-100	<0.001 ppb

Table from:
Langdon, JS, 1988, Investigation of fish kills, *Fish diseases,* proceedings 106, pp.167-223.

Definitions:
(ppb)- parts per billion = (µg/L) micrograms per litre
(ppm)- parts per million = (mg/L) milligrams per litre

Appendix 9.14: Pesticides, herbicides, algicides, fungicides, de-mosser products and organochlorine pesticides

Pesticides, herbicides, algicides, fungicides and de-mosser products.

Parameters (metals)	Levels in water associated with fish kills (ppm) or as specified.	Acceptable continuous exposure levels in water for culture (ppm) or as specified.
Acrolien	>0.14	No information
Carbamates Dithiocarbamates	>0.17	<0.0001ppb
Carbaryl	>0.5-10	0.02ppb
Copper sulphate	>0.14-0.50	<0.002
Chlorthalonil	>10-20	No information
Diquat	>90-723	No information
Diuron	>4-152	No information
Glycophosphate	>12-130	No information
Ivermectin anthelmintic	>0.1	<0.001ppb
Malathion	>0.1-30	<0.008ppb
Organophosphate-Diazinon	>0.2-5.2	<0.002ppb
Paraquat	>840	No information
Parathion	>0.3-1.6	<0.001ppb
Pyrethrins	>0.005-0.001	<0.001ppb
Rotenone piscicides	>0.5-4 (at 16-22o^{C})	No information
Simazine	>10	<0.01
Trichlorphon	>0.8-100	<0.001ppb
2,4-D	>2.0-96.5	<0.004ppb

Organochlorine pesticides

Parameters Therapeutic and agricultural chemicals	Levels in water associated with fish kills (ppm) or as specified.	Acceptable continuous exposure levels in water for culture (ppm) or as specified.
Aldrin	>0.013-0.05	<0.01ppb
Chlordane	>0.02-0.08	<0.004ppb
Chlordecone (Kepone)	>0.004-0.07	<0.001ppb
DDT	>0.008-0.027	<0.003ppb
Dieldrin	>0.008-0.05	<0.005ppb
Endosulphan	>0.01	<0.01ppb
Endrin	>0.0003-0.002	<0.003ppb
Heptachlor	>0.019-0.25	No information
Lindane (BHC)	>0.23-0.8	<0.02ppb
Pentachloraphenate	>0.1	<0.1ppb
Toxaphene (camphenes)	>0.003-0.018	<0.01ppb

Appendix 9.15: Fertilisers, detergents and petrochemical charts

Fertilisers, detergents and petrochemical charts

Parameters Therapeutic and agricultural chemicals	Levels in water associated with fish kills (ppm) or as specified.	Acceptable continuous exposure levels in water for culture (ppm) or as specified.
Aniline, toluidine	>100	No information
Benzene	>10-260	No information
Detergents-sodium dodecyl sulphate	>28-32	<0.1
Dodecyl benzosulphonate **Sulphonates**	>5 >4	<0.1 No information
Hexachlorobenzene	More toxic than benzene	No information
Lime (calcium oxide & calcium hydroxide)	Causing pH >9-10	No information
Napthalene	>3.0	No information
Petrochemicals-diesel or car oils	>50-100	No information
Phenanthrene	>1-2	No information
Phenols, cresols	>5-45	No information

Appendix 9.16: List of antimetabolites causing malnutrition

List of antimetabolites causing malnutrition

Parameters **Antimetabolites**	**Source**	**Clinical signs of malnutrition**
Alkaloids	Green parts of potato plants	Hepatotoxic
Antibiotins (avidin)	Raw egg white Soybean meal Kidney and haricot beans	Anti-vit D Anti-vit D Anti Vit E
Cyclopropenoid acids	Kapok & cottonseed oils	Cause growth inhibition, fatty liver and are carcinogenic with aflatoxins.
Gossypol	Cottonseed	Hepatoxic
Haemagglutinins	Soyabean meal	Affects the uptake of several nutrients and minerals such as calcium
Linamarin **Dhurrin**	Linseed meals, lima beans, cassava Damaged/aged sorghum or maize	Cyanide poisoning
Linatine (Anti	Expeller linseed meal	Anti-vit B_6
Lipoxidase	Soybean meals	Anti-vit A
Mimosine	Tropical legume ipil-ipil	Growth inhibitor (anti-thyroid from goitrogen)
Phytates	Soybean, sesame, groundnut, cottonseed meal, cereal hulls	Form complexes with proteins, phosphorus, calcium, zinc, copper, magnesium causing malnutrition's of these nutrients
Protease inhibitors (Globulin)	Unheated/underheated soyabean	Protein concentrates inhibit the activity of trypsins leading to sulphur-amino acid deficiencies
Thiaminase	Raw freshwater fish, herrings, mussels, clams & shrimps	Anti-vit B_1

Appendix 9.17: List of minerals, use in the fish's body and signs of deficiency

List of minerals, use in the fish body and signs of deficiency

Minerals	Function in fish body	Clinical signs of deficiency
Calcium	For skeletal development, muscle contraction, blood clotting, nerve transmission and acid base regulation	Reduced growth, poor feed conversion and bone mineralisation, anorexia
Chloride	For osmoregulation	
Copper	For respiration	Reduced growth, reduced heart cytochrome c oxidase activity
Iodine	For thyroid function	Goitre
Iron	For respiration and function of haemoglobin and cytochrome c oxidase functions	Hypochromic, microcytic anaemia
Magnesium	Involved in enzyme activity, osmoregulation, neuromuscular transmission.	Nephrocalcinosis, cataracts, skeletal deformities, sluggishness and mortality
Phosphorus	For skeletal development & bone mineralisation, energy reactions, and nutrient metabolism	Skeletal/cranial deformities, reduced growth, poor feed conversion and bone mineralisation
Potassium	Osmoregulation	
Selenium	For Vit E functions as antioxidants by glutathione peroxidase activity in cell membranes	Reduced growth, myopathy, anaemia, cataracts (if Vit E also depleted)
Sodium	For osmoregulation	
Zinc	For enzyme function including dehydrogenases, aldolases, peptidases and phosphatases	Reduced growth, short body dwarfism, fin erosion, cataracts, mortality

Appendix 9.18: List of Chemical carcinogens, sources and typical clinical signs

Chemical carcinogens, sources and typical clinical signs

Chemical carcinogens	Source	Typical clinical signs
Cyclopropenoid fatty acids	Oils in feedstuff act as synergistic partners with aflatoxins.	Cocarcinogen with Aflatoxin B
Dehydroepiandrosterone	An adrenal steroid (carcinogenic through its function as a precursor of sex steroids or peroxisome proliferation).	Hepatic neoplasm
Halogenated compounds	Chronic exposure to chlorine	Oral papilloma's in Black-bullhead fish
Mycotoxins	Aflatoxins	Hepatic carcinomas and hepatomas
N-nitroso compounds	Diethyl nitrosamine	Hepatocellular carcinoma, cholangiocarcinoma, hemangiopericytomas
Polycyclic Aromatic hydrocarbons (PAHs)	From crude oil products	Mummichogs fish with hepatocellular neoplasms, exocrine pancreatic neoplasms, Niagara Falls dermal neoplasms in Freshwater-drum fish and oral papilloma's in White-sucker fish

Appendix 9.19: Infectious disorders of finfish

Infectious disorders of finfish **(Definitions:** * present in Australia [E] emerging disease)

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable parasitic diseases				
Platyhelminth	**Gyrodactylosis (*Gyrodactylus salaris*)**	Lethargy, scrubbing (rubbing against objects in response to skin irritation) and flashing (darting and twisting of fish and erratic swimming) and gathering in low-current waters when heavily infected. Skin ulcers, sloughing of the skin, greyish appearance; as disease progresses, dorsal and pectoral fins may have a whitish appearance due to thickening of the epidermis, excess mucous on skin, frayed fins. High mortality up to 100% in farmed Atlantic salmon.	Obligate freshwater ectoparasite that may be present for years in farmed salmonids, especially rainbow trout, without the fish showing any clinical signs of disease. *G. salaris* is a freshwater parasite that cannot survive in seawater; however, it can survive a few days at salinity of up to 20 parts per thousand. The parasite can survive 5–6 days detached from the host but cannot survive drying out. Transmission is horizontal (directly via the water column) by contact between infected and uninfected fish, or by contact between host fish and detached parasites on the substrate. The parasite is readily spread between farms during transportation.	Treatment: Praziquantel as a bath or prolonged immersion treatment (2–10 mg/L). Organophosphates (to which some fish species are very sensitive), mebendazole, formalin, and potassium permanganate.
Myxosporean	**Whirling disease (*Myxobolus cerebralis*)**	Whirling swimming, black tail and skeletal deformity. Spores are very resistant (20-30 years in streams).	Fish <4 mths old. Trout and salmons affected although some species are immune. Earthen ponds are required for the tubificid worm and parasite development cycle. Can spread by importation of infected frozen fish. Affects salmon and trout. Non-zoonotic. Infective triactinomyxons (TAMS) are released from tubicid worms and enter the fish. Spores form in uncalcified skeletal cartilage of young fish causing deformities, vestibular organ damage and nerve impingement.	Treatment: none available. Control: avoid earthen ponds as housing.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Ectoparasites				
Monogeneans				
(Annelids)	**Piscicola**	Piscicola leeches are visible and attach anywhere on the host body (mostly on or under the opercula, in the mouth, along the jaw and at the bases of fins). Mild cases do not cause serious harm since most tissue damage is localised at the sites of attachment, but heavy infestations cause extensive tissue damage including epidermal erosion/ ulceration, haemorrhaging, necrosis and anaemia. External epidermal erosions may serve as portals of entry for secondary bacterial or fungal pathogens.	Piscicola is a freshwater leech that can be abundant in some freshwater lakes, ponds and streams. Piscicola attach to the skin of freshwater fish and suck their blood/other tissue fluids for a few days before dropping off. Adults suck blood a few times prior to producing the oval encased 'cocooned' eggs which fall to the bottom of the pond. Juveniles hatch and find a new host fish. Leeches of this genus have been implicated as possible vectors of the Infectious haematopoietic necrosis virus (IHNV).	No treatment required apart from treating secondary infections with antibiotic or antifungal medications.
(Crustacea)	**Argulus**	Erratic swimming patterns and rubbing against tank walls are common clinical signs. Heavy infestations cause inflammation of the skin, open haemorrhaging wounds, increased production of mucous, loss of scales, and corrosion of the fins. Secondary infections with bacteria and fungi, further degrade the skin layers. The fish become stressed, anaemic, inappetent have a reduced growth rate and can die. It can cause the severe disease state argulosis in a wide variety of fish species. It is responsible for epizootic outbreaks that have led to the collapse of aquaculture operations.	It is sometimes called the common fish louse and is common and widespread crustacean ectoparasite in marine, brackish, and freshwater environments. It affects a variety of hosts species. All life stages of both sexes are parasitic. It attaches to its host, usually a fish, via its suction cups, pierces the skin with its sharp stylet, and feeds on blood. It may live in the gills. The common fish louse is also a vector for pathogens, introducing organisms such as bacteria, flagellates, and the virus that causes spring viraemia of carp. It is an intermediate host to nematodes of the family Skrjabillanidae.	Treatment: Organophosphates given as 2 or 3 doses at 1-wk intervals to kill emerging larvae and juveniles. Salt (NaCl), formaldehyde, potassium permanganate, formalin and trichlorfon.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Ectoparasites				
Monogeneans				
(Crustacea)	**Caligus**	Sea lice cause physical and enzymatic damage at their sites of attachment and feeding, producing abrasion-like lesions varying in severity depending on host species, age, and general health of the fish. Sea-lice infection causes chronic stress and reduced immunity by: changing the mucous consistency, damaging the epithelium causing loss of blood, fluids/electrolyte changes, and cortisol release leading to disease susceptibility and poor growth rates.	Intense farming infestations can spread to wild populations of fish. A sea louse (plural sea lice) are marine ectoparasites that feed on the mucous, epidermal tissue, and blood of host marine fish and can be host specific. The copepodid stage is the infectious stage and it searches for an appropriate host, likely by chemo- and mechanosensory clues. Currents, salinity, light, and other factors also assist copepodites in finding a host. Preferred settlement on the fish occurs in areas with the least hydrodynamic disturbance, particularly the fins and other protected areas.	Treatment: Freshwater baths, pyrethroids (Cypermethrin (Excis, Betamax) and deltamethrin (Alphamax)). Resistance to pyrethroids has been noted. Bathing fish with hydrogen peroxide (350–500 mg/L for 20 min) will remove mobile sea lice from fish but can be toxic to some fish. In-feed treatments such as avermectins and emamectin benzoate. Cleaner fish are used in some fish farms in Norway and other northern hemisphere countries.
(Crustacea)	**Lepeoptherius (Sea lice)**	Small white-grey skin erosion patches around the head, base of dorsal fins and perianal region. Heavy infestations can cause mortality especially in smolts post transfer to sea water cages. It is the most economically important parasite in salmon aquaculture systems.	Flat fish ectoparasite. It feeds on the mucous, skin, and blood of the fish, with egg-producing females infecting the pectoral and pelvic fins of the host, while immature individuals and males are found on the rest of the body.	Treatment: Organophosphates, pyrethroids, emamectin benzoate and hydrogen peroxide. Sea lice develop resistance to the same treatments being used repeatedly.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Ectoparasites				
Monogeneans				
(Crustacea)	**Lernaea (anchor worms)**	Frequent rubbing or "flashing" Localised redness/inflammation on the fish's body. Tiny white-green or red worms in wounds. Breathing difficulties & general lethargy.	Parasites of freshwater fish.	Treatment: Potassium permanganate as a tank treatment or a "dip". Salt dip, a formalin dip, and modern antiparasitic may help. Salt in the aquarium at 1 to 2 tablespoons may help prevent secondary infections. Manual removal of the whole parasite (including the head).
(Molluscs)	**Glochidia**	Lethargy, altered behaviour and reduced expression of sexual traits including poor sperm production. Dysfunction of liver, kidneys and gills. Can cause mortality in cases of heavy infestation.	Glochidia is the name of the larval stage of mussels and clams. They are a temporary parasitic inhabitant of fish. Some molluscs can lure fish close by exposing a piece of their mantle in the water and dose the fish with spawn when it strikes. Glochidia then encyst in the gill epithelium and grow, before dropping off within 10-30 days. In this manner the mussel can extend its range using the fish for transportation. Glochidia can resemble trematode metacercarial cysts.	Surviving fish hosts develops acquired immunity once affected.
Monogenean trematodes (Flukes)	***Diplostomum spathaceum* (eye flukes)**	Blindness and cataracts. Red localised patches on skin, fins or gills during early stages. Heavy infestation can cause obstruction of lamellar blood vessels and death from hypoxia. Poor growth and death due to inability to find food.	Zoonotic potential via skin or mouth during cleaning of tanks. *D. spathaceum* cercariae penetrate fish via gills and fins, migrate to the eye lens, vitreous or retina where the metacercaria stage develop and live for up to 2 years causing blindness through parasitic cataract, keratoglobulins, herniation and tumour formation. Birds eat the infected fish where the adult stage develop in the bird's gut. Eggs are laid, released from the bird hatch into miracidia stage, infest the intermediate host (mollusc snails) and release cercariae back into the water.	Treatment: Molluscides such as unchelated copper at night when snails are most active. Treat fish with praziquantel at 50mg/kg fish weight orally. Remove fish from ponds/tanks prior to treatment.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Ectoparasites				
Monogeneans				
Monogenean trematodes (Flukes)	**Benedinia**	Lethargy, swim near the surface, have clamped fins, seek the corners of aquaria or the sides of the pond, and have diminished appetite. Rubbing the bottom or sides (flashing) of the tank. Scale loss may occur where the monogeneans are attached, and the skin may vary in colour where the parasites have fed. Heavy gill infestations result in respiratory disease, swollen and pale gills, increased respiratory rate & piping (gulping air at the water surface). Fish will be less tolerant of low-oxygen conditions. Heavy infestations of the skin or gills can cause mortality especially in farmed fish. Secondary bacterial or water mould infections are common on tissue that has been damaged by monogeneans. In marine fish, the capsalid monogeneans may infest the skin, eyes, and gills, resulting in extreme irritation to the host. Grey patches and open wounds may appear on the skin and the eyes may be swollen and appear cloudy. Sharks pull sand into their gills to rid the parasites.	Captive fish held in crowded tanks allows hatching monogeneans to easily find host fish, along with aggressive behaviour by tankmates, poor nutrition, and poor water quality impacting on the ability of the fish's immune system to respond to the presence of the parasites. Monogeneans found on wild fish seldom cause disease or death in free-ranging populations unless natural factors lower their immune system. Parasitic flatworms are common and browse on the fish's body feeding on mucous and epithelial cells of the skin and gills; however, a few adult monogeneans will remain permanently attached to a single site on the host. Some monogenean species invade the rectal cavity, ureter, body cavity, and even the blood vascular system. They are found on fish in fresh and saltwater and in a wide range of water temperatures. Transmission of monogeneans from fish to fish is primarily by direct contact. Monogeneans have a direct life cycle, which means that no intermediate host is required for the parasite to reproduce.	Treatment: Dipping fish in fresh or saltwater, depending on the fish species for 10 minutes. (less useful for euryhaline fish species which are often tolerant of varying salinity, so they are not as likely to be affected by this method). Monitor fish closely. The sticky eggs may however persist. Other treatment: praziquantel under veterinary direction in separate tank. Dissolve powdered form in alcohol prior to use as per instructions. Potassium permanganate, formalin, hydrogen peroxide, copper can also be used but have side effects for some species.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Amoeba)	**Thecamoeba**	Attach to the gill lamellae and cause mucous build-up on the gills of infected fish and hyper-plastic lesions, causing white spots and eventual deterioration of the gill tissue leading to respiratory distress and heart failure.	Ambient water temps above 16^{o}C (more common in summer), overcrowding and poor water circulation are contributing factors to the disease.	Treatment: Freshwater bath.
(Ciliate)	**Brooklynella**	Loss of appetite, skin lesions, sloughing off excessive body mucous, heavy respiration/gasping at surface for oxygen, body rubbing. Mortality occurs around 30 hours after infections has been identified.	Brooklynella is a ciliate parasite with a direct life cycle: It lives, feeds and reproduces directly on fish (no encysted stage). Horizontal transmission possible through water. Protozoa targets skin and gills. Outbreaks of *Brooklynella* are correlated with environmental stress due to trauma, high concentrations of ammonia, elevated temperatures, overcrowding, etc.	Treatment: Formalin, malachite green and hyposalinity baths.
(Ciliate)	**Chilodonella**	Lethargy, mottled grey skin due to increased mucous production, gill lesions, scale loss, slow swimming near the surface/edges of the tank, slim/malnourished appearance. Many fish die when infestations become moderate.	Histopathology: shows ciliates attacking the gills and skin causing gill epithelial hypertrophy, hyperplasia and lamellar fusion. This is followed by lymphocytic infiltration leading to necrosis and mild oedema. Lymphocytic dermatitis is also noted.	Treatment: Formalin, malachite green and hyposalinity baths.
(Ciliate)	***Cryptocaryon irritans and Icthyopthirius multifiliis*) Ich or white spot.**	Fish rub or flash due to the irritation. Skin ulceration and erosion noted. Respiratory problems are noted when gills are infected. White spots appear later on skin and gills causing severe gill and skin damage leading to osmotic stress. Mortality of up to 100%.	Common in freshwater species and aquarium marine fish. Brought in by infected new stock. Outbreaks occur at 15-24^{o}C, with a rapid life cycle in 3-6 days (slower in cooler water temps). Non-zoonotic. Protozoan ciliate on gills & skin forming trophonts (white spots). Infective tomite stage - reinfects fish while in water. Rapid multiplication results in large numbers of free tomont being released into the water where they are free swimming.	Treatment: Salt at 2ppt for *I. multifiliis* continuous bath for 7 days at 24-26^{o}C (up to 6 weeks at cooler water temps). Freshwater for 3-15 mins or hyposalinity bath for *C. irritans*. Reduce salinity by 5-10ppt/day until <16ppt. Return to normal salinity after 3 weeks. Formalin baths are also an option. Repeat treatments are essential to catch all stages.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Ciliate)	**Scyphidians**	Affects the gills and skin. Colonies of it may extend out of the gills to the edge of the operculum and into the buccal cavity. Ulcerative lesions of the skin are noted.	Commonly on fish from static ponds with water high in organic matter and containing large number of the aquatic bacteria on which they feed.	Treatment: Formalin, malachite green and hyposalinity baths.
(Ciliate)	**Trichodina**	Inappetence, malnutrition. Weakened fish become susceptible to opportunistic bacterial pathogens in the water leading to secondary infections.	Trichodina is one of the most common ciliates present on the skin and gills of pond-reared fish. Low numbers (less than five organisms per low power field) are not harmful, but when fish are crowded or stressed, and water quality deteriorates, the parasite multiplies rapidly and causes serious damage.	Treatment: Formalin, malachite green and hyposalinity baths.
(Ciliate)	**Tetrahymena**	Tetrahymena on the body surface in low numbers (less than five organisms per low power field) is probably not significant. Skin shows white spots and epidermal damage.	Tetrahymena is a protozoan commonly found living in organic debris at the bottom of a freshwater aquarium or tank. Tetrahymena is a teardrop-shaped ciliate that moves along the outside of the host. It is commonly found on dead material and is associated with high organic loads. Infections are often associated with poor environmental conditions, concurrent disease, or an immunocompromised fish host. Gross pathology: Tetrahymena can invade skin, muscle, and internal organs, causing extensive necrotic changes.	Treatment: Formalin, malachite green and hyposalinity baths.
(Flagellates)	**Ichtyobodo**	Lethargy, “flashing”, listlessness, loss of appetite, the grey/blue layer on the skin is a result of cellular destruction and excessive mucus production. Massive infections on skin and gills can cause epithelial hyperplasia or hypertrophy and may result in severe or fatal osmoregulatory or respiratory problems.	Worldwide infections in freshwater and marine fish. The flagellates spread rapidly between hosts in fish farms, by both direct contact or through free-swimming parasites. Young fish are most prone to the disease and have a higher mortality rate.	Treatment: Salt and formalin baths.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Flagellates)	**Crytobia**	*C. branchialis*, lives on the skin or gills. It can deform the skin and cause anorexia and death. *C. iubilans*, an endoparasite that lives in the intestines and causes granulomatous inflammation of the abdominal organs, resulting in weight loss and death. *C. salmositica*, *C. borreli*, and *C. bullocki*, are blood parasites that lead to anaemia and lesions in the haematopoietic tissues.	There are 52 species of *Cryptobia* known from fish. 40 of these live in the blood, 7 in the gut, and 5 on the body surface. In fish, the disease is most important in salmonids. Marine and freshwater fish can be infected. These protozoans can be found on most continents. Blood feeding leeches are implicated in the transmission of the bloodborne species. Morbidity and mortality rates are linked to husbandry standards and the presence of bacteria and other parasites.	Treatment: Isometamidium chloride, dimetridazole bath treatments with (80 mg/L for 24 hours, repeated daily for 3 days), 2-amino-5-nitrothiazol (10 mg/L for 24 hours, repeated daily for 3 days) has been effective. Control: There is a vaccine against *C. salmositica* which lasts up to 2 years.
(Flagellates)	**Ooddinium (Velvet disease - also called gold-dust, rust and coral disease)**	Dusty, brownish-gold colour. Flashing, rubbing and darting behaviour. Lethargy and clamp their fins very close to their body. If untreated, a 'dusting' of particles (which are in fact the parasites) will be seen all over the infected fish, ranging in colour from brown to gold to green. In the most advanced stages, fish will have difficulty breathing, will often refuse food, and will eventually die of hypoxia due to necrosis of their gill tissue. Rapid death of the whole tank is common in untreated fish.	The disease occurs most commonly in tropical fish, and to a lesser extent, marine aquaria. Spread by contaminated tanks, fish, and tools (such as nets or testing supplies). Rarely, can be spread by the fish eating frozen live foods (such as bloodworms) containing dormant forms of the species. Frequently, however, the parasite is endemic to a fish, and only causes a noticeable "outbreak" after the fish's immune system is compromised for some other reason. The disease is highly contagious and can prove fatal to fish.	Treatment: Salt/freshwater baths suppress the reproduction of the parasite but does not eradicate the disease. Other treatments include: copper sulphate, methylene blue, formalin, malachite green, acriflavin, Chloroquine phospate/ pyrimethamine, metronidazole and chloroquine. Additionally, because velvet parasites derive a portion of their energy from photosynthesis, leaving a tank in total darkness for seven days provides a helpful supplement to chemical curatives and raising water temperatures as long as the zone of comfort is within the normal levels for the species of fish.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Flagellates)	**Trypanosomiasis**	Anaemia due to haemolysis with pale gills. Damage to haematopoietic tissues. Lethargy, emaciation, splenomegaly. Mortality (>60%) in younger fish.	Uncommon in cultured fish but common in wild fish of cold fresh or marine water areas. *Trypanosoma*, which includes 184 species in fishes. No zoonosis recorded in fish trypanosome infections unlike other species of the parasite. Caused by a haemflagellate called Trypanosoma. Requires leeches for its life cycle who feed on the fish. 3 days post feeding small trypomastigotes appear in the fish's blood and continue to grow. Takes 62 days at 0-1^{o}C or 42 days at 4-6^{o}C to complete parasitic development.	Treatment: Trichlorphon 2ppm bath for 60 minutes. Isometamidium chloride (trypanocidal drug) used at 1mg/kg IM for *C. salmositica* and pyrimethamine (antimalarial drug) effective against *Cryptobia spp.*
Endoparasites				
Metazoan species				
(Acanthocephala)	**Pomphorhynchus Thorny-headed worms, or spiny-headed worms**	Found as adults in the intestine of fish. Juvenile worms of many other species occur in the viscera, especially the mesentery and liver, of fish that act as paratenic hosts. Not generally regarded as an economically important disease but they have been linked to local extinction of natural populations. There is little knowledge of the more subtle effects the parasite has on host populations, except underdeveloped musculature and disproportionately large heads suggesting malnutrition, but mortality might be significant when hosts are stressed.	Freshwater and marine water fish worldwide. *Acanthocephala spp*. have an eversible proboscis, armed with spines, which it uses to pierce and hold the gut wall of its host causing mucosal tissue damage which extends into the muscle tissues of the gut leading to perforation and poor food absorption. Acanthocephalans have complex life cycles, involving at least two hosts, which may include invertebrates, fish, amphibians, birds, and mammals. Gross pathology: Fibrotic nodules on the intestinal surface. Some discoloured, enlarged or inflamed viscera. Pyloric caeca is larger than normal. Acanthocephalans occasionally perforate the intestinal wall and protrude into the coelom or attach their proboscides to another organ.	Treatment: Fenbendazole.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Endoparasites				
Metazoan species				
(Cestodes)	**Diphyllobothrium Fish tapeworm" or "broad tapeworm"**	No clinical signs in most cases.	Zoonotic: Infects humans who consume raw fish and considered the most important fish-borne zoonosis. The tapeworm can reach up to 12 metres in the human body where it absorbs food from the human gut. Freshwater fish in cooler climates serve as the second intermediate hosts of broad tapeworms; they become infected after consuming copepods with procercoids.	Treatment: Fenbendazole.
(Cestodes)	**Ligula**	Emaciation, anaemia, discoloration of the scales and susceptibility to secondary infection. Infections sterilises the fish and reduces normal growth.	The tapeworm *Ligula intestinalis* occurs in the body cavity of its cyprinid second intermediate host. The mechanism by which infected fish are prevented from reproducing is unknown but is presumed to act upon the brain–pituitary–gonadal axis of the fish to inhibit further development of reproductive organs.	Treatment: Fenbendazole, praziquantel, control copepods in tanks.
(Nematodes)	**Anisakis**	Deformed body shape, haemorrhage, mortality, traumatic enteritis, loss of balance through damage to their swim bladder, reduced swimming performance, lethargy, reduced sexual display rate, ulceration of gill cover, fraying of fins, large nodules on the ventral surface of the skin and fish can be seen swimming or floating on their sides. Infected fish can be more susceptible to decreased oxygen content in the water. Severe disease can arise when a nematode colonises a new host species.	Final definitive hosts of these nematodes are marine mammals following a complex life cycle. These nematode parasites use different crustaceans/fish species as intermediate or paratenic hosts. Humans are accidental hosts. Freshwater, marine and brackish water fish species. Gross pathology: Damage to the mucosa & submucosa by nematode migration and proteolytic damage from nematode enzymes. Larger parasites can cause deformation of organs and body shape, mesenteric and visceral adhesions, granulomas, haemorrhage, deep nodules within the stomach wall and general inflammation.	Treatment: Fenbendazole, praziquantel, control copepods in tanks.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Endoparasites				
Metazoan species				
(Nematodes)	**Camallanus**	Red or pink worm protruding from the anus. And retract again when fish start moving. Fish may become listless, bloated, inappetent leading to emaciation and have decreased brood stock production. Faeces are white/clear/pale or mucous in appearance. However, fish can be infected but not show any clinical signs.	Freshwater or marine wild caught fish. Juvenile fish are more likely to show clinical signs and have reduced growth rates. Gross pathology: Nodules may be noted in the skin and muscle from encysted parasites. The parasite can penetrate deep into the intestinal wall, and into the circular muscle layer. The attachment site shows flask-shaped ulcers in the mucosal and submucosal layers. Granulomatous tissue surrounds these ulcers along with, extensive fibrosis at the surface (the fibrosis probably reduces the loss of body fluids and blood cells from the intestinal wall).	Treatment: Fenbendazole, levamisole. or piperazine for all fish because once you see this worm, likely the other fish have it and are in various stages of worm infestation. Treat all new fish in a quarantine tank over the course of 4 weeks before introducing them to your main tank, because Humane killing of the infected fish may be more humane to prevent months of suffering.
(Nematodes)	**Contracaecum**	Fish eat well, but are emaciated, brood stock production declines overtime and juveniles have stunted growth. Nematodes infect other tissues/organs resulting in signs related to the organ system affected and degree of damage (mainly muscle, liver, and tissues surrounding the internal organs). Visible signs of infection include haemorrhaging, external lumps or nodules, inflammation, necrosis and cysts or granulomas.	Life cycle involves invertebrates, freshwater fish and finally fish-eating birds or mammals. 3rd stage larvae usually found in the body cavity, branchial chambers, and mesenteries of the fish. Nematodes migrate within the body of a fish causing Worm tracks/tunnels can be noted throughout affected tissue as larvae migrate causing physical damage to the fish. Nematodes are usually considered as the most economically important helminth parasites of fish of the world. Zoonotic through eating raw fish.	Treatment: Fenbendazole and levamisole for gut infections. Surgical removal is the only way to rid fish of internal worms that are not found in the intestine. Control: Reduce level of invertebrates in tanks/ponds and prevent contact with fish eating birds.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Endoparasites				
Metazoan species				
(Trematodes)	**Clinostomum** **Yellow grub disease**	Metacercaria are large, often yellow and encyst in a variety of sites in the body such as the oral cavity, gills, intestines, tail, muscles and eye sockets of fish or amphibians. When present, the parasites are highly visible to the unaided eye after skinning or filleting because of their size and colour. The unpleasant appearance of the fish is unappealing to the consumer, resulting in discard of carcasses during inspection and economic losses in fish farming.	*Clinostomum marginatum* is a species of parasitic fluke (class Trematoda). It is commonly called the "yellow grub". It is found in many freshwater fish. It is also found in frogs and some species can also be found in the mouth and oesophagus of aquatic birds such as herons and egrets. Eggs of these trematodes are shed in the faeces of aquatic birds and released into water. Aquatic birds become hosts of this parasite by ingesting infected freshwater fish. The metacercaria are found right beneath the skin or in the muscles of host fish and are surrounded with the connective tissue. Infiltration of inflammatory cells with mild haemorrhage in some muscular fibres as well as hyaline degeneration, indicating myositis. Zoonotic through humans eating raw fish.	Treatment: None available. Control invertebrates in tanks/ponds and prevent contact with fish eating birds.
(Trematodes)	**Cryptocotyle**	The fish surrounds the raised cystic nodules (usually less than 1mm in diameter) in the skin with black pigmented melanin in response to the foreign organism. The black spots are often visible to the naked eye. High levels of this parasitic infection will kill the fish host.	The parasite has a complicated life cycle with several hosts: snail, fish, and, finally, a bird or a mammal. These worms are present in both fresh and marine fish. Tissue sections reveal a thick, fibrinous capsule around the encysted metacercaria with the periphery of the capsule containing numerous melanocytes. Zoonotic through humans eating raw fish.	Treatment: None available. Control invertebrates in tanks/ponds and prevent contact with fish eating birds.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Endoparasites				
Metazoan species				
(Trematodes)	**Diplostomum (eye flukes)**	Exophthalmia, local haemorrhage, lens cataract, thickening or complete destruction of the lens, reduced fish growth, emaciation and deformities of the vertebral column. Fish become blind, which appear as fish becoming dark, unable to feed, losing condition and having increased susceptibility to predation. Acute mortality with haemorrhages, resembling a serious bacterial infection are signs of the cercarial syndrome form of the disease. Metacercariae may be visible in the fish's eye.	The parasite has a complicated life cycle with several hosts: snail, fish, and, finally, a fish-eating bird. Metacercariae of *Diplostomum* spp. in the eye tend to be site specific, restricted to the lens, vitreous humour or retina. The cercariae mostly penetrate through the flank of the fish, often along the lateral line and they migrate through the tissues to the eye.	Treatment: None available. Control invertebrates in tanks/ponds and prevent contact with fish eating birds.
(Trematodes)	**Nanophyetus (Fish flu)**	Cysts or sites of irritation from penetration of the cercariae through the scales and muscle layers. 'Grain of salt' appearance in the organs such as kidneys.	The lifecycle involves snails which release free-living cercariae that penetrate the skin of salmon, lose their tails, and become metacercariae. These develop in a number of tissues of the salmon with heavy concentrations in the kidneys, liver, heart, and tail. The life cycle is completed when the adult trematode develops in the intestine of mammals or birds that eat fish. Zoonotic by eating raw fish (Salmon sp.). The parasite is most known for its association with "salmon poisoning disease", which, left untreated, is fatal to dogs and other canids. The canids are affected by the *Neorickettsia helminthoeca* bacteria, for which *N. salmincola* acts as a vector, and not by the parasite itself.	Treatment: None available. Control invertebrates in tanks/ponds and prevent contact with fish eating birds. Take precautions when handling fish (prevent hand-to-mouth transmission of the metacercariae and be wary of hand contamination from heavily infected fish).

