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FOR THE NSW DPI (FISHERIES) ANIMAL CARE AND ETHICS COMMITTEE

A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research

4th Edition

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More information

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1. Foreword

Research with vertebrates in New South Wales, Australia, is governed by the Animal Research Act, 1985. Under this act, all research must be covered by a current Animal Research Authority, issued by an accredited Animal Care and Ethics Committee.

The NSW Animal Research Act 1985 was introduced to protect the welfare of animals, by ensuring their use in research is always humane, considerate, responsible and justified. The Animal Research Regulation 2010 incorporated the Australian Code for the Care and Use of Animals for Scientific Purposes into the legislation.

Any organisation or person that uses or supplies vertebrate animals for research or teaching in NSW must comply with the Animal Research Act. The Act applies to all individuals, groups, institutions, organisations, schools and companies which use animals.

Organisations which conduct research with vertebrate animals must (under the Animal Research Act, administered by the Animal Welfare Branch of NSW DPI and the NSW Animal Research Review Panel) become an accredited research establishment. Independent scientists or researchers must obtain an Animal Research Authority from an Animal Care and Ethics Committee (ACEC) of an accredited research establishment or from the NSW DPI Secretary's ACEC (formerly known as the Director-General's Animal Care and Ethics Committee).

This document provides a guide to procedures and practices for collecting and maintaining fish, research techniques and to the operation of aquaculture facilities for the purpose of scientific research that are consistent with the objectives of the Australian Code for the Care and Use of Animals for Scientific Purposes. Information has also been provided to assist scientists complete the application form necessary to obtain an Animal Research Authority.

Basic information for most topics is presented and more detailed information provided for topics that are most frequently raised by researchers or members of Animal Care and Ethics Committees who must approve applications. Emphasis has been placed on describing basic fish husbandry requirements, maintaining water quality, using chemicals legally, recognising and treating some of the most common diseases and legal obligations relating to the reporting of notifiable aquatic diseases and transporting live fish. Methods for collecting and sampling fish in the wild and practical details of methods for anaesthesia and euthanasia are also described.

This document does not attempt to cover all aspects or methods that may be available to the researcher in any given circumstance. Full details of methods, even if they are included in this guide, must be provided in the application for an Animal Research Authority.

Examples of a New Proposal Form for an Animal Research Authority, for an Annual Renewal, for a Major-Three Year Renewal and the form for Reporting Unexpected Adverse Events are attached. Also attached are copies of an Animal Research Authority and an example of a final report that must be provided to the NSW DPI (Fisheries) ACEC on completion of research. Finally, the NSW DPI (Fisheries) ACEC Terms of Reference, Grievance Procedures and Terms of Appointment are provided.

2. Animal Research Authorities

2.1 What is animal research?

Animal research includes the use of animals in:

- Experimental research
- Surgical, medical, psychological, biological, chemical or physical treatment
- Abnormal husbandry or dietary conditions
- Electric shock or radiation studies
- Collection of blood, tissue or other body samples
- Product Testing
- Teaching
- Diagnosis
- Field surveys
- Production of biological material
- Feeding trials

An 'animal' includes any vertebrate that is any of the following:

- mammal
- bird
- reptile
- amphibian
- fish and cephalopods

2.2 Why is an Animal Research Authority required?

The use of animals for scientific purposes is regulated by the NSW Animal Research Act 1985. The following extracts from the Australian Code for the Care and Use of Animals for Scientific Purposes 8th Edition (NHMRC, 2013) outline the responsibilities and justifications for conducting animal research:

- Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. This respect is demonstrated by:
  - i using animals only when it is justified
  - ii supporting the wellbeing of the animals involved
  - iii avoiding or minimising harm, including pain and distress, to those animals
  - iv applying high standards of scientific integrity
  - v applying Replacement, Reduction and Refinement (the 3Rs) at all stages of animal care and use:
    - a the *Replacement* of animals with other methods
    - b the *Reduction* in the number of animals used
    - c the *Refinement* of techniques used to minimise the adverse impact on animals
  - vi knowing and accepting one's responsibilities.
- The care and use of animals for scientific purposes must be subject to ethical review.
- A judgement as to whether a proposed use of animals is ethically acceptable must be based on information that demonstrates the principles in Clause 1.1, and must balance whether the potential effects on the wellbeing of the animals involved is justified by the potential benefits.
- The obligation to respect animals, and the responsibilities associated with this obligation, apply throughout the animal's lifetime, including acquisition, transport, breeding, housing, husbandry, use of the animal in a project, and provisions for the animal at the conclusion of their use.

2.3 Animal Care and Ethics Committees

Under the NSW Animal Research Act 1985, all experiments with vertebrates, need to be approved by an accredited Animal Care and Ethics Committee. The NSW DPI (Fisheries) Animal Care and Ethics Committee must approve all applications for research using fish by NSW DPI scientists before they commence. This Committee also assesses applications for research on fish from Sydney Water and Water NSW (via a Memorandum of Understanding) and on occasion from other agencies.

The NSW DPI (Fisheries) ACEC's role is to review and approve new and ongoing projects and provide advice and recommendations on the care and use of animals. The Committee ensures that all fisheries research conducted complies with the legislation and Code of Practice (http://www.nhmrc.gov.au/guidelines-publications/ea28). The Committee is responsible for monitoring the care and use of animals within the department, including inspections of animals and facilities.

The Committee must consider and evaluate written protocols to conduct research, on the basis of the researchers' responses to a comprehensive set of questions, including the justification for the research; its likely impact on the animals; and procedures for preventing or alleviating pain or distress. The NSW DPI (Fisheries) ACEC has the power to stop inappropriate research and take action regarding unexpected adverse events and non-compliance with the Code by withdrawing research approvals. The ACEC requires that measures for adequate care, including emergency care are described in the protocol. The ACEC also provides guidance and support to researchers on matters relevant to the care and use of animals, through preparation of guidelines, and dissemination and/or direction to relevant scientific literature. The Committee must also submit an Annual Report on its operations to the Animal Welfare Branch of NSW DPI.

2.3.1 ACEC Membership

The membership and duties of ACECs are laid down in the Animal Research Regulation 2010. The requirements are based on the Australian Code of Practice, which also provides the benchmark against which the Committee judge research, when the research is proposed and carried out. The categories are:

Category A — A person with qualifications in veterinary science that are recognised for registration as a veterinary surgeon in Australia, and with experience relevant to the institution's activities or the ability to acquire relevant knowledge.

Category B — A suitably qualified person with substantial and recent experience in the use of animals for scientific purposes relevant to the institution and the business of the AEC. This must include possession of a higher degree in research or equivalent experience. If the business of the AEC relates to the use of animals for teaching only, a teacher with substantial and recent experience may be appointed.

Category C — A person with demonstrable commitment to, and established experience in, furthering the welfare of animals, who is not employed by or otherwise associated with the institution, and who is not currently involved in the care and use of animals for scientific purposes. Veterinarians with specific animal welfare interest and experience may meet the requirements of this category. While not representing an animal welfare organisation, the person should, where possible, be selected on the basis of active membership of, and endorsement by, such an organisation.

Category D A person not employed by or otherwise associated with the institution and who has never been involved in the use of animals in scientific or teaching activities, either in their employment or beyond their undergraduate education. Category D members should be viewed by the wider community as bringing a completely independent view to the AEC, and must not fit the requirements of any other category.

More than one person may be appointed to each category. The Committee also has a Chairperson and Executive Officer. See Appendix 9.1 for the list of NSW DPI (Fisheries) ACEC members as of 2015.

2.3.2 Procedures of ACECs

The NSW DPI (Fisheries) ACEC conducts quarterly meetings to consider new research projects, renewals, amendments and other issues associated with animal research or teaching. A meeting quorum requires the attendance of at least one member of each category. Only in exceptional circumstances will research projects be considered at other than a scheduled meeting. The Committee must:

- Review, discuss, approve, reject or revoke proposals to perform animal research or teaching
  Request modifications and / or additional information
- Document their consideration of proposals
- Have guidelines on procedures for animal research or teaching
- Inspect animals and facilities used in research or teaching

Investigators (researchers) are ultimately responsible for the care, management and well being of their research animals. People working within NSW DPI in any role, including as: an employee, a consultant, a post graduate student or an animal attendant; who wish to use animals in research must hold an Animal Research Authority issued by the NSW DPI (Fisheries) ACEC. The NSW DPI (Fisheries) ACEC also issues Animal Research Authorities under agreement for defined research undertaken by Sydney Water and Water NSW. Animal Research Authorities for other individuals, independent researchers, groups or agencies (outside NSW DPI, Sydney Water and Water NSW) wishing to undertake research with fish are not usually assessed by the NSW DPI (Fisheries) ACEC. Approval for these groups should be obtained from the NSW DPI Secretary's ACEC (formerly known as the Director-General's Animal Care and Ethics Committee).

All proposals to undertake animal research must be submitted in writing to the NSW DPI (Fisheries) ACEC and approved before the research is commenced. Full information should be provided of:

- The justification for the use of animals
- The justification for the number and species of animals used
- The procedures to be used
- The expected impact on the welfare of the animals and the methods which will be used to avoid and alleviate pain and distress

Investigators must keep detailed records of the health and experimental use of animals in their charge. The records may be examined at any time by the ACEC or inspectors of the Animal Welfare Branch, NSW DPI.

2.4 Where an Animal Research Authority application form can be obtained

Applications are available from Ms Jo Pickles, NSW DPI (Fisheries) ACEC, Port Stephens Fisheries Institute, Locked Bag 1, Nelson Bay, NSW, 2315. Tel: 0249 163901. Email: jo.pickles@dpi.nsw.gov.au or the DPI intranet site. An example of the form is attached at Appendix 9.2.

2.5 Animal Research Authority renewals

Animal Research Authorities are valid for a period of 12 months. Any scientist or investigator running a project for longer than one year is required to have their Authority renewed each year by submitting an 'Annual Renewal form for an Animal Research Authority' (see Appendix 9.3). Some projects run for longer than three years. If a research project will continue after three years, a major revision of the project and methods is required. The researcher must submit the Major-Three Year Renewal form for an Animal Research Authority. The Executive Officer will notify the Principal Investigator when the Authority for a project is due to expire and, if a Standard Renewal or Major-Three Year Renewal is sought. The Principal Investigator will be asked to either apply for a renewal or notify the Executive Officer that a renewal is not required. In that case a Final Report will need to be submitted. Final Reports need to include details of relevant aspects, including the number and fate of animals used and the condition of animals remaining after the project. A brief summary of the outcomes or findings of the project is also required (see Appendix 9.5 for an example of a completed Final Report).

2.6 Reporting Unexpected Adverse Events

An unexpected adverse event is defined as an event that may have a negative impact on the wellbeing of animals and was not foreshadowed in the approved project or activity. Reporting of an adverse event should be carried out as soon as possible to the ACEC Executive Officer, ideally within 72 hours.

An unexpected adverse event may result from different causes, including but not limited to:

- death of an animal, or group of animals, that was not expected (e.g. during surgery or anaesthesia, or after a procedure or treatment)
- adverse effects following a procedure or treatment that were not expected
- adverse effects in a larger number of animals than predicted during the planning of the project or activity, based on the number of animals actually used, not the number approved for the study
- a greater level of pain or distress than was predicted during the planning of the project or activity
- power failures, inclement weather, emergency situations or other factors external to the project or activity that have a negative impact on the welfare of the animals.

Our ACEC has always understood a small number of fish will occasionally die unintentionally despite every attempt to handle them as careful as possible. We believe unintentional and unexpected are the same thing. Our Committee will use a 'common sense approach' in this area and understand how smaller fragile species are more likely to not survive and therefore this is seen as unintentional but an expected outcome. Therefore, these events will not warrant the Reporting Form for Unexpected Adverse Events.

2.7 When to do a Post Mortem Exam?

i When no cause of death is readily apparent eg. no water quality crashes.
ii When no cause is apparent and the health of more animals is under threat ie. infectious disease/toxicities etc.

iii When there is any possible public health implications.

Detailed information as a guide to conducting a post mortem can be found here. http://www.agriculture.gov.au/biosecurity/risk-analysis/ira/current-animal/ornamental-finfish/ornamental-fish-testing-project-final-report/appendix-2

2.8 Inspection obligations

2.8.1 NSW DPI (Fisheries) ACEC

Inspections of all animal housing, laboratory areas and facilities must be conducted regularly by members of the ACEC and appropriate records maintained to ensure compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes.

Policy of NSW DPI states that inspections by ACECs may be used to:
- Ensure the Code of Practice and ACEC directions are being complied with
- Familiarise ACEC members with what is happening 'in practice' as opposed to 'on paper'
- Promote interaction between the ACEC members, researchers and animal care staff

All ACEC members should participate in inspections, though it is not necessary for every member to attend every inspection. NSW DPI (Fisheries) ACEC inspects each research location on an annual basis and includes a calendar on every agenda describing the inspection schedule for the year.

Inspections should include examination of:

- Animal holding facilities
- Animals (health and welfare)
- Research procedures (on occasion)
- Animal identification and care records
- Research records (on occasion)

Inspections are made to ensure animals are held in current and approved protocols and that the procedures being conducted are in accord with the approved protocol. Discussion with animal carers and researchers during inspections is encouraged as a means of obtaining information and providing an opportunity for contact between these people and ACEC members. Research procedures should be viewed by ACEC members on occasion. ACEC members are encouraged to view procedures as often as possible as an additional means of assessing the impact of projects on animals. Procedures to be viewed may be chosen on the basis of criteria including:

- Contentious research project or procedure
- Convenience (procedure coinciding with inspection)
- Commonly occurring procedure
- Operator has not been seen performing procedure by the ACEC
- ACEC member(s) have not previously seen procedure performed

A written report is submitted to the Executive Officer after each inspection, discussed at the next available meeting and attached to the minutes.

