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

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for the
Primary Industries (Fisheries) Animal Care and Ethics Committee

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1. SUMMARY

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 2005 incorporated the Australian Code of Practice for the 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 Emergencies and Animal Welfare Branch of Industry & Investment NSW 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 ACEC of the Director-General of Industry & Investment NSW.

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 of Practice 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 completed Application Form for an Animal Research Authority, and for an Annual Renewal are attached. Also attached are copies of an Animal Research Authority and an example of a final report that must be provided to the Primary Industries (Fisheries) ACEC on completion of research. Finally, the Primary Industries (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

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 of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2004) outline the responsibilities and justifications for conducting animal research:

i Scientific and teaching activities using animals may be performed only when they are essential to:
   - obtain and establish significant information relevant to the understanding of humans or animals;
   - the maintenance and improvement of human or animal health and welfare;
   - the improvement of animal management or production; or
   - the achievement of educational objectives.

ii Studies using animals may be performed only after a decision has been made that they are justified, weighing the scientific or educational value of the study against the potential effects on the welfare of the animals.

iii People who use animals for scientific purposes have an obligation to treat them with respect and consider their welfare as an essential factor when planning and conducting studies.

iv The acquisition, care and use of animals for all scientific purposes in Australia must be in accord with the Australian Code of Practice (NHMRC, 2004) and with Commonwealth, State and Territory legislation.

v Investigators have direct responsibility for all matters relating to the welfare of the animals they use.

vi Institutions such as I&I NSW are required to establish an Animal Care & Ethics Committee (ACEC) to ensure that all animal use conforms to the NSW Animal Research Act 1985 and to the standards of the Australian Code of Practice.

vii Investigators must submit written proposals for all animal studies to an ACEC which must take into account the expected value of the knowledge to be gained, the justification for the study, and all ethical and animal welfare aspects.

viii Scientific and teaching activities must not commence until written approval has been obtained from the appropriate ACEC that will supervise the research or teaching study.

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 Primary Industries (Fisheries) Animal Care and Ethics Committee must approve all applications for research using fish by Primary Industries scientists before they commence. This Committee also assesses applications for research on fish from Sydney Water, Department of Environment, Climate Change and Water (DECCW) and on occasion from other agencies.

The Primary Industries (Fisheries) ACEC's role is to advise, monitor, discipline and control animal research. The Committee ensures that all fisheries research conducted complies with the legislation and Code of Practice. The Committee is responsible for monitoring research 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 Primary Industries (Fisheries) ACEC has the power to stop inappropriate research and to discipline researchers by withdrawing their 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 animal welfare, through preparation of guidelines, and dissemination and/or direction to relevant scientific literature.

2.3.1 ACEC Membership

The membership and duties of ACECs are laid down in the Animal Research Regulation 1995. 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:

A A veterinarian or person with similar expertise relevant to the species being studied
B An animal researcher
C An animal welfare representative who is not involved with the establishment or with animal research or supply
D An independent person who is not a researcher and preferably is not employed by the establishment

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 Primary Industries (Fisheries) ACEC members as of 2008.

2.3.2 Procedures of ACECs

The Primary Industries (Fisheries) ACEC conducts quarterly meetings to consider new research protocols, protocol renewals, protocol 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 protocols be considered at other than a scheduled meeting. The Committee must:

- Review, discuss, approve, reject or revoke proposals to perform animal research or teaching
- 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 Primary Industries 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 Primary Industries (Fisheries) ACEC. The Primary Industries (Fisheries) ACEC also issues Animal Research Authorities under agreement for defined research undertaken by Sydney Water and sections of the Department of Environment, Climate Change and Water (DECCW). Animal Research Authorities for other individuals, groups or agencies (outside Primary Industries, Sydney Water or DECCW) wishing to undertake research with fish are not usually assessed by the Primary Industries (Fisheries) ACEC.

All proposals to undertake animal research must be submitted in writing to the Primary Industries (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 Emergencies and Animal Welfare Branch, Primary Industries.