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Coccidia)	**Eimeria**	Clinical signs depend on target organ affected but may include: general malaise, poor reproductive capacity, and chronic weight loss.	Intracellular organisms of the epithelium (of the gut, gall bladder, swim bladder and kidney tubules) and tissues (liver) in marine and freshwater fish which is most prevalent in spring. Transmission via predation and necrophagy seems to be the route of transmission not only for extraintestinal coccidia but for endogenously sporulating intestinal coccidia, while still in their host gut tissue or in the intestinal lumen. Gross pathology: Excess of mucoid material within the gastrointestinal tract, sloughed intestinal epithelial cells, loss of intestinal surface due to the large numbers of parasites in an epicellular position, and multifocal areas of intestinal necrosis associated with sloughing of parasites are found on post mortem.	Treatment: Coccidiostats or coccidiocides such as sulphonamides, nitrofurazone, or amprolium.
(Coccidia)	**Goussia**	Water influx (due to environment barriers being damaged) - entering across all permeable surfaces like gill lamellae and oral mucosa, or by ion losses across these membranes causing a dilution of bodily fluids. The weak clinical symptoms of this coccidiosis might be explained by the rapid repair of destroyed tissue and the absence of dehydration in freshwater fishes. Compared to coccidia of birds and mammals, the tissue damage caused by *G. carpelli* indicates high pathogenic capacity.	Mainly freshwater fish as hosts, with some members of *Goussia* parasitising fish which swim in brackish waters. The parasites reside in the cranial gastrointestinal tract (mainly) of the host, and also in the gallbladder & liver. During feeding, some water is swallowed and enters the intestinal tract. Loss of intestinal epithelium surface cells (during the merogonic and gamogonic development stage), causes more of this ingested water to be absorbed. Enterocytes harbouring *G. carpelli* oocysts migrate into subepithelial tissue. Phago-cytic activity was seen in all granulocyte types in the gut.	Treatment: Coccidiostats or coccidiocides such as sulphonamides, nitrofurazone, or amprolium.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Flagellates)	**Hexamita**	Weight loss/emaciation, anorexia and weakness where the fish appears slender with sunken abdomen and the head is enlarged. Skin becomes darker in colour; gills appear pale with hyperplasia. Exophthalmia. Anaemia and yellowing of intestinal contents. Watery/jelly-like with excessive mucous and minimal faecal matter. There will be a decreased egg hatchability and death of young fry. In severe cases, fish lie horizontally and have distended abdomens. Morbidity rate is 100% and mortality rate can be 100% in young fish without therapy.	Transmission is through the water within faecal matter. Confounding factors for the disease include stress from malnutrition, transportation, overcrowding and poor water quality. Gross pathology: Swollen liver with petechial haemorrhages, ascites with blood clots in the visceral cavity. Enlarged spleens, kidneys and gall bladders.	Treatment: Metronidazole oral or bath.
(Flagellates)	**Trypanoplasma (Cryptobia)**	Anaemia, anorexia, splenomegaly, general oedema and abdominal distension with ascites. Metabolic function and swimming performance are reduced. Fish are susceptible to hypoxia and their immune system is depressed during acute cryptobiosis.	Freshwater or marine fish species. Transmitted by the freshwater leech.	Treatment: Trypanocidal drugs such as isometamidium chloride for both treatment and prophylactic therapy. Control: Attenuated live vaccination.
(Flagellates)	**Trypanosoma**	Most infected fish are asymptomatic, but with heavy burdens symptoms include anaemia, leucocytosis, hypoglycaemia and splenomegaly.	Freshwater and marine fish are affected. Aquatic leeches are both hosts and vectors of fish.	Treatment: None available. Control leeches in tanks.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Microsporidia)	**Glugea**	As the disease progresses, muscle cells are destroyed and replaced by connective tissue, resulting in an emaciated or concave appearance and skeletal deformities. Subcutaneous cysts are also noted. **The most common pathogen found in zebrafish research facilities.**	Freshwater or marine water fish. Microsporidia are obligate intracellular eukaryotic parasites. The infectious spore stage has a thick, chitinous endospore, making it very resistant to environmental stress and lysis, allowing the organism to maintain viability for extended periods in the aquatic environment. Fish are exposed to the parasitic spores by consumption of an infected host or spores in the water column.	Treatment: Toltrazuril for 6 treatments and albendazole. Note: the use of chlorine to disinfect eggs is not very effective.
(Microsporidia)	**Heterosporis**	Destruction and eventual widespread necrosis of the host skeletal muscles. Important microsporidian disease worldwide, impacting wild & farmed raised fishes (marine & freshwater environments).	**Common in research laboratories with zebra fish due to close proximity of animals within the tank.** Fish are exposed to the parasitic spores by consumption of an infected host or spores in the water column.	Treatment: None available.
(Microsporidia)	**Pleistophora.** **It is the causative agent of 'Neon Tetra disease'.**	Infections in the skeletal muscle, with no involvement of smooth or cardiac muscle, brain tissue and visceral organs.	Transmission initiated by ingestion of spores. Spores released from dead fish are a likely source of infection, but could also be released from the intestines, skin or urinary tract of infected fish.	Treatment: None available.
(Myxozoa)	**Ceratomyxa**	In fish, the main mucosal sites include the intestine, skin and gills. While most myxozoan are not highly pathogenic for their fish hosts, some cause severe disease and many others cause economic losses in aquaculture as a result of decreased fish growth, fecundity or flesh quality.	Myxozoan are abundant parasites in nature. Their life cycles involve two hosts: an invertebrate, (e.g., annelid), and a vertebrate, usually a fish. Direct fish to fish transmission via ingestion of infected tissue and exposure to contaminated water or individuals shedding spores has been demonstrated in marine species; affecting wild & aquaculture fish populations. The parasite infects intestinal tissues, and although it can cause problems in culture facilities with unprotected water supplies, it is largely a problem of free-ranging salmonids	Recently the finding of a specific mucosal immunoglobulin in teleost (IgT), analogous to mammalian IgA, and the capacity of fish to develop a specific mucosal immune response against different pathogens, has highlighted the importance of studying immune responses at mucosal sites.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant parasitic diseases				
Protozoan species				
(Myxozoa)	**Henneguya (Proliferative gill disease)**	Gills have branchial inflammation, epithelial hyperplasia, lamellar fusion, and lysis of chondrocytes, leading to suffocation causing high mortality.	Gross pathology: Lytic areas in the cartilage of the primary lamellae on microscopic examination and is confirmed histologically by the presence of the organism.	Treatment: None available.
(Myxozoa)	**Hexacapsula Hoferellus Sphaerospora Thellohanellus**	Most myxozoans are not highly pathogenic for their fish hosts, some cause severe disease and many others cause economic losses in aquaculture as a result of decreased fish growth, fecundity or flesh quality (soft flesh).	Life cycles involve two hosts: an invertebrate (an annelid), and a vertebrate, usually a fish. They parasitise and damage in multiple organs, including mucosal tissues, which they use also as portals of entry (Stomach, intestine, buccal cavity, skin and gills). Species inhabiting kidney tubules are excreted in urine, those is scale pockets slough from skin from living fish. Spores that form in the brain, cartilage, muscle and visceral organs are released into the environment after the death of their host. Once released the spores sink to encounter their benthic invertebrate hosts or are filtered out of the water column by filter-feeding invertebrate hosts.	Treatment: None available.
(Myxozoa)	**Kudoa (soft flesh)**	During the fish's life it seems to have few clinical signs. *Kudoa* sp. produce enzymes after their host dies that cause muscle breakdown, making fillets soft and watery (soft flesh).	Life cycle as above. Gross pathology: fish muscle with obvious white cysts, known as post-mortem muscle liquefaction.	Treatment: None available.
(Myxozoa)	***Tetracapsuloides bryosalmonae* Proliferative kidney disease**	The cause of proliferative kidney disease, also has a broad distribution, infecting wild and cultured salmonids mainly in Europe and North America. Anaemic at the late stage of the disease.	Occurs mostly during higher water temperatures. Gross pathology: Kidneys are enlarged into swollen, greyish, bulbous ridges, splenomegaly, bloody ascites, and pale gills (anaemia). Histolopathology: Haematopoietic hyperplasia and diffuse/chronic inflammation. The parasites may be surrounded by coalescing whorls of inflammatory cells.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable bacterial diseases				
***Aeromonas salmonicida*-atypical strains**	**Goldfish ulcerative disease, Carp erythrodermatitis, Ulcer disease of flounder, eel or salmon.**	Blood filled furuncles which turn into deep tissue ulcerations. Lethargic/abnormal swimming and disorientation. Loss of appetite. Increased mortality. Atypical strains affects mainly non-salmonids (wild and cultured, marine and freshwater). Infection with 'Atypical A. salmonicida' does not necessarily result in the acute mortality and septicaemia that are characteristic of the typical furunculosis strain, but manifests more as external lesions and ulceration, often involving secondary infection. A new atypical strain that has recently been described in Australia, *A. salmonicida biovar Acheron,* causes Marine Aeromonad disease of Salmonids (MAS) in Atlantic salmon.	Transmission occurs horizontally (between fish via the water). Susceptibility to the disease increases with damaged mucous and skin, which occurs when fish are handled with nets. Outbreaks occur at water temperatures above 10°C (i.e. summer months in Australia) and are be precipitated by stress (i.e. handling, overpopulation and rapid temperature fluctuations). Secondary infection with other bacteria results. Fish that survive disease outbreaks are recognised as carriers of the disease and may continue to infect the remaining population without themselves exhibiting signs of infection. Gross pathology: white raised patches on skin progressing to ragged-edged red ulcers, haemorrhages on the skin 7 fin bases (usually the paired fins), fingernail-sized ulcers found anywhere on the fish but most often on the upper side of the lateral line behind the head or at the base of the tail fin, pale gills with petechial (pinpoint) haemorrhages, intestinal protrusion through the abdominal wall following severe ulceration, haemorrhages in muscle and internal organs, swollen kidneys and spleen. Microscopic pathological: hyperplasia of the gills, containing bacterial colonies, ulcerated areas that show oedema, hyperaemia, leukocyte infiltration and degenerative changes. Hyperaemia & haemorrhage in spleen and kidneys. Fibroblast-like cells, which may produce granulomas in the dermis, spleen and kidneys.	Treatment: Antibiotics have been used but resistance issues limit their efficacy. Control: Vaccine available. Tank cleaning/disinfecting will help prevent infections including: Desiccation (drying out) temperatures above 50°C for over 2 minutes, 2 mg/L chlorine for 1 minute 300 mg/L benzalkonium chloride for 2 minutes 2.6 mg/L iodine for 5 minutes 0.5% Virkon S for 10 minutes UV light >6 mJ/cm2 or 0.5 mg/L/min ozone.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable bacterial diseases				
(*Edwardsiella ictalurid*)	**Enteric septicaemia of catfish**	Meningio-encephalitis causing intermittent listlessness then chaotic swimming. Swellings on the head due to erosion of the connective tissues which ulcerates exposing brain tissues ('hole in the head disease'). Very important infectious disease in commercial fishing in many countries. Also found in ornamental fish.	Limited temp zone of 18-28°C with disease outbreaks mainly in spring and autumn. Two forms of the disease occur: Chronic encephalitis where bacteria enters olfactory system and migrates to the brain causing granulomatous inflammation. Acute septicaemia of the intestines and other internal organs.	Treatment: Oxytetracycline, sulfadimethoxine-ormetoprim (some resistance issues) or florfenicol, as antibiotic feeds.
(*Renibacterium salmoninarum*)	**Bacterial kidney disease (BKD)**	BKD is a slow, progressive and frequently fatal infection of cultured and wild salmonids in both fresh and marine waters. Lethargy, pale gills, exophthalmos, haemorrhagic lesions around the vent, on skin (blisters/spawning rash), fins and in musculature. Muscular cystic cavities also visible. Swollen abdomen Important: Animals with this disease may show one or more of these signs, but the pathogen may still be present in the absence of any signs. Advanced infection becomes apparent only after the first year of the fish's life. Other non-salmonid species have been demonstrated susceptible to infection with *R. salmoninarum*, but only when raised in proximity to highly infected salmonids.	The bacterium is transmitted both horizontally (between fish via the water) and vertically (parent to offspring via eggs). The causative bacterium is likely to persist only within salmonids and not in the environment. Gross pathological: Creamy-white, granulomatous, nodular lesions in the kidney and sometimes in the liver and spleen, which may be encapsulated, ascites (fluid in the abdominal cavity), haemorrhages on the abdominal wall and in the viscera, diffuse, white membranous layer on one or more internal organs, splenomegaly, Microscopic pathology: focal or diffuse granulomatous reaction in the kidneys, liver and spleen, small, rod-shaped bacteria (*Renibacterium salmoninarum*) in histological sections of skin lesions.	Treatment: Erythromycin and Oxytetracycline antibiotics injections or oxytetracycline orally given to fry and broodstock. Note: Erythromycin resistance is increasing. Surface disinfection of eggs does not prevent vertical transmission. Control: An intraperitoneal vaccine is also available and can be used as a treatment in the face of an outbreak of BKD for fish >10g in weight.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable bacterial diseases				
Rickettsia/chlamydias	**(*Piscirickettiosis salmonis)***	Multiple white to haemorrhagic skin-muscle ulcers. Causes a systemic and chronic infection in all ages of fish mainly in sea cage farming. However, acute mortality cases are possible with minimal clinical signs. Mainly affects salmonids but can affect other species. Mortality in some salmon species of 30-90%.	Non zoonotic. Gross pathology: anaemia, multifocal liver lesions, ascites and petechial haemorrhages. Liver lesions may rupture resulting in cavities and damage to vascular integrity.	Treatment: Oxolinic acid antibiotic. Note: It will not eradicate the disease totally.
***Yersinia ruckeri*-(Hagerman strain)**	**Enteric redmouth disease**	Primarily found in rainbow trout and other cultured salmonids. The disease is characterized by subcutaneous haemorrhaging that presents as reddening of the throat, mouth, gill tips, and fins, and eventual erosion of the jaw and palate. Exophthalmia and darkening of the skin are also common signs. The fish often demonstrate abnormal behaviour and anorexia. Mortality rates can be high. It is most commonly seen in fish farms with poor water quality.	*Y. ruckeri* infections transmitted by direct contact between fish. The bacteria can then be released when the carrier fish become stressed. Shedding of the bacterium in the faeces is also likely to play an important role in transmission, and *Y. ruckeri* can survive at least 4 months outside the host. Gross Pathology: Petechial haemorrhages on the surfaces of the liver, pancreas, pyloric caeca, swim bladder and in the lateral muscles. The spleen is enlarged and almost black in colour. The lower intestine can become reddened and filled with an opaque, yellowish fluid. Histopathology: Gills show hyperaemia, oedema and desquamation of the epithelial cells in the secondary lamellae have been described. Focal areas of necrosis can be present in the spleen, kidney and liver. In the kidney, degenerated renal tubules, glomerular nephritis and a marked increase in melano-macrophages may be observed.	Treatment: Amoxicillin; oxolonic acid; oxytetracycline; sulphadiazine in combination with trimethroprim and florfenicol antibiotics. (Resistance issues have been noted, therefore culture and sensitivity testing must be carried out prior to treatment). Control: Vaccines available for prevention. Some success has been demonstrated for the use of probiotics to fight *Y. ruckeri*: oral administration of *Bacillus subtilis* and *Bacillus licheniformis* protects rainbow trout against subsequent infections.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Aeromoniasis	**(*Motile aeromonad septicaemia*-MAS, *Aeromonas hydrophila)***	'Bruise' like skin lesions progressing to the loss of scales and dermal tissue, exposing underlying muscle or cartilage. Secondary invasion of the ulcer by bacteria/fungi and generalised erythema of the skin suggesting progression to systemic disease. Superficial ulcers are a primary feature of disease in koi and any internal lesions are a secondary effect following bacterial invasion infection. The severity of the disease varies but significant mortality may occur.	Disease in koi may be influenced by environmental stressors including: overcrowding, trauma, transport, temperature fluctuation and poor water quality. Zoonotic: Humans infected with *Aeromonas spp*. may show a variety of clinical signs but the commonest syndromes are gastroenteritis and localised wound infections from contamination of skin lesions.	Treatment: Debridement of the lesion and antibiotic medication. Bacterial resistance is common therefore bacterial sensitivity tests must be performed. (Initial injectable antibiotic, then in-feed treatments). Baths or dips are ineffective/have adverse environmental effects. Efficacy depends on the stage of the disease, bacterial resistance and overall condition of the fish. Control: Vaccine is available.
Botulism	**(*Clostridium botulinum* type E)**	Botulism has been recognized as a major cause of fish mortality. Initial clinical signs of disease are loss of colour and hyperventilation of the gills, followed by sluggish random swimming. As a result of muscular paralysis and loss of equilibrium, the fish float on the surface, swimming on their backs and swimming into the sides of the fish tanks. Eventually they would sink until death with signs of extended gill covers.	Clostridium botulinum, an anaerobic spore-forming gram-positive bacillus with toxic spores are among the most powerful neuroparalytic poisons known. Type E botulism toxin is produced by bacterium that naturally persist as harmless spores in the marine and freshwater sediments of wetlands, rivers, and lakes. The bacterium produces the toxin when there is a rich nutrient source (i.e. dead animal), low water levels with pH of 7.5 to 9.0, a lack of oxygen & an opt temp (usually warmer >20^{o}C). Spores in soil are mobilized by heavy rain and dust carried away by wind. Once established, a botulism outbreak is self-perpetuating. The toxin then enters the aquatic food chain, affecting fish that eat molluscs or other fish. Fish are carriers of *C. botulinum*, but botulism outbreaks in fish populations may lead to death on a large scale.	Treatment: Antibiotics are available, but are often too late once clinical signs are noted.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Edwardsiellosis	**(*E. tarda* infection/*Edwardsiella* septicaemia** **Fish gangrene** **Emphysematous putrefactive disease of catfish** **Red disease of eels**	All life stages of fish are affected by *E. tarda* and haemorrhaging of the body cavity, muscle, and organs including liver and kidneys are commonly seen. General behavioural changes include: loss of balance, bursts of abnormal activity, and increased food consumption. The skin that covers muscle abscesses may be pale or petechial. Morbidity rate from 5-70%. Mortality may be acute, but in most cases is chronic depending on how stressful the environmental conditions are for the fish.	*Edwardsiella tarda* infects freshwater and marine fishes, reptiles and amphibians and mammals throughout the world. *E. tarda* resides in the intestine of fish and other aquatic animals and in the mud of ponds/waterways. Transmission is through infected water and mud from carrier animal faeces, and most probably infect susceptible fish through trauma of the epithelium or via the intestines. The infection is enhanced by water temperatures of 20-30°C. Zoonotic: In humans it causes diarrhoea and gastroenteritis. Extraintestinal infections produce typhoid-like illness, peritonitis with sepsis, cellulitis and meningitis. Humans can get infected with *E. tarda* by eating infected fish meat. Gross pathology: Kidneys and spleen show necrotic white/grey lesions on the surface of the organs. Adult fish can have organomegaly, pale inflamed gills, exophthalmia and cataracts, haemorrhagic red lesions (ecchymosis) on the skin and fins, erosion of the skin, systemic oedema and ascites. The anal region of certain species can become swollen and hyperaemic and rectal prolapses can occur. Microscopic pathology: Suppurative interstitial nephritis. Small abscesses which enlarge and liquefy, spreading bacteria to surrounding tissues and vessels, causing ulceration of the dermis and emboli and infecting the spleen, liver, epicardium, stomach, gill and musculature. Hepatitis with micro-abscesses.	Treatment: Oxytetracycline, sulfadimethoxine or methoprim. The latter two can cause cessation of feeding in some fish species. There is some antibiotic resistant strains, so culture and sensitivity testing is necessary. Resistant strains can be treated with the addition of oxalinic acid or miloxacin in their feed. Control; Vaccination is available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Flexibacteriosis	**(*Flexibacter columnaris, Tenacibaculum maritimum, Flavobacteria spp.)*** **Saddle back disease** **Fin and tail rot** **Columnaris infection**	Tail and fin rots with red line moving up the body. Whitish areas of skin which ulcerate such as areas over the saddle area of the dorsal fin. Gills have yellowish mucous. Bacteria eat away at the tail and fins of fish which ulcerate, and the fish become septic. Common in ornamental fish. Disease is rapid (24 hrs).	Outbreaks occur in stressed fish (overhandled or diseased). Mortality occurs more when water temps are >15^{o}C. Mineral content of the water (e.g. hardness 33-70mg/L $CaCO_{3)}$ promotes the disease. Low D.O., high nitrite, physical injury such as net damage, overstocking, uneaten feed all favour outbreaks. No zoonosis known. Common disease of the skin, gills and tail of freshwater fish. F. columnaris (Freshwater) and *Tenacibaculum maritimum* (marine) live in aquatic environments and become an opportunistic pathogen.	Skin infection treatment: Salt bath 10-30ppt for 30 minutes depending on species tolerance. Benzalkonium chloride bath at 1-2mg/L for one hour with aeration. Systemic infection treatment; Oxytetracycline 55-83mg/kg fish/day in the feed for 10 days or 10-100mg/L bath for 1-3 days. Withholding periods apply. Control: good management practises are a better way of reducing this disease.
Mycobacteriosis	**(*M. fortuitum, M. chelonae, M. marinum*)**	Emaciated, poor growth rates, deformities due to granulomas, skin ulcers. Most common chronic disease (weeks to months) found in aquarium fish for both fresh and marine species.	Ubiquitous bacteria in water, from contaminated sediment and can last up to 2 years. Bacteria are released from infected fish, amphibians and reptiles. Feeding of infected trash fish and skin abrasions all promote the disease. Bacteria is shed from infected skin ulcers and cannibalism of dead fish is the main pathway for infection. Zoonotic. Signs includes: Localised non-healing ulcers ('fish tank/swimming pool granulomas'), which are difficult to heal due to antibiotic resistance. Serious in immune suppressed people. Gross pathology: White granulomatous nodules in internal organs such as kidney, spleen and liver.	Treatment: Antibiotic resistance is an issue. Control: This bacterium is very resistant to antibacterial cleaning agents including chlorine bleach and quaternary ammonium compounds but is sensitive to alcohol at 60-85%.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Norcardiosis	***(N. asteroids, N. kampachi)***	Affects both freshwater and marine fish species and may develop undetected for months in juvenile fish prior to clinical presentation. Clinical signs: 'flashing/ rubbing flanks on the tanks' sides, skin lesions varying from pits 2mm diameter in the opercular region and elsewhere, to raised granulomatous lesions up to 30mm diameter on ventral surfaces of the fish. Fish with advanced lesions are lethargic, poor performers, emaciated, have poor feed conversion rates and eventually die.	Transmission is through fry eating uncooked/unpasteurised fish tissue (live, raw or frozen) or horizontal transmission from sick fish. Possibly also through leaf matter from local tree cover. Histopathology: white -yellow granulomas 1-2mm in size in the spleen, kidney and liver. If fish mount an immune response, the spots heal and form hard black spots. Microbiology: thread-like, beaded and branching acid fast (pink) gram stained bacterium grown on Lowenstein Jensen agar isolates the bacteria take 4-10 days at 25-35o^{C}.	Treatment: Antibiotics. Control: Vaccination research is underway.
Pasteurellosis	**(*Photobacterium damsela)***	A bacterial disease affecting wild and farm fish, responsible for economic losses in cultured fish worldwide due to its wide host range, high mortality rate, and ubiquitous distribution. Acute form: minimal clinical signs. Chronic form: Anorexia, darkening of the skin, focused necrosis of the gills, reddened operculum and enlarged spleen 7 kidney are the only external clinical signs often observed. Outbreaks on fish farms are explosive and are characterized by sudden reduction in feeding response and rapid onset of mortality.	Transmission: Horizontal transmission from fish to fish within a culture unit and carriers under stressful conditions could suffer from reinfection. Outbreaks of photobacteriosis are correlated with water temperatures of 18-25^{o}C (spring and autumn) and salinities of 5-25 ppt. Below this temperature, fish can harbour the pathogen as subclinical infection and become carriers for long periods Histopathology: Chronic form- Creamy-white granulomatous nodules or whitish tubercules in several internal organs, composed of masses of bacterial cells, epithelial cells, and fibroblasts. The nodules in internal viscera, particularly kidney and spleen, with widespread internal necrosis and septicaemia.	Treatment: Oxytetracycline, sulfadimethoxine with an ormetoprim potentiator (Romet® 30), oxolinic acid, ampicillin, amoxicillin and florfenicol medicated feeds) have been used in fish aquaculture to control photobacteriosis outbreaks, but after only a few years the pathogen acquired resistance to various antibiotics. Control: Vaccination available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Pseudomoniasis	***(P. fluorescens, P. anguilliseptica) (red spot disease, sekiton-byo disease.***	The bacteria affect a variety of fish species cultured in marine and brackish waters. Clinical signs: typical septic condition with petechial haemorrhages in the skin of the ventral side of the body, mouth and the area around the vent. Affected rainbow trout also show haemorrhages at the fin bases. Some species become lethargic and have mainly eye lesions and fin erosion.	Histopathology: petechial haemorrhages in the skin, peritoneum, adipose tissue and liver. A granulomatous inflammation of connective tissues surrounding the skeleton/cartilage of the head region, oedematous lesions in the liver tissue with cloudy swelling of liver cells and focal necrotic areas. Oedematous changes are also seen in kidney glomeruli and tubuli with detachment of tubular epithelium	Treatment: Ampicillin or trimethoprim/sulfamethoxazole gives a good response. Control: Vaccination is not commercially available.
Rickettsia/chlamydias	**(*Epitheliocystis microcystis)***	Clinical signs: First signs include haemorrhages and lesions of the skin. The lesions range from small areas to shallow ulcers up to 2 cm in diameter. In acute cases, death may be the only gross sign of disease. Moribund fish collect at the water surface along the edges of the sea cages. They are lethargic, dark, and show loss of appetite, with pale gills. Haematocrits are frequently 25% or less.	Transmission is thought to be horizontal but it has not been confirmed. Gross pathology: Internally, the kidneys are swollen and the spleen enlarged. Petechial haemorrhages are found on the swim bladder and viscera. Diagnostic ring-shaped, cream-colored lesions are present on the livers of chronically infected fish. Piscirickettiosis produces marked pathologic changes in most internal organs of infected fish, where severe changes occur in the intestine, kidney, liver, and spleen. Histopathology: Necrosis and inflammation may occur throughout the body, especially in cells adjacent to blood vessels. Epithelial hyperplasia results in lamellar fusion of the gills. The bacterium is commonly observed within macrophages and in the cytoplasm of infected host cells.	Treatment; Antibiotic levels may not reach sufficient concentrations within the host cells in vivo, to terminate replication of the pathogen. Control: No vaccines available as yet.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Streptococcosis	**(*S. iniae, Lactococcus garviae, Enterococcus seriolida*)**	Popeyes, cloudy/reddened eyes. Haemorrhage of fin bases, anal area, body ulceration. Common in both freshwater and marine water species especially barramundi.	Transmission occurs where there is overcrowding, water temps above 20°C, a change of feed, increased handling and transportation and poor husbandry. Rapid spread in culture systems. The bacteria can survive 6 months in infected frozen fish diets. Found in aquatic environments and sediment. Potential zoonotic disease. Gross pathology: Internal haemorrhagic disease (Septicaemia). Blood congestion and necrosis of the liver, spleen, intestines and kidney.	Treatment: Culture and sensitivity required to identify the correct type of antibiotic to use due to current resistance issues with this bacterium. Options: erythromycin, doxycycline, lincomycin. Control: Autonomous vaccination used in Australia and commercial vaccine is also available.
Vibriosis	**(*V. anguillarum, V. alginolyticus, V. vulnificus, V. parahemolyticus*)**	Darkening of fish, anaemia, ulceration and haemorrhage of skin, eye ulceration, exophthalmos and corneal oedema. Sudden death. Considered the most significant bacterial disease of marine aquaculture affecting the sea cage industry. High mortality (>50%) in juveniles and chronic debilitation in older fish.	Vibrio is ubiquitous in aquatic environments in warm or cool water temps, so act as opportunistic pathogens. High water temps (20-28°C) increase risk as does high salinity, organic pollution, husbandry stresses such as high stocking density, handling and transportation, environmental changes (bad weather) in warm weather species. Cold water vibriosis occurs at 2-5°C and is associated with poor hygiene, lack of water exchange. Damage to the integument/intestines allows the bacteria to rapidly colonise, releasing proteases causing necrotising dermomyositis and septicaemia. Zoonosis is associated with this bacterium causing gastroenteritis, wound infections and potential septicaemia. Gross pathology: Necrosis of the liver, spleen, kidney, intestine and heart. Visceral petaechiation also noted. Chronic disease presents as granulomatous muscle lesions and eye lesions.	Treatment: Antibiotic resistance is an issue in this disease. Control: Vaccination in juvenile fish by injection or bath.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant bacterial diseases				
Yersinosis	**Enteric redmouth-(ERM)**	Clinical signs vary with fish age with a more chronic condition in older/larger fish. Disease outbreaks start with low level mortalities that are sustained over time, resulting in high cumulative stock losses. Fish behavioural changes include: swimming near the surface, lethargic movements and loss of appetite. Other signs include: exophthalmia and darkening of the skin, subcutaneous haemorrhages in and around the mouth and throat, which give the disease its common name. This is a serious septicaemic bacterial disease affecting a wide host range with a broad geographical distribution, causing significant economic losses in the fish aquaculture industry.	Transmission: By direct contact. A carrier state where infectious fish can survive 2 months carrying the bacteria in their intestines can also occur prior to the bacteria being released if the carrier fish become stressed. Shedding of the bacterium in the faeces is also likely to play an important role in transmission, and *Y. ruckeri* can survive at least 4 months outside the host. The bacterium enters the fish via the secondary gill lamellae and from there it spreads to the blood and internal organs. Affects all ages of fish but it is most acute in young fish (fry and fingerlings). Histopathology: Gram-negative, rod-shaped, facultative anaerobes *causing* general septicaemia and focal necrosis with inflammation in most organs. Also areas with petechial haemorrhage, hyperaemia, oedema and desquamation of the epithelial cells in the secondary lamella. In the kidney, degenerated renal tubules, glomerular nephritis and a marked increase in melano-macrophages may be observed	Treatment: Amoxicillin; oxolinic acid; oxytetracycline; sulphadiazine in combination with trimethoprim and florfenicol. This narrow range of options may facilitate the emergence of antibiotic resistance. Screening of *Y. ruckeri* isolates has shown that only 1 out of 50 was resistant to florfenicol. Control: Vaccine available. The use of probiotics to fight *Y. ruckeri by* oral administration of *Bacillus subtilis* and *Bacillus licheniformis* may protect rainbow trout against subsequent infections.
Notifiable fungal diseases				
Aphanomyces invadens	**Epizootic ulcerative syndrome**	Increasing mortalities from severe ulcers and damage to the skin and muscles followed by secondary bacterial infection. Bleeding lesions or spots which deepen into raw ulcers exposing muscles and underlying tissues. Major cause of fish loss in fish culture systems. Secondary bacterial disease raise the mortality rates.	Intensive stocking leading to skin abrasions, poor water quality with high organic wastes, cooler water temps which delay healing or damage skin and depress the immune system. Acidic waters e.g. acid sulphate soils favour infection. The fungus is widespread in both freshwater and estuarine fish habitats and invade the skin causing ulceration and necrosis. Spores are very resistant in the environment. Non-zoonotic	Treatment: Maintain continuous salt baths 5-10ppt until ulcers heal in freshwater species. May take weeks. Treat secondary bacterial infections. Control: Remove moribund fish as they remain a source of spores.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant fungal diseases				
Branchiomyces sp.	**Gill rot.**	*Branchiomyces sp.* primarily infect the gill tissue of fish so preventing oxygen take up. Fish appear lethargic and may be seen gulping air at the water surface. Damaged gills appear marbled with pale areas instead of uniformly bright red. High mortalities are often associated with this type of infection.	Transmission: As the dead tissue from the affected gills dies and falls off, the spores are released into the water and transmitted to other fish. Gill rot is caused by the fungi *B. sanguinis* and *B. demigrans.* Associated with environmental stressors such as low pH (5.8 to 6.5), low dissolved oxygen, or a high algal bloom. *Branchiomyces sp.* grow at temperatures between 13.9-35°C but grow best between 25-32°C.	Treatment: Formalin and Copper sulphate have been used to help stop deaths.
Exophila sp.		It has low host specificity and is abundant in harsh environments like soil contaminated with heavy metals. It affects freshwater and seawater fish. Clinical signs: abnormal swimming behaviours, depression and darkening of skin. Black nodules form on the skin, and gills. Mortalities more common in cooler water 11–15°C. It can cause deadly infections in Atlantic salmon where the hyphae invade the brain causing chronic inflammation.	A mesophilic and saprotrophic black yeast and member of the dark septate endophytes which is found in marine and soil environments. *E. pisciphila* forms symbiotic relationships with various plants by colonizing on roots, conferring resistance to drought and heavy metal stress. Transmission: An opportunistic pathogen causing infections in captive fish. Captive fish are especially susceptible due to the confined space of aquariums and accumulation of fungal particles. Decorative pieces, stones or contaminated food in aquariums can all be reservoirs of *E. pisciphila*. Ideal growth conditions for *E. pisciphila* occur between 20–30 °C , where maximum growth occurs at 37 °C. This differentiates it from *E. jeanselmei* which otherwise has similar physiology. Histopathology: Fungal hyphae are extensively distributed in musculature and internal organs forming nodule like lesions. Multifocal chronic inflammatory lesions displaces structures in all affected organs.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant fungal diseases				
Icthyophonus hoferi		Fungal disease of both freshwater and marine fish. Sand-paper skin, emaciation and body deformity. Causes disease and mortality in several species.	Life cycle of fungus involves a spherical, double walled resting stage (spore) in the infected tissue germination and hyphae formation occurs by herniation through the wall. Transmission: Ingestion of infected raw fishes or fish products or other food transmits disease, skin abrasions or damaged gills, carriers through faecal discharge, and direct contact. Non-zoonotic. Histopathology: Causes granuloma formation in internal viscera (grey white nodules).	Treatment: None available.
Phoma sp.		Abnormal swimming behaviour (swim on one side or in a vertical position with tail down or may rest on one side at the bottom of the rearing container), exophthalmia, multiple rounded areas of muscle softening, protruded haemorrhagic vents, and abdominal swelling. Fish may also exhibit haemorrhage of the caudal fin and/ or petechial haemorrhages on the lateral and ventral body surfaces. Those fish that are infected will eventually die. In natural infections, cumulative mortality is generally low (<2-5%) but can be up to 20%.	Transmission: It is a weakly infectious facultative fish pathogen that likely invades either by entrance of conidia or hyphae into the air bladder via the pneumatic duct connecting the oesophagus, or by entering with food into the lower gastrointestinal tract where the primary focus of infection may develop. Therefore, transmission of infectious stages is suspected to be oral with food or with gulping air to inflate the air bladder. Histopathology: swim bladders were filled with whitish creamy viscous fungal mass, surrounded by dark red areas in swim bladder walls, kidneys, and musculature. The fungus infection is characterized by mycelial invasion of the air bladder and/ or digestive tract. The fungus invades other organs becoming systemic resulting in gut obstruction and peritonitis.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant fungal diseases				
Saprolegnia sp.	**Saprolegniasis**	Kills fish eggs. Cotton wool-like growths on abraded skin and gills or fish eggs. Fish can be completely covered with the fungus.	Widespread disease in freshwater environments. Increasing organic waste in the culture environment, overcrowding, organically enriched waters in colder months. Rapid spread of skin and gill infection causing mortalities in days through abraded skin lesions. Non-zoonotic. Histopathology: Superficial necrosis of tissues leading to osmotic imbalance and death. Accumulation of organic matter in the fungal matt (E.g. green with algae or brown with sediment).	Treatment: Salt bath 5ppt continuously and treat secondary bacterial infections. Note: Hard to treat once established.
Notifiable viral diseases				
Aquabirnavirus	**Infectious Pancreatic Necrosis (IPN) and other non-notifiable Aqaubirnaviruses: Milkfish, Striped bass, Common carp, Loach irnaviruses, Japanese eel virus, European eel virus, Yellowtail ascites virus**	Progressive mortality increasing each day in faster growing fish. Darkening of pigmentation, pronounced distended abdomen, corkscrewing/spiral swimming. Highly contagious disease of young salmon within intensive rearing conditions. Viral strain, quantity host and the environment affect the mortality rate of 10-90%. Subclinical disease can be present in estuarine and freshwater fish species.	Young fish are more susceptible but smolts can be affected as they transition from fresh to seawater. Higher mortality in warmer water. Fish can be carriers for 6 years. No zoonosis. Histopathology: Pancreas, stomach and oesophagus become ulcerated and haemorrhagic. Viral particles found in cytoplasm of infected cells. Intestine are empty or full of mucous and causes necrosis of other organs such as kidney, GIT and liver.	Control: Vaccines available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Herpesvirus	**Channel Catfish virus disease**	Is highly virulent among young naïve populations of cultured channel catfish – usually up to about 6 months of age. Initially inappetence, then convulsive erratic swimming including a "head-up" posture, lethargy, exophthalmia, distended abdomen, and haemorrhages at the base of the fins and ventral abdomen. Mortality can approach 100% in outbreaks in higher water temperatures. Survivors may lose weight initially but then become carriers.	Overstocking and poor water quality predispose farmed fish populations to outbreaks of disease. Transmission: Horizontally and vertically from brood fish to young fish and eggs. The virus is shed via urine, and virus entry is through the gills. The incubation period can be as short as 3 days. Most outbreaks occur in the summer months at higher water temperatures (e.g. 30°C). Histopathology: Haemorrhaging of the musculature, liver and kidneys, dark and enlarged spleen, fish include yellow/red-tinged fluid in the peritoneum, pale, enlarged kidneys, which may be the only internal indication of disease in infected fish. Microscopic lesions are characterised by oedema, and severe and generalised necrosis of the haemopoietic tissues of the kidney and spleen. Necrosis and haemorrhage also occur in the liver and digestive tract.	Treatment: None available. Control: No vaccine available.
Herpesvirus	[E] **Infection with koi herpesvirus**	Lethargy, disorientation, gasping. Extensive gill necrosis and haemorrhage. Skin haemorrhage and excess mucous. Sunken eyes and congestion of fins. Severe mortality in koi & common carp (80-100% in 2-4 days).	Outbreaks in water 17-28^{o}C. Adults are more susceptible. Survivors become carriers until virus is reactivated up to 30 weeks post exposure where water temp is >20oC causing disease and shedding. Commonly transmitted by transportation to fish shows around the world. No zoonosis.	Control: Vaccination by co-habitation using water temperature manipulation to prevent the outbreak of disease in the young naïve fish.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Herpesvirus	***Oncorhyncus masou* virus disease**	Mainly in salmon and trout at 1 month of age. Oedema and haemorrhages and some ulcerative skin lesions. Virus multiplication in endothelial cells of blood capillaries, haematopoietic tissue and hepatocytes underlies the clinical signs. Some have no clinical signs. Survivors are persistently infected with virus and retain the virus until maturation where it is shed via the sexual products at the time of spawning.	Transmission is horizontal and possibly 'egg-surface associated'. Horizontal transmission may be direct or vectorial (other fish species, parasitic invertebrates and piscivorous birds and mammals). Infectious virus is shed via faeces, urine, sexual products and probably skin mucus, while the kidney, spleen, liver and tumours are the sites where virus is the most abundant during the course of overt infection. Gross pathology: Internally, intestinal haemorrhage and white spots on the liver were observed.	Treatment: Virus is susceptible to ultraviolet irradiation, ozone or iodophor treatment.
Irridovirus	**Epizootic Haematopoietic Necrosis (EHNV) (European catfish virus) (ECN)**	Clinical signs can be non-specific and vary between species. In perch, sudden death is common with darkened body surface, ataxia, lethargy & erythema around the nostrils and brain region. Haemorrhages may occur in the gills and at the base of the fins. Suspect EHNV in redfin perch if an epidemic is characterised by sudden high mortality (regardless of husbandry conditions) and histological evidence of necrosis in the renal hematopoietic tissue, spleen and liver. In Trout - darkening of the body surface, lethargy, inappetence, abdominal distension and loss of equilibrium. Less fish are generally affected in outbreaks and it is associated with poor husbandry.	Oral, gill or skin transmission may occur, as can fomite transmission. Birds act as mechanical vectors, where the virus can survive in their gut for a few hours and they shed the virus in regurgitated food, on their feathers, feet and bill. EHNV can remain infective for more than 97 days in the water and for at least 113 days in dried fish tissues. It can also survive for more than 300 days in cell cultures at 4°C and for two years in fish tissues stored at –20°C. The incubation period is 3-10 days in water temperatures of 19-21°C. No zoonosis. Gross pathology: Necrosis and swelling of the liver, hematopoietic tissues within the kidney. Haemorrhages at the base of the fins and focal haemorrhages in the gills. Spleen is swollen/pale or shrunken. Petechiae on the viscera. Multiple white to yellow areas of focal necrosis are sometimes found in the liver.	Treatment: None available. Control: Vaccines are not available for any species.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Iridovirus	**Grouper iridoviral disease**	Weakness, floaters, darker colour. Die with mouth open. High mortality in sea cage facilities especially in older larger & juvenile fish. Survivors are carriers and shedders.	Horizontal transmission associated with post transport stress and recent stocking. No zoonosis. Histopathology: Necrosis of haematopoietic tissues, liver, heart, kidney and gill hyperplasia due to enlarged/hypertrophied basophilic cells containing cytoplasmic virions.	Treatment: None available.
Iridovirus	**Red sea bream iridoviral disease**	Animals with this disease may show one or more of these signs, but the pathogen may still be present in the absence of any signs. Lethargy, obvious opercular movement (increased respiratory effort), severe anaemia, petechiae of the gills, and splenomegaly. Wide host range. Low to high mortality (O-100%)	Highly contagious with juveniles more susceptible than adults. Horizontal transmission through the water column from other infected fish. Vertical transmission has yet to be confirmed. Mortality can depend on water temperature, with higher mortalities occurring at higher water temperatures. Outbreaks of disease occur at water temperatures greater than 20°C, with viral multiplication increasing with water temperatures up to at least 28°C. The virus is stable within tissue to –80°C, and can be inactivated by ether, chloroform and formalin. Gross pathology: dark skin (change in skin colour is a significant gross sign,) petechial (pinpoint) haemorrhage of the gills, pale gills and enlarged spleen. Histopathology: enlarged cells, deeply giemsa positive, in the spleen, heart, kidney, liver, intestine and gills of infected fish, which are characteristic of this disease. Small dark spots within fresh wet mounts of gill lamellae (melano-macrophage centres).	Treatment: None available Control: Vaccine is available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Iridovirus	**White sturgeon iridoviral disease**	Erratic or slow swimming. The virus infects the skin, gills and upper alimentary tract and fish stop feeding when the olfactory organ epithelium and oral mucosa are infected, leading to emaciation; dying 2-3 weeks later. Haemorrhages on the abdomen and the ventral scuta may be present along with swollen and slightly pale gills. Mortality of up to 95% has been reported in hatchery aged fish increasing when secondary infections with external protozoa or bacteria contribute to the overall illness.	Transmission is by contaminated water, contaminated equipment and infected fish. Wide host range. No zoonosis. Histopathology: Focal to diffuse hyperplasia with characteristic amphophilic to basophilic enlarged Malpighian cells is evident on stained tissue sections of the integument.	Treatment: None available. Control: No commercial vaccine available.
Nodavirus	***Viral encephalopathy and retinopathy**	Erratic corkscrew swimming pattern, loss of balance. Colour changes. Emaciated. Acute mortalities in many hatchery sized marine fish species.	Vertical transmission in water from infected fish. Native fish species very susceptible. No zoonosis. Histopathology: Vacuolative encephalopathy, retinopathy & myelopathy in fry.	Treatment: None available. Control: No commercial vaccine available.
Orthomyxovirus	**Infectious salmon anaemia**	Clinical signs show 2-4 weeks post infection including: Pale gills and lethargy. Affects salmon, trout and eel (northern hemisphere). Mortality rate 2-50%.	Horizontal transmission via blood, mucous, faeces, urine, equipment and nosocomial spread. Presence of sea lice increases risk greatly within 5 km radius of an infected farm as does high density stocking. No zoonosis. Histopathology: Severe anaemia, liver congestion, splenomegaly, exophthalmia, visceral petechiae and leukopaenia.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Rhabdovirus	**Infectious haematopoietic necrosis (IHNV)**	Mainly in salmonid sp. Lethargy interspersed with bouts of frenzied, abnormal activity (flashing and spiral swimming), darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages internally and externally. A trailing faecal cast is observed in some species. Spinal deformities are present among some of the surviving fish. Virus multiplication in endothelial cells of blood capillaries, haematopoietic tissues, and cells of the kidney underlies the impairment of osmotic balance causing oedema and haemorrhage. Mortality is common especially in stressed fish with bacterial co-infections. Acute outbreaks cause daily deaths and cumulative mortality may reach 90–95%. In chronic cases, losses are protracted and fish in various stages of disease can be observed in a facility.	Horizontal transmission by direct exposure &by invertebrate vectors. Water temp plays an important part of the transmission, with temps between of 8°C and 15°C causing most clinical signs. Virus is shed via urine, sexual fluids and external mucous, whereas kidney, spleen and other internal organs are the sites in which virus is most abundant during the course of overt infection. Virus entry is through the gills & the bases of fins. Fry are the most susceptible stage, with older fish being more resistant, but susceptibility varies between fish species. However, once they reach spawning, they become susceptible again and shed large amounts of virus. The virus will survive in fresh water for at least 1 month at cooler temps, especially if organic material is present. Gross pathology: Darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages internally and externally. Internally, fish appear anaemic and lack food in the gut. The liver, kidney and spleen are pale. Blood sampling of fry: Reduced haematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large amounts of cellular debris. Histopathology: Degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract. Necrosis of eosinophilic granular cells in the intestinal wall is pathognomonic of infection with IHNV.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Rhabdovirus	**Viral haemorrhagic septicaemia (VHSV)**	Initial clinical signs are non-specific: lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins, gills, eyes and skin, and a distended abdomen due to oedema in the peritoneal cavity. However, a rapid onset to mortality (which can reach up to 100% in fry) can occur. In the chronic state of infection, affected fish do not generally exhibit external signs. VHSV can also occur in a nervous form, characterised by severe abnormal swimming behaviour, such as constant flashing and/or spiralling. In septic stages of the disease, the virus is abundant in all tissues including skin and muscles. Target organs are kidney, heart and spleen as these are the sites in which virus is most abundant. In chronic stages, virus titres can become high in the brain. Survivors become carriers. Younger fish are more susceptible.	Affects a wide range of fish species (freshwater and marine) and at temperatures ranging from 2 to 20°C (commonly 4°C and 14°C). Infected fish are the reservoirs. Piscivorous birds acts as external mechanical vectors. Transmission is horizontal through contact with other infected fish or contaminated water, etc. Virus is shed from infected fish via the urine and reproductive fluids. Incubation time is dependent on temperature and dose; it is 5–12 days at higher temperatures. The virus survives for longer periods at 4°C (up to 1 year) in freshwater especially if organic matter is available but in seawater the virus can be inactivated within 4 days. Freezing fish and then thawing does not completely kill the virus. Gross pathology: Generalised petechial haemorrhaging in the skin, muscle tissue (especially in dorsal muscles) and internal organs. Petechial bleeding in the dorsal musculature is a very common sign of VHSV infection. The kidney is dark red in the acute phase but can demonstrate severe necrosis in moribund fish. Swollen spleen. Pale mottled liver. The gastrointestinal tract, especially the hind gut, is pale and devoid of food Blood sampling; The red blood cell level is very low in the acute phase of VHSV and the blood appears light red and transparent.	Treatment: none available. Control: No vaccine available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Notifiable viral diseases				
Rhabdovirus	**Spring viraemia of carp**	Dark colour, exophthalmia, pale gills, ascites, mucoid faecal cast trails from protruding and inflamed vent. Lethargy and weakness. Petechiae and ecchymoses in gills and skin. High mortality in various species of carp in Europe, Asia and Middle east.	Young fish show severe disease. High fatality in water <10°C. Survival rate increases as water is >18°C because immunity strengthens. Gross pathology: Oedema of internal organs, peritonitis and enteritis. Histopathology: Virus replicates in liver, kidney and spleen.	Control: Vaccination available in endemic areas with live-attenuated or inactivated viral vaccines.
Significant viral diseases				
Adenovirus	**Cod, Dab & Sturgeon adenoviruses**	Epidermal hyperplasia and tumours.	Sturgeon adenovirus with mortalities in epizootics associated with flavobacterial infection. No zoonosis. Histopathology: Tumours seen in epidermal hyperplasia without mucous cells.	Treatment: None available.
Herpesvirus **(Note: there are also many more types of known fish herpesvirus)**	**Anguillid (Eel herpesvirus)**	Lethargic and swim near the surface or the water's edge. Reddened fins and a mottled appearance to the skin. The main damage caused by this virus is to the gills, with severe necrosis (cell death) and loss of normal gill structure. The latter changes are often seen in combination with bacterial and fungal infection. The internal organs can also be affected with inflammation and further necrosis, leading to organ failure, debilitation and death. Mortality ranges from 1 to 10%.	A warm water virus that is most active between 10°C and 26°C. Disease is usually triggered by underlying stress from: poor water quality, high stocking levels and barriers to migration. Gross pathology: Haemorrhages in the skin, fins, and gills, congested gill epithelium, and pale liver.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Herpesvirus	***Goldfish herpesvirus haematopoietic necrosis**	Listless fish stay on the bottom of tank/pond. No external clinical signs. Severe epizootics causing mass acute mortality in goldfish.	Disease transmits via importation of ornamental fish trade from carrier fish to naïve fish. Affects any aged fish during at water temperatures of 15-25^{o}C. Less severe in water temps >25^{o}C. No zoonosis.	Treatment: None available.
Herpesvirus	***H. cyprini (Type 1, 2, 3)*** **(Carp pox virus)**	Type 1 -Benign papillomatous lesions in the epithelium of common carp, Adults- infected fish show no behavioral or clinical signs. Juveniles- show clinical disease and high mortality. Infected juvenile carp develop appetite loss, distended abdomens, exophthalmia, darkened skin pigmentation and haemorrhages on the operculum and abdomen. Type 2 - causes herpesvirus haematopoietic necrosis in goldfish (*Carassius auratus*). The affected fish present no characteristic external signs except for apathy and pale gills. 50–100% mortality. Type 3 - Severe epizootic disease and mass mortality among *C. carpio*, causing large economic losses in carp industries worldwide. Affected fish exhibit apathy, gill epithelium necrosis, pale patches on the skin, pale and irregularly coloured gills, increased mucous production, and exophthalmia.	Type 1 -The disease is seasonal (water temperatures less than 15°C, and it regresses when water temperature increases). Histopathology; type 1 is present in nervous and subcutaneous tissues after disease regression, suggesting that the virus becomes latent, which might explain the recurrence of lesions. Type 2 – Disease outbreaks depend on water temperature and occur between 15–25°C Histopathology: shows discoloration and necrosis in the spleen and kidney, and hypertrophy and hyperplasia in the gill epithelium Type 3 - Outbreaks appear seasonally when the water temperature is 18–28°C . Fry are more susceptible to infection than mature fish, but larvae appear resistant. Horizontal transmission through fish excrement into the water allows virus penetration of the fish's skin. Birds may also be mechanical vectors.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Herpesvirus	***Pilchard herpesvirus**	Fish die rapidly following respiratory distress due to hypoxaemia and hypercapnia. Gill lesions are focal progressing to generalised over 4 days. It has caused several massive epizootic events in Australia. Estimates of mortality rates ranged as high as 75%.	Latent infections may periodically breakdown in individuals, resulting in clinical disease. It is believed that the virus is now endemic in Australia, either in a latent form or as a cause of unrecognized low-grade disease in an immune population. Gross pathology: Lesions in the gills and comprised acute to subacute inflammation followed by epithelial hypertrophy and hyperplasia.	Treatment: None available
Herpesvirus	**Salmonid: Type I, Type II (Oncorhynchus masou virus) & Type III herpesvirus**	Type 1- Mild disease, with few clinical signs. It causes darkened pigmentation, apathy, pale gills, exophthalmia & distended abdomens. Type 2 – Large economic losses of farmed and wild salmonid sp. in Japan. Clinical signs depend on the fish's age. Juveniles: Acute infection with apathy, exophthalmia, skin ulcers, external haemorrhages, hepatic necrosis and up to 100% mortality. Age four months-1 year post infection: 12–100% of surviving fish develop tumours, located around the mouth and head. Type 3 –Non-specific clinical signs: Lethargy interspersed with periods of hyperexcitability, spiral swimming, epithelial hyperplasia, haemorrhages in the eyes, mouth, base of the fins. Acute disease up to 100% mortality in juveniles at 6-15^{o}C.	Type I- Disease outbreaks occur when the water temperature is 10 °C or less. Adult fish shed the virus in ovarian fluids. Type II- Horizontal transmission through the water and vertically via ovarian fluids. Type III– Horizontal transmission in colder water temperatures by contact. Survivors become long term carriers. Histopathology: Hyperplasia, hypertrophy, and necrosis of epidermal cells.	Treatment: None available