In 2007, the Committee introduced self-audit reports to be conducted by OICs of that particular centre to augment on-site physical inspections by ACEC members. The ACEC usually inspect centres like the Narrandera Fisheries Centre (NFC) and National Marine Science Centre (NMSC) annually and over the past decade inspections have confirmed the Centres are well run, fish are well cared for and acceptable practices for research used. In recognition of previous 'performance' and to optimise the time available for inspections a decision was made to conduct physical inspections at the NFC and NMSC every 2-3 years with self-audit reports to be

completed by the OIC in the intervening years. Where self-audits are carried out, the report is submitted to the ACEC. OICs were consulted in the development of this form to ensure it was user friendly and informative as a self-audit report. While facility inspections are important, inspections of field practices such as electrofishing, netting, blood sampling, inserting tags in fish and viewing fishways has also been undertaken by the Committee.

2.8.2 NSW DPI Emergencies and Animal Welfare Branch / NSW Animal Research Review Panel

The Animal Welfare Branch of NSW DPI conducts its inspections under the Animal Research Act (usually by a veterinary officer of the Animal Welfare Branch and a member of the Animal Research Review Panel). There are three different types of visits:

i Accreditation site visits
ii Inspection of animal research authority and animal supply licence holders' facilities
iii Investigation of complaints

These visits are to ensure that animal research and supply complies with the legislation and Code of Practice. Accreditation, authorities and licences are issued subject to a satisfactory inspection and can be suspended or cancelled. The Code of Practice provides criteria against which institutions are assessed.

This includes assessing the membership, procedures and activities of the ACEC, animal care procedures, animal research procedures and the physical facilities for housing and using animals. Central to this is an evaluation of the well-being of the experimental or breeding animals.

Assessment commences with an examination of written material provided by the institution or individual. This includes a list of the protocols considered by the ACEC and the people issued with animal research authorities, ACEC minutes, annual report, records of inspections conducted and information about the procedures of the Committee's operation and decisions.

A detailed report is prepared by the Animal Welfare inspection team as soon as possible after the inspection. The report covers an evaluation of the ACEC, assessment of the well-being of the animals, housing and holding facilities and animal care and monitoring, including emergency procedures. Once the Panel has considered the report, recommendations may arise which will alter the terms of accreditation or licence. Conditions of previously approved accreditation requirements may have been met or the Panel may impose additional conditions.

3. Do fish feel pain

The question of whether fish feel pain, or experience the type of negative physical and/or emotional responses that humans associate with pain, is a subject of considerable debate. That debate is germane to the discussion about whether ethics are relevant when considering research with fish. If fish do not feel pain or 'suffer' it may be less important to consider their welfare. For fish to be given welfare considerations they must demonstrate cognitive characteristics of sentient beings (Chandroo et al., 2004). (Sentient beings are those endowed with feelings and unstructured consciousness [wordnet.princeton.edu/url/weburn]).

Do fish have the ability to experience pain, fear and psychological stress? There is no doubt that fish are aware of their surroundings and actively avoid potentially harmful situations. They respond to external stimuli, are capable of learning and have the anatomy and physiology similar to other sentient animals. Grandin & Deesing (2003) proposed that animals that protect injured body parts, reduce activity when sick or self-administer opiates or pain-reducing drugs are capable of suffering from pain. Fish have demonstrated these behaviours. They 'pain guard', by reducing feed intake when they have inflamed guts and avoid places where they have previously been shocked or hooked (Chandroo, et al, 2004). In addition, in a study where fish were injected in the lips with either a saline solution or acetic acid, those injected with the acid solution engaged in pain related behaviours such as rubbing their lips on the gravel and rocking. Those behaviours were reduced when morphine was administered (Sneddon, 2003 – cited in Grandin and Deesing, 2003). Fish also display a clear physiological response to stress including handling, high stocking density and sub-optimal salinity and temperatures, as evidenced by elevated blood cortisol and glucose concentrations (Mazeaud, et al., 1977). The responses above are consistent with those experienced by higher vertebrates and led Chandroo et al. (2004) to conclude this implies that fish have the capacity to suffer and therefore welfare considerations should be applied. Grandin and Deesing (2003) concluded that fish were in a 'grey area' and that more research was needed particularly on whether fish genuinely 'suffer' from pain.

The contrasting view refers to the development of the cerebral cortex. In humans, awareness of pain relies on functions of specific regions of the cerebral cortex. These regions are absent in fish, and, as there is no functional equivalent, fish are unable to experience pain as understood by humans (Rose, 2002). In his review of the neurobehavioral nature of fishes, Rose (2002) cautions against anthropomorphic thinking and concludes that fish 'display robust, nonconscious, neuroendocrine, and physiological stress responses to noxious stimuli'. These responses have been misinterpreted as evidence of 'pain and suffering' but that given their neuroanatomy, genuine awareness of fear in fish is impossible.

This very brief discussion addresses the question of whether fish feel pain and presents a couple of the key academic references on the topic. It is clear that functional anatomy in fish is very different to that in mammals and that fish do not experience pain in the way humans understand it. However, considering the welfare of fish is also important for other reasons. Firstly from a practical perspective, fish performance is improved when stress is reduced (this is a basic tenet of successful aquaculture). Secondly, reducing stress during harvest and slaughter improves flesh quality (Bosworth et al., 2007). Finally, regardless of how animals respond to noxious stimuli, humans are affected (usually negatively) by imposing stressful conditions on other animals. Taken together, these are compelling arguments for reducing the imposition of stressful conditions on all animals and therefore that welfare of fish is worthy of consideration.

4. Animal husbandry

4.1 Basic husbandry

4.1.1 Facilities

'Facilities' includes the ponds, raceways, tanks, cages and aquaria in which animals are kept. Scientists and the investigators, ACEC's and fisheries research institutions are responsible for ensuring that facilities are appropriately staffed, designed, constructed, equipped, operated and maintained to achieve a high standard of animal care and to fulfil scientific requirements. The overall condition and management of facilities must permit effective maintenance and servicing and be compatible with maintaining the animals in good health.

Operation of facilities

All fish-holding facilities must be operated in a manner that optimises conditions for fish. Guidelines for the design and operation of fish hatcheries and aquaculture facilities are given in Rowland and Tully (2004) and Rowland et al. (2007).

Appropriate stocking densities, aeration and water management must be used. All facilities should be aerated; tanks and aquaria continuously with diffused air or oxygen and ponds nightly for around 8 h/day with diffused air or mechanical aerators such as paddlewheels. At very high temperatures and feeding rates, or on overcast, still days, ponds may need to be aerated for longer periods or continuously. Cages should be located in aerated ponds.

In circular, self-cleaning tanks, a constant flow of water is used to facilitate the removal of solids and dissolved wastes (eg. ammonia) and to supplement aeration. If tanks need to be static, eg. during chemical treatment, fish should not be fed and water (10-30 %) should be exchanged daily. Tanks should be placed under cover or in a building out of direct sunlight to provide an environment with relatively low light intensity. It is beneficial to some species, eg. Murray cod, to partially cover tanks to reduce stress.

Static ponds should be managed (stocking densities, aeration, water quality, diseases, feeding etc.) according to guidelines for particular species, eg. techniques for the culture of silver perch have been published (Rowland and Bryant, 1995; Rowland et al., 2007).

Stocking densities

Optimal stocking densities vary with a number of factors including culture unit (pond, tank, cage), species, size of fish, culture phase, water quality etc. The following table gives optimal and upper densities for the different units.

Housing	Optimal	Upper density[1]
Tanks	10 kg/m^3	100 kg/m^3
Cages	20 kg/m^3	100 kg/m^3
Ponds	5 t/ha	20 t/ha

[1] Requires greater control of water quality, more experience and entails higher risk.

Monitoring requirements

All fish holding facilities and support systems must be inspected every 24 hours. Things to observe include changes to the fishes' external body, especially any signs of diseases, abnormal swimming behaviour, abnormal feeding behaviour, as well as unexpected changes in

the appearance of the water. Water quality variables and fish health need to be monitored regularly (see following).

4.1.2 Nutrition and feeding

Commercial diets are available from a number of feed manufacturers in Australia and overseas for marine and freshwater fish including diets for larvae, fry, fingerlings, juveniles and adults.

Wherever possible, manufactured fish diets should be stored for as short a time as possible before use. If the diets are to be stored for longer than a month or two, they should be kept in cool (<15°C), dry conditions, or frozen. At all times, manufactured diets should be kept cool and dry. If there is any sign of fungal contamination, diets should be discarded.

The manufactured diet should be designed for the target species, life-stage and size. The nutritional requirements of silver perch have been determined and practical diets formulated for silver perch (Allan and Rowland, 2002). Fresh or frozen bait fish or other aquatic plant or animal material are often used as a food source. They usually need to be stored frozen and care must be taken to ensure they are not contaminated and do not deteriorate.

Fish should be fed to optimise survival, health and growth. Appropriate feeding strategies should be followed for each species, where available. Guidelines for feeding silver perch on restricted rations have been published (Rowland et al., 2001) and these would be good guidelines for other species. Under feeding will reduce growth and potentially compromise health, and excess feeding can adversely affect water quality. At such times feeding rates can be reduced or feeding suspended until water quality improves. Fish held in quarantine should not be fed.

4.1.3 Water quality variables

The water quality variables alkalinity, hardness, conductivity and metals are relatively stable and 'characterise' the water in which fish are held and grown. Dissolved oxygen, pH, ammonia and nitrite are unstable variables that are influenced by culture activities and can change rapidly. Other important variables are; temperature, salinity, nitrogen, hydrogen sulphide and turbidity. Each species will have an optimal range for each variable, as well as lethal limits. Details of each of these variables and their importance for holding and growing fish can be found in Rowland (1998) and much of the following summary is taken from that publication.

Temperature

Water temperature influences chemical and biological procedures. Fish are cold-blooded (poikilothermic) and so water temperature affects their metabolism, digestion, growth, sexual maturity and reproduction. Rates of chemical and biological reactions roughly double for every 10°C increase in temperature. As water temperature increases, fish become more active, consume more food, use more oxygen and grow faster. However, when the temperature exceeds the critical level for a particular species, fish become stressed, more vulnerable to disease, may stop growing and can die.

Salinity

Salinity refers to the total concentrate of all dissolved ions. As salinity rises, the ability of water to conduct electricity also increases and conductivity is therefore often used to measure or estimate salinity. In general, freshwater is 0-500 mg/L salinity and full seawater is 35 000 mg/L (or 35 g/L; the units are sometimes presented as 'parts per thousand', ppt or ‰). Many Australian native freshwater fish, such as silver perch, golden perch, Murray cod and catfish can tolerate long-term exposure up to at least 5 g/L salt, while many estuarine species such as mulloway and snapper can tolerate salinities down to as low as 10 g/L. Rainbow trout,

Australian bass and barramundi can tolerate salinities of 0-35 g/L. When changing salinity, fish should be allowed to adjust slowly (eg. 1-5 g/L/day). Salt reduces stress, increases mucus production, promotes healing of damaged skin and kills some ectoparasites in freshwater fish.

Dissolved oxygen

Dissolved oxygen is the most critical and limiting variable in fish husbandry and aquaculture. Like all animals, fish cannot live without oxygen and lethal levels vary from just less than 1 mg/L to about 3 mg/L. Sub-lethal levels (eg. 2-4 mg/L) can stress fish, reduce growth and increase susceptibility to disease. Oxygen enters water through diffusion at the air-water interface and as a result of photosynthesis when there are plants (eg. algae) in the water. In ponds and natural waters, dissolved oxygen undergoes significant diurnal and seasonal fluctuations (see Rowland 1998). In aquaria, tanks and raceways, dissolved oxygen is usually maintained by aeration of the water using low pressure compressors or blowers (through diffusers like air stones). In ponds, paddle-wheel aerators are among the most efficient methods of transferring oxygen from the air to the water. Mechanical aeration creates currents and so assists with mixing water throughout the pond.

pH and carbon dioxide

The pH of water is the measure of the hydrogen ion concentration and indicates whether it is acidic (pH < 7), neutral (pH = 7) or alkaline (pH > 7). The desirable range for most species of fish is 6-9. A pH of 4 is lethal for most species, while prolonged exposure to pH levels of above 10 can be lethal.

Carbon dioxide affects pH because it has an acidic reaction in water. Phytoplankton and other aquatic plants remove carbon dioxide (and produce oxygen) from water during photosynthesis in daylight hours and all organisms add carbon dioxide through respiration. Typically, in ponds or water bodies with algal blooms (phytoplankton) or other aquatic vegetation, the pH will rise during the day, peaking in the afternoon, then decline to a minimum around dawn. As with many water quality variables, the interaction of pH with other variables can be critically important. The inter-relationship of pH with ammonia is one of the most obvious examples (see following).

Alkalinity

Alkalinity is the total quantity of ‘bases’ present in water (primarily carbonate and bicarbonate ions) and is measured as mg/L of equivalent calcium carbonate (CaCO3). The bases ‘buffer’ water against changes in pH. Waters of low alkalinity (eg < 20 mg/L) are poorly buffered and are relatively unproductive for fish culture. A desirable range of alkalinity is 50-200 mg/L, but fish survive in waters up to 400 mg/L.

Hardness

Is the total concentration of metal ions (mainly calcium and magnesium) in water and as it is also expressed as mg/L CaCO3. Waters of low hardness (eg. < 20 mg/L) are called ‘soft’ while those above 200 mg/L are ‘hard’. Most productive waters are between about 20 and 250 mg/L CaCO3. In general, where the alkalinity is derived from calcium or magnesium carbonate, hardness and alkalinity values are similar. However, if alkalinity is derived from sodium bicarbonate (NaHCO3), it is possible to have soft water with a high alkalinity. Waters with hardness values over 400 mg/L or less than 20 mg/L are unsuitable for most fish.