2.4 Where an Animal Research Authority Application Form can be obtained

Applications are available from the Executive Officer, Primary Industries (Fisheries) ACEC, Port Stephens Fisheries Institute, Locked Bag 1, Nelson Bay, NSW, 2315, (facsimile [02] 4982 1107, telephone [02] 4982 1232), or the Primary Industries intranet site. An example of a completed 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 'Application for Annual Renewal of Proposal to Use Animals' (see example of completed renewal form at Appendix 9.3). Some projects run for longer than three years. If a research project will continue after three years, in addition to completing an 'Application for Annual Renewal of Animal Research Authority', a major revision of the project and methods is required. The researcher must also complete a 'New Application for an Animal Research Authority'. Any major changes to the project including research methods must be described in the new application. The Executive Officer will notify the Principal Investigator when the Authority for a project is due to expire and, if a renewal is sought, whether a 'New Application for Approval to Use Animals' must be completed (ie. after three years). 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 Inspection obligations

2.6.1 Primary Industries (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 of Practice for the Care and Use of Animals for Scientific Purposes.

Policy of NSW Department of Primary Industries 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. Primary Industries (Fisheries) ACEC inspects each research location regularly 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 GAC, NFC and Wakool 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 GAC, NFC and Wakool 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 and blood sampling, etc have not routinely been done. Inspections during 2008 were introduced to address this deficiency.

2.6.2 *Primary Industries (Emergencies and Animal Welfare Branch/NSW Animal Research Review Panel)*

The Emergencies and Animal Welfare Branch of Industry & Investment NSW conducts its inspections under the Animal Research Act (usually by a veterinary officer of the Emergencies and 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 aquaculture 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 (NHMRC, 2004, p. 34).

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	Optimum	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^{o}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^{o}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 ($CaCO_3$). 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 $CaCO_3$. 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 $CaCO_3$. 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 ($NaHCO_3$), 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 (NH_3) and a much less toxic ionised form (NH_4^+). 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 (e.g. 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.

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

I&I NSW 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 present of any *Declared Disease*, they "…. must notify a fisheries officer as soon as practicable of the infection or suspected infection." unless the infection or suspected infection has already been notified to a fisheries officer. More specifically, notifications of the presence of suspected or confirmed *Declared Diseases* should be made to the Manager,

Aquatic Biosecurity and Risk Management NSW I&I who may be contacted through the office contacts for the Port Stephens Fisheries Institute and who 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 September 2009 the Declared Diseases list is as follows:

- Epizootic haematopoietic necrosis—EHN virus
- Epizootic haematopoietic necrosis—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)
- Infectious salmon anaemia (Ex)
- Epizootic ulcerative syndrome (*Aphanomyces invadans*)
- 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)
- White sturgeon 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)

- Diseases marked (Ex) are considered to be exotic to Australia
- Diseases marked (A) are considered to be present in Australia, but absent from NSW
- Diesases not marked are known to exist in some areas of NSW, but remain notifiable to NSW I&I 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. (trichodonsis), 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 Department of Primary Industries aquaculture scientists.

4.3.4 Bacterial infections

The incidence of bacterial diseases in native freshwater fish in 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 can not 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	1 hour (tanks only)
100	2 hours (tanks only)
15-30	Repeat each 2 days for ichthyophthiriosis (ponds and tanks)
30	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.

4.3.7a 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 4.2 and 5.5 of the Australian Code of Practice (NHMRC, 2004). 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 (NHMRC, 2004). 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, 2004).

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.

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. Quarantine involves holding fish in isolation in tanks

under low-stress conditions where they are readily observed, treated and handled. Diseased fish in ponds should remain in the pond until the disease is controlled. Fish should be checked for disease as soon as possible after being placed in quarantine. Water should not be exchanged or the tanks/ponds drained until the disease status of the fish is known. Appropriate chemical therapeutics should be used if fish are diseased. Hold fish in tanks with 2 – 5 g/L salt for at least 5 days to reduce stress and prevent fungal infection. Do not feed fish in quarantine tanks. Fish should be checked immediately prior to stocking or dispatch to ensure they are disease-free.

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.

4.5 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.

4.6 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 4.1.

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.

4.7 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.

5.2 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.

5.3 Registered or permitted anaesthetics

5.3.1 Benzocaine (Ethyl-p-amino benzoate)

Benzocaine is probably the most commonly used anaesthetic in fisheries research applications as it is very 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.