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Herpesvirus	**White-Sturgeon, Angelfish, Smooth Dogfish & Japanese Flounder herpesviruses, Acipenserid herpesvirus 1**	Clinical signs can be minimal even in dead fish. Mortality rate 35%.	Histopathology: Epidermal lesions and diffused dermatitis.	Treatment: None available.
Iridovirus (Note: many types of species specific iridoviruses in fish)	***[E] Gourami & Murray cod iridovirus**	Often no clinical signs even in dead fish.	The international trade of juvenile fish for food and ornamental aquaculture has aided the spread of these viruses. Horizontal transmission in water to naïve fish.	Treatment: None available.
Iridovirus	**Large-mouth bass iridovirus**	Minimal clinical signs unless wild caught bass are kept in captivity, where mass mortality can occur. Other signs: Lethargy and swimming near the surface, then a loss of equilibrium and floating in a laterally recumbent position. The swim bladder may be reddened or overinflated.	Horizontal transmission but most infected fish do not show signs of disease.	Treatment: none available.
Iridovirus	***Lymphocystis**	Infection of abraded skin with nodular appearance that may coalesce and appear tumour-like. Affects many species of fish (Not salmon, catfish or cyprinids). Low mortality but body disfigurements are noted.	It is the most common viral infection of aquarium fish and stressed fish are most susceptible (E.g. after transportation). Long incubation of weeks to months. No zoonosis. Histopathology: Virus affects dermal fibroblasts leading to hypertrophy of cells with a nodular appearance.	Treatment: Most lesions self-resolve. Treat secondary infections when nodules slough off.
Iridovirus	**Viral erythrocytic necrosis (originally termed 'Piscine erythrocytic necrosis')**	Affects red blood cells of many species of marine and anadromous fishes causing a severe anaemia that can reduce their stamina, predispose them to other infections or increase the impact of other stressors.	Horizontal transmission.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Paramyxovirus	**Chinook salmon paramyxovirus**	No clinical signs of disease. The virus is of low virulence and not associated with disease or mortality.	The viral agent is generally isolated from asymptomatic carrier fish during routine viral screening Transmission is horizontal by water or fish to fish. A marine reservoir for the virus is suspected.	Treatment: None required. The prognosis for the host is good regarding the non-pathogenic nature of the virus.
Picornavirus	**Smelt & salmonid picornavirus**	Some fish have no symptoms, but young fish can have neurological symptoms. Other signs: Focal hepatitis and haematopoietic necrosis, skin lesions including tumours, increased mucous production and mucous ulcers, reddened skin, haemorrhaging on the ventral surfaces and fin bases.	Found is many fish species. This is a wild based disease and not normally found in farmed fish. There are no pathopneumonic gross pathological signs for this disease.	Treatment: None available.
Reovirus	**There are many species of fish aquareoviruses** **(Grass carp haemorrhage virus)**	Aquareoviruses normally have low or no pathogenicity for fish. Clinical signs: Haemorrhages on the body surface, the base of the fins, and eyes. Hepatitis and pancreatitis. The virus causes haemorrhagic disease and is the most pathogenic Aquareovirus. Mortality may reach 80%.	Fish, shellfish, and crustacean species serve as natural hosts.	Treatment: None available. Control: Vaccine is available.
Retrovirus	**Atlantic salmon swim bladder sarcoma**	Poor physical condition, lethargy, swollen abdomens and multifocal haemorrhages on the body and fins.	Gross pathology: Grossly, coalescing, smooth, firm, pale-tan masses, up to 2cm in diameter, expand the wall of the swim bladder and impinge on the luminal space. Histopathology: Well-demarcated, unencapsulated, multinodular masses expand within the swim bladder wall within the luminal and serosal basal laminae. Tumours are composed of neoplastic spindle cells organised into long interlacing streams subdivided by thin bands of collagen, consistent with the diagnosis of leiomyosarcoma.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Retrovirus	**Atlantic salmon papilloma**	Epidermal masses, plaque-like or exophytic and verrucous, grow to 4 cm size and are distributed on the opercula, fins, and tail. Masses increase in size until later summer and then appear to slough off, occasionally leaving remnant ulcers prone to secondary infections by opportunistic pathogens. Found in cultured and wild Atlantic salmon inhabiting both the freshwater and marine environments. Minimal morbidity and mortality. Lesions may reduce carcass value in farmed fish.	The disease is seasonal (summer). Histopathology: Well-demarcated proliferations of Malpighian cells (nonkeratinizing epithelial cells that comprise the outer layers of the piscine epidermis) that expand the epidermis up to 15 times normal thickness. Plaque-like subtypes have abrupt margins and a flat surface. Exophytic, verrucous masses have deep rete pegs with intervening fibrovascular stalks. Anaplasia, pleomorphism, and bizarre mitotic figures have been reported in both subtypes and dermal invasion is not a feature. In a small proportion of masses, proteinaceous, eosinophilic inclusions and rarely intranuclear inclusions have been reported in degenerate cells. The regressive stage is characterized by neovascularisation and leukocyte infiltration, followed by necrosis, ulceration, and then rapid epithelialisation.	Treatment: None available. Self-limiting in most cases.
Retrovirus	**Damselfish neurofibromatosis**	A neurofibromatosis-like disease in bicolour damselfish *(Stegastes partitus).* Focal hyperpigmented plaques initially, eventually coalesce into rugose, flat masses that cover a large surface area of the body. Later non-pigmented subcutaneous & dermal nodules characterised as neurofibromas and malignant peripheral nerve sheath tumours. Progressive disease ending in death.	Gross pathology: Multicentric tumours that track cutaneous nerves on the body and fins, spinal nerves (trigeminal and facial nerves), and nerves of internal organs in advanced stages of the disease. Two subtypes: 1. Hyperpigmented plaque-like epidermal masses that invade the dermis and bones causing erosion/distortion of scales and fin rays and 2. Larger, non-pigmented subcutaneous nodules that invade skeletal muscle, internal organs, and bone and eventually erupt through the skin. Microscopy: Nerve sheath tumours are unencapsulated, invasive proliferations of spindle cells and plump pleomorphic cells.	Treatment: None available.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Retrovirus	**Esocid lymphosarcoma**	Initially tumours are found in the skin, but progress to internal organs. Tumours are colourless skin protrusions, several centimetres in size.	A transmissible tumour which affects two species of fish: *Esox Lucius* and *Esox masquinongy*, in North America and Europe by physical contact between fish during spawning.	Treatment: None available.
Retrovirus	**Plasmacytoid leukaemia of chinook salmon**	Darkened fish, lethargic and swim near the water surface. Bilateral exophthalmia and anaemia. Mortality in salmon stock in pen culture.	Wild salmon carry the retrovirus. No zoonosis. Histopathology: Anaemia from proliferation of virus infected plasmablasts infiltrating blood and major organs including spleen and pancreas. Accumulation of hyperaemic tissue behind the eyeballs. Distal intestinal thickening.	Treatment: None available.
Retrovirus	**Viral erythrocytic infection of seabass**	Affects many fish species. Anaemia, increased susceptibility to secondary infection, and direct mortality.	Microscopically: Erythrocytes have single, double or multiple intracytoplasmic inclusions and vacuoles. Clinically infected sea bass have low haematocrit values ranging from 2% to 29% and low RBC counts.	Treatment: None available.
Retrovirus	**Walleye dermal sarcoma**	Spontaneous dermal sarcomas in walleye are multifocal to coalescing, white to pale pink dermal nodules, up to 1 cm in diameter, with a smooth to cobblestone surface, occurring anywhere on scaled skin. Tumour regression occurs by a progressively intense mononuclear cell infiltration and epidermal ulceration followed by tumour necrosis and involution.	The lesions are seasonal mainly in cooler months and regress in warmer months. Histopathology: the skin is elevated by a well-demarcated, pseudo encapsulated neoplasm within the dermal stratum spongiosum (scale bed) (composed of spindle cells that whorl around a central scale).	Treatment: None available, self-limiting disease.