Ammonia and Nitrite

Ammonia is the major product excreted when fish (and other aquatic animals) catabolise protein. It is excreted across the gills and in urine and faeces. Ammonia is also a by-product of the decomposition of organic matter by bacteria. In water, total ammonia exists in two forms; a

highly toxic unionised form (NH3) and a much less toxic ionised form (NH4+). The proportion of ammonia in each form depends on pH, temperature and salinity. For example, at a pH of 8.0, 1.6% of ammonia is in the toxic unionised form at 8^{o}C, but 8.8% at 32^{o}C. Similarly, at 24^{o}C, 0.5% of ammonia is in the unionised form at a pH of 7.0 compared with 34.4% at a pH of 9.0. Fortunately, ammonia is very soluble and is readily used as a nutrient by plants. So, when pH rises in ponds with algal blooms, ammonia concentrations tend to be low.

Ammonia is 'nitrified' to nitrite and then to nitrate through a two-step process by the 'nitrifying' bacteria Nitrosomonas and Nitrobacter. These bacteria occur naturally and are the main agents responsible for removing ammonia and nitrite in biological filters. At high levels, nitrite replaces oxygen in the blood to form methaemoglobin. Gill filaments become brown giving rise to the condition 'brown blood disease'. Nitrite toxicity is reduced when chloride is present in the water. Adding salt at 5 g/L is very effective in reducing toxicity and explains why marine and estuarine fish are much less susceptible than freshwater species.

Sensitivity of fish to ammonia and nitrite varies between species and age/size of fish. Lethal levels (acutely toxic concentrates that are predicted to kill 50% of the population over 4 days) are around 1.0 mg/L unionised ammonia and 160 mg/L nitrite. However, growth of silver perch is reduced when concentrations are above 0.36 mg/L unionised ammonia or 1.4 mg/L nitrite over three weeks.

Nitrogen is not normally toxic to fish, but if the water is supersaturated, the nitrogen can cause gas bubble disease which can kill fish. Bubbles form under the skin, behind the eyes or in blood vessels, in the same way 'the bends' occur in humans. Supersaturation can occur when water is pumped under pressure or derived from under-ground. Such water should be passed through 'degassing' columns and/or vigorously aerated before use.

Hydrogen sulphide

This compound is produced by the bacterial decomposition of organic matter under anaerobic (without oxygen) conditions. Hydrogen sulphide smells like rotten-eggs and is often called 'rotten-egg gas'. Concentrations less than 1 mg/L can be lethal to fish. It can be removed from the water by vigorous aeration or by adding potassium permanganate.

Turbidity

This refers to the amount of suspended material such as clay, organic material or plankton (including plants, phytoplankton or zooplankton) in water. Turbidity is not usually harmful to fish. Turbid water can help prevent colonisation of the pond bottom by aquatic plants that can interfere with harvest operations. Excessive and/or persistent clay turbidity can interfere with development of beneficial algal blooms in ponds, while excessive organic turbidity can increase biological oxygen demand and lead to problems with low levels of dissolved oxygen.

4.1.4 Water quality management

Maintenance of good water quality is one of the most important aspects of fish husbandry, and the majority of problems occurring in a hatchery/aquarium are associated with poor water quality. Water quality in freshwater aquaculture is discussed in detail by Rowland (1998). Maintenance of good water quality requires the regular monitoring of dissolved oxygen, temperature, pH and ammonia, and for marine and brackish water species, salinity. Because dissolved oxygen is a key limiting factor, all culture units are aerated; tanks continuously and ponds nightly (for details see Rowland 1998; Rowland et al. 2007)

Water exchange

The three basic systems of water exchange/circulation are a) static, b) flow-through and c) recirculating systems.

a Static systems

Static systems do not receive continuous water inflow. They rely on relatively low stocking densities and biomass, and/or in the case of ponds, natural processes within the system to maintain water quality. Most earthen ponds are run as aerated, static systems, with periodic additions of water to replace that lost through evaporation and seepage. Water exchange can be achieved by over-flow or by partially draining the pond or tank and then replacing lost water. Water exchange can be used to improve water quality when a 'crisis' occurs (eg. algal bloom crashes followed by low dissolved oxygen).

b Flow-through systems

In flow-through systems there is a 'single-pass' of water, ie. water is only used once, and the tank, raceway or pond usually remains full with water entering and leaving the system at the same time from different locations. For tanks and ponds, water inflow should be designed to maximise mixing and help concentrate solids near the drain or outflow (eg. used to generate a circular water current). Out-flowing water leaves via an overflow (eg. monk, external standpipe) and in tanks is drawn from the bottom; such tanks are 'self-cleaning'.

c Recirculating aquaculture systems

Tank-based recirculating systems (RAS) can be use for a range of purposes including quarantine, broodfish and over-wintering fingerlings at elevated temperatures. Some species such as Murray cod and barramundi can be grown to market-size (> 500 g). RAS are characterised by: re-conditioning and re-use of water; mechanical filtration to remove solids; biological filtration to convert ammonia to nitrite and nitrate; high stocking densities and production rates; low water and land requirements; and potential for good control of water quality, wastes, temperature and culture conditions. Depending upon the complexity of the system, RAS can also include foam fractionation (air or another gas is bubbled through a column of water to trap and remove organic particles) and methods to increase dissolved oxygen (eg. pure oxygen injection). Treatments to remove or reduce potential pathogens, such as ozonation or UV filtration are sometimes included in RAS.

NOTE: Biofilters must also be managed as a living, breathing organism. Most importantly, it takes time for surface bacteria or biofilters to establish. The time depends upon the amount of nutrients supplied, temperature of the water and water-flow characteristics of the system. Bacterial preparations and food (nutrients) are available and can be used to reduce this 'start-up' time that typically is between 1-3 months. Care must be taken to ensure the filter is not starved of nutrients or that chemicals used to treat fish (eg. formalin) do not affect the biofilter. Careful monitoring should be undertaken when changes in the filter-loading rates (ie. when fish are added or removed) are made or when any chemicals are used.

Turbulence

Turbulence in tanks is caused by aeration or water inflow and keeps solids suspended in the water column. Turbulence also mixes water, but needs to be kept as low as possible when rearing larvae up to 10 days old. Larvae must not be prevented from reaching the surface during this period to enable swim-bladder inflation. After this period, increased turbulence assists in food distribution, enabling uniform growth and potentially reducing the incidence of cannibalism.

Skimmers

If rearing larvae, surface skimmers should be in place during swim-bladder inflation to reduce oil scum, which may prevent larvae from gulping air on the water surface. Skimmers are not necessary after this stage, but certainly aid in the removal of excess lipids and proteins from the tank.

Cleaning

Self-cleaning tanks reduce the need for cleaning. Static tanks should be cleaned regularly, by siphon or vacuum pump, to reduce problems with the accumulation of organic matter (uneaten food, faeces) and fouling organisms, bacteria and algae. Tanks not in use should be left dry. Sand filters need to be backwashed regularly, and cartridge filters should be cleaned and dried periodically to prevent build up and decomposition of accumulating waste material and to ensure efficient operation. Floors, drains, etc., associated with tank rooms should be cleaned and sterilized on a regular basis. Dilute pool chlorine or sodium hypochlorite (NaOCl 20 ppm) or caustic soda (NaOH 1%) are suitable cleaning agents for this purpose.

4.2 Using chemicals in research to treat fish

It is illegal to supply fish or other animal products for human consumption if they contain residues of chemicals above the legal limits (Maximum Residue Limit (MRL)) set in the Food Standards Code (as adopted into the *NSW Food Act 2003*). If there is no limit set in the Standard then no residue at any detectable level is permitted.

In order to ensure that such contamination does not occur the *Stock Medicines Act 1989* and the *Pesticides Act 1999* regulate the use of chemical treatments for animals and plants. These acts require that only chemicals which are registered for the specific treatments are used except that, in the case of animal treatments, some flexibility is provided to veterinarians for animal treatment.

There are very few chemicals approved for the treatment of fish, but treatment of fish strictly within research facilities does not require use of approved chemicals provided **fish which may end up being consumed by humans are not treated**. In addition a small number of permits have been issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) to allow certain unregistered treatments to be used on fish. These permits can provide additional advice in relation to treating fish which may be consumed by humans.

All issued permits may be found on the website of the APMVA at: http://www.apvma.gov.au/permits/permits.shtml by entering ‘fish’ (or ‘silver perch’) under ‘Crop/Animal’, though some will also come up incorrectly if ‘fish’ is entered under ‘Pest/Purpose’. It is possible to limit the search to current permits only.

4.2.1 When use of unregistered treatments is allowed

Small-scale trial permit PER7250, issued by the APVMA, says that:

- All screening tests, laboratory assessments and other research involving chemicals such as residue, efficacy and crop or animal safety trials done within the confines of a research facility are covered.
- If the trials are done within the confines of the research facility, and unregistered or off-label treatments are used for animals within the research facility, there are no restrictions on the treatment provided that **no produce is supplied for human consumption**.

4.2.2 When use is not allowed

Any use of unregistered products, or products used off-label, outside the research facility or to treat fish/animals within the research facility and which may in future be consumed by humans is not covered by PER7250 and is not permitted. Just because the use occurs within a research facility does not exempt the use from the controls if fish are eventually to be eaten.

Use of unregistered stock medicines (veterinary chemical products) or use of either registered stock medicines or pesticides off-label in these situations is illegal (except if registered stock medicines are used off-label under the written directions of a veterinarian—see below).

4.2.3 Complying with PER7250

In order to comply with the conditions of PER7250 in relation to the use of unregistered treatments within research facilities, departmental staff are required to meet all the following terms and conditions of the permit.

i Trials carried out on research facilities can only be conducted by persons who are trained or experienced in the handling and use of agricultural and veterinary chemicals and who handle and use such chemicals as part of their normal duties.

ii The trials can only involve constituents and chemical products that are NOT:
- genetically manipulated organisms;
- veterinary biologicals for use outside the confines of a research facility;
- a stock medicine (eg. chloramphenicol) or pesticide that is prohibited by NSW legislation;
- an active constituent or chemical product whose use has been prohibited under the Agricultural and Veterinary Chemicals (Administration) Regulations 2004.

iii Disposal of any produce from plants or animals treated during the trials cannot result in direct or indirect consumption by humans or animals.

iv All trials involving animals must comply with conditions laid down in animal welfare legislation or guidelines.

v Detailed records must be maintained for 2 years listing:
- the date the trial is conducted;
- for trials conducted within the confines of a research facility, the name and address of the research facility;
- for trials conducted outside the confines of a research facility, the jurisdiction and specific location within each jurisdiction that the trials are conducted;
- the trial details, including plants, animals or items treated, the pest controlled or reason for treating, the rates and frequency of application;
- the active constituents or chemical products used plus the total amounts used;
- the method of disposal of produce from treated plants or animals; and
- the names of the persons conducting or controlling the trials.

vi These detailed records of trials must be made available to the APVMA upon request.

It is important to remember that **all** terms and conditions must be followed in order to be covered by this permit. If all terms and conditions cannot be complied with, then it will be necessary to apply for and receive a trial permit from the APVMA prior to commencing trials.

Failure to comply with any of these conditions would mean that the person conducting the trial is not covered by permit PER7250 and is therefore committing an offence under the Stock Medicines Act or the Pesticides Act. While this could result in prosecution the more likely outcome is that either the individual or the whole research facility would be required in future to apply for a permit every time they wished to undertake a trial that involved chemical treatment.

Any use of unregistered chemical products, or products used off-label, **outside the research facility** or to treat fish/animals **within the research facility and which may in future be**

consumed by humans is not covered by research permit PER7250 and is not permitted. Use that occurs within a research facility does not exempt the use from the controls if fish are eventually to be eaten.

4.2.4 Record keeping

In order to fulfil the APVMA requirement for keeping detailed records of all pesticide research done under this permit, each research facility will need to devise a system for collecting and recording this information, although this usually forms part of any trial protocol.

Data can be stored in either hard copy or electronic database format. The Biological and Chemical Risk Management (BCRM) Unit in Head Office may monitor these records to ensure they are being kept, as APVMA Inspectors can legally audit this information at any time.

4.2.5 Veterinarians' Right to Prescribe

NSW DPI administers the Stock Medicines Act 1989 which controls the use of stock medicines and the rights of veterinarians to use or recommend use of some stock medicines contrary to label directions.

Stock medicines include the majority of products used to treat animals including fish. Permits for off-label use are required from the APVMA for treatments of animals (fish) **outside a research facility** under the following circumstances:

- to use a product which is not registered on a food-producing species (whether it is intended to be eaten or not);
- to trial a product where the details cannot be supplied on the label because of the need for blind treatments; and
- to use a pesticide product off-label.

While it is illegal in NSW to use a registered stock medicine contrary to the label directions, a permit for such use is usually not required because a veterinary surgeon can legally authorise ('prescribe') the use. To do so they must have real, and not nominal, responsibility for the animals they are treating or for which they are recommending treatment.

Veterinarians can undertake or 'prescribe' the use of products registered for one food-producing species: on any other species; at a different rate, by a different route of administration or otherwise contrary to the label directions for use.

If such changes are made by a veterinary surgeon, they must supply their own 'label' (i.e. written instructions) which provides all the details for use, in particular a revised and appropriate withholding period statement. In this case no permit is required, but failure to comply with the directions would be a breach of the Stock Medicines Act. All responsibility for the off-label use they authorise rests entirely with the prescribing veterinarian.

4.2.6 Safe use of chemicals

While use of unregistered chemicals within a research facility can be quite legal from the chemical control perspective, many of the chemicals which might be used are industrial chemicals with no label safety directions for their use. Due care must be taken in their use and all Material Safety Data Sheets held and read for those chemicals.

4.2.7 Treating wild caught fish to be released

Use of unregistered products on fish which might be consumed by humans cannot be authorised by a veterinarian. In some circumstances, wild fish may be caught and brought into research

facilities for breeding purposes and subsequently released. Such fish often require hormone or antibiotic treatments and in some cases there are registered treatments available.

If full-label withholding periods cannot be observed before releasing treated fish, then researchers should always seek to maximise the withholding period before releasing such fish back into the wild. The health and residue risks from small numbers of treated fish released in this way are not high.

This does not apply to large numbers of fish bred within a research facility for either release to the wild or for provision to growers and ultimately for human consumption.