Usually applied during egg and milt stripping and for transportation purposes. Clearance rates are rapid, as is the animal's recovery to normal behaviour or response to stimuli.

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**.

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/mL)	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 Rates) for Benzocaine

Handling (Sedation)	20-35 mg/litre Immersion Bath
Surgery (Anaesthesia)	50-75 mg/litre Immersion Bath
Euthanasia	100 or > 100 mg/litre 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 (but there is a 500 degree day withholding period—see APVMA permit PER11262). For research use (not involving human consumption—see above) no withholding period is required and off-label use is allowed.

5.3.2 *Tricaine methanesulphonate (MS222)*

This is a commonly used anaesthetic, however it is relatively expensive. It is available in powder form. It is very unstable in sunlight and should be stored in sealed, light proof containers.

MS222 causes marked brachycardia, associated with low rates of opercular movement. Forced irrigation of the gills is thus useful during prolonged anaesthesia.

Used in stripping eggs at low dosages, light anaesthesia and euthanasia but the margin between anaesthesia and acutely toxic concentrations is small. It has a low persistence in the aquatic environment.

Administration and preparation of MS222

MS 222 powder is very soluble in water and may be added directly to anaesthetic baths.

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**.

5.3.3 *Aqui-S®*

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.

5.4 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	Not Recommended

[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.

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.

5.5 Euthanasia of fish

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.

5.6 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 poikilotherms, their core body temperature varies, often matching the ambient temperature of the water around them. They do not direct a large metabolic effort to keeping warm. Fish are also 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 PRIMARY INDUSTRIES (Fisheries) ACEC that may be helpful.

5.7 Methods of Euthanasia

5.7.1 Chemical euthanasia (most commonly anaesthetic overdose)

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

ii At present the most appropriate agent is benzocaine, administered at an immersion dose rate of greater than or equal to 100 mg/litre. This is a guide as the dose may vary between species. Fish should be left in solution for ten minutes after opercular movement (respiration) has stopped. If necessary, as long as consciousness is lost, death can be confirmed by physical means such as pithing/spiking/iki-jimi/cervical dislocation.

iii MS222 has been used in the past at an immersion dose rate of greater than or equal to 100mg/litre but is difficult to obtain and not in common usage.

iv Aqui-S, a popular sedative/anaesthetic agent, may not always be suitable on its own for euthanasia. Although it is considered to be suitable for euthanasia of non-salmonid fish when used at the rate of 100 mg/L, it is commonly used to sedate fish prior to physical euthanasia methods (AGDAFF, 2009)..

v The intraperitoneal injection of pentobarbitone (a barbiturate) is used but generally requires removal of fish from the water, or restraint in say a cradle in situ. Its use may present operator safety issues and requires appropriate disposal methods of euthanised fish. A suitable dose rate may be greater than or equal to 60 mg per kilogram animal bodyweight. The Committee does not recommend its use at this stage.

5.7.2 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.

5.7.3 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, and 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. Many researchers have found it to be a humane method of destruction. 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 zero degrees Celsius. A salt water slurry should be minus four degrees Celsius. 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, 2004).

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 Department of Primary Industries 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 Department of Primary Industries 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.

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.

NSW Department of Primary Industries research staff conduct electrofishing following the guidelines outlined in the Australian Code of Electrofishing Practice[1], which states in Section 5.5, Care of Fish:

‘Only the minimum power necessary to attract and stun the fish effectively should be used. Contact of fish with live anodes should be avoided, as the resulting shock will be much greater. If threatened species that are not being targeted are affected, appropriate measures must be taken to minimise disturbance and stress to these fish. Commercial operations must also take action to minimise harm to any species that they are not authorised to catch under the conditions of their licence.’

Also, Section 5.6 of the Code, Care of Other Fauna, requires that:

i ‘Electrofishing must be halted within 15 m of any animals standing in or about to drink from the water, or in contact with a wire fence line that enters the water.
ii The utmost care possible should be taken to avoid shocking platypus, birds and other native aquatic animals.’