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Rhabdovirus	**Lyssavirus-like group Snakehead rhabdovirus (SHRV), Eel virus B-12, Carpione rhabdovirus**	Necrotic ulcerations containing fungal hyphae (*A. invadans*) with granulomatous reactions. *A. invadans* is the primary causative agent of EUS outbreaks and these infections are thought to be facilitated by environmental factors, such as infection by viruses like SHRV or cutaneous damage.	Affects warm water wild and pond-cultured fish of various species in SE Asia. Viral replication occurs at both 15°C and 28°C with an optimal temperature range for viral replication is between 28°C and 31°C. Histopathology: Infected embryos and juvenile fish reveal vascular monocyte accumulation, accumulation of cellular debris in the gas bladder, necrosis of hepatocytes, and necrosis of pharyngeal epithelial cells. Adults: form mycotic epithelial granulomas as a result of an infection of the muscle tissue and sometimes multinucleated giant cells.	Treatment: None available. Control: Virus is inactivated following treatment with acid (pH = 3), chloroform (50%), and heat (56 °C), 12.5 ppm chlorine, 50 ppm iodine, or a 1:2000 dilution of peroxygen disinfectant.
Rhabdovirus	**Japanese Flounder Hirame rhabdovirus**	Abdominal distension, fin reddening and yellow ascitic fluid in the abdominal cavity. Causes acute haemorrhage disease in fish culture, resulting in a great economic loss in parts of Asia and Europe.	Histopathology: Cell degeneration and necrosis in the kidney.	Treatment: none available. Control: DNA vaccine?
Rhabdovirus	**Vessiculovirus-like group (Pike fry rhabdoviruses)**	Acute infection of the vascular system. Petechial haemorrhages in the brain, spinal cord, spleen and kidneys	Horizontal and vertical transmission from adults to eggs and by water. Incubation period is 5 days at 14^{o}C and only 1-2 days at 21-24^{o}C. Gross pathology: Extensive degeneration and necrosis in the kidney.	Treatment: None available