4.3 Diseases and health management

Infectious diseases are common in intensive aquaculture and can cause significant mortalities. Regular monitoring and appropriate management are essential for the maintenance of good fish health. Information is available on aquatic animal diseases, particularly regarding diseases of native freshwater fish, and is described in Rowland and Ingram (1991), Callinan and Rowland (1995), Ingram et al. (2005) and Read et al. (2007) (this latter publication provides additional detail relating to the diagnosis, treatment and prevention of silver perch diseases). However in comparison with terrestrial animal diseases, much still remains unknown about many aquatic animal health issues. Therefore, while some information is provided below in relation to some of the more commonly encountered fish health issues this is provided for information only and must not be considered as an authoritative guide to the diagnosis and treatment of fish diseases. It is therefore very important that disease events in fish be appropriately investigated, particularly where the cause of mortality or disease event is not clear, and appropriate veterinary advice should be sought and/or material submitted to a diagnostic laboratory for further investigation.

4.3.1 Notifiable aquatic diseases

Under the Fisheries Management Act 1994, a number of aquatic animal diseases are listed as *Declared Diseases*, and there are obligations relating to the notification of these diseases that researchers need to be aware of. Specifically, whenever a researcher knows or has reason to suspect the presence of any *Declared Disease*, they "…. must notify a Fisheries Officer as soon as practicable of the infection or suspected infection." Notifications of the presence of suspected or confirmed *Declared Diseases* should be made to the Strategy Leader, Aquatic Biosecurity, NSW DPI who may be contacted through the office directory for the Port Stephens Fisheries Institute. Biosecurity staff can advise regarding appropriate submission of samples to a diagnostic laboratory to either confirm or rule out involvement of a suspected *Declared Disease*.

These obligations to report the suspected or confirmed presence of *Declared Diseases* not only relate to fish that are cultured or housed in experimental facilities, but also extend to fish populations in the wild that may be encountered by a researcher in the course of their work.

The NSW list of Declared Diseases under the Fisheries Management Act, 1994 in relation to finfish (additional diseases are listed for molluscs and crustaceans) can be downloaded from www.legislation.nsw.gov.au. As at February 2015 the Declared Diseases list is as follows:

- Epizootic haematopoietic necrosis—EHN virus
- European catfish virus, European sheatfish virus (Ex)
- Infectious haematopoietic necrosis (Ex)
- *Oncorhynchus masou* virus disease (Ex)
- Spring viraemia of carp (Ex)
- Viral haemorrhagic septicaemia (Ex)
- Channel catfish virus disease (Ex)
- Viral encephalopathy and retinopathy
- Infectious pancreatic necrosis (Ex)

- Infection with HPR-deleted or HPRO infectious salmon anaemia virus (Ex)
- Bacterial kidney disease (*Renibacterium salmoninarum*) (Ex)
- Enteric septicaemia of catfish (*Edwardsiella ictaluri*) (A)
- Piscirickettsiosis (*Piscirickettsia salmonis*) (Ex)
- Gyrodactylosis (*Gyrodactylus salaris*) (Ex)
- Red sea bream iridoviral disease (Ex)
- Furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*) (Ex)
- *Aeromonas salmonicida*—atypical strains
- Whirling disease (*Myxobolus cerebralis*) (Ex)
- Enteric redmouth disease (*Yersinia ruckeri—Hagerman strain*) (Ex)
- Koi herpesvirus disease (Ex)
- Grouper iridoviral disease (Ex)
- Infectious spleen and kidney necrosis virus- ISKNV like viruses (Ex)
- Infection with salmonid alphavirus (Ex)

- Diseases marked (Ex) are considered to be exotic to Australia
- Diseases marked (A) are considered to be present in Australia, but absent from NSW
- Diseases not marked are known to exist in some areas of NSW, but remain notifiable to NSW DPI under the Fisheries Management Act, as *Declared Diseases*

4.3.2 Ectoparasitic infestations

Ectoparasites are pathogens that commonly cause diseases in both freshwater and marine fishes. If left untreated, ectoparasitic diseases can cause very high mortalities. Parasitic diseases account for around 80% of all disease records for silver perch (Rowland et al. 2007). The parasites and their etiology, diagnosis and treatment in Australian native freshwater fishes are described in detail by Rowland & Ingram (1991), Callinan and Rowland (1995) and Read et al. (2007).

The most common parasites of native freshwater fish are the ciliate protozoans *Ichthyophthirius multifiliis* (which causes the disease ichthyophthiriosis or white spot), *Chilodonella hexasticha* (chilodonellosis) and *Trichodina* sp. (Trichodinosis), the flagellate *Ichthyobodo necator* (ichthyobodosis), and monogenean trematodes or gill flukes. The species of gill fluke infesting silver perch is *Lepidotrema bidyana*. All these parasites infest gill and skin tissues, and can be readily diagnosed using a microscope. Outbreaks can usually be controlled by using formalin in ponds, or salt or formalin in tanks (for details see Read et al. 2007).

4.3.3 Fungal infections

Water moulds (Class Oomycetes) are ubiquitous and cause fungal infections of fish. There are several common fungal diseases of freshwater and marine fishes; two examples are fungus (cotton wool-like growths on skin or gills) caused by *Saprolegnia parasitica*, and Epizootic Ulcerative Syndrome (EUS or red spot disease) caused by *Aphanomyces invadans*. Fungi are frequently opportunists that normally feed on dead tissue and are able to infect fish when the epidermis is damaged and/or the immune system is suppressed. They are generally considered secondary pathogens. Fungal infections can usually be avoided by good husbandry, careful handling and the maintenance of good water quality. The prophylactic use of 2-5 g/L NaCl in tanks will prevent many fungal infections, and the application of formalin may reduce the severity of an outbreak once an infection has commenced in ponds or tanks. Prevention of fungal diseases is generally much easier than control.

Listed below are treatments for bacterial and fungal infections and for ectoparasites that are well established and commonly used by NSW DPI (Fisheries) scientists.

4.3.4 Bacterial infections

The incidence of bacterial diseases in native freshwater fish is low (< 3%) and they cause few problems at facilities where good fish husbandry and health management are used. Bacterial infections usually follow periods of severe stress caused by rough handling and poor water quality, particularly low dissolved oxygen and high ammonia; a combination of these factors will predispose fish to infection. In fish larvae, bacteria can also be ingested with food and may proliferate in the gut.

Bacterial diseases can be treated with antibiotics. As the action of antibiotics is often pathogen-specific, it is important to accurately diagnose a bacterial disease to ensure that the most effective antibiotic and dosage is administered (Rowland & Ingram, 1991). Antibiotics should only be used to treat a diagnosed bacterial disease, and should not be used prophylactically because prolonged or repeated application, at dosages insufficient to kill bacteria, may lead to the development of resistant strains that will subsequently prove difficult to treat (Rowland & Ingram, 1991). For most antibiotics no detectable residue is allowed in food fishes in Australia. Since 'research' fish should not be entering the food chain this should not be an issue.

Signs and Diagnosis

External bacterial infections are easily recognised by skin inflammation (redness and swelling), fin erosion, lesions and ulcers and/or gross colonies of bacteria (eg. *Flexibacter*) of the epidermis on the torso or fins. Other signs may be exophthalmia (pop-eye), swelling of the abdomen, fluid in the body cavity and lesions in tissues such as spleen, liver and kidney. The bacteria may be identified by sending fish to a histology/pathology laboratory for analysis. The best samples are moribund (dying) fish which should be sent live to the laboratory if possible. Otherwise recently-dead or freshly euthanased fish can be placed, in a plastic bag, on ice for preservation and may be suitable for post-mortem analysis if live moribund fish cannot be sent live to the laboratory. It is important that such samples are kept chilled but are not frozen

In NSW, the Elizabeth Macarthur Agricultural Institute (EMAI) Menangle, NSW is a diagnostic laboratory that accepts submissions of fish samples for disease diagnosis.

For further information relating to descriptions of symptoms and treatment for some common specific fish bacterial diseases refer Read et al. (2007) .

4.3.5 Treatment of bacterial infections

Broad-spectrum antibiotics such as oxytetracycline (OTC) are utilized in the treatment of some bacterial infections in fish. OTC is an artificially engineered antibiotic used sparingly to combat bacterial infection, but it can also be used as a biological tagging agent in age/growth experiments (skeletal chronology). OTC is available in powder and liquid forms and may be applied to fish externally (by bathing or immersion), orally (by ingestion) or by intraperitoneal injection. Because there are registered or permitted products available OTC may be used in accordance with those permits even for food fish. For research use (not involving human consumption—see above) no withholding period is required and off-label use is allowed.

Other antibiotics, such as florfenicol, have also been permitted for food fish under certain conditions.

Bathing / Immersion

During bathing or immersion the OTC acts at the site of infection, and is absorbed through the gill filaments into the blood stream. This method has generally been more effective than all other methods of antibiotic treatment. OTC solution is added directly to tanks at rates of 20 mg/L

active ingredient for 7 days at 20°-30°C and 10 days at < 20°C. During treatment water should be well aerated and fish should not be fed.

Medicated feed

For details of antibiotic treatment using pelleted, medicated feed see Read et al. (2007). For marine fish, a soft pellet can be prepared by combining 2 kg of minced pilchard and 3 kg of pellet crumble with OTC powder. The recommended pellet concentration of OTC is 50-100mg of OTC/kg of pelleted feed. Pellets can also be directly coated with OTC. Vitamin C (ascorbic acid) is often added at a concentration of 0.5 - 1.0 g/5 kg to pelleted feed to assist in the healing process and to reduce associated stress. Oxolinic acid too can be used as a treatment itself and is also a good agent for prophylactic purposes.

Intraperitoneal injection

Treatment with OTC by injection into the intraperitoneal cavity is used for skeletal chronology (to mark the bones of fishes for aging or tagging purposes), but it may also be used therapeutically, especially for larger fish or when it isn't possible to feed or immerse the animal requiring treatment. There are several liquid forms of OTC available for injection. Recommended OTC injection rates are 50 - 100 mg/kg body weight.

4.3.6 Treatment of Ectoparasitic and Fungal infections – Formalin

Formalin is used in ponds or tanks for marine and freshwater fish both as an antifungal agent and for the control of ectoparasitic diseases of fish, and fish eggs (GESAMP, see reference list). Baths may be short-term and high concentrations or long-term, low concentrations (see Table below). Formalin removes oxygen from water, and therefore ponds and tanks must be aerated during treatment (Rowland & Ingram, 1991; Rowland et al. 2007). Formalin is rapidly depleted to below therapeutic levels within 48 h in ponds, and so control of some diseases such as ichthyophthiriosis which has a complex life cycle depends on applications every second day until the disease is controlled. Some gill flukes lay eggs and control of these parasites is dependent on at least three consecutive applications, 1-3 weeks apart.

Formalin concentrations and application to control some fish diseases.

Formalin concentration (mg/L)	Application
200 mg/L	1 hour (tanks only)
100 mg/L	2 hours (tanks only)
15-30 mg/L	Repeat each 2 days for ichthyophthiriosis (ponds and tanks)
30 mg/L	3 treatments, 1-3 weeks apart (ponds and tanks)

Because there are registered or permitted products available formalin may be used in accordance with those permits even for food fish. For research use (not involving human consumption—see above) no withholding period is required and off-label use is allowed.

WARNING: Formalin is a potential carcinogen and should be handled carefully as to avoid skin contact, eye irritation and inhalation (Katz, 1989).

4.3.7 Treatment of Ectoparasitic infections – Trichlorfon

Trichlorfon (Neguvon®) is an organophosphate that controls infestations of ectoparasites such as gill flukes and anchor worm (*Lernaea* sp.). Treatment with a Neguvon (anthelmintic powder) bath over a twelve to fourteen day period is often a successful treatment for dactylogyrid gill flukes. If fish show signs of stress due to ectoparasites or if a treatment appears unsuccessful (ie. the fish continue to display symptoms of stress, such as continuous flashing) further diagnosis is necessary. Fish should be examined after all treatments to evaluate effectiveness. For use on freshwater finfish refer to APVMA permit PER9750 before use.

Recommended Neguvon bath treatment.

Day	Concentration (mg/L)
1	1
2	0
3	1
4	0
5	1
(continue schedule for twelve to fourteen days)	

NB: There is no treatment required on days 2, 4 and 6, etc.

Neguvon breaks down quickly. During treatment it is also advised to maintain the 10-20 % exchange water rate. It is possible for the treatment concentration to be doubled to 2.0 mg/L if required, however 1.0 mg/L is the preferred concentration. As the required concentration of Neguvon is quite low, preparing a larger volume of treatment solution will ensure greater accuracy of treatment concentration.

Fish should be closely monitored for signs of distress during treatments. Neguvon can become toxic to fish if stored incorrectly or if used past its expiry date.
NB. Read the label safety directions and refer to APVMA permit PER9750 before use.

Because there are registered or permitted products available Neguvon may be used in accordance with those permits, or under written veterinary direction, even for food fish. For research use (not involving human consumption—see above) no withholding period is required and off-label use is allowed.

4.4 Transport protocols

Changes in environment, noise, movement and confinement all contribute to the stress that fish experience. Animals are particularly susceptible to transport stress. The ability to cope with stress during transport will depend on the fishes' state of health, species, age, sex, stocking density, period without food, the duration of the trip, the mode of transport, and water quality, particularly temperature, oxygen, pH and ammonia.

It is imperative that all sources of distress are identified, minimized or avoided to ensure that the health and well being of transported fish are not unduly compromised.

The general principles given below are derived from sections 3.2.5 to 3.2.8 of the Australian Code for the Care and Use of Animals for Scientific Purposes 8th Edition (NHMRC, 2013). Stress during transport can be minimized by:

i appropriate size, design and construction of transport containers;
ii maintenance of good water quality, including use of pure oxygen;
iii limiting exposure to extremes of temperature, noise, visual disturbance and vibration;
iv providing, if appropriate for the species, an inner shelter within the transport container;
v ensuring that animals are separated where there is incompatibility of species, age, size, sex, or reproductive status;
vi preventing unnecessary handling;
vii administering anaesthetics, if appropriate, during handling, by appropriately trained persons;
viii application of NaCl (2-5 g/L) for freshwater fish.