[1]Australian Code of Electrofishing Practice, 1997. NSW Fisheries Management Publication No. 1

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. The following handling techniques for sampling and returning animals are taken directly from ACP (NHMRC, 2004).

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 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.

vi Release should be at the site of capture, unless an alternative site is justified in the project proposal.

vii 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.

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.

<u>T-Bar Tags</u>

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 into the fin spines 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.

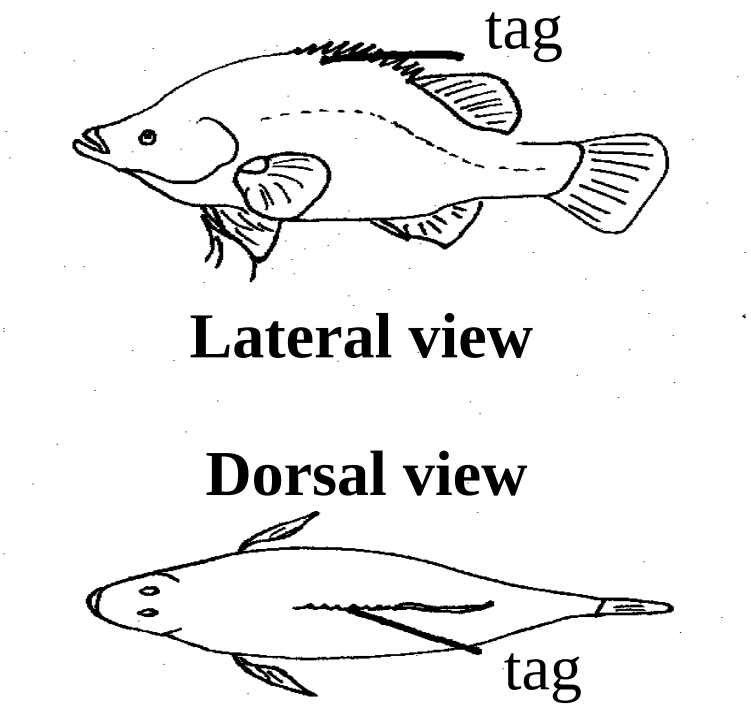

Figure 1 Correct location of dorsal tag placement (Supplied by Dr Ivor Stuart, Kingfisher Research). On the lateral view, the tag should not be implanted further than 5mm from the top of the fish to avoid interfering with the lateral line (sensory organ). Note the dorsal view demonstrates that the tag should not protrude from the fish at an angle less 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 (11mm) or half duplex (23mm) 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 (This reduced the possibility of obtaining duplicate numbers). Tags are either encapsulated in glass (traditional) or plastic (food safe). To reduce the risk of injury if a tagged fish is consumed, any tagging undertaken in NSW now must use food-safe tags as standard practice.

PIT tags are inserted by using a special applicator needle. Applicators are usually specific to the type of tag being used (i.e. Full or half duplex) and manufacturers generally provide applicators for their tags. Tags can be planted in either the peritoneal (gut) cavity or into the dorsal musculature. Implantation into the gut cavity can be problematic as it can damage internal organs. Unless staff are specifically trained in gut cavity tagging, implantation into the dorsal musculature will give best results. Tags should be implanted into the shoulder region of the fish, as close to the head as possible (to minimise risk of ingestion if the fish is subsequently recaptured and filleted). The needle should firstly be used to remove scales in the insertion area and then inserted to a depth no more than 10mm. The tag is then inserted as close to the interface between the skin and muscle as possible (i.e. very shallow). This is necessary because regular muscular contractions are known to dislodge tags in some species. 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 5 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. 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 (e.g. following electrofishing) or euthanased. Any other samples should only be collected from euthanased fish.

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 1). 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 5.5).

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 2) 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. .