Disease type	Common name	Clinical signs & impact	Risk factors, pathogenesis & pathological signs	Treatment methods
Significant viral diseases				
Togavirus	**Erythrocytic inclusion body syndrome (EIBS)**	May be asymptomatic, or fish are lethargic, anorexic and anaemic with chronic mortality often associated with secondary infections by other pathogens. In severe cases of uncomplicated anaemia, cumulative fish mortality over 20% has been reported with haematocrits less than 20%. Five stages of EIBS have been described: preinclusion, inclusion body formation, cell lysis with low haematocrits, recovery with increasing haematocrits and full recovery.	The disease can be transmitted horizontally. Surviving fish develop an acquired immunity against reinfection that is transferable by passive immunisation. No zoonosis.	Treatment: None available, often a self-limiting disease.
Togavirus	**Pancreas-disease in Atlantic salmon**	Emaciated from malnutrition and poor feeding. Hanging in corners of tank/sea cage. Darkened dorsally with white faecal casts. Poor growth rate in farmed salmon. Mortality <5%, morbidity 30%.	Disease incubation 7-14 days at 12-15^{o}C. Possible wild reservoir of virus. No zoonosis. Histopathology: Necrosis of exocrine pancreas with little inflammation. Myopathy of heart and skeletal muscle.	Treatment: None available. Control: Potential for vaccination.

Appendix 9.20: Diseases and their common diagnostic pathways

Diagnostic test definitions

Test	Abbreviation	Test	Abbreviation
Transmission electron microscope	TEM	Immunohistochemistry test	IHC
Enzyme-linked immunosorbent assay	ELISA	Cell culture	CC
Polymerase chain reaction	PCR	Viral neutralisation assay	VNA
Real time Polymerase chain reaction	RT, PCR	IHCT & hemagglutination inhibition	IHCHI
In situ hybridisation	ISH	Viral culture	VC
Immunofluorescence antibody test	IFAT	PCR test using RSIV primers.	PCR-RSIV

Diagnostic test definitions: staining techniques

Test	Abbreviation	Test	Abbreviation
Ziehl Neelsen stain	ZNS	Klein's silver stain	KS
Giemsa stain	GS	Trichrome (Gomori's or Wheatly-modified) staining	TC
Löwenstein–Jensen medium	LJM	Fungal special stain- Grocotts silver	GRS
Periodic Acid Schiff	PAS	Toluidine blue stain	TB
Methylene blue	MB	Fluorescence brighteners (fluorochromes)	FB
Gram stain	GRAM	Gomori methenamine silver	GMS

Diagnostic test definitions: Culture

Test	Abbreviation	Test	Abbreviation
Blood agar	BA	Sabourad's agar	SA
Thiosulphate citrate bile salt media for bacterial culture of fish kidney	TCBS	Marine agar for bacterial culture	MA
Czapek Dox agar for fungal culture	CDA	Brain heart infusion	BHI
Trypticase soy agar	**TSA**		

Diseases by category		Diagnostic pathway																					
		Husbandry/history review (pH, temp, diet, water quality, soil quality)	Light or dissection microscopy	Transmission electron microscopy (TEM)	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity testing	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and stains	RT-PCR	PCR	ELISA	In situ hybridisation (ISH)	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation test	Cell culture of embryos	Haemagglutination test	Biochemical typing
Notifiable parasitic diseases																									
Platyhelminth	Gyrodactylosis (*Gyrodactylus salaris*)						x	x				X wet													
Myxosporean	Whirling disease (*Myxobolus cerebralis*)						x	x																	
Significant parasitic diseases																									
Ectoparasites																									
Monogeneans																									
(Annelids)	Piscicola		x																						
(Crustacea)	Argulus		x																						
(Crustacea)	Caligus		x																						
(Crustacea)	Lepeoptherius		x																						
(Crustacea)	Lernaea		x																						
(Molluscs)	Glochidia														x										
Monogenean trematodes (Flukes)	Benedinia		x		x																				
Monogenean trematodes (Flukes)	*Diplostomum spathaceum* (eye flukes)		x				x	x																	
Protozoans																									
(Amoeba)	Thecamoeba		x		x			x										x							
(Ciliate)	Brooklynella		x									x			x										
(Ciliate)	Chilodonella		x KS/ GS									x			x										x
(Ciliate)	*Cryptocaryon irritans and Icthyopthirius multifiliis*) Ich or white spot.		x																						
(Ciliate)	Scyphidians		x																						
(Ciliate)	Trichodina		x																						

Diseases by category		Diagnostic pathway																						
		Husbandry/history review (pH, temp, diet, water quality, soil	Light or dissection microscopy	Transmission electron microscopy (TEM)	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity testing	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and stains	RT-PCR	PCR	ELISA	In situ hybridisation	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation test	Cell culture of embryos	Haemagglutination test	Biochemical typing
Protozoans																									
(Ciliate)	Tetrahymena		x																						
(Flagellates)	Crytobia			x	x						x					x		x							
(Flagellates)	Ichtyobodo		x	x											x										
(Flagellates)	Ooddinium		x		x			x				x			x										
(Flagellates)	Trypanosomiasis										x M B														
Endoparasites																									
Metazoan species																									
(Acanthocephala)	Pomphorhynchus						x	x																	
(Cestodes)	Diphyllobothrium		x				x	x																	
(Cestodes)	Ligula		x				x	x																	
(Nematodes)	Anisakis		x				x	x																	
(Nematodes)	Camallanus		x				x	x																	
(Nematodes)	Contracaecum		x				x	x																	
(Trematodes)	Clinostomum		x				x	x																	
(Trematodes)	Cryptocotyle		x				x	x																	
(Trematodes)	Diplostomum		x				x	x				x													
(Trematodes)	Nanophyetus		x				x	x				x													
Protozoan species																									
(Coccidia)	Eimeria		x GS	x				x				x													
(Coccidia)	Goussia		x GS	x																					
(Flagellates)	Hexamita	x					x	x																	
(Flagellates)	Trypanoplasma		x	x				X			X GS				x										
(Flagellates)	Trypanosoma		x	x							X GS				x										
(Microsporidia)	Glugea		x	x				x							x										
(Microsporidia)	Heterosporis		x	x				x TB							x										
(Microsporidia)	Pleistophora		x FB	x				x							x										

Diseases by category		Diagnostic pathway																						
		Husbandry/history review (pH, temp, diet, water quality	Light or dissection microscopy	Transmission electron microscopy	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and	RT-PCR	PCR	ELISA	In situ hybridisation	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation	Cell culture of	Haemagglutination	Biochemical typing
(Myxozoa)	Ceratomyxa							x GS				x			x									
(Myxozoa)	Henneguya							x GS				x			x									
(Myxozoa)	Hexacapsula							x GS				x			x									
(Myxozoa)	Hoferellus							x GS				x			x									
(Myxozoa)	Kudoa							x GS				x			x									
(Myxozoa)	Myxobolus (myxosoma)							x GS				x			x									
(Myxozoa)	Proliferative gill disease							x GS				x			x									
(Myxozoa)	Proliferative kidney disease							x GS				x			x									
(Myxozoa)	Sphaerospora							x GS				x			x									
(Myxozoa)	Thellohanellus							x GS				x			x									
Notifiable bacterial diseases																								
*Aeromonas salmonicida-atypical strains	Goldfish ulcerative disease, Carp erythrodermatitis, Ulcer disease of flounder, eel or salmon.							x							x									
(Edwardsiella ictalurid)	Enteric septicaemia of catfish							x							x	x		x						
(Renibacterium salmoninarum)	Bacterial kidney disease							x	x						x	x		x						x	
Rickettsia & chlamydias	(Piscirickettiosis salmonis)						x	x			x GS				x			x		x					
Yersinia ruckeri- (Hagerman strain)	Enteric redmouth disease								x							x								x	
Significant bacterial diseases																								
Aeromoniasis	Goldfish ulcer disease Atypical Aeromonas salmonicida)		x						x															
Botulism	(Clostridium botulinum type E)	x													x									
Edwardsiellosis	(E. tarda infection)								x						x	x								
Flexibacteriosis	(Flexibacter columnaris, Tenacibaculum maritimum, Flavobacteria spp.)								x			x												

Diseases by category		Diagnostic pathway																					
		Husbandry/history review (pH, temp, diet, water quality, soil	Light or dissection microscopy	Transmission electron microscopy (TEM)	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity testing	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and stains	RT-PCR	PCR	ELISA	In situ hybridisation	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation test	Cell culture of embryos	Haemagglutination test	Biochemical typing
Mycobacteriosis	(M. fortuitum, M. chelonae, M. marinum)								x L JM		x ZN				x										
Norcardiosis	(N. asteroids, N. kampachi)											x													
Pasteurellosis	(Photobacterium damsela)								x BA BHI						x			x						x	
Pseudomoniasis	(P. fluorescens, P. anguilliseptica)								x BA TSA																
Rickettsia & chlamydias	(Epitheliocystis microcystis)														x										
Streptococcosis	(S. iniae, Lactococcus garviae, Enterococcus seriolida)								x BA						x										x
Vibriosis	(V. anguillarum, V. alginolyticus, V. vulnificus, V. parahemolyticus)								x MA/ TCB S																x
Yersinosis	(Enteric redmouth-ERM)								x BA TSA						x	x		x							
Notifiable fungal diseases																									
Aphanomyces invadens	Epizootic ulcerative syndrome						x	x	x CDA			x GS			x										
Significant fungal diseases																									
Branchiomyces sp.							x	x GMS				x gills													
Exophila sp.								x	x SA						x										
Icthyophonus hoferi							x	x GS PAS	x SA																

Diseases by category		Diagnostic pathway																						
		Husbandry/history review (pH, temp, diet, water quality, soil quality)	Light or dissection microscopy	Transmission electron microscopy (TEM)	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity testing	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and stains	RT-PCR	PCR	ELISA	In situ hybridisation (ISH)	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation test	Cell culture of embryos	Haemagglutination test	Biochemical typing
Significant fungal diseases																									
Phoma sp.									x SA																
Saprolegnia sp.	Saprolegniasis	x					x	x	x SA																
Notifiable viral diseases																									
Aquabirnavirus	Infectious pancreatic necrosis														x	x	x					x	x		
Herpesvirus	Channel catfish virus disease														x										
Herpesvirus	E Infection with koi herpesvirus		x	x											x										
Herpesvirus	Oncorhyncus masou virus disease														x	x		x			x	x			
Iridovirus	Grouper iridoviral disease	x					x	x							x RSI			x							
Irridovirus	Epizootic haematopoietic necrosis (European catfish virus)			x										x	x	x									
Iridovirus	Red sea bream iridoviral disease														x			x							
Iridovirus	White sturgeon iridoviral disease			x										x	x			x							
Nodavirus	*Viral encephalopathy and retinopathy							x							x			x	x				x		
Orthomyxovirus	Infectious salmon anaemia							x						x		x		x	x		x			x	
Rhabdovirus	Infectious haematopoietic necrosis							x							x	x		x				x			
Rhabdovirus	Viral haemorrhagic septicaemia			x										x		x		x			x				
Rhabdovirus	Spring viraemia of carp							x						x		x		x			x	x			

Diseases by category		Diagnostic pathway																						
		Husbandry review (pH, temp, diet, water quality, soil quality)	Light or dissection microscopy	Transmission electron microscopy (TEM)	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity testing	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and stains	RT-PCR	PCR	ELISA	In situ hybridisation (ISH)	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation test	Cell culture of embryos	Haemagglutination test	Biochemical typing
Significant viral diseases																									
Adenovirus	Cod, Dab & Sturgeon adenoviruses			x																					
Birnavirus	Milkfish, striped bass, common carp, loach birnaviruses														x	x	x					x	x		
Birnavirus	Japanese eel (eel virus European)														x	x	x					x	x		
Birnavirus	Yellowtail ascites virus														x	x	x					x	x		
Herpesvirus	Anguillid herpesvirus			x											x						x				
Herpesvirus	*Goldfish herpesvirus haematopoietic necrosis			x				x													x				
Herpesvirus	*H. cyprini													x	x										
Herpesvirus	*Pilchard herpesvirus			x				x							x										
Herpesvirus	Salmonid types I,II & III herpesvirus			x				x							x										
Herpesvirus	White-sturgeon, Angelfish, Smooth dogfish & Japanese flounder herpesviruses			x				x							x										
Iridovirus	*EGourami & Murray cod iridovirus														x										
Iridovirus	Large-mouth bass iridovirus	x	x	x											x										
Iridovirus	*Lymphocystis			x				x																	
Iridovirus	Viral erythrocytic necrosis		x	x							x GS														
Paramyxovirus	Chinook salmon paramyxovirus			x				x							x			x			x			x	

Diseases by category		Diagnostic pathway																						
		Husbandry review (pH, temp, diet, water quality, soil quality)	Light or dissection microscopy	Transmission electron microscopy (TEM)	Biopsy	Clinical signs are diagnostic	Gross pathology	Histopathology	Bacterial/fungal culture & sensitivity testing	Blood culture	Blood smear	Skin scrape/squash (wet or dry mount)	Fungal assay and stains	RT-PCR	PCR	ELISA	In situ hybridisation (ISH)	Immunofluorescence antibody test (IFAT)	Immunohistochemistry test (IHCT)	Immunoperoxidase	Viral culture	Viral neutralisation test	Cell culture of embryos	Haemagglutination test	Biochemical typing
Significant viral diseases																									
Picornavirus	Smelt & salmonid picornavirus			x											x						x				
Reovirus	30 species of fish aquareoviruses													x											
Retrovirus	Atlantic salmon papilloma							x							x										
Retrovirus	Atlantic salmon swim bladder sarcoma			x				x							x										
Retrovirus	Damselfish neurofibromatosis							x																	
Retrovirus	Esocid lymphosarcoma			x																					
Retrovirus	Plasmacytoid leukaemia of chinook salmon			x				x										x							
Retrovirus	Viral erythrocytic infection of seabass			x																					
Retrovirus	Walleye dermal sarcoma													x											
Rhabdovirus	Lyssavirus-like group (Snakehead rhabdovirus, eel virus B-12, Carpione rhabdovirus)			x				x										x				x			
Rhabdovirus	Japanese flounder Hirame rhabdovirus			x										x							x				
Rhabdovirus	Vessiculovirus-like group (Pike fry rhabdoviruses)																				x				
Togavirus	Erythrocytic inclusion body syndrome			x																					
Togavirus	Pancreas-disease in Atlantic salmon			x				x																	

Appendix 9.21: Diseases and their Management/control option

Disease by category		Management options																		
		Assess and quarantine fish/fish eggs on arrival	Test for carrier fish	Test prior/restrict movement of fish between facilities	Destock, clean, disinfect, dry out tanks/ponds	Clean up organic waste and biofilm	Use separate catch nets for each tank (clean & disinfected between use)	Avoid earthen ponds	Remove excessive sediment	Evaluate and treat water source	Increase feed quality	Decrease feeding rate /feed waste	Avoid trash feeding	Reduce stocking rate and avoid overcrowding	Reduce husbandry stressors such as rough handling & over	Grade fish regularly to reduce aggression	Increase water exchange rate	Remove sick and dead fish and cull affected fish for pathology assessment	Drop/raise water temperature	Avoid feeding live feeder fish
Platyhelminth	Gyrodactylosis (*Gyrodactylus salaris*)	x					x													
Myxosporean	Whirling disease (*Myxobolus cerebralis*)		x	x	x		x	x										x		
Significant parasites																				
Ectoparasites																				
Metazoan species																				
(Annelids)	Piscicola	x							x											
(Crustacea)	Argulus	x			x		x			x										
(Crustacea)	Caligus	x			x		x											x		
(Crustacea)	Lepeoptherius	x																		
(Crustacea)	Lernaea	x			x															
(Molluscs)	Glochidia	x																		
Monogenean trematodes	Benedinia (flukes)	x								x	x			x	x	x				
Monogenean trematodes	Diplostomum spathaceum (eye flukes)	x																		
Protozoan species																				
(Amoeba)	Thecamoeba	x								x				x	x					
(Ciliate)	Brooklynella	x			x					x				x	x					
(Ciliate)	Chilodonella	x			x					x				x	x					
(Ciliate)	*Cryptocaryon irritans & Icthyopthirius multifiliis*) Ich or white spot.	x																		
(Ciliate)	Scyphidians	x			x		x			x				x	x					
(Ciliate)	Trichodina	x			x		x			x				x	x					
(Ciliate)	Tetrahymena	x			x		x			x		x		x	x					
(Flagellates)	Crytobia	x									x					x				
(Flagellates)	Ichtyobodo	x								x	x			x	x					
(Flagellates)	Ooddinium	x								x										
(Flagellates)	Trypanosomiasis	x								x										