Conditions for transport

Containers must be escape and tamper-proof and should be protected from sudden movements and extremes of climate. The water quality should be of the highest possible standard at the start of the journey. The temperature should be kept constant. Relatively low or high temperatures should be avoided. A constant air or oxygen supply to the holding tanks should maintain dissolved oxygen concentrations >5 mg/L. Pure oxygen is preferable and is necessary when fish are stocked at high densities. Fish should be transported in a dark environment, with very low light intensity to reduce stress.

Handling

Injury and stress-induced disease can occur to animals, which are handled or crowded during transportation. Therefore, handling should be kept at a minimum. Appropriate handling techniques should be used including sedation with anaesthetics if necessary. Wherever possible, the short and long-term consequences of capture and handling should be recorded (NHMRC, 2013).

The frequency of inspection stops throughout a journey will depend on the stocking density and duration of a journey. Generally, inspections occur every 2-4 hours of the journey, more heavily stocked tanks may require checking more frequently.

4.5 Quarantine

Quarantine procedures restrict the movement of fish into, or out of a facility. They are a tool for preventing pathogens and diseases entering a facility; preventing the spread of diseases; and preventing diseased fish leaving a facility. The following batches of fish should be quarantined: 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 fish suspected or known to be diseased.. Diseased fish in ponds should remain in the pond until the disease is controlled.

The following quarantine measures should be observed when transporting or transferring aquatic animals between facilities

- Ensure that there are adequate and suitable facilities available with adequate lead time to ensure that appropriate quarantine measures can be taken.
- The tanks should be biologically separated from other holding animals. Quarantined animals must have their separate nets, hoses and cleaning equipment allocated. Best practice should be observed by staff interacting with quarantined animals i.e. hands should be thoroughly washed prior to and after handling animals. Footbaths should be used when entering and leaving the quarantined area.
- Once animals are transported they must be placed into quarantine tanks for a minimum period of two weeks. Prior to transfer environmental monitoring of quarantine tanks

should be carried out to ensure optimal conditions. Animals must not be offered food until they have acclimatised for at least 24 hours.

- Following the holding period fish should be assessed to be healthy before releasing into a project. Most potential disease issues should start to show signs of infection within that period. If disease is found during the quarantine period it may be necessary to extend the quarantine period for treatment.
- At the end of the Quarantine period all tanks must be cleaned with either 70% or 99% ethanol, or sodium hypochlorite, floors and surrounding areas should be cleaned. Dilute pool chlorine or sodium hypochlorite (NaOCl 20 ppm) or caustic soda (NaOH 1%) are suitable cleaning agents for this purpose.

4.6 Signs and management of stress

Stress is a general physiological reaction to trauma, or to a physical or psychological threat to the body that impairs normal functioning, and reduces performance and chances of survival. Stress may be acute (eg. netting, rough handling, low dissolved oxygen) or chronic (eg. very high stocking densities, aggression, poor nutrition). Stress can lead to reduced feed intake, slower growth, and lower resistance to infectious diseases. Signs of stress include: changes in feeding behaviour, including loss of appetite; abnormal or unusual colour (pale, dark, blotchy); abnormal behaviour (flighty, erratic swimming, swimming slowly, gasping at surface); lack of response to stimuli; congregation near surface or edges. Stress is minimised by: good water quality; appropriate stocking densities; adequate quantities of a nutritionally-complete diet for fish receiving artificial feed; a stable environment; limited physical disturbance; careful handling (facilitated by knotless nets, anaesthetics); and protection from bird predation in ponds.

4.7 Hatchery requirements

Marine and freshwater hatcheries are facilities that generally house and maintain a variety of animals including algae, invertebrates (as live feeds), larvae, fry, fingerlings and broodstock. It is important that hatchery procedures and protocols are developed, recorded and made readily available to all staff working within the unit. Design and operation of freshwater fish hatcheries are described in detail in Rowland and Bryant (1995) and Rowland and Tully (2004).

The following are key areas that should be addressed by hatchery managers.

Culture tanks

Tanks should be appropriate size and design, details of ACEC requirements are listed in 3.1.

Management of tanks

Water supply and drainage, aeration, screens, general management, standard hygiene and sterilization procedures.

Stocking of larvae

Quarantine and acclimation procedures.

Record keeping

Records of individual ponds, tanks and other facilities; date, daily events, feeding, treatments, water quality, mortalities, animal numbers, carer in charge.

Feeding

Live feeds, (rotifers and brine shrimp), weaning, artificial diets, nutrition, food storage and preparation.

Harvesting / Size grading

Procedures associated with harvesting/grading regarding methods, handling, destination of animals harvested etc.

4.8 Carers

Record Carer

Details of staff responsible for animals, at time of action, should be recorded.

Qualifications / Experience

Of staff caring for animals/operations should be obtained and judged if appropriate.

5. Anaesthetics and Euthanasia

5.1 Indications for anaesthesia

Anaesthetics play a key role in the welfare and health of fish by reducing stress and physical damage. Anaesthetics are used to facilitate:

- fish handling
- post-harvest transportation
- diagnostic procedures
- surgery to sedate and calm fish
- artificial breeding – broodfish anaesthetised to enable gamete sampling, hormone injection, and egg and milt stripping
- to euthanase animals.

Anaesthetics are employed at low doses, such that their limited use in coastal aquaculture presents no significant environmental risk, although there may be many hazards to users (GESAMP). Where there is a potential that previously anaesthetised fish may be captured and consumed within a short period of time after anaesthesia (eg. within 3 days), the anaesthetic AQUI-S should be used. AQUI-S is the main anaesthetic approved for use in the harvesting of fish for human consumption, but it is registered only for use on salmonids. Written veterinary directions are required for off-label use (such as in non-salmonids) but the food residue limits apply to all fish species not just salmonids. For research use (not involving human consumption—see above) no withholding period is required and off-label use is allowed.

Levels of anaesthesia.

Level	Description	Signs displayed
0	Normal behaviour	Reactive to stimuli. Good muscle tone, normal equilibrium and operculum rate.
1	Sedation	Equilibrium maintained. At lighter levels there is some reaction to external stimuli and normal opercular rates. Deeper levels show no reactivity to mild external stimuli and reduced opercular rates.
2	Light anaesthesia	Opercular rate increases initially, then decreases as anaesthesia deepens. Progressive loss of equilibrium. Reacts to only deep pressure stimuli. Colour changes may be seen.
3	Surgical anaesthesia	No reaction to any stimuli. Slow opercular rate, with operculum spread. No muscle tone, no equilibrium control.
4	Medullary collapse	Cessation of operculum movements, followed some time later by cardiac arrest.

5.2 Registered or permitted anaesthetics

Any use of unregistered products, or products used off-label, **outside the research facility** or to treat fish/animals **within the research facility and which may in future be consumed by humans** is not permitted. Just because the use occurs within a research facility does not exempt the use from the controls if fish are eventually to be eaten. See comments above under **Treating wild caught fish to be released.**

The two anaesthetics that have fallen into most common use for fin fish are AQUI-S (iso-eugenol) and Benzocaine (ethyl-p-amino benzoate). More recently the use of AQUI-S has become widespread due to its ease of use and effectiveness as an anaesthetic agent.

5.2.1 Benzocaine (Ethyl-p-amino benzoate)

Benzocaine is a commonly used anaesthetic for a variety of fisheries research applications. It is effective, relatively safe and easy to use, and relatively inexpensive. There is a Minor Use Permit for the use of benzocaine in aquaculture in Australia.

Preparation and administration of benzocaine

Ethyl-p-amino benzoate is dissolved in 100% ethanol at a recommended concentration of 1 gram per 10 millilitres (1 g/10 mL) to form a stock solution. This solution should be kept refrigerated, preferably in a brown glass container, and not exposed to sunlight. One millilitre of this stock solution contains 100 milligrams(mg) of benzocaine ie. 1 mL stock = 100 mg benzocaine. This stock solution is then used via immersion bath for either anaesthesia or euthanasia. To give an immersion bath concentration of one milligram per litre (1 mg/L) stock solution is added at the rate of 0.01 millilitre per litre of bath water (0.01 mL/L), or 1 mL/100 litres of immersion bath.

Dilution rates for Benzocaine Stock Solution.

Dose of Benzocaine required in immersion bath (mg/L)	Dilution rate of Stock Solution (mL/100 litres of immersion bath)
25 mg/L	25 mL/100 litres
50 mg/L	50 mL/100 litres
75 mg/L	75 mL/100 litres
100 mg/L	100 mL/100 litres

Dose rate guidelines (immersion rate) for Benzocaine.

Handling (Sedation)	20-35 mg/L immersion bath
Surgery (Anaesthesia)	50-75 mg/L immersion bath
Euthanasia	100 or >100 mg/L immersion bath

Note:
a These immersion rates are a guide.
b Always consult colleagues experienced with the species and anaesthetic agent being used.
c For sedation/anaesthesia it is advisable to start with low end doses and ‘titrate to effect’.

Because there are registered or permitted products available benzocaine may be used in accordance with those permits, or under written veterinary direction, even for food fish (withholding period — do not use less than 500 degree days before harvesting fish for human consumption, eg. 500 degree days = 50 days at 10°C water temperature or 25 days at 20°C water temperature) - see APVMA permit PER14638). For research use (not involving human consumption - see above) no withholding period is required and off-label use is allowed.

5.2.2 AQUI-S®

The Committee recommends the use of ‘AQUI-S’, for fish anaesthesia and euthanasia. AQUI-S does not have a withholding period it is effective at a wide range of concentrations, water soluble, biodegradable and non toxic.The anaesthetic mechanism of AQUI-S and associated clove oil derivatives has been poorly studied, but they appear to act similarly to other local anaesthetics by inhibition of voltage-sensitive sodium channels within the nervous system (Leary et al 2013).

AQUI-S has some advantages that have led to its increased use. These include:
- a wide therapeutic safety margin
- freshwater or seawater soluble (benzocaine is ethanol soluble) making it easy to work with, and
- a smooth dose related induction and rapid recovery are reported.

A useful concentration or dosage calculator together with a download on the preparation of anaesthetic baths can be found at www.aqui-s.com. Any use of unregistered chemical products, or products used off-label, **outside the research facility** or to treat fish/animals **within the research facility and which may in future be consumed by humans** is not covered by research permit PER7250 and is not permitted. Use that occurs within a research facility does not exempt the use from the controls if fish are eventually to be eaten.

AQUI-S is registered for use as an anaesthetic for salmonids in aquaculture, and is available commercially. The active ingredient is iso-eugenol. It is a very effective anaesthetic for freshwater fish. AQUI-S is water soluble, and a stock solution can be prepared by diluting concentrated AQUI-S to 100 mg/L in distilled water. The stock solution can be added directly to anaesthetic baths.

Concentrations required for handling, surgery and euthanasia[1].

Anaesthetic	Concentration (mg/L)[1]		
	Handling	Surgery	Euthanasia
Benzocaine	20-35	50-75	100
AQUI-S[1]	25	60	150 (see section 5.5.1)

[1] Refer to the label directions for use. These concentrations are for silver perch (see Stone and Tostin, 1991) and those required for other fish may differ. In the absence of other information, researchers are advised to start with lower concentrations and increase the dose if necessary. Dose rate variations between species can be marked (see Becker et al., 2013)

AQUI-S is approved for use in the sedating of fish to be used for human consumption, but such use only applies to salmonids. Written veterinary directions are required for off-label use (such as in non-salmonids) but the food residue limits apply to all fish species not just salmonids. For research use (not involving human consumption - see above) no withholding period is required and off-label use is allowed.

5.2.3 Other agents for finfish

The use of AQUI-S or Benzocaine should cover the vast majority of sedation and anaesthesia requirements, and it is these agents that the Committee advises most strongly. If the use of other anaesthetic agents is planned please advise the committee and help disseminate any information you may have. Other anaesthetic agents are used including MS222. For a review of these please refer to Neiffer et al., 2009.

5.2.4 Magnesium Chloride

Magnesium chloride is an effective anaesthetic for a variety of cephalopods (Messenger et al 1985). Magnesium Chloride acts centrally on the nervous system to induce anaesthesia

The recommended anaesthetic bath concentration is 27 g/L (Goncalves et al., 2012). Please refer to Andrews et al., 2013 for an overview of pain, distress and euthanasia in Cephalopods.

5.3 Euthanasia of fish and cephalopods

Euthanasia is the act of inducing a death that is pain and distress free. Death is the irreversible cessation of brain function. Euthanasia is performed with respect for the animals entrusted to our care.

Methods of euthanasia cause cessation of brain function by two basic mechanisms: (1) the direct destruction of brain tissue particularly the centre controlling respiratory function located in the brainstem. Example: pithing (Iki-jimi); and (2) the depression of brain tissue causing loss of consciousness followed by paralysis of the respiratory control centre. Death then results from cardiac arrest and or hypoxaemia (low blood oxygen). Example: lethal anaesthetic dose.

For euthanasia to be humane, the loss of consciousness should precede the loss of motor activity (muscle movements).

It is important to understand that the loss of motor activity does not necessarily mean the absence of distress; hence the use of neuromuscular blocking agents alone as a means of animal euthanasia is seldom advised.

5.4 Special considerations for fish euthanasia

Establishing universal guidelines for the euthanasia is difficult because of the marked anatomical and physiological diversity between species; for example some fish due to their behaviour and anatomy may be humanely stunned by clubbing (concussive stunning) prior to cervical dislocation; tropical species may be more susceptible to ice slurry euthanasia than temperate species.

Fisheries research is conducted in a wide range of working environments from land based or laboratory/aquaria, to deep sea commercial vessels sometimes in difficult conditions. Land based research will often allow access to more euthanasia options.

Fish are generally more tolerant of hypoxia; it is for this reason that exsanguination (bleeding to death), on its own, or decapitation on its own, are generally not acceptable methods of euthanasia.

This Committee encourages researchers to seek help from colleagues whenever necessary and to communicate any information to the NSW DPI (Fisheries) ACEC that may be helpful.