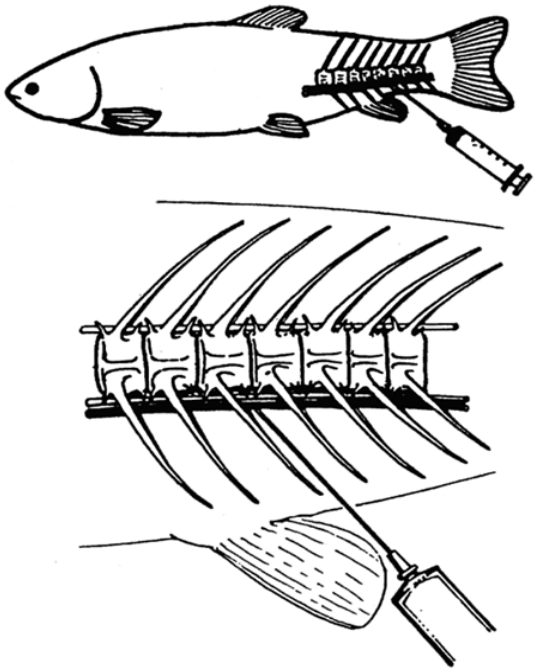

Figure 1 Site for blood collection using needle and syringe.
(http://www.fao.org/docrep/field/003/AC160E/AC160E09.htm)

Figure 2 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 (by-catch drowning)

This Committee asks researchers to report, in their applications, 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 loss which the following material addresses. We are indebted to Dr Tom Grant of the University of NSW for the following information. Dr Grant has worked in the field of platypus ecology for thirty years.

Platypus

- Platypuses do not have long term diving adaptations. They may die within a few minutes of oxygen deprivation.
- Platypuses have little subcutaneous fat insulation and can rapidly become hypothermic.
- Spurring mortalities may occur in confined animals. 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. It is notable though that in some streams they are often seen in the middle of the day.
- Platypuses normally avoid an area of disturbance/human activity, however Dr Grant has also observed 'inquisitive' individuals.

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 locally very significant. Electrofishing, using the following recommendations, would be the preferred sampling method, when appropriate to the experimental design and purpose.

Electrofishing

- There is no published work on the effects of electrofishing on the electroreceptive cells in the platypus bill, however the electric fields of the strength used may have the potential to cause damage. Dr Grant observed a backpack stunned platypus to recover consciousness within a minute and to be active within a few minutes more.
- Where possible electrofishing should be conducted during daylight hours (which is normally the case), 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 to be diurnally active.

Fyke Nets

- Nets should be checked several times during an 18-24 hour period.
- Greater emphasis should be placed on checking nets at night, and in winter conditions especially in highland areas.

Gill Nets

- Use unweighted nets. It is not possible to monitor nets which follow the bottom and have no surface line.

- Use frequent observation and lifting of nets. When Dr Grant is using unweighted gill nets to capture platypuses the nets are checked every 10 to 15 minutes with a spotlight, and are lifted every 1 to 2 hours to remove snags, turtles or fish.
- Do not use sinking nets(nets with no floating top line).

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 his 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 wrapped in a thick material such as a hessian bag.

Conclusion

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

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, 2004).

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, 2004).

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 I&I NSW Primary Industries (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 exotic / 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. It is currently legal under NSW legislation to immediately release any captured fish – whether native or exotic – back into the water from which they were captured. However, I&I NSW Primary Industries encourages euthanasia of captured exotic fish (in accordance with the guidelines in section 5 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 I&I NSW is required to release any live fish into natural waters.

Any proposal to keep or culture an exotic fish 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

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9. ACKNOWLEDGMENTS

We would like to thank some immediate past members of the Primary Industries (Fisheries) Animal Care and Ethics Committee: Kevin Taplin, Kevin Burke and Daniel Large who had long and valued input to the Committee. As well, to Jo Pickles who is a tireless fisheries management officer, a special thanks, and also to Helena Heasman and Sarah-Jane Day.

We also wish to thank Dr Tom Grant of the University of NSW for the information on by-catch drowning, and managing platypus in particular.

We would like to acknowledge the contributions from departmental staff, namely;
Dr Lee Baumgartner, Mr Jeffrey Go, Mr Lee Cook and Ms Rebecca Chapman for providing significant input in the revision of this 3rd edition.