Disease by category		Management options																		
		Assess and quarantine fish/fish eggs on arrival	Test for carrier fish	Test prior/restrict movement of fish between facilities	Destock, clean, disinfect, dry out tanks/ponds	Clean up organic waste and biofilm	Use separate catch nets for each tank (clean & disinfected between use)	Avoid earthen ponds	Remove excessive sediment	Evaluate and treat water source	Increase feed quality	Decrease feeding rate/feed waste	Avoid trash feeding	Reduce stocking rate and avoid overcrowding	Reduce husbandry stressors such as rough handling & over handling	Grade fish regularly to reduce aggression	Increase water exchange rate	Remove sick and dead fish and cull affected fish for pathology assessment	Drop/raise water temperature	Avoid feeding live feeder fish
Endoparasites																				
Metazoan species																				
(Acanthocephala)	Pomphorhynchus	x								x										
(Cestodes)	Diphyllobothrium	x								x							x			
(Cestodes)	Ligula	x			x															
(Nematodes)	Anisakis	x						x												
(Nematodes)	Camallanus	x																		x
(Nematodes)	Contracaecum	x			x															
(Trematodes)	Clinostomum	x			x															
(Trematodes)	Cryptocotyle	x			x															
(Trematodes)	Diplostomum	x			x					x										
(Trematodes)	Nanophyetus	x			x															
Protozoan species																				
(Coccidia)	Eimeria	x			x															
(Coccidia)	Goussia	x			x															
(Flagellates)	Hexamita	x			x									x	x					
(Flagellates)	Trypanoplasma	x																		
(Flagellates)	Trypanosoma	x						x										x		
(Microsporidia)	Glugea	x			x	x		x	x	x							x			
(Microsporidia)	Heterosporis	x			x												x			
(Microsporidia)	Pleistophora	x			x												x			
(Myxozoa)	Ceratomyxa	x			x			x												
(Myxozoa)	Henneguya	x			x			x												
(Myxozoa)	Hexacapsula	x			x			x												
(Myxozoa)	Hoferellus	x			x			x												
(Myxozoa)	Kudoa	x			x			x												
(Myxozoa)	Myxobolus (myxosoma)	x			x			x							x					
(Myxozoa)	Proliferative gill disease	x		x	x			x												
(Myxozoa)	Proliferative kidney disease	x		x	x			x												
(Myxozoa)	Sphaerospora	x			x			x												

Disease by category		Management options																		
		Assess and quarantine fish/fish eggs on arrival	Test for carrier fish	Test prior/restrict movement of fish between facilities	Destock, clean, disinfect, dry out tanks/ponds	Clean up organic waste and biofilm	Use separate catch nets for each tank (clean & disinfected between use)	Avoid earthen ponds	Remove excessive sediment	Evaluate and treat water source	Increase feed quality	Decrease feeding rate /feed waste	Avoid trash feeding	Reduce stocking rate and avoid overcrowding	Reduce husbandry stressors such as rough handling & over handling	Grade fish regularly to reduce aggression	Increase water exchange rate	Remove sick and dead fish and cull affected fish for pathology assessment	Drop/raise water temperature	Avoid feeding live feeder fish
Protozoan species																				
(Myxozoa)	Thellohanellus	x			x			x												
Notifiable bacterial diseases																				
**Aeromonas salmonicida*-atypical strains		x			x															
(*Edwardsiella ictalurid*)	Enteric septicaemia of catfish	x								x				x	x					
(*Renibacterium salmoninarum*)	Bacterial kidney disease	x			x															
Rickettsia/chlamydias	(*Piscirickettiosis salmonis)*	x					x			x				x	x	x	x	x		
Yersinia ruckeri-(Hagerman strain)	Enteric redmouth disease	x								x				x						
Significant bacterial diseases																				
Aeromoniasis	Goldfish ulcer disease Atypical *Aeromonas salmonicida*)	x			x		x			x										
Botulism	(*Clostridium botulinum* type E)	x			x				x	x								x		
Edwardsiellosis	(*E. tarda* infection)	x								x				x	x				x	
Flexibacteriosis	(*Flexibacter columnaris, Tenacibaculum maritimum, Flavobacteria spp.)*	x				x	x					x		x	x	x	x	x		
Mycobacteriosis	(*M. fortuitum, M. chelonae, M. marinum*)	x			x													x		
Norcardiosis	*(N. asteroids, N. kampachi)*	x											x							x
Pasteurellosis	(*Photobacterium damsela)*	x												x	x					
Pseudomoniasis	*(P. fluorescens, P. anguilliseptica)*	x			x									x	x					
Rickettsia/chlamydias	(*Epitheliocystis microcystis)*	x			x															
Streptococcosis	(*S. iniae, Lactococcus garviae, Enterococcus seriolida*)	x				x						x		x	x	x	x	x		
Vibriosis	(*V. anguillarum, V. alginolyticus, V. vulnificus, V. parahemolyticus*)	x							x		x			x	x					
Yersinosis	(Enteric redmouth-ERM)	x																		

Disease by category		Management options																	
		Assess and quarantine fish/fish eggs on arrival	Test for carrier fish	Test prior/restrict movement of fish between facilities	Destock, clean, disinfect, dry out tanks/ponds	Clean up organic waste and biofilm	Use separate catch nets for each tank (clean & disinfected between use)	Avoid earthen ponds	Remove excessive sediment	Evaluate and treat water source	Increase feed quality	Decrease feeding rate /feed waste	Avoid trash feeding	Reduce stocking rate and avoid overcrowding	Reduce husbandry stressors such as rough handling & over handling	Grade fish regularly to reduce aggression	Increase water exchange rate	Remove sick and dead fish and cull affected fish for pathology assessment	Drop/raise water temperature	Avoid feeding live feeder fish
Notifiable fungal disease																			
Aphanomyces invadens	Epizootic ulcerative syndrome	x			x	x				x					x	x			
Significant fungal disease																			
Branchiomyces sp.		x		x	x		x			x									
Exophila sp.		x																	
Icthyophonus hoferi		x			x								x					x	x
Phoma sp.		x																	
Saprolegnia sp.	Saprolegniasis	x												x	x	x		x	
Notifiable viral diseases																			
Aquabirnavirus	Infectious pancreatic necrosis	x								x				x					x
Herpesvirus	Channel catfish virus disease	x	x							x				x					
Herpesvirus	[E] Infection with koi herpesvirus	x	x																
Herpesvirus	*Oncorhyncus masou* virus disease	x	x											x	x				
Iridovirus	Grouper iridoviral disease	x			x		x							x	x				
Irridovirus	Epizootic haematopoietic necrosis (European catfish virus)	x																	
Iridovirus	Red sea bream iridoviral disease	x								x				x	x				
Iridovirus	White sturgeon iridoviral disease	x																	
Nodavirus	*Viral encephalopathy and retinopathy	x	x	x	x														
Orthomyxovirus	Infectious salmon anaemia	x			x	x													
Rhabdovirus	Infectious haematopoietic necrosis	x			x					x				x	x		x		x
Rhabdovirus	Viral haemorrhagic septicaemia	x																	
Rhabdovirus	Spring viraemia of carp	x			x														
Significant viral diseases																			
Adenovirus	Cod, Dab & Sturgeon adenoviruses	x			x														

Disease by category		Management options																		
		Assess and quarantine fish/fish eggs on arrival	Test for carrier fish	Test prior/restrict movement of fish between facilities	Destock, clean, disinfect, dry out tanks/ponds	Clean up organic waste and biofilm	Use separate catch nets for each tank (clean & disinfected between use)	Avoid earthen ponds	Remove excessive sediment	Evaluate and treat water source	Increase feed quality	Decrease feeding rate /feed waste	Avoid trash feeding	Reduce stocking rate and avoid overcrowding	Reduce husbandry stressors such as rough handling & over handling	Grade fish regularly to reduce aggression	Increase water exchange rate	Remove sick and dead fish and cull affected fish for pathology assessment	Drop/raise water temperature	Avoid feeding live feeder fish
Significant viral diseases																				
Birnavirus	Milkfish, striped bass, common carp, loach birnaviruses	x																		
Birnavirus	Japanese eel (eel virus European)	x																		
Birnavirus	Yellowtail ascites virus	x																		
Herpesvirus	Anguillid herpesvirus	x												x	x					
Herpesvirus	*Goldfish herpesvirus haematopoietic necrosis	x												x	x					
Herpesvirus	*H. cyprini	x												x	x				x	
Herpesvirus	*Pilchard herpesvirus	x												x	x					
Herpesvirus	Salmonid types I,II & III herpesvirus	x												x	x					
Herpesvirus	White-sturgeon, Angelfish, Smooth dogfish & Japanese flounder herpesviruses	x												x	x					
Iridovirus	*EGourami & Murray cod iridovirus	x																		
Iridovirus	Large-mouth bass iridovirus	x																		
Iridovirus	*Lymphocystis	x												x	x					
Iridovirus	Viral erythrocytic necrosis	x																		
Paramyxovirus	Chinook salmon paramyxovirus	x												x	x					
Picornavirus	Smelt & salmonid picornavirus	x												x	x					
Reovirus	30 species of fish aquareoviruses	x																		
Retrovirus	Atlantic salmon papilloma	x												x	x					
Retrovirus	Atlantic salmon swim bladder sarcoma	x																		
Retrovirus	Damselfish neurofibromatosis	x																		

Disease by category		Management options																			
		Assess and quarantine fish/fish eggs on arrival	Test for carrier fish	Test prior/restrict movement of fish between facilities	Destock, clean, disinfect, dry out tanks/ponds	Clean up organic waste and biofilm	Use separate catch nets for each tank (clean & disinfected between use)	Avoid earthen ponds	Remove excessive sediment	Evaluate and treat water source	Increase feed quality	Decrease feeding rate /feed waste	Avoid trash feeding	Reduce stocking rate and avoid overcrowding	Reduce husbandry stressors such as rough handling & over handling	Grade fish regularly to reduce aggression	Increase water exchange rate	Remove sick and dead fish and cull affected fish for pathology assessment	Drop/raise water temperature	Avoid feeding live feeder fish
Retrovirus	Esocid lymphosarcoma	x																			
Retrovirus	Plasmacytoid leukaemia of chinook salmon	x		x																	
Retrovirus	Viral erythrocytic infection of seabass	x																			
Retrovirus	Walleye dermal sarcoma	x																			
Rhabdovirus	Lyssavirus-like group (Snakehead rhabdovirus, eel virus B-12, Carpione rhabdovirus)	x			x																
Rhabdovirus	Japanese flounder Hirame rhabdovirus	x																			
Rhabdovirus	Vessiculovirus-like group (Pike fry rhabdoviruses)	x																			
Togavirus	Erythrocytic inclusion body syndrome	x												x	x						
Togavirus	Pancreas-disease in Atlantic salmon	x			x																

References

- Adams, A (ed.), 2016, Fish vaccines, Birkhaüser advances in Infectious Diseases, (ISBN 978-3-0348-0980-1)
- Ageze, N & Menzir, A, 2018, Prevalence Of Nematode (Contracaecum) And Cestode (Ligula Intestinalis) Parasites Infection In Two Fish Species At Lake Tana, *International Journal of Advanced Research and Publications,* vol. 2, no. 3, http://www.ijarp.org/published-research-papers/mar2018/Prevalence-Of-Nematode-contracaecum-And-Cestode-ligula-Intestinalis-Parasites-Infection-In-Two-Fish-Species-At-Lake-Tana-.pdf
- Alaskan Department of Fish and Game, *Black Spot Disease (Neascus and Heterophyids),* viewed 5 February 2020, https://www.adfg.alaska.gov/static/species/disease/pdfs/fishdiseases/black_spot_disease.pdf
- Alaskan Department of Fish and Game, *Phoma herbarum,* viewed 5 February 2020, https://www.adfg.alaska.gov/static/species/disease/pdfs/fishdiseases/phoma_herbarum.pdf
- Alvrez-Pellitero, P, 2004, *Report about fish parasitic diseases*, viewed 27 January 2020, http://om.ciheam.org/om/pdf/b49/04600222.pdf
- Asean University Network, Fish diseases -Hexamitiosis, viewed 27 January 2020, http://www.aun.edu.eg/developmentvet/fish%20diseases/5_3.htm
- Australian Government, Department of Agriculture, Fisheries & Forestry, 2012, *Aquatic Animal Diseases Significant to Australia: Identification Field Guide 4th Edition Biosecurity,* viewed 10 April 2020, https://nswaqua.com.au/blog/wp-content/uploads/2020/03/aquatic-animal-diseases-significant-aus-id-field-guide-4ed.pdf
- Australian Government, Department of Agriculture, 2014, *Importation of freshwater ornamental fish: review of biosecurity risks associated with gourami iridovirus and related viruses Final import risk analysis report ,* viewed 27 January 2020, https://www.agriculture.gov.au/sites/default/files/style%20library/images/daff/__data/assets/pdffile/0004/2404309/gourami-ira.pdf
- Australian Government, 2020, *Aquatic Animal Diseases Significant to Australia: Identification Field Guide 5th Edition,* viewed 27 April 2020, https://www.agriculture.gov.au/animal/aquatic/guidelines-and-resources/aquatic_animal_diseases_significant_to_australia_identification_field_guide#viral-diseases-of-finfish
- Australian Government, 2019, *Australia's National List of Reportable Diseases of Aquatic Animals,* viewed 27 January 2020, https://www.agriculture.gov.au/animal/aquatic/reporting/reportable-diseases
- Becker, CD, 2020, *Flagellate parasites of fish*, viewed 27 April 2020, https://www.cabi.org/ISC/abstract/19772504718
- Biosecurity New Zealand, 2009, *Import risk analysis: Tropical, subtropical and temperate freshwater and marine ornamental fish and marine molluscs and crustaceans,* viewed 27 January 2020, https://www.mpi.govt.nz/dmsdocument/2753/direct
- CABI, 2020, *Hirudinea as vectors and disease agents in fish*, viewed 27 January 2020, https://www.cabi.org/isc/datasheet/79600#tooverview
- CABI, 2020, *Invasive Species Compendium, Edwardsiella septicaemia (Edwardsiella tarda infection),* viewed 24 April 2020, https://www.cabi.org/isc/datasheet/84398
- Carter, V, Pierce, R, Dufour, S, Arme, C & Hoole, D, 2005, The tapeworm Ligula intestinalis (*Cestoda: Pseudophyllidea*) inhibits expression and puberty in its teleost host, *Rutilus rutilus*, *Society for reproduction and Fertility*, vol. 30, no. 6, pp. 939-945, viewed 27 January 2020, https://rep.bioscientifica.com/view/journals/rep/130/6/1300939.xml
- Centre for Agriculture and Bioscience International, 2020, *Animal science database*, 2020, viewed 27 January 2020, https://www.cabi.org/animalscience/ebook/20173129435
- Chong, RSM, 2009, Aquaculture production, VETS 4021 lecture notes, University of Queensland

- Clausen, JH, Madsen, H, Murrell, KD, Thi Van, P, Thi Thu, HN, Do, DT, Thi, LAN, Manh, HN & Dalsgaard, A, 2012, Prevention and Control of Fish-borne Zoonotic Trematodes in Fish Nurseries, Vietnam, *Emerging Infectious Diseases*, vol. 18, no. 9, pp.1438-1445, viewed 27 January 2020, https://wwwnc.cdc.gov/eid/article/18/9/pdfs/11-1076.pdf
- Crane MStJ & Moody MJG, 2018, Australian and New Zealand standard diagnostic procedures (ANZSDP) for Megalocytivirus infections of Finfish, viewed 27 January 2020, https://www.agriculture.gov.au/sites/default/files/documents/anzsdp-megalocytivirus_0.pdf
- Dave, D, Wloga, D & Gaertig, J, 2009, Tetrahymena, *Methods in Cell Biology*, vol. 93, pp.1-20, viewed 27 January 2020, https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/tetrahymena
- De Hoog, GS, Vicente, VA, Najafzadeh, MJ, Harrak, MJ, Badali, H & Sevedmousavi, S, 2016, Waterborne *Exophiala* species causing disease in cold-blooded animals, *Persoonia,* vol. 27, pp. 46–72, Doi: 10.3767/003158511X614258, viewed 27 January 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3251318/
- Espelund, M & Klaveness, D, 2014, Botulism outbreaks in natural environments – an update, *Frontiers in Microbiology,* vol. 5, pp. 287, doi: 10.3389/fmicb.2014.00287, viewed 27 January 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052663/
- Ferguson, JA, Watral, V, Schwindt, AR & Kent, ML, 2007, Spores of two fish microsporidia (Pseudoloma neurophilia and Glugea anomala) are highly resistant to chlorine, *Diseases of aquatic Organisms*, vol. 76, no. 3, pp. 205-214, DOI: 10.3354/dao076205, viewed 27 January 2020, https://www.ncbi.nlm.nih.gov/pubmed/17803106
- Food and Agriculture Organization of the United Nations, *Coccidioses,* viewed 27 January 2020, http://www.fao.org/3/v9551e/V9551E09.htm
- Food and Agricultural Organization of the United Nations, Fisheries and Aquaculture Department, *15. Fish disease prevention and treatment,* viewed 10 September 2019, http://www.fao.org/tempref/FI/CDrom/FAO_Training/FAO_Training/General/x6709e/x6709e15.htm
- Fryer, JL & Mauel, MJ, 1997, The Rickettsia: an Emerging Group of Pathogens in Fish, Emerging Infectious Diseases, vol. 3, no. 2, pp. 137-144, viewed 27 January
- Francis-Floyd, R & Floyd, MR, 2011, *Amyloodinium ocellatum, an Important Parasite of Cultured Marine Fish*, viewed 27 January 2020, http://agrilife.org/fisheries/files/2013/09/SRAC-Publication-No.-4705-Amyloodinium-ocellatum-an-Important-Parasite-of-Cultured-Marine-Fish.pdf
- Kibenge, FSB & Godoy, MG (eds.), 2016, *Aquaculture Virology*, Elsevier Inc. London, UK
- Klinger, RE & Floyd, RF, 2013, *Introduction to Freshwater Fish Parasites*, viewed 27 January 2020, http://fisheries.tamu.edu/files/2013/09/Introduction-to-Freshwater-Fish-Parasites.pdf
- Klinger, R, Francis-Floyd, R & Riggs A, 2001, International Association for Aquatic Animal Medicine, *A nonlethal approach to diagnosing bacterial disease, in: 2001 Proceedings,* Annual Conference, Tampa, Florida
- Lalitha, KV & Gopakumar, K, 2008, Sensitivity of Tilapia (*Oreochromis mossambicus*) to *Clostridium botulinum* toxins, Aquaculture Research, vol. 32, no. 9, pp. 761-764, Doi.org/10.1046/j.1365-2109.2001.00616.x, viewed 27 January 2020, https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2109.2001.00616.x
- Lane, RL & Morris, JE, 2010, Prevention, and Effects of Common Grubs (Digenetic trematodes) in Freshwater Fish, *NCRAC Technical Bulletins. 14*, viewed 5 February 2020, https://pdfs.semanticscholar.org/2f0c/f821fd89be7db7e5e815c1cebac91be4bd54.pdf
- Lemos, M, fermion, BR, Rodrigues, CS, Hoffman, L, Silva, R, Camargo, EP, Teixeira, MMG & Souto-Padrón, T, 2015, Phylogenetic and morphological characterization of trypanosomes from Brazilian armoured catfishes and leeches reveal high species diversity, mixed infections and a new fish trypanosome species, Parasites & Vectors, vol. 8, no. 573, Doi.org/10.1186/s13071-015-1193-7, viewed 27 January 2020, https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-015-1193-7#citeas

- Lovy, J & Friend, SE, 2015, Intestinal coccidiosis of anadromous and landlocked alewives, *Alosa pseudoharengus*, caused by *Goussia ameliae* n. sp. and *G. alosii* n. sp. (Apicomplexa: Eimeriidae), *International Journal for Parasitology: Parasites and Wildlife,* vol. 4, no. 2, pp. 159-170, https://doi.org/10.1016/j.ijppaw.2015.02.003, viewed 27 January 2020, https://www.sciencedirect.com/science/article/pii/S2213224415000115
- Maclachlan, NJ & Dubovi, EJ (eds.), 2017, *Fenners Veterinary Virology 5th ed.,* Elsevier Inc., Oxford, UK
- Merck Sharp & Dohme Corp, 2020, *Aquarium Fishes,* MSD and the MSD Veterinary Manual, viewed 27 January 2020, https://www.msdvetmanual.com/exotic-and-laboratory-animals/aquarium-fishes
- Min, E, 2003, Nanophyetiasis, viewed 27 January 2020, http://web.stanford.edu/group/parasites/ParaSites2003/Nanophyetiasis/Nanophyetiasis%20Home%20Page.htm
- Mitchell MA & Tully TN, 2009, Manual of Exotic pet Practice, Saunders Elsevier, St. Louis, Missouri
- Stilwell, J & Yanong, RPE, 2020, Myxosporidiosis (Myxozoan Infections) in Warmwater Fish, *University of Florida*, viewed 10 April 2020, https://edis.ifas.ufl.edu/pdffiles/FA/FA20100.pdf
- Nabi, S, Tanveer, S, Ganaie, SA, Ahad, S, Niyaz, U & Abdullah, I, 2015, Acanthocephalan infestation in fishes –A review, *The Journal of Zoology Studies*, vol. 2, no. 6, pp. 32-37, viewed 27 January 2020, https://www.journalofzoology.com/volume2/v2i6/pdf/5.1.pdf
- Noga, E, 2010, *Fish Disease: diagnosis and treatment,* Wiley and Blackwell, Hoboken, new Jersey, ISBN: 9781119949466
- Padros, F, Knudsen, R & Blasco-Costa, I, 2018, Histopathological characterisation of retinal lesions associated to *Diplostomum* species (Platyhelminthes: Trematoda) infection in polymorphic Arctic charr *Salvelinus alpinus, International journal for Parasitology: Parasites and Wildlife, vol. 7, no. 1, pp. 68-74,* viewed 27 January 2020, doi: 10.1016/j.ijppaw.2018.01.007, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6032039/
- Phelps, NBD, Mor, SK, Armién, AG, Pelican, KM & Goyal, SM, 2015, Description of the Microsporidian Parasite, *Heterosporis sutherlandae* n. sp., Infecting Fish in the Great Lakes Region, USA, *PLoS One*, vol. 10, no. 8, pp. e0132027, Doi: 10.1371/journal.pone.0132027, viewed 27 January 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4526549/
- Pridgeon, JW & Klesius, PH, 2012, Major bacterial diseases in aquaculture and their vaccine development, *CABI*, Doi: 10.1079/PAVSNNR20127048 viewed 27 January 2020, https://naldc.nal.usda.gov/download/55894/PDF
- Quigley, DTG & McArdle, JF, 1998, Management and control of proliferative kidney disease (PKD) in a freshwater Atlantic salmon (Salmo salar L.) farm in Ireland: a case history, *The Journal of the Fish Veterinary Society*, no. 2, pp. 1-12, viewed 27 January 2020, https://www.fishvetsociety.org.uk/wp-content/uploads/2017/01/fvsjournalissue2.pdf
- Read, P, Landos, M, Rowland, SJ, Mifsud, C, NSW Department of Primary Industry, 2007, *Diagnosis, Treatment & Prevention of the Diseases of the Australian Freshwater Fish Silver Perch (Bidyanus bidyanus)*, viewed 27 January 2020, https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/638416/Silver-Perch-Diseases-Manual.pdf
- Reed, P, Francis-Floyd, R, Klinger, E & Petty, D, *Monogenean Parasites of Fish,* viewed 27 January 2020, http://animal-world.com/encyclo/fresh/information/Diseases.htm#Brooklynella
- Sanders, J L, Watra,l V, & Kent, ML, 2012, Microsporidiosis in zebrafish research facilities, *ILAR journal*, vol. *53, no. 2, pp.*106–113, doi:10.1093/ilar.53.2.106, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4117576/
- Scholz, T & Kuchta, R, 2016, Fish-borne, zoonotic cestodes (*Diphyllobothrium* and relatives) in cold climates: A never-ending story of neglected and (re)-emergent parasites*, Food and Waterborne Parasitology*, vol. 4, pp. 23-38, https://doi.org/10.1016/j.fawpar.2016.07.002, viewed 27 January 2020, https://www.sciencedirect.com/science/article/pii/S2405676616300117