5.5 Methods of euthanasia

5.5.1 Chemical euthanasia (most commonly anaesthetic overdose)

The euthanasia of fish by a lethal dose of anaesthetic is the preferred method.

i **AQUI-S**

AQUI-S® is a water-dispersible liquid anaesthetic for fin fish, crustacea and shellfish. It can be used for humane harvesting, transportation, grading, handling and other husbandry procedures as well as for euthanasia. Dose and exposure time depend on the species, application and the condition of the fish. AQUI-S is effective in both freshwater and saltwater aquaculture operations.

Because there is the potential for contact irritation at higher concentrations (which may be species dependent), the committee advises induction of narcosis first with a dose of 40 mg/L before increasing the bath concentration to >=150 mg/L. The time to medullary collapse (and subsequent death) will vary but would be in the order of 30 minutes. Again fish should be left in

solution for at least 10 minutes after showing no signs of life (cessation of opercula movement), and if unsure extend the soak time or consider two stage euthanasia as long as the fish has lost consciousness.

ii **Benzocaine**

Benzocaine has been the most commonly used drug, administered via an immersion bath at a dose rate of >or=100 mg/L. This is a guide only as doses will vary between species. Fish should be left in solution for 10 minutes after opercular movements and other signs of life have ceased. Note that respiratory paralysis can precede cardiac paralysis by some time (fish tending to be tolerant of hypoxia). As well ram ventilators (eg. Tuna) may show little respiratory movement under anaesthesia. If needed, as long as consciousness has been lost, death can be confirmed by a physical means such as pithing or cervical dislocation (ie. two stage euthanasia).

iii **Magnesium chloride for cephalopods**

Magnesium salts may also be mixed in water for use as immersion euthanasia agents for cephalapods. In these animals, magnesium salts induce death through suppression of neural activity. The recommended immersion bath dose rate is 75 g/L stock solution diluted 1:1 with seawater. This is then followed by rapid decapitation (Andrews et al., 2013)

5.5.2 Non chemical/physical methods

Sometimes circumstances are such that chemical euthanasia is not possible. The use of euthanasia drugs may in some way compromise the veracity of the research, or simply not be possible in a field research or industry based setting. In such circumstances it may be acceptable to use other methods of euthanasia. The method used will vary according to the research setting, the fish species, and the experience of the researcher in a particular technique.

5.5.3 Direct physical destruction of brain tissue

The aim is to cause death by irreversibly damaging the respiratory control centre in the brain stem (high spinal) area. It is often best to use a two stage euthanasia protocol, but again the final method may be species dependent.

Clubbing

A well placed firm blow to the head will induce concussive stunning in some species. This could then be followed by cutting the throat to cause exsanguination and then bending the head back to sever the spine (cervical dislocation). Clubbing could also be followed by pithing.

Pithing/Iki-jimi/Spiking

These are essentially the same, causing irreversible brain damage. Depending on the anatomy of the species, a sharp instrument is inserted just behind the eye and sometimes rotated around, or back and forth. The fish may twitch and flare its gills and then die. Some people will also cut the throat and bend the head back to sever the spine. Depending on the species a two stage process may be better from the start. The fish is first rendered senseless by clubbing or sedation prior to pithing. As an example of sedation a suitable AQUI-S dose for immersion of Mulloway is about 10 ppm.

Decapitation or ‘Spinal Transection’

Used alone decapitation is generally not an acceptable method of euthanasia because fish may remain conscious for some time. Cervical dislocation, spinal transection and spinal ablation are essentially synonyms and neurologically speaking no different to decapitation. Under some

circumstances, ‘spinal transection’ as a sole procedure may be acceptable because in some species, when performed by some people it may cause enough brainstem (high spinal) injury to result in a rapid painless death. Spinal transection may also be acceptable for fish of a particular age and or size.

Consider a two stage process when using physical methods of euthanasia (eg. AQUI-S followed by decapitation). Always seek advice from other colleagues who are experienced with the species being euthanased.

Chilling/Ice Slurry

The use of ice slurry euthanasia has been a common fisheries practice. Some researchers have found it to be a humane method of euthanasia for individual species (Blessing et al., 2010). This Committee does note however that this opinion is not universal. The Committee prefers the use of lethal anaesthetic dosage whenever possible.

Suggested Ice Slurry Protocol:

i Fish should not come in direct contact with ice. Crushed ice may be preferable to block ice.

ii Add ice to at least 50% of volume. Use a thermometer. A fresh water slurry should be 0°C . A salt water slurry should be -4°C Continually monitor the temperature and add further ice as needed. Fish numbers should be low enough to enable the maintenance of the above temperatures.

iii Generally tropical species will be more susceptible to chilling. Allow the fish to remain in the bath for twenty minutes, or for ten minutes after respiratory movements have ceased. If unsure about death and if appropriate, use a two stage process, and follow up with for example cervical dislocation or pithing.

Conclusion

When unavoidable, the destruction of the animals in our care must be humane. Together we should continue to assess euthanasia protocols so that best practice is always used to minimize distress and suffering. Feedback to the Animal Care and Ethics Committee from fish researchers is an essential part of this process.

6. Sampling and Collection

6.1 Acceptable methods of fishing for field-based sampling and collection

A wide range of minor procedures are used in the field. These may involve only capture and release, often facilitated by the use of an anaesthetic. Such procedures could include tagging, examination, measurement, and sampling.

Where it is proposed to use chemicals such as anaesthetics, note that unregistered treatments should not be used. See the comments under **Treating wild caught fish to be released** above.

The following list shows methods of fish sampling and collection that may be used by investigators, subject to ACEC approval and only if the following requirements are met:

i All procedures are conducted by appropriately qualified and experienced persons, using clean equipment;
ii Equipment necessary to provide for health and welfare of the animals and relief of pain is readily available;
iii Uneventful recovery to full consciousness should occur in an area where animals can be readily observed, can maintain normal body temperature and are protected from injury or predation;
iv The potential impact of procedures on dependent young is minimised and
v The methods and equipment used are appropriate for the species.

Methods of fishing

i Gill Netting
ii Trapping
iii Hauling/seine netting
iv Trawling
v Electrofishing (refer to Australian Code of Electrofishing Practices)
vi Drop/hand lining
vii Fyke Netting
viii Dredging sediments

Studies are often associated with commercial fisheries, therefore commercial harvesting practices may often be used for catching fish. In these cases, practices which ensure rapid loss of consciousness, such as stunning or anaesthesia should be used wherever possible (NHMRC, 2013).

6.2 Electrofishing summary and effects on captured fish

Boat and backpack electrofishing have been the main sampling tools for freshwater fish research conducted by NSW DPI staff in recent years. With the boat-mounted units, a pulsed DC electrical field is generated in the water around the boat affecting fish within up to 4 m under ideal conditions. The standard protocol uses a pulsed DC current with voltage set at a low initial level of 400 V, but this may be varied depending on the conductivity of the water. The effectiveness of the initial settings is assessed by checking whether any fish are caught and by the behaviour of shrimps. Fish within the electric field are temporarily immobilised, captured with dip nets, and placed into a live well on the boat and subsequently inspected, measured and weighed, etc depending on the requirements of the project being conducted. Backpack units produce a similar but much smaller field, usually only affecting fish within 0.5 m of the hand-held anode.

Fish recover in several minutes, and can be released back into the water. NSW DPI has conducted fish injury studies investigating the effects of electrofishing on several species of native fish. These showed that a very small percentage of fish suffered injuries such as bleeding, nerve damage and opercular damage, but the incidence of injury was much less than that caused by more conventional survey capture methods. These results are supported by overseas research that show electrofishing causes fewer injuries to fish if performed correctly than other techniques such as gill nets. There have been no reports of any injury to platypus or other aquatic vertebrates resulting from electrofishing in NSW. Platypuses and other aquatic vertebrates stunned as a result of electrofishing in NSW have recovered and been released. However any lasting injury particularly to the sensory system of the platypus, which detects electrical fields in the milli or microvolt range through its bill, has not been investigated.

In addition to surveying fish populations in rivers and dams, research staff also use electrofishing for capturing broodstock for its freshwater fish breeding programs. This technique is preferred for broodstock collection as fish receive fewer injuries than with other capture methods and the process does not affect their reproductive success.

Research staff conduct electrofishing following the NSW DPI Electrofishing Procedures 2013 (http://intranet.dpi.nsw.gov.au/divisions/fisheries/electrofishing-procedures-dpi.pdf), which states in Section 4.6.1; Care of Fish:

'Only the minimum power necessary to attract and capture fish effectively should be used. Fish should be removed from the field as quickly as possible. If adverse effects are observed in sampled fish, electrofishing settings should be adjusted accordingly. Contact of fish with live anodes should be avoided, as the resulting shock will be much greater. If threatened species are observed that are not being targeted, appropriate measures must be taken to minimise disturbance and stress to these fish.'

Also, Section 4.6.2 of the Electrofishing Procedures; Care of Other Fauna, requires that:

i Electrofishing must cease a minimum of 10 metres from any animals standing in or about to drink from the water.
ii Electrofishing must cease a minimum of 10 metres from any metal structure within the water or wire fence lines that enter the water.
iii Care should be taken to avoid shocking platypus, birds, reptiles and other aquatic animals. The species and fate (ie. died, obviously injured, apparently uninjured) of all wildlife affected by sampling practices need to be noted on data sheets and documented in ACEC reporting.
iv Care should be taken in ensuring that physical variables as high water temperatures or low O2 do not conspire to result in excessive mortality.

6.3 Handling techniques for sampling and returning animals

The principle aim of handling techniques is to minimize stress experienced by fish as far as possible and to prevent any further damage. There must be sufficient staff to restrain animals in a quiet environment and prevent injury to animals and handlers. The following handling techniques for sampling and returning animals are taken directly from the Code (NHMRC, 2013).

i The time for which the fish is held should be minimal and consistent with the aims of the study.
ii Fish must be held in such a way as to minimize stress and/or injury. 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.
iii Wherever possible, fish must be sampled whilst still in the water. This is particularly relevant when using any trapping or netting sampling methods.
iv Holding areas must be safe, quiet and hygienic.

v Fish must be assessed regularly if prolonged restraint or confinement is required.
vi Close confinement devices must:
- allow fish to rest comfortably;
- minimize the risk of escape or injury;
- be adequately aerated;
- maintain constant temperature; and
- minimize the risk of disease transmission.

vii Release should be at the site of capture, unless an alternative site is justified in the project proposal.
viii 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.
ix When releasing fish from holding tanks, fish must be supported by both hands and gently lowered into the water.
x If handling or restraint is likely to cause harm, including pain and distress, to the animal, the use of chemical restraint (eg. sedatives) should be considered.
xi If any adverse impact is detected, the animal must be released, or the method of restraint must be modified to minimise that impact.

At the time of release all reasonable steps must be taken to protect animals from injury and predation.

6.4 Tagging protocol

Fish are tagged to provide information on movement patterns, total population size and accumulation rates below weirs. It is important that any tagging program has a well-defined set of research questions that are designed to answer a specific objective. Tagging is an invasive procedure and scientists 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 program 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 staff have adequate and regular training to ensure techniques are correct which will minimise stress on fish and also ensure a high probability of tag retention.

6.4.1 External Tags

Tag selection depends on a range of factors which would be determined before commencing the program. These include the species of interest, the size of fish, experience of staff undertaking the work, length of the tagging program, project budget and the type of information being collected. External tags come in a range of designs but the most commonly-used are T-bar and Dart tags.

T-Bar Tags

T-bar tags are used for fish, crustaceans and shellfish species where large numbers of fish may need to be tagged in a short space of time and/or holding time is critical for fish survival. Tags can be varied in length and the minimum length should be determined by the amount of print required on the tag.

Information from tag supplier Hallprint Pty Ltd suggests that as a general rule, fine anchor tags (type TBF) are suitable for most finfish and crustaceans according to the following optimal size ranges:

- Finfish (between dorsal or anal fin rays) 15 cm TL - 30 cm TL
- Rock lobster (through moult suture) 3 cm CL - 6 cm CL

- Crabs (through moult suture) 4 cm CW - 7 cm CW

Standard anchor tags (type TBA) are suitable for most finfish and crustaceans according to the following optimal size ranges:

- Finfish 20 cm TL - 50 cm TL
- Rock lobster 6 cm TL to max. size
- Crabs 7 cm CL to maximum size

T-bar tags are implanted using an applicator gun. The gun contains a magazine which can hold up to 20 tags and a needle for applying into the fish. Tags are usually administered between the pterygiophores of either the dorsal or anal fin. 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. The needle should be inserted, at a 45 degree angle, between two pterygiophores, preferably the 3rd and 4th dorsal spine. The gun is then deployed and the gun rotated clockwise before removing the needle to ensure the tag lodges correctly. The needle is then withdrawn and the tag can be checked for correct placement. The fish should then be placed into aerated water for recovery before release. It is important not to tag any fish that are showing signs of stress.

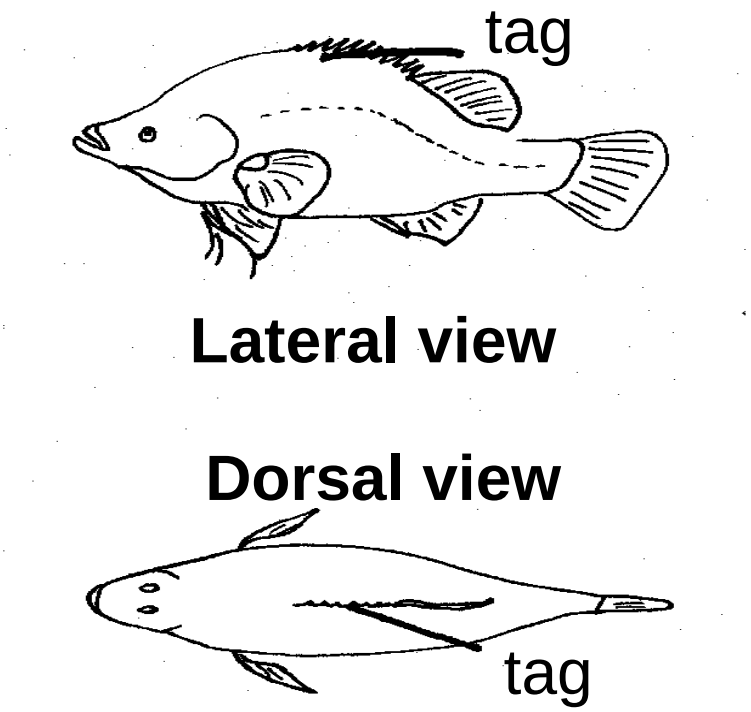

Figure 1

Correct location of dorsal tag placement (Supplied by Dr Ivor Stuart, Kingfisher Research). On the lateral view, the tag should not be implanted further than 5mm from the top of the fish to avoid interfering with the lateral line (sensory organ). Note the dorsal view demonstrates that the tag should not protrude from the fish at an angle greater than 45 degrees as angles greater than this can create drag and inhibit swimming ability.