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

10. APPENDICES

10.1 Primary Industries (Fisheries) Animal Care and Ethics Committee members - 2009

The current members of the Primary Industires (Fisheries) Animal Care and Ethics Committee are:

Name	***Category***
Dr Geoff Allan (Primary Industries), Chair	Animal Research
Mr Tony Gregory	Animal Welfare
Mr John Kennedy	Veterinarian
Ms Sherry Mead	Community
Mr Joe Pera (Sydney Water)	Animal Research
Ms Jo Pickles (Primary Industries), Executive Officer	Not applicable
Mr Vinod Reddy	Community
Dr Peter Scanes (DECCW)	Animal Research
Dr Malcolm Smeal	Veterinarian
Ms Eliza Walker	Animal Welfare

10.2 Example of a completed application for an animal research authority

ACEC Ref:	03/09

Project Title:	Impacts of electrofishing on larval native fish

Principal Investigator:	Dr Lee Baumgartner

NEW APPLICATION for an Animal Research Authority
[Animal Research Act 1985 and Regulation 1995]
This form also needs to be completed by applicants for major three yearly renewal

Please note

1 Submit this proposal with a copy of your research protocol/preschedule.

2 Append any other information that you think the ACEC should consider with your proposal.

3 To reduce the risk of delayed approvals, send protocols to reach the Executive Officer at least two (2) weeks before the scheduled ACEC meeting at which you want your proposal considered. This will allow distribution to members and discussion of clarifications/modifications with you before the meeting. If further clarification is needed you may be asked to attend the meeting. ACEC meetings will normally be held in March, June, September and December.

4 Send to: The Executive Officer
Animal Care and Ethics Committee
Port Stephens Fisheries Institute
Taylors Beach Road
TAYLORS BEACH NSW 2316
Tel: (02) 4982 1232
Fax: (02) 4982 1107

GUIDELINES FOR APPLICATIONS TO ANIMAL CARE AND ETHICS COMMITTEE
For **NEW** applications the ACEC requires clear justification that the work needs to be done. The Committee also requires that the researchers propose to use the minimum number of animals to produce a scientifically satisfactory result.
For **RENEWALS** the ACEC requires evidence that management can adequately service the research with adequate housing, feed and staffing.

Office Use:
Received: Considered: Amended:
Approved: Authority Issued:

1 **Expected Date of Commencement:** Oct 03 **Expected Date of Completion:** Ongoing

2 **Names and Qualifications**

2a **Principal Investigator:** Dr Lee Baumgartner
Qualifications: BSc (Biological Science), BSc Hons (Aquatic Science) PhD
Address: Narrandera Fisheries Centre, Buckingbong Rd, Narrandera, 2700
Telephone: 02 6959 9021
Facsimile: 02 6959 2935

2b **Other Investigators and Animal Care Staff:**

Names:	Qualifications:	Experience:
Dr lee Baumgartner	Bsc PhD 9	11 years
Mr Tim MCGarry	Bsc	2 years
Mr Ian Wooden	Bsc	17 years
Mr Martin Asmus	Bsc	12 years
Mr Jamie Hutchison	Bsc	2 years
Mr Mick Bettanin	Bsc	6 years
Mr Jarrod McPherson	Bsc	3 years
Ms Prue McGuffie	Bsc	2 years
Ms Frances Cory	Bsc	2 years
Mr Garry McLean	Bsc	2 years
Mr Lachie Jess	Bsc	4 year

2c **Other Research Collaborators (outside NSW DPI):** No
If Yes, please provide details and evidence of their animal research authority for their component of the research if it is not covered by this application:

3 **Please explain, in simple terms that will be easily understood by people without scientific training, the following**

3a **Briefly describe the background and justification to your proposed research** (list 1 or 2 key relevant published papers if appropriate) (250 words max):
Electrofishing is a major sampling tool used by freshwater researchers worldwide. Both the ACEC committee and national electrofishing working group have expressed an interest in determining the impacts of electrofishing on larval fish. There currently exists an opportunity to assess any potential effects using larval fish produced by the native fish hatchery at the Narrandera Fisheries Centre. Live animals must be used in this type of research because we are specifically aiming to determine the effect of electrofishing on live larval fish.