- Stoskopf, MK, 1993, *Fish medicine*, Elsevier Health Sciences, London UK
- Wang, ML, Chen, HY & Shih, HH, 2017, Occurrence and distribution of yellow grub trematodes (*Clinostomum complanatum*) infection in Taiwan, *Parasitology Research,* vol. 116, no. 6, pp. 1761-1771, doi: 10.1007/s00436-017-5457-3, viewed 27 January 2020, https://www.ncbi.nlm.nih.gov/pubmed/28474185
- Weiss, LM & Becnel, JL, 2014, *Microsporidia: Pathogens of Opportunity*, Wiley Blackwell, Chichester, West Sussex
- Whipps, CM & Kent, ML, 2006, Polymerase chain reaction detection of *Pseudoloma neurophilia*, a common microsporidian of zebrafish (Danio rerio) reared in research laboratories, *Journal of the American Association for Laboratory Animal Science*, vol. 45, no. 1, pp. 36-39, viewed 27 January 2020, https://www.ingentaconnect.com/content/aalas/jaalas/2006/00000045/00000001/art00004?crawler=true
- Wikivet, 2012, *Diplostomum spathacaeum,* viewed 5 February 2020, https://en.wikivet.net/Diplostomum_spathacaeum
- Woo, PTK, 2003, *Cryptobia (Trypanoplasma) salmositica* and *Salmonid cryptobiosis, Journal of Fish Diseases, vol. 26, no. 11-12, pp. 627-46,* doi: 10.1046/j.1365-2761.2003.00500.x, viewed27 January 2020, https://www.researchgate.net/publication/8929285_Cryptobia_Trypanoplasma_salmositica_and_salmonid_cryptobiosis
- Woo, PTK, 2014, *Diseases and disorders of finfish in cage culture 2nd ed*., CABI International, Oxfordshire UK
- World Organisation for Animal Health, 2019, *Manual of Diagnostic Tests for Aquatic Animals*, viewed 27 January 2020, https://www.oie.int/standard-setting/aquatic-manual/
- Yanong, RPE, 2017, Nematode (Roundworm) Infections in Fish, University of Florida, *IFAS extension*, viewed 27 January 2020, http://edis.ifas.ufl.edu/fa091

Fisheries/aquaculture general glossary

The following fisheries glossary is included to aid in the interpretation of specific terms used in the aquaculture industry and parts of these guidelines. This glossary has been referenced from many online dictionary resources.

A	Abundance	Is a measure of how many fish are in a population or a fishing ground. See relative abundance and absolute abundance.
	Acoustic survey	A systematic gathering of information on fish availability and abundance using underwater sound.
	Acoustical oceanography	The use of underwater sound to map underwater topography and the contents of the sea.
	Aerial survey	A method of gathering information on surface fish movement and density by visual observation and photography from low-flying aircraft.
	Aggregation	Any grouping of fish, for whatever reason (or unknown reason) they are concentrating. See shoaling.
	Agricultural runoff	Surplus water from agricultural land, often draining into rivers and then into the sea, and often enriched with nutrients, sediment, and agricultural chemicals.
	Alginate production	A gel substance extracted from brown algae and used industrially as a thickening agent for food and paint.
	Algal bloom	A rapid excessive growth of algae, generally caused by high nutrient levels, particularly phosphorus. When the algae die, algal blooms can deplete oxygen to the point where fish cannot survive.
	Artisan fishing	A term sometimes used to describe small scale commercial or subsistence fishing practices. The term particularly applies to coastal or island ethnic groups using traditional techniques and traditional fishing boats.
	Anadromous	Fish that live their adult lives in the ocean but migrate up freshwater rivers to spawn. Examples are Pacific salmon. Fish that migrate in the opposite direction are called *catadromous*.
	Anoxic sea water	Sea water depleted of oxygen. See hypoxia.
	Anoxic sediments	Sediments depleted of oxygen.
	Antarctic convergence	A line encircling Antarctica where cold, northward-flowing Antarctic waters meet and sink beneath the sub-Antarctic waters, creating an upwelling zone which is very high in marine productivity, especially in Antarctic krill.
	Aquaculture	The farming of freshwater and saltwater organisms including molluscs, crustaceans and aquatic plants. See also fish farming and mariculture.
	Availability	(1) the proportion of a fish population living where it can be fished. (2) catch per unit effort. (3) a term sometimes used to describe whether a given fish of a given size can be caught by a given type of gear in a given fishing area.
B	Bait fish	Are small fish caught for use as bait to attract large predatory fish. See forage fish.
	Bathypelagic	The open ocean or pelagic zone that extends from a depth of 1000 to 4000 meters below the ocean surface.
	Beach	A geological landform along the shoreline of a body of water, consisting of loose particles composed of rock, such as sand, gravel, shingle, pebbles, or cobble, or of shell fragments or coralline algae fragments.

	Beam trawling	The simplest method of bottom trawling. The mouth of the trawl net is held open by a solid metal beam attached to two solid metal plates, welded to the ends of the beam, which slide over and disturb the seabed. This method is mainly used on smaller vessels, fishing for flatfish or prawns, relatively close inshore.
	Bed	The bottom of a river, or watercourse, or any body of water, such as the seabed.
	Benthic zone	The ecological region at the lowest level of a body of water such as an ocean or a lake, including the sediment surface and some sub-surface layers. Organisms living in this zone are called benthos.
	Benthos, benthic or demersal	Aquatic organisms which live on or in the seabed, also known as the benthic zone. Included are both mobile animals, such as crabs and abalone, and non-mobile animals, such as corals and sponges.
	Billfish	Large, predatory fish characterised by their long sword-like bill. Billfish include the sailfish, marlin and swordfish. They are important apex predators feeding on a wide variety of smaller fish and cephalopods.
	Bioacoustics	In underwater acoustics and fisheries acoustics this term is used to mean the effect of plants and animals on sound propagated underwater, usually in reference to the use of sonar technology for biomass estimation
	Bimodal	A bimodal distribution is a distribution with two different modes which appear as distinct peaks. An example in fisheries is the length of fish in a fishery, which might show two or more modes or peaks reflecting fish of different ages or species.
	Biodiversity	Is the variation of life forms within an area. In the context of fisheries, the number and variety of organisms found within a fishery.
	Biomass	The total weight of a fish species in each area. Can be measured as the total weight in tons of a stock in a fishery or can be measured per square metre or square kilometre. The most successful species worldwide, in terms of biomass, may be the Antarctic krill, with about five times the total biomass of humans.
	Biotone	A region where a distinctive transition from one set of biota to another occurs. An example is the region where tropical and temperate waters mix.
	Biotoxins	Natural toxins produced by organisms, often for use as a defense mechanism.
	Bony fish	Fish that have a bony skeleton and belong to the class Osteichthyes. Basically, this is all fish except for sharks, rays, skates, hagfish and lampreys.
	Bottom trawling	A fishing method that involves towing trawl nets along the sea floor. Bottom trawling can cause serious damage to sea floor habitats.
	Brackish water	Water that has more salinity than fresh water, but not as much as seawater. It may result from mixing seawater with fresh water, as in estuaries.
	Breach	A whale's leap out of the water.
	Breaker zone	The zone where ocean surface waves approaching the shore commence breaking, typically in water depths between five and ten metres.
	Brood	The collective offspring of a species produced in a time span. See also cohort.
	Buoy	A floating object usually moored to the bottom. Buoys can be used as temporary markers, called dans, during Danish seine fishing to mark the anchor position of a net, or when fishing with lobster pots to mark the position of the pots.

	Bycatch	Bycatch is the harvest of marine life and seabirds during fishing operations when other fish were the target. For example, bycatch might consist of a species which was not the targeted species, such as a shark caught on a tuna longline. Or it might consist of fish of the targeted species, but not of the targeted age or size. Some shrimp fisheries have a bycatch five times the weight of the caught shrimp. See also incidental catch.
C	Carapace	A calcified protective cover on the upper frontal surface of crustaceans. It is particularly well developed in lobsters and crabs.
	Carrying capacity	The supportable population of a species, given the food, habitat conditions and other resources available within a fishery.
	Casting	The act of throwing bait or a lure over the water, using a fishing rod.
	Catadromous	Fish that live their adult lives in freshwater lakes or rivers but migrate down rivers to spawn in the sea. An example are freshwater eels of genus *Anguilla*, whose larvae drift on the open ocean, sometimes for months or years, before travelling thousands of kilometres back to their original rivers (see eel life history). Fish that migrate in the opposite direction are called *anadromous*.
	Cephalopods	(From the Greek for "head-feet") animals such as squid and octopus where tentacles converge at the head. Cephalopods are the most intelligent of the invertebrates with well-developed senses and large brains.
	Cetacean	Member of the group of marine mammals that includes whales, dolphins and porpoises. They are the mammals most fully adapted to aquatic life and are noted for their high intelligence.
	Cetacean bycatch	The incidental capture of non-target cetacean species by fisheries. Bycatch can be caused by entanglement in fishing nets and lines, or direct capture by hooks or in trawl nets.
	Chondrichthyan	Cartilaginous fish, including sharks, rays and chimaeras.
	Cohort	Those individuals of a stock born in the same spawning season. For annual spawners, a year's recruitment of new individuals to a stock is a single cohort or year-class. See brood.
	Commercial fishery	An umbrella term covering fisheries resources and the whole process of catching and marketing fish, molluscs and crustaceans. It includes the fishermen and their boats, and all activities and resources involved in harvesting, processing, and selling.
	Conspecific	Organisms or populations that belong to the same species. Organisms that don't belong to the same species are *heterospecific*.
	Continental margin	The zone of the ocean floor that separates the thin oceanic crust from the thick continental crust. Continental margins constitute about 28% of the oceanic area.
	Continental rise	Is below the slope, but landward of the abyssal plains. Extending as far as 500 kilometres from the slope, it consists of thick sediments which have cascaded down the slope and accumulated as a pile at the base of the slope.
	Continental shelf	The seabed from the shore to the edge of the continental slope, covered by relatively shallow seas (known as shelf seas) and gulfs.
	Continental slope	The slope which starts, usually abruptly at about a 200-metre depth, at the outer edge of the continent shelf and dips more steeply down to the deep-ocean floor (abyssal plain).
	Coriolis effect	Due to the Earth's rotation, freely moving objects on the surface of the earth veer right in the northern hemisphere and left in the southern hemisphere. This effect is called the Coriolis effect, and works on winds and ocean currents. The effect varies with latitude and is zero at the equator and increases towards the poles.
	Cottage industry	Small, locally owned businesses usually associated in fishing with traditional methods and low relative yield.
	Crab pot fishery	A fishing technique where crabs are lured by bait into portable traps, sometimes called pots.

	Crustaceans	A group of freshwater and saltwater animals having no backbone, with jointed legs and a hard shell made of chitin. Includes crabs, lobsters, crayfish, shrimp and krill.
D	Danish seine	A widely used commercial fishing technique which uses a small trawl net with long wire warps. The seine boat drags the warps and the net in a circle around the fish. The motion of the warps herds the fish into the central net. Danish seining works best on demersal fish which are either scattered on or close to the bottom of the sea or are aggregated (schooling). See also purse seine.
	Dead zone	An area in an ocean or large lake where oxygen levels are extremely low, often due to eutrophication. Dead zones have been increasing since the 1970s.
	Deep ocean currents	Currents in the deep ocean, also known as thermohaline circulation or the "conveyor belt", are driven by density and temperature gradients. They can be contrasted with surface ocean currents, which are driven by the wind.
	Demersal zone	The zone at or near the bottom of a sea or lake. Inhabitants of the demersal zone feed off the bottom or off other demersal fish. See also pelagic zone.
	Demersal fish	Fish that live in the demersal zone. Examples are cod, flounder and snapper. Compared to pelagic fish, demersal fish contain little oil. See also bottom feeder.
	Demersal trawling	Trawling on or near the bottom of a sea or lake. See also *bottom trawling*.
	Depletion	Reducing the abundance of a fish stock through fishing.
	Delisted	A species which is no longer listed under the ESA. See also *recovered species*.
	Detritivores	Detritivores (also known as detrivores, detritus feeders, detritophages,), are heterotrophs that obtain nutrients by consuming detritus including decomposing animal and plant parts as well as faeces.
	Diatoms	Minute planktonic unicellular or colonial algae.
	Downwelling	A downward movement (sinking) of surface water caused by onshore Ekman transport, converging currents or when a water mass becomes more dense than the surrounding water.
	Dorsal	Relating to or situated near or on the animal's back.
	Dredging	Dredge designed to catch scallops, oysters or sea cucumbers are towed along the bottom of the sea by specially designed dredge boats.
	Driftnet	A gillnet suspended by floats so that it fishes the top few metres of the water column. Drift nets can be many kilometres long. Because drift nets are not anchored to the sea bottom or connected to a boat, they are sometimes lost in storms and become ghost nets.
	Dropline	A fishing line with one or more hooks, held vertically in the water column with weights and generally used on the continental shelf and slope. Several droplines may be operated by a vessel, either on manually or mechanically operated reels.
E	Echinoderms	A group of marine animals that includes sea stars, sea urchins and sea cucumbers, abundant on the floor of the deep sea, as well as in shallower seas.
	Economic rent	The profit that could be earned from a fishery owned by an individual. Individual ownership maximizes profit, but an open entry policy usually results in so many fishermen that profit barely matches opportunity cost. See *maximum economic yield*.
	Ectothermic, poikilotherms	Animals that control body temperature through external means, using the sun, or flowing air or water.

	Ekman transport	Resultant flow at right angles to and to the right of the wind direction in the northern hemisphere, to the left in the southern hemisphere.
	Elasmobranch	Cartilaginous fish that includes sharks, skates and rays (but not chimaeras)..
	Electrophoresis	A technique used by fisheries scientists. Tissue samples are taken from fish, and electrophoresis is used to separate proteins such as enzymes, based on their different mobilities in an electric field. This information is used to differentiate between morphologically similar species and to distinguish sub-populations or stocks.
	El Niño	Large scale, cyclical (generally three to seven years), ocean warming and cooling episodes across the equatorial Pacific. Warm water pools in the east in El Niño conditions and in the west during La Niña conditions. It begins around Christmas (El Niño means Christ child). These changes disrupt weather patterns and the migration habits of fish.
	Endangered species	An endangered species is a population of an organism which is at risk of becoming extinct. The IUCN has calculated the percentage of endangered species as 40 percent of all organisms based on the sample of species that have been evaluated through to 2006.
	Endemic	Native to a certain region, often a fairly small local area.
	Endothermic	Animals which maintain a body temperature which is above ambient temperature. See Ectothermic.
	Epibenthos	Invertebrates that live on top of the seabed. Compare benthos.
	Epifauna	Animals living on the surface of the seabed or a riverbed, or attached to submerged objects or aquatic animals or plants.
	Epipelagic	The top layer of the ocean from the surface down to about 200 metres. This is the illuminated zone where there is enough light for photosynthesis. Nearly all primary production in the ocean occurs here. See photic zone.
	Escapement	The percentage of a spawning anadromous fish population that survives all obstacles during their migration, including fishing pressure and predation, and successfully reach their spawning grounds.
	Estuary	A semi-enclosed coastal body of water with one or more rivers or streams flowing into it, and with a free connection to the open sea. Healthy estuaries can have high rates of biological productivity.
	Eulittoral zone	Another name for the intertidal zone or *foreshore*, extending from the spring high tide line to the neap low tide line.
	Euryhaline	Fish that are tolerant to a wide range of salinities.
	Eutrophication	An increase in chemical nutrients – typically compounds containing nitrogen or phosphorus – in an ecosystem. Eutrophication in water often results in an increase in algae growth and decay, which can lead to decreased levels of oxygen and fish populations.
	Exclusive Economic Zone (EEZ)	A sea zone under the law of the sea over which a state has special rights to the exploration and use of marine resources. Generally, a state's EEZ extends to a distance of 200 nautical miles (370 km) out from its coast.
F	Farmed fisheries	Are fisheries where the fish are farmed using aquaculture techniques. They can be contrasted with wild fisheries.
	Fecundity	The number of eggs a fish produces each reproductive cycle; the potential reproductive capacity of an organism or population. Fecundity changes with the age and size of the fish.
	Fish	A true fish is a vertebrate with gills that lives in water. However, in the context of fisheries, the term "fish" is generally used more broadly to include any harvestable animal living in water, including molluscs, crustaceans and echinoderms. the term "shellfish" refers to molluscs the term "finfish" refers to bony fishes, sharks and some rays the term "scale fish" refers to fish bearing scales

		the term "fish" can refer to more than one fish, particularly when the fish are from the same species the term "fishes" refers to more than one species of fish
	Fishery	The activities leading to and resulting in the harvesting of fish. It may involve capture of wild fish or raising of fish through aquaculture. A fishery is characterised by the species caught, the fishing gear used, and the area of operation.
	Fishing effort	A measure of how much work is needed by fisherman to catch fish. Different measures are appropriate for different kinds of fisheries.
	Fishmeal	Protein-rich animal feed product based on fish.
	Fishing trip	Usually performed by using a vessel, fishing trip starts when departing a port and ends when returning to port or in some cases when landing the catch. Fishing trip consists of different types of activities performed when fishing, for example - setting traps, recovering traps, crossing a fishing zone, relocating catch, discarding catch etc.
	Fishing vessel	Any vessel normally used for the harvesting of living aquatic resources or in support of such activity. This includes vessels which provide assistance to other fishing vessels such as supply, storage, refrigeration, transportation or processing (mother ships).
	Fishing fleet	An aggregation of fishing vessels of a particular country, such as the Russian fishing fleet, or using a particular gear, such as purse seine fleet.
	Flashing	Abnormal behaviour pattern including darting and twisting of fish and erratic swimming
	Flushing time	The time required to replace all the water in an estuary or harbour by the actions of currents and tides.
	Fork length	In fishes with forked tails, this measures from the tip of the snout to the fork of the tail. It is used in fishes when is difficult to tell where the vertebral column ends.
	Forage fish	Are small fish which are preyed on by larger predators. Typical ocean forage fish are small, filter-feeding fish such as herring, anchovies and menhaden. They compensate for their small size by forming schools. See bait fish.
	Foreshore	Intertidal area between the highest and lowest tide levels.
	Founder effect	The loss of genetic variation when a new colony is established by some individuals moving to a new area that is unoccupied. As a result, the new population may be distinctively different from its parent population.
	Free-diving	Diving under water without the assistance of breathing apparatus to collect oysters, abalone, corals, sponges, crayfish etc. The gear usually includes a snorkel, face mask, flippers, weight belt and wet suit.
	Front	Region of sharp gradient in temperature or salinity, indicating a transition between two current systems or water masses. Fronts are usually associated with high biological activity and high abundance of highly migratory resources such as tuna. They are actively sought as fishing areas and can be monitored by satellite remote sensing.
	Fry	Recently hatched fish that has reached the stage where its yolk-sac has almost disappeared, and its swim bladder is operational to the point where the fish can actively feed for itself.
G	Gametes	Eggs and sperm.
	Gear	The equipment used by fishermen when fishing. Some examples are hooks, lines, sinkers, floats, rods, reels, baits, lures, spears, nets, gaffs, traps, waders and tackle boxes.
	Gene flow	The movement of genes from one population to another by individuals moving between the populations.
	Ghost nets	Fishing nets and other gear that has been left or lost in the ocean and continues to capture and kill fish.

	Gillnet	Fishing nets constructed so that fish are entangled or enmeshed, usually in the gills, by the netting. According to their design, ballasting and buoyancy, these nets can be used to fish on the surface, in midwater or on the bottom. The mesh size of the net determines the size of fish caught, since smaller fish can swim through the mesh. See also drift net.
	Global positioning system	A device which uses satellite signals to accurately determine a fishing vessel's position and course.
	Groundfish	Fish that lives most of its life on or near the sea bottom, such as cod, haddock, or flounder.
	Gulf	A large area of water bordered by land on three sides.
H	Habitat	The place where an organism lives.
	Halocline	A zone in which salinity changes rapidly.
	Harmful algal bloom (HAB)	An algal bloom that produces toxins detrimental to plants and animals. Scientists prefer this term to red tide, since not all algal blooms are harmful, nor do all algal blooms cause discoloration, and the blooms are not associated with tides.
	Harvest	The number or weight of fish caught and retained from a given area over a given period of time. Note that landings, catch, and harvest are different.
	Hatchery	The process of cultivating and breeding a large number of juveniles in an enclosed environment. The juveniles are then released into lakes, rivers or fish farm enclosures.
	High seas	Waters outside national jurisdictions.
	Highly migratory species	A term which has its origins in the United Nations Convention on the Law of the Sea. It refers to fish species which undertake ocean migrations and also have wide geographic distributions. It usually denotes tuna and tuna-like species, shark, marlins and swordfish. See also transboundary stocks and straddling stocks.
	Husbandry	The farming practice of breeding and raising fish stock.
	Hypoxia	Occurs in aquatic environments when dissolved oxygen becomes depletion to a level which is harmful to aquatic organisms.
I	Individual transferable quota (ITQ)	A management tool by which the total allowable catch quota is allocated to individual fishers or companies who have long-term rights over the quota or can transfer it to others by sale, lease, or will. See also *quota.*
	Intertidal	The intertidal zone is the region of land which is submerged during high tide and exposed during low tide.
	Introduced species	Species brought into an area where it does not naturally occur but is able to survive and reproduce there.
	Invertebrates	Animals without a backbone, such as octopus, shellfish, jellyfish and corals. See also vertebrates.
	Isobath	A contour line linking regions of the same depth.
	Isopleth	Contour line joining points corresponding to similar values. Often used to plot yield-per-recruit values on a graph showing the changes as a function of size at first capture and fishing mortality.
	Isopods	Group of small crustaceans that includes fish lice.
	Isotherm	Contour line connecting points with the same temperature.
	IUCN Red List	The IUCN Red List of Threatened Species (also known as the IUCN Red List or Red Data List), created in 1963, is the world's most comprehensive inventory of the global conservation status of plant and animal species.