Dart Tags

Dart tags are larger than T-bar tags and have been developed for use in studies on larger-bodied species. Small dart tags are applied with needles with an approx. 2.4 mm outside diameter. These are suited to finfish species between about 20 cm and 30 cm and are popular for small fish likely to undergo fast growth rates such as juvenile tuna or for fish such as Murray cod, carp or golden perch. Medium dart tags are also available and are applied with needles of an approx. 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 approx. 4.0 mm outside diameter needle and are most suitable for with finfish and sharks from about 60 cm including tuna, barramundi, tarpon, adult Murray cod and Spanish mackerel.

Prior to tagging the fish should be under control. Researchers should consider anesthetisation for large fish or fish with spines to minimise stress on the fish and the handler. Applicator guns are not available for dart-tags, so these are applied using a hand-held needle. The needle should be used to firstly remove scales in the tagging area. 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 (Figure 2). The tag can then be tugged slightly to ensure it is set. Fish should be allowed to recover in an aerated tub prior to release.

Figure 2

Correct placement of a dart tag showing barb pushed through spines and lodging between pterygiophores.

6.4.2 Internal Tags

Passive integrated transponders (more commonly known as PIT tags or microchips) are becoming increasingly popular in fish migration studies 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 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.

PIT tags are inserted by using a specialised applicator needle. Applicators are usually specific to the type and size of tag being used (i.e. full or half duplex) and manufacturers generally provide applicators for their tags. Tag retention trials undertaken at Narrandera Fisheries Centre using Murray cod, golden perch and silver perch indicate that inserting tags into the coelomic (peritoneal) cavity results in the highest rates of tag retention and lowest mortality (Lee Baumgartner, Unpublished data). Tagging in the shoulder, cheek or pelvic region is not recommended for these species, and the method should be generally transferable to other species. Fish should be at stage 3 of anaesthesia prior to tagging and tags should be inserted between the body wall and internal organs (a needle angle close to parallel with the fish will reduce the risk of piercing organs). Tagging should ideally be conducted outside of predictable reproductive windows for species and additional care should be taken when tagging gravid females as rupturing ovaries can result in additional shedding during spawning. The needle can then be removed and the tagged fish placed into an aerated tub for recovery prior to release. A hand-held PIT reader should then be used to verify that the tagging was successful.

6.5 Techniques for collection of animals for laboratory analysis

To minimise the distress to animals being kept for analysis or preservation it is essential that animals be euthanased as soon as possible after capture.

Animals should not be euthanased by placing directly into preservative. All vertebrate animals should be correctly euthanased prior to fixation; for suitable procedures in euthanasia refer to Section 4 of this Guide.

When sorting the catches of haul nets, traps, etc. it is recommended that the net or trap 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. Discarded animals to be returned to the wild should be released in suitable places, safe from advantaged predators or unsuitable water conditions which may be found close to sorting areas.

6.6 Techniques for collection of blood and other samples from finfish

Blood should only be collected from fish that have been firmly restrained in a foam cradle, preferably heavily anaesthetised, heavily stunned (eg. following electrofishing) or euthanased. Any other samples should only be collected from euthanased fish. Other procedures that may include the recovery of material for genetic analysis (eg fin clips or field based biopsy) would be assessed by the committee on a case by case basis.

Appropriate labelling of blood samples is important to ensure meaningful data is obtained. An example of a possible labelling scheme is outlined below:

Label all samples from each individual fish with the same number, unique to that individual fish. The date and location of capture should also be recorded for each individual fish. Sites within each location should be numbered separately (e.g. if there are a number of sampling sites at Turner's pond, then all samples from the first individual fish caught at Turner's pond site 1 would be numbered TP1/1, samples from the second fish TP1/2, samples from the first fish at Turner's pond site 2 would be TP2/1, etc.)

Blood collection should only be performed by individuals who have been trained in this method. All equipment for blood collection needs to be prepared in advance of collection in order to minimise the time fish are restrained. The necessary equipment includes appropriately-sized needles and syringes, sample vials for blood, plastic bags for holding samples, waterproof marker pens, labels and pencils, paper towel, anaesthetic, tubs for anaesthetising fish and for recovery, dip nets, ice and esky for storing samples (if remote from a fridge or freezer). After fish are restrained in the foam cradle, an appropriately size needle and syringe should be selected and the needle inserted behind the anal fin at the ventral midline, and directed cranial and dorsal

until the spine is reached. The needle is then withdrawn slightly and blood drawn into the syringe (see Figure 3). A new needle and syringe should be used for each fish and the foam cradle rinsed with clean water and wiped with a new sheet of paper towel between each fish. Following blood sampling, fish should be euthanased or immediately placed in a recovery tub with well aerated (or oxygenated) clean water. Recovery must be carefully observed and any fish experiencing difficulties with swimming or orientation after the usual recovery period should be euthanased (see section 4.5).

Blood may also be sampled from freshly euthanased fish. Sample vials, a sharp knife or scalpel and all equipment should be prepared prior to sampling. Immediately following euthanasia, use a sharp knife or scalpel and cut off the tail behind the anal fin (Figure 4) and allow the blood to drain from the caudal blood vessels into the blood collection tubes (fill tubes to no more than 1 cm from the top of the tube). The caudal vessels are located directly beneath the spine. The knife or scalpel used for blood collection need to be wiped clean then rinsed with clean water and wiped with a new sheet of paper towel between each fish to minimise any cross contamination between samples.

Blood samples will need to be handled according to the desired end use and the experimental protocol to be employed. For procedures where clotted blood is required, blood should be stored in a cool, shaded area until clotted and then placed on ice.

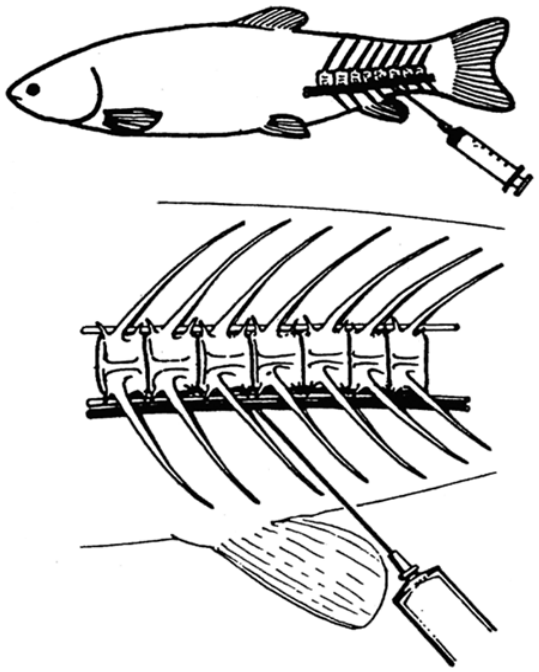

Figure 3

Site for blood collection using needle and syringe. (http://www.fao.org/docrep/field/003/AC160E/AC160E09.htm).

Figure 4

Site for removal of tail (indicated by dashed line) for blood collection, following euthanasia of fish. (modified from http://www.fao.org/docrep/field/003/AC160E/AC160E09.htm) .

6.7 Improving Platypus survival (reducing by-catch drowning)

This Committee asks researchers to report, in their applications, renewals and final reports any wildlife species unintentionally affected by their research. Most commonly this concerns non-target species caught or harmed by fish community sampling. Whilst all by-catch animals are of concern, it is the occasional platypus by-catch mortality, which the following material addresses.

Platypus

- Platypuses do not have long-term diving adaptations. They will die within a few minutes of being restrained underwater in a net or a trap .
- Platypuses have little subcutaneous fat insulation and can rapidly become hypothermic.
- Spurring mortalities may occur if more than a single male is confined in a trap or fyke net. This will be more likely in the mating season (July to October), when the male venom glands increase in size and output.
- The death of a lactating female could result in the loss of one to three juveniles in the burrow. The lactating period is September to March.
- Platypuses are mainly nocturnal, but individuals are commonly seen an hour or so before dark and after dawn. However, in some streams they can be seen in the middle of the day.
- Platypuses normally avoid an area of disturbance/human activity; however 'inquisitive' individuals have been reported in the wild.

General

Larger numbers of platypuses were once drowned in fisheries operations when greater reliance was placed on netting, particularly gill netting. The number of animals affected by fisheries sampling may be small in total now, but the deaths of only a few lactating females in a lower order stream could be very significant to a local population. Electrofishing, using the following recommendations, would be the preferred sampling method, when appropriate to the experimental design and purpose.

Electrofishing

- On occasions where platypuses have been stunned by electrofishing, they have recovered after a few minutes and have swum away when released.
- However, there is no published work on the effects of electrofishing on the electroreceptors in the platypus bill and the electric fields of the strength used may have the potential to cause damage to these sensory receptors.

- Where possible electrofishing should be conducted during daylight hours, also avoiding the two hours before darkness and the two hours after first light.
- Where possible electrofishing (especially the more powerful boat based form) should be organized to avoid areas where platypuses are observed or locally known to be diurnally active.

Fyke Nets

- The cod ends of fyke nets must be fixed (eg. to star picket, pegged to bank, attached to overhanging branch) above the water level. Captured platypuses will climb into the elevated cod-end and survive.
- Allowance must be made for any possible expected rainfall events that may cause the level of the water body to rise. The fyke nets should be removed, or the cod-ends further elevated, in the case of unexpected high flow events.
- Nets should be checked several times during each 24-hour period to release any captured platypuses. During the use of fyke nets to capture platypuses for research, nets are checked every 1-3 hours, depending on how far apart they are deployed.
- Greater emphasis should be placed on checking nets at night, especially in winter conditions, particularly in highland areas.

Gill Nets

- Unless no alternative capture method is available only use unweighted nets with a buoyant floating surface line. This enables captured platypuses to bring the net to the surface and be additionally supported by the float line. If undisturbed, air trapped in the fur also provides buoyancy to a platypus on the surface.
- Use frequent observation and lifting of nets to remove snags, turtles or fish, which can hold the net down and result in captured platypuses being unable to reach the surface.
- Unless the importance of the research is considered to outweigh the risk and/or no alternative capture method is available, do not use sinking nets. It is not possible to safely monitor sinking nets that follow the bottom and have no float line on the surface.

Platypus Handling

The fear of being spurred by a platypus may lead to inappropriate handling of an animal. Always assume a platypus is an adult male. Females do not have spurs. A juvenile male cannot deploy its spurs and they have no venom for their first year. If an animal has to be handled it should be picked up by the rear half of the tail so a spur injury cannot be inflicted, and then restrained in a thick material such as a hessian bag.

Captured individuals should be securely housed out of the water in a quiet, dark location and returned to the water once the nets have been removed from the water body. Out of the water, platypuses are very susceptible to heat stress and should be kept at temperatures at or below 25^{o}C. When the fur is not disturbed (out of water platypuses will groom themselves dry), an uninjured platypus can maintain its body temperature at air temperatures as low as 5^{o}C, although more energy is required to maintain homeothermy at such low temperatures, increasing physiological stress on a captive animal.

Conclusion

Electrofishing appears to have less impact than netting; avoid night sampling; use unweighted nets; avoid using sinking nets; check and lift nets often especially at night, and during winter; be especially careful in lower order waterways; avoid the female lactating period (late September to mid-March in NSW).

7. Environmental Considerations

7.1 Disposal of chemicals

For information regarding the disposal of chemicals, please examine the following reference:

(GESAMP, 1997) (IMO/FAO/UNESCO-IOC/WHO/IAEA/UN/UNEP) Joint group of Experts on the Scientific Aspects of Marine Environmental Protection, 1997. Towards safe and effective use of chemicals in coastal aquaculture. Rep. Stud. GESAMP, 65.

7.2 Disposal of animal carcasses

Appropriate provision must be made for prompt and sanitary disposal of animal carcasses and waste material in accordance with any commonwealth, state or territory legislation, local council by-laws and community standards (NHMRC, 2013).

7.3 Translocation and quarantine

There are a number of requirements governing the import, capture, handling and transport of animals. Some requirements are listed below. It should be noted that this list is not comprehensive and it is the responsibility of the investigator to consult the relevant state and territory authorities to ensure compliance with all requirements (NHMRC, 2013).

i Under quarantine and fauna laws and formal agreements, the Commonwealth and individual States and territories regulate the movement of animals or animal tissues into Australia and across State and Territory borders within Australia.
ii A Certificate of Health may be required to accompany animals travelling interstate. This is normally issued by State or Territory Departments of Agriculture or their equivalent.
iii For native fauna, the appropriate State or Territory Fauna Authority may require further certification that animals will be taken legally.
iv Permits must be obtained from The Biodiversity Group (Environment Australia) formerly ANCA, for the importation of live animals, except those species which are specifically exempt. The Australian Quarantine and Inspection Service (AQIS) should also be contacted.
v Permits are also required by The Biodiversity Group (Environment Australia) and AQIS for the importation of dead specimens and tissues.
vi Permits must be obtained from The Biodiversity Group (Environment Australia) for the export of both live and dead specimens of all native Australian fauna. Prior approval is also required from The Biodiversity Group (Environment Australia) for the export of some animal species not native to Australia.