		 Summary of 2006 IUCN Red List categories.
	IUCN	The International Union for the Conservation of Nature and Natural Resources is the world's main authority on the conservation status of species. Their system divides threatened species into three categories: critically endangered (CR), endangered (EN), and vulnerable (VU). They also list extinctions that have occurred since 1500 AD and taxa that are extinct in the wild.
J	Jigging	A method of fishing which uses lures on a vertical line that is moved up and down or jigged. Jigging can be done manually with hand-operated spools. It is also done automatically using machines when fishing for arrow squid.
	Jukung	A traditional fishing boat used in Indonesia.
	Juvenile	A young fish or animal that has not reached sexual maturity.
K	Krill	A small shrimplike planktonic crustacean of the open seas. It is eaten by a number of larger animals, notably the baleen whales.
L	La Niña	A condition involving an excessive pooling of cool water which occurs in the equatorial Eastern Pacific Ocean. See El Niño.
	Lagoon	A body of shallow salt or brackish water separated from the deeper sea by a shallow or exposed sandbank, coral reef, or similar feature.
	Land runoff	Rainfall, snow melt or irrigation water that runs off the land into streams and other surface water, and ultimately into the ocean. Land runoff can carry pollutants, such as petroleum, pesticides, and fertilisers.
	Landing	The amount of fish (usually in tons) harvested from the sea and brought to the land. May be different from the catch, which includes the discards. Landings are reported at the points at which fish are brought to shore. Most often, landings provide the only record of total catch, i.e. landings plus discards. Note landings, catch, and harvest define different things.
	Line fishing	A general term for fishing methods which use fishing lines. It includes handlines, hand reels, powered reels, pole-and-line, droplines, longlines, trotlines and troll lines.
	Littoral	The shallow water region around lake or sea shores where significant light penetrates to the bottom. Typically occupied by rooted plants. On sea shores it includes the intertidal zone.
	Logbook	An official record of catch and its species composition, fishing effort and location, recorded on board the fishing vessel. In many fisheries, logbooks are a compulsory condition of licensing.
	Longlines	A long fishing line with many short lines, called snoods and carrying hooks, attached at regular intervals. Pelagic longlines are suspended horizontally at a fixed depth using surface floats. Demersal longlines are weighted at the seabed and have closer-spaced hooks. A longline can be miles long with several thousand hooks.
M	Mariculture	A branch of aquaculture where marine organisms are cultivated in the open ocean, or an enclosure of the ocean, or in tanks, ponds or raceways filled with seawater. Examples are the farming of marine fish, prawns, oysters and seaweed.
	Marine mammal	Mammals that are primarily ocean-dwelling or depend on the ocean for food, such as porpoises, whales, seals, walrus and polar bears.
	Marine protected area (MPA)	Marine area with some level of legal restriction to protect living, non-living, cultural, and/or historic resources.

	Maximum economic yield (MEY)	The total amount of profit that could be earned from a fishery if it were owned by one individual. An open entry policy usually results in too many fishermen, so profits are barely higher than opportunity costs. See *economic rent*.
	Maximum sustainable yield (MSY)	The maximum catch that can be taken from a species' stock over an indefinite period. Under the assumption of logistic growth, the MSY will be exactly at half the carrying capacity of a species, as this is the stage at when population growth is highest. The maximum sustainable yield is usually higher than the optimum sustainable yield. Studies have shown that fishing at the level of MSY is often not sustainable. See also long-term potential yield.
	Meristics	A series of measurements on a fish, such as scale counts, which are used to separate different populations or races of fish.
	Mesopelagic	Ocean depths extending from 200 to 1000 metres (650 to 3280 feet) below sea level.
	Migration	A systematic (as opposed to random) movement of individuals in a fish stock from one place to another.
	Minimum landing size	The smallest length at which it is legal to keep or sell a fish. Sizes vary with the species of fish and also vary in different places around the world.
	Mollusc/Mollusk	A group of freshwater and saltwater animals with no skeleton and usually one or two hard shells made of calcium carbonate. Includes oysters, clams, mussels, snails, conches, scallops, squid and octopus.
	Morphometrics	Measurements which characterise the form, shape and appearance of an animal or plant. Difference in morphometrics, such as colouration, can be used to distinguish different stocks of the same species.
	Mortality	Mortality is a death rate from various causes, such as the proportion of a fish stock dying annually. See also natural mortality and fishing mortality.
	Mud flat	Are coastal wetlands that form when mud is deposited by the tides or rivers, sea and oceans. They are found in sheltered areas such as bays, bayous, lagoons, and estuaries.
N	Natal	Relating to birth, such as many salmon that return to their place of birth to spawn.
	Nearshore waters	Relatively shallow inshore waters that do not extend beyond the continental shelf. See also sublittoral zone.
	Neritic zone	The shallow pelagic zone over the continental shelf. See also nearshore waters.
	Nitrate	A water-soluble molecule made up of nitrogen and oxygen, commonly found in agricultural fertilizers, and therefore in land runoff. Too much nitrate concentration is can be toxic to marine life.
	Nursery	The part of a fish or animal habitat where the young grow up.
	Nutrient upwelling	Nutrient upwelling is the 'welling-up' of deeper water that is usually richer in nutrients than surface water.
O	Ocean basin	Geologically an ocean basin is a large geologic basin which is below sea level.
	Ocean currents	Oceanic currents can be divided into surface and deep ocean currents. Surface currents are generally wind driven and develop typical clockwise spirals in the northern hemisphere and counter-clockwise spirals in the southern hemisphere. Surface currents can operate to a depth of 400 meters and apply to about ten percent of water in the ocean. Deep ocean currents are driven by density gradients in water due to temperature (thermo) and salinity (haline) differences. This thermohaline circulation, occurs at both deep and shallow ocean levels and moves much slower than tidal or surface currents. Upwelling and downwelling areas in the oceans are areas where significant vertical currents of water are observed. Ocean currents can be contrasted with the tidal currents that occur in coastal areas.

	Ocean surface waves	Are surface waves that occur on the free surface of the ocean. They usually result from wind and are also referred to as wind waves. Some waves can travel thousands of miles before reaching land.
	Ocean Tracking Network	A research effort using implanted acoustic transmitters to study fish migration patterns.
	Oceanodromous	Fish that migrate only within salt (ocean) waters.
	Oceanography	The branch of earth sciences that studies the ocean, including marine organisms and ecosystem dynamics; ocean currents and waves; plate tectonics and the formation of underwater topography; and movements of various chemical substances and physical properties within the ocean and across its boundaries.
	Otoliths	Calcareous deposits or bones found in chambers at the base of the skull in fish. Sectioned, these bones often show rings or layers which can be used to determine age.
	Otter trawl	An otter trawl is a demersal trawl which uses large rectangular otter boards to keep the opening of the trawl net from closing. Otter trawls are towed by a single trawler.
	Overfishing	Occurs when fishing activities reduce fish stocks below an acceptable level. This can occur in any body of water from a pond to the oceans.
P	Pair trawling	Occurs when two trawlers tow the same net. Otter boards are not needed and very large nets can be held open and towed in this manner.
	Panmictic	Refers to random mating where all individuals within a population are potential partners.
	Parameter	Parameter in fisheries is a characteristic measure of some aspect of a fish stock. it is usually expressed as a numerical value, such as the "natural mortality rate".
	Pelagic zone	Any water in the sea that is not close to the bottom.
	Pelagic fish	Fish that spend most of their life swimming and feeding in the pelagic zone, as opposed to resting on or feeding off the bottom. Examples are tuna and most sharks.
	Phosphate	A chemical compound containing phosphorus and oxygen, commonly found in agricultural fertilizers and land runoff.
	Photic zone	"Sun lit" zone extending downward from a lake or ocean surface to the euphotic depth where the light intensity falls to one percent of that at the surface. The photic zone exposed to sufficient sunlight for photosynthesis to occur. The depth of the photic zone can be greatly affected by seasonal turbidity. Typical euphotic depths vary from only a few centimetres in highly turbid eutrophic lakes, to around 200 metres in the open ocean. About 90% of all marine life lives in this region.
	Piscivore	A carnivorous animal that eats primarily fish.
	Plankton	Consist of any drifting organisms (animals, plants, archaea or bacteria) that inhabit the pelagic zones, particularly the surface areas, of oceans or bodies of fresh water.
	Poikilotherms	An organism that cannot regulate its body temperature except by behavioural means such as basking or burrowing, therefore its internal temperature varies considerably.
	Population	See stock.
	Population dynamics	The study of fish populations and how fishing mortality, growth, recruitment, and natural morality affect them.
	Population model	A hypothesis of how a population functions. It often uses mathematical descriptions of growth, recruitment and mortality.

	Primary Productivity	A measurement of plant production that is the start of the food chain. Much primary productivity in marine or aquatic systems is made up of phytoplankton, which are tiny one-celled algae that float freely in the water.
	Purse seine	A fishing technique capable of harvesting large quantities of surface-schooling pelagic fish by surrounding the school with a net. A line which passes through rings on the bottom of the net can be tightened to close the net so that the fish are unable to escape. See also Danish seine.
	Phytoplankton	Tiny, free-floating, photosynthetic organisms in aquatic systems.
Q	Quota	Quota is the amount of catch that can be legally landed in a time period. It could refer to a fishery as a whole (total allowable catch) or to an amount allocated to an individual or company. See also individual transferable quota.
	Quota management system (QMS)	A system that limits the amount of fish that can be taken by commercial fishers. The QMS sets a quota that can be taken by each commercial fisher.
R	Recruitment	The number of new young fish that enter a population in a given year. More pragmatically, it can be defined as the number of young fish that attain a size where they can be legally caught or become susceptible to being caught by a given fishing gear.
	Red tide	Discolouration of surface waters, most frequently in coastal zones, caused by large concentrations of micro-organisms. See harmful algal bloom.
	Risk analysis	Evaluates the possible outcomes of various harvesting strategies or management options.
S	Salinity gradient	Salinity gradient: Change in salinity with depth, expressed in parts per thousand per metre. See halocline.
	Sample	A relatively small part of a fish stock which is removed for study, and which ideally is representative of the whole. The greater the number and size of the samples, the greater the confidence that the information obtained accurately reflects the status (such as abundance by number or weight, or age composition) of the stock.
	Scrubbing	Abnormal behaviour pattern where fish rub against objects in response to skin irritation
	Seamounts	Underwater mountains rising at least 1000 metres above the sea floor.
	Sea grass	Members of marine seed plants that grow chiefly on sand or sand-mud bottom. They are most abundant in water less than 10 metres deep. Common types are eel grass, turtle grass and manatee grass.
	Selectivity	Ability of a type of fishing tackle or gear to catch a certain size or kind of fish, compared with its ability to catch other sizes or kinds.
	Seashore	The coast or that part of the land adjoining or near the ocean. See intertidal zone.
	Shelf break	Where the continental shelf and continental slope meet. At the shelf break, the more gently sloping region of the seabed adjacent to a landmass rather abruptly slopes more steeply down towards the ocean depths, commonly around depths of 200 metres.
	Shellfish	General term for aquatic invertebrates (molluscs, crustaceans and echinoderms).
	Shoal or sandbar	Is a somewhat linear landform within or extending into a body of water, typically composed of sand, silt or small pebbles. Bars can appear in the sea, in a lake, or in a river.
	Shoaling	Describes the behaviour of fish which aggregate together, including mixed species groups. Fish derive many benefits from shoaling behaviour including defense against predators through better predator detection and by diluting the chance of capture, enhanced foraging success, and higher success in finding a mate. It is also likely that fish benefit from shoal membership through increased hydrodynamic efficiency.

	Shore	A shore or shoreline is the fringe of land at the edge of a large body of water, such as an ocean, sea, or lake. A shore of unconsolidated material is usually called a beach. See intertidal zone.
	Simulation	An analysis that shows the production and harvest of fish using a group of equations to represent the fishery. It can be used to predict events in the fishery if certain factors change. See population dynamics.
	Smolt	Is a stage of a salmon life cycle that is getting ready to go out to sea. As they begin to mature, they adapt for life in salt water in an intermediary stage known as smolts. This process marks the beginning of their first migration from their home stream to the ocean.
	Socioeconomics	A word used to identify the importance of factors other than biology in fishery management decisions. For example, if management results in more fishing income, it is important to know how the income is distributed between small and large boats or part-time and full-time fishermen.
	Spawning	The production or depositing of large quantities of eggs in water.
	Species	A group of organisms capable of interbreeding and producing fertile offspring.
	Species density	The number of species in a sampled area.
	Species group	A group of similar species. Similar species are often difficult to differentiate without detailed examination.
	Sport fishery	See recreational fishery.
	Stakeholder	Anyone who has a stake or interest in the outcome of the project, as well as anyone one who is affected by the project.
	Standardisation	Procedures which maintain methods and equipment as constant as possible. Without standardization one cannot determine whether measurements of yearly differences in relative abundance are caused by actual fluctuations in stock abundance or by differences in the measurement procedure used. Lack of standardization is one reason why surveys using different commercial fishing vessels in different years do not produce comparable information. For example, if two vessels of different horsepower are used in separate years, the results can't be compared unless vessel mensuration experiments are performed. This would involve comparing the two vessels' catches to determine the influence of their fishing power on the size of the catch, and a determination of a correction factor.
	Stock	Group of fish of the same species (for example, snapper) that occupy a defined area of the ocean. Fish stocks are the basis of fisheries' management. Not to be confused with stockfish.
	Straddling stocks	A term defined by the United Nations as "stocks of fish such as pollock, which migrate between, or occur in both, the economic exclusion zone of one or more states and the high seas". They can contrasted with transboundary stocks. A stock can be both transboundary and straddling.
	Sub-Antarctic waters	Waters adjacent to, but not within, the Antarctic circle (about 66030'S).
	Subtropical waters	Waters adjacent to, but not within, the tropics.
	Super seiner	A large purse seiner, usually over 70 metres long, with freezing and storage facilities, and capable of fishing for an extended period in open oceans.
	Surf	Collective term for breakers. Also, the wave activity in the area between the shoreline and the outermost limit of breakers.
	Surf zone	As ocean surface waves come closer to shore they break, forming the foamy, bubbly surface called surf. The region of breaking waves defines the surf zone.

	Surface ocean currents	Surface currents are generally wind driven and develop typical clockwise spirals in the northern hemisphere and counter-clockwise rotation in the southern hemisphere. In wind driven currents, the Ekman spiral effect results in the currents flowing at an angle to the driving winds. Surface currents make up about ten percent of the water in the ocean and are generally restricted to the upper 400 meters. They can be contrasted with deep ocean currents, which are driven by density and temperature gradients.
	Surplus production	Surplus production is the inherent productivity of a fish stock that can be harvested on a sustainable basis. Based on the theory that, at large stock size, reproductive rates and rate of stock growth are slowed by self-regulating mechanisms, and that stock growth rates are faster after removals, as the stock attempts to rebuild. In theory, fishing can be moderated to take advantage of the more productive stock growth rates, provided that it does not exceed the stock recovery capacity.
	Sustainable fishing	Fishing activities that do not cause or lead to undesirable changes in biological and economic productivity, biological diversity, or ecosystem structure and functioning, from one human generation to the next.
	Sustainable yield	Sustainable yield is the catch that can be removed over an indefinite period without causing the stock to be depleted. This could be either a constant yield from year to year, or a yield which is allowed to fluctuate in response to changes in abundance.
T	Tag and release	Marking or attaching a tag to a fish so that it can be identified on recapture. Used for the study of fish growth, movement, migration, and stock structure and size.
	Teleosts	The teleosts or Teleostei are by far the largest infraclass in the class Actinopterygii, the ray-finned fishes, and make up 96% of all extant species of fish. Teleosts are arranged into about 40 orders and 448 families. Over 26,000 species have been described.
	Threatened species	Threatened species are species which are vulnerable to extinction in the near future. The IUCN further divides them into three categories: vulnerable, endangered, and critically endangered.
	Tidal current	Alternating horizontal movement of water in coastal areas, associated with the rise and fall of the tide as the earth rotates. The rise and fall is caused by gravitational forces exerted by the moon and the sun. Unlike ocean currents, tidal currents change in regular patterns that can be predicted for future dates.
	Tidal flats	Are coastal wetlands that form when mud is deposited by tides or rivers. Also called *mudflats*.
T	Transboundary stocks	Are fish stocks which range across the EEZs of two or more countries. They can be contrasted with straddling stocks. A stock can be both transboundary and straddling.
	Trap fishing	Fishing by means of traps, often designed to catch a particular species, such with lobster pots.
	Trash fish	Catch with no commercial value which is discarded, especially when trawling. Also called *rough fish*. See also *coarse fish*.
	Trawling	Is fishing with a large bag-like net, called a trawl, which is drawn along behind a boat called a trawler. The net can be dragged along the sea bottom in order to target demersal fish, or pulled through clear water in order to target pelagic fish. Trawling along the sea bottom can result in significant bycatch and habitat destruction.
	Trophic level	The position that a species occupies in a food chain. The species it eats are at a lower trophic level, and the species that eats it are at a higher trophic level.
	Trolling	A method of fishing where one or more fishing lines, baited with lures or bait fish, are drawn slowly through the water behind a boat. Trolling is used to catch pelagic fish such as mackerel and tuna species.

	Turbidity current	A current of rapidly moving, sediment-laden water that is heavier than clear water and therefore flows downslope along the bottom of the sea or a lake. The term is most commonly used to describe underwater currents in lakes and oceans, which are usually triggered by earthquakes or slumping.
	Turtle excluder device (TED)	A specialized device that allows a captured sea turtle to escape when caught in a trawl net.
U	Underwater acoustics	Due to its excellent propagation properties, underwater sound is used as a tool to aid the study of marine life, from microplankton to the blue whale. See also Ocean Tracking Network.
	Upwelling	The process by which water, usually cold and nutrient-rich, rises from a deeper to a shallower depth. This is often a result of offshore surface water flow, particularly when persistent wind blows parallel to a coastland and the resultant Ekman transport moves surface water away from the coast.
V	Vertebrates	Animals with a backbone, including fish (sharks, rays and bony fish), amphibians, reptiles and mammals. See also invertebrates.
	Vessel monitoring system (VMS)	Technology used in commercial fishing to allow environmental and fisheries regulatory organizations to track the location of vessels.
V	Virtual population analysis (VPA)	An analysis of fish population numbers that uses the number of fish caught at various ages or lengths and an estimate of natural mortality to estimate fishing mortality in a cohort. It also provides a back estimate of the number of fish in a cohort at various ages.
	Vulnerable species	A species which is likely to become an endangered species unless the circumstances threatening its survival and reproduction improve.
W	Wetlands	Areas of land where the soil is saturated with moisture, such as swamps and mangrove forests.
	Whirling	Fish display a whirling/tail chasing abnormal behaviour with erratic swimming patterns.
	Wild fish	Are fish which live free, not penned in, in lakes, rivers or the sea. They can be contrasted with farmed fish.
	Wild fisheries or "capture fisheries"	Are fisheries which target wild fish. They can be contrasted with farmed fisheries.
	Wind currents	Currents created by the action of the wind. Surface ocean currents are generally wind driven and develop typical clockwise spirals in the northern hemisphere and counter-clockwise rotation in the southern hemisphere. In wind driven currents, the Ekman spiral effect results in the currents flowing at an angle to the driving winds. Surface currents make up about ten percent of the water in the ocean and are generally restricted to the upper 400 meters.
Z	Zoobenthos	The aquatic fauna of the region at or near the bottom of the sea; the animal component of the benthic community.
	Zoobenthivore	Any creature that feeds on the zoobenthos
	Zooplanktivore	Any organism that consumes zooplankton
	Zooplankton	Zooplankton are weakly swimming/floating animals. They use water currents to move great distances. They are usually larger than phytoplankton, ranging from tiny copepods, less than a centimetre long, to much larger jellyfish and salps.

Definitions

General terms

Animal carer- can be an investigator, chief investigator or animal handler/laboratory technician/animal facility manager.
Animal handler- trained/deemed competent person/s other than the investigator who care for the animals on a day to day basis. They should be named on the AEC application.
Chief investigator- has overall responsibility for a scientific project and supervises investigators.
Distress - a negative mental state that indicates when an animal is unable to cope with a degree of underlying pain, anxiety, fear, stress. In animals, it is defined by changes in behaviour and physiology.
Housing facility or aquaculture room/laboratory – the room or area where fish housing tanks are kept and fish procedures are undertaken.
Housing tanks- where fish are kept during the scientific project (Note: these can be aquariums, ponds, sea cages).
Investigator- can be a teacher or researcher who manages and is responsible for a scientific project on a day to day basis.
OH&S- Occupational health and safety.
PPE- Personal protective equipment.

Specific terms

Aerator tank- a separate tank set up with an oxygen supply to allow recovery of fish following handling or other procedures
Ammonia - Ammonia is the major waste product of protein or nitrogenous metabolism in fish and other aquatic organisms.
Broodfish/broodstock- A group of mature individuals used in aquaculture for breeding purposes.
Fingerlings- a fish that has reached the stage where the fins can be extended and where scales have started developing throughout the body.
Fry- Recently hatched fish that has reached the stage where its yolk-sac has almost disappeared, and its swim bladder is operational to the point where the fish can actively feed for itself.
CO^2- carbon dioxide
D.O.- dissolved oxygen
Metabolic rate- A measurement of the number of calories that the fish burns at rest.
Tempering- to align one type of fish housing with another as closely as possible with regards to water quality and general set up
Water buffers- products (acid or bases/alkaline) added to water to maintain a stable pH
Zoonotic-a disease that has transmitted from an animal to a human