7.4 Noxious and pest fish issues

A list of aquatic species declared as noxious fish or noxious marine vegetation under the Fisheries Management Act is available on the NSW DPI (Fisheries) website. Class 1 noxious species cannot be possessed or sold without a specific permit. Class 2 noxious species can only be kept in enclosed aquaria.

Both noxious fish and other non–native / pest fish species can cause significant ecological damage in the wild. Researchers conducting fish sampling in the wild frequently capture common pest species such as carp and redfin perch, and less frequently may encounter more exotic species such as cichlids or other ornamental fish. The Code (Section 3.3.44) states captive feral and pest species must be killed humanely unless the aims of a project require their release, or the study involves death as an endpoint. NSW DPI encourages euthanasia of

captured exotic fish (in accordance with the guidelines in section 4 of this document) on biosecurity grounds, to reduce harm to the native fish community.

Other than the immediate re-release of fish to the waters where they were captured, a permit from NSW DPI is required to release any live fish into natural waters.

Any proposal to keep or culture a non-native species for research purposes will require consideration of biosecurity issues, such as security of the facilities to prevent escape and appropriate disposal of fish once research has concluded.

8. References

Allan, G. L. and Rowland, S. J., 2002. Silver perch, Bidyanus bidyanus. In: Nutrient Requirements and Feeding of Finfish for Aquaculture. C. Webster and C. Lim (Editors), pp. 358-373. CABI Publishers, UK.

Andrews, P.L.R., Darmaillacq, A-S., Dennison, N., Gleadall, I.G., Hawkins, P., Messenger, J.B., Osorio, D., Smith, V.J. and Smith, J.A., 2013. Journal of Experimental Marine Biology and Ecology, 447: 46-64.

AVMA Guidelines on Euthanasia. (June 2007)

Australian code for the care and use of animals for scientific purposes, 8th Edition (2013). NH&MRC.

Australian Government Department of Agriculture, Fisheries and Forestry 2009, Operational procedures manual: Destruction (Version 2.0), Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT.

Becker, J., Baumgartner, L. and Hay, A., 2013. Optimisation of efficacious dosage of Benzocaine and AQUI-S in to immobilise selected freshwater fish during routine handling and surgical procedures. Conference paper, 6p.

Blessing, J.J., Marshall, J.C. and Balcombe, S.R., 2010. Humane killing of fishes for scientific research: a comparison of two methods. Journal of Fish Biology, 76: 2571-2577.

Bosworth, B. G., Small, B. C., Gregory, D., Kim, J., Black, S. and Jerrett, A., 2007. Effects of rested-harvest using anesthetic AQUI-S™ on channel catfish, Ictalurus punctatus, physiology and fillet quality. Aquaculture, 262: 302-318.

Callinan, R.B. and Rowland, S.J., 1995. Diseases of silver perch. In: Silver Perch Culture, Proceedings of Silver Perch Aquaculture Workshops, April 1994. S.J. Rowland and C. Bryant (Editors), pp. 67-75. Austasia Aquaculture, Sandy Bay, Tasmania, Australia.

Chandroo, K. P., Duncan, I. J. H. and Moccia, R. D., 2004. Can fish suffer?: perspectives on sentience, pain, fear and stress. Applied Animal Behaviour Science, 86: 225-250.

Culloty, S.C., and Mulcahy, M.F., 1992. An evaluation of anaesthetics for *Ostrea edulis* (L.). Aquaculture, 107(2): 249-252.

GESAMP (IMO/FAO/UNESCO-IOC/WHO/IAEA/UN/UNEP), 1997. Joint group of Experts on the Scientific Aspects of Marine Environmental Protection, Towards safe and effective use of chemicals in coastal aquaculture. Rep. Stud. GESAMP.

Goncalves, R.A., Aragao, C., Frias, P.A. and Sykes, A.V., 2012. The use of different anaesthetics as welfare promotors during short-term human manipulation of European cuttlefish (*Sepia officinalis*) juveniles. Aquaculture, 370-371: 130-135.

Grandin, T. and Deesing, M., 2003. Distress in animals: is it fear, pain or physical distress? American Board of Veterinary Practitioners – Symposium 2002, May 17, Manhattan Beach, California. 12pp.

Ingram, B.A., Gavine, F. and Lawson, P., 2005. Fish health management guidelines for farmed Murray cod. Fisheries Victoria, Research Report Series No. 32.

Katz, S., 1989. Environmental impact assessment for the use of formalin-F in the control of external protozoa on penaeid shrimp. Unpublished report to US FDA, August 1989, 12pp.

Leary, S., 2013. AVMA guidelines for the euthanasia of animals: 2013 edition.

Mazeaud, M. M., Mazeaud, F. and Donaldson, E. M., 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc., 106(3): 201-212.

Messenger, J.B., Nixon, M. and Ryan, K.P., 1985. Magnesium chloride as an anaesthetic for cephalopods. Comp Biochem Physiol C., 82(1):203-5.

Neiffer, D.L. and Stamper, M.A., 2009. Fish sedation, analgesia, anesthesia, and euthanasia: considerations, methods, and types of drugs. ILAR Journal, 50(4):343-60.

NHMRC (National Health and Medical Research Council), 2013. Australian code for the care and use of animals for scientific purposes. 8th edition.

NSW DPI Electrofishing Procedures, 2013. NSW DPI, Port Stephens.

Read, P., Landos, M., Rowland, S.J. and Mifsud, C., 2007. Diagnosis, treatment and prevention of the diseases of the Australia freshwater fish silver perch (Bidaynus bidyanus). NSW DPI, Orange.

Rose, J. D., 2007. Anthropomorphism and the Mental Welfare of Fishes. Diseases of Aquatic Organisms.

Rose, J. D., 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Reviews in Fisheries Science, 10(1): 1-38.

Rowland, S.J., 1998. Water quality in freshwater aquaculture. Fishfact 1, NSW Fisheries.

Rowland, S.J. and Bryant, C., 1995 (Editors). Silver Perch Culture, Proceedings of Silver Perch Aquaculture Workshops, April 1994. S.J. Rowland and C. Bryant (Editors). Austasia Aquaculture, Sandy Bay, Tasmania, Australia.

Rowland, S.J. and Ingram, B.A., 1991. Diseases of Australian native freshwater fishes. Fisheries Bulletin No 4. NSW Fisheries, Sydney.

Rowland, S.J., Landos, M., Callinan, R.B., Allan, G.L., Read, P., Mifsud, C., Nixon, M., Boyd, P. and Tully, P., 2007. Development of a health management strategy for the silver perch aquaculture industry. Final Report to Fisheries Research and Development Corporation for Project Nos 2000/067 and 2004/089. NSW Department of Primary Industries – Fisheries Final Report Series No. 93. NSW Department of Primary Industries, Cronulla.

Rowland, S.J., Read, P., Glendenning, D. and Allan, G.L., 2001. Guidelines for feeding silver perch. NSW Fisheries, Sydney.

Rowland, S.J. and Tully, P., 2004. Hatchery quality assurance program for Murray cod (Maccullochella peelii peelii), golden perch (Macquaria ambigua) and silver perch (Bidyanus bidyanus). NSW Department of Primary Industries, Sydney.

Sladky, K.K., Swanson, C.R., Stoskopf, M.K., Loomis, M.R. and Lewbart, G.A., 2001. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). Am. J. Vet. Res., 62: 337–342.

Stone, D. and Tostin, N., 1999. Clove oil a big yawn for silver perch. Fisheries NSW Magazine, Spring 1999, p. 19.

Other resources:

Protocol for Fish Euthanasia(2005), SOP NoX, PIR, Victoria

Guidelines for Euthanasia (2004). University of Minnesota

Internet Site: Animal Pain Website, University of Edinburgh (accessed 9-4-2007)

Internet Site: Catch Care Tips for Recreational Fishers, Department of Fisheries Western Australia (accessed 4-1-2008)

Internet Site: Guidelines for the use of ice water immersion for the euthanasia of temperature sensitive small fish, Texas A&M University (accessed 4-1-2008)

Internet Site: UW policy for the euthanasia of fish species (2002). University of Washington (accessed 9-4-2007)

Internet Site: Fish and amphibian euthanasia, Cornell University (accessed 4-1-2008)

Internet Site: Haematological Examination of Fish. From Diagnostics, Prevention and Therapy of Fish Diseases and Intoxications. Manual for International Training Course on Fresh-Water Fish Diseases and Intoxications: Diagnostics, Prophylaxis and Therapy (1991). Edited by Z. Svobodová and B. Vykusová, Research Institute of Fish Culture and Hydrobiology, Vodňany, Czechoslovakia (accessed 12-2-09).

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This Committee is indebted to the scientific community of NSW DPI (Fisheries) for their continued cooperation and feedback. They show compassion and respect for the animals in their care.

10. Appendices

10.1 NSW DPI (Fisheries) Animal Care and Ethics Committee members as at 2015

Name	Category
Ms Tracey Friend	D; Independent (Community)
Mr Tony Gregory	C; Animal Welfare
Ms Gillian Hay	C; Animal Welfare
Mr John Kennedy	A; Veterinarian
Dr Michael Lowry	B; Animal Researcher (and Chairperson)
Mr Joe Pera	B; Animal Researcher
Mr Vinod Reddy	D; Independent (Community)
Dr Malcolm Smeal	A; Veterinarian

10.2 Example of the new proposal form for an Animal Research Authority

ACEC New Proposal form (2015 version).p

10.3 Example of the annual renewal form for an Animal Research Authority

ACEC Annual Renewal form (2015 \

10.4 Example of the major three-year renewal form for an Animal Research Authority

ACEC Major Three-Year Renewal

10.5 Example of an Animal Research Authority certificate

NSW GOVERNMENT Department of Primary Industries

ANIMAL RESEARCH AUTHORITY

Names of Applicants:	Location of Research:	Conditions of Authority:
Karina Hall Anna Scott Amanda Beasley	National Marine Science Centre, Coffs Harbour, Camp Cove, Chowder Bay, Little Manly Cove and/or Quarantine Bay, Sydney Harbour.	As per application.

are authorised by

NSW Department of Primary Industries

to conduct the following type of research

BIOLOGY AND COMMERCIALLY IMPORTANT CUTTLEFISH IN NSW
ACEC REF 14/15 – COFFS HARBOUR

as approved by and in accordance with the establishment's
Animal Care and Ethics Committee

Primary Industries (Fisheries) Animal Care & Ethics Committee

This authority remains in force from

4 DECEMBER 2014 to 4 DECEMBER 2015

unless suspended, cancelled or surrendered.
(Major three yearly review due in 2017)

JO PICKLES
EXECUTIVE OFFICER

MICHAEL LOWRY
CHAIR

19 December 2014

10.6 Example of an Animal Research Authority Final Report

ACEC Final Report form (2015 version).p

10.7 Example of the Reporting Form for Unexpected Adverse Events

ACEC Reporting form
(unexpected events)

10.8 NSW DPI (Fisheries) ACEC Terms of Reference

NSW DPI - AEC Terms of Reference s

10.9 NSW DPI (Fisheries) ACEC Grievance Procedures

The following Grievance Procedures have been drawn up to follow the requirements and recommendations of the Australian Code for the Care and Use of Animals for Scientific Purposes and the Animal Research Act.

Procedures have been developed to handle the following:

i complaints from independent people who are not associated with research;

ii complaints by staff of NSW DPI;

iii disputes between investigators and NSW DPI AECs;

iv serious disagreements between members of an AEC; and

v disagreements between an AEC and management of NSW DPI Institutes and research stations.

Types i) and ii) are to be referred to the relevant AEC for investigation. The investigation may take the form of interviews of the complainant, the subject of the complaint, witnesses; unannounced inspection of the facility or animals in question; seeking expert advice on technical matters from outside the institution.

Report to the Director, Research Business with recommendations for any action considered appropriate. This may include cancellation or suspension of the Animal Research Authority, formal reprimand or dismissal. The complainant will be advised in general terms of the outcome. Confidentiality must be maintained at all times to protect the complainant.

It is essential that staff may raise concerns without jeopardising employment, entitlements or general working conditions.

Any outsider with a grievance may report it to a member of the AEC who is obliged to raise the matter with the AEC as soon as possible to allow the complaint to be resolved quickly.

Types iii), iv) and v) may need referral to the Director, Research Business, NSW DPI. Guidance on policy matters may be sought from the Animal Research Review Panel (ARRP). In cases where investigators wish to lodge an appeal, it is to be lodged direct with the Director, Research Business. The Director, Research Business may investigate procedural matters, may request the AEC to re-examine the matter or refer new information to the AEC for consideration but may not overrule a decision of the AEC made on the basis of correct procedure in compliance with the legislation.

Where necessary, the Director, Research Business may request the AEC to seek further technical advice or may appoint an independent mediator to assist resolution of such matters.

All complaints are to be treated confidentially.

10.10 NSW DPI (Fisheries) ACEC Terms of Appointment

The following Terms of Appointment have been prepared for the NSW DPI (Fisheries) ACEC:

i Each member of the Committee shall be appointed for a term of four years.

ii Nominations for vacant positions will be advertised through NSW DPI (Fisheries) website and in newspapers or newsletters relevant for the category being recruited. When a position for a Veterinary Surgeon is vacant, a notice will be put in the Australian Veterinary Association NSW Newsletter. Positions for Welfare representatives will be advertised via the RSPCA, in the Australian and New Zealand Association of Animal Societies Newsletter (or other appropriate publication). Positions for Community representatives will be advertised via the Department of Premier and Cabinet; Boards and Committees website.

iii Candidates may serve two or more terms but must nominate each time.

iv Animal researcher categories filled by nomination by NSW DPI (Fisheries) Executive for a period of three years.

v Chair position should be for period of three years.

vi Chair position may be filled by representative for any category provided the nomination is endorsed by NSW DPI (Fisheries) Executive.

Notwithstanding the above, positions for Animal Welfare and Community representation need to be endorsed by Animal Welfare Unit.