3b **Objectives of the project:**
i) To determine the effect of electrofishing on the survival of larval fish
ii) To test larval survival over a range of different electrical field strengths

3c **Location of field studies or where research will be conducted:**
Narrandera Fisheries Centre

3d **Research procedures** (include experimental design and statistical analyses to be used):
Initially, the electric field around the vessel will be mapped using a peak field strength meter. This will determine the strength of the electric field at various distances from the boat. A total of 1,000 larvae or juveniles from each of Murray cod, Golden Perch and Silver Perch will be obtained from the hatchery at the Narrandera Fisheries Centre. The larvae will be divided into groups of 25 and placed into 500ml plastic containers that have been perforated to allow an electrical current to pass through.

The containers will be anchored at specific points around the electrofishing vessel that coincide with four different field strengths ('treatments') of 0 - 0.1 Volts per square centimetre, 0.1 – 0.5 V/cm^2, 0.5-1.0 V/cm^2 and 0 V/cm^2 (control). These are the standard ranges used when mapping a field around an electrofishing boat.

A total of 5 replicates (of 25 larvae) will be placed within each field range the electrofisher will be operated for a total of 15 seconds (representing a standard electrofishing 'shot') and the larval fish will be collected. The process will then be repeated using a 30-second electrofishing shot to determine the effects of longer exposure to the electric field.

At the conclusion of each experiment the condition of larvae is recorded. The larvae will then be transferred to larval holding troughs in the Narrandera hatchery and monitored for a period of four days to observe if there are any delayed electrofishing injuries.

The effects of electrofishing will be determined for each species through a two-way ANOVA (with replication) using electrofishing field strength and electrofishing shot time as factors.

3e **Numbers and species of animals to be used (even if only rough estimates are available)** *(You mush answer this question – please use common as well as scientific species names)***:**
1000 larvae of Murray Cod (*Maccullochella peelii*)
1000 larvae of Golden perch (*Macquaria ambigua*)
1000 larvae of Silver perch (*Bidyanus bidyanus)*

500 eggs of Murray cod (*Maccullochella peelii*)

1000 juveniles of Murray Cod (*Maccullochella peelii*)
1000 juveniles of Golden perch (*Macquaria ambigua*)
1000 juveniles of Silver perch (*Bidyanus bidyanus)*

3f **Justification for the use of these numbers:**
These numbers were selected for two reasons-
(i) They will provide enough individuals for valid statistical analysis.
(ii) This is the maximum number that could be obtained from the hatchery

4 **4a** **Has this work been undertaken previously?** Yes
If so, why is it necessary to repeat it? Please provide assigned ACEC reference number(s).
A small component using larval golden perch and Murray cod was previously undertaken but has not been reported. Work on other species is yet to be initiated and has been difficult to undertake due to a lack of funding.

4b **Has this application previously or simultaneously been submitted to this or another ethics committee?** Yes
If so, provide reasons for re-submission or simultaneous submission and the name of the ACEC(s).

The project is due for a three year renewal.

4c **Have any of the people participating in the project had any animal research authority or animal supplier's licence cancelled?** No
If so, provide details include the name of the person, details of the authority and the reason for cancellation..

5 **Identify procedures that may cause pain or distress:**

5a **Procedures:**
Electrofishing may cause undue pain and distress

5b **Justification for these procedures:**
This experiment is necessary to fully determine the extent of pain and distress that electrofishing causes to larval and juvenile fish

5c **What steps will be taken to avoid or minimise this pain or distress?**
One of the major outcomes of this experiment will be to understand how pain and distress whilst undertaking electrofishing operations. This can then be used to develop more appropriate electrofishing practices.

6 **Will any animals be euthanased during or after this research?** No *(Please delete one*)

6a **If Yes, approximately how many animals will be euthanased?**

6b **Drugs or process for euthanasia**

6c **Dose rate (if applicable)**

6d **Routes of administration**

6e **Is death intended to be the specific endpoint of the experimental procedure?** *(ie. do you intend for animals to die to provide results)* No *(Please delete one*)

6f **If death is <u>not</u> the endpoint, what is the planned endpoint?**
The endpoint is loss of equilibrium during electrofishing operations. Once fish have lost equilibrium electrofishing will cease and recovery will be monitored. In wild electrofishing operations, equilibrium is the point where fish are collected by research staff. So exposure beyond loss of equilibrium is not necessary for these experiments.

7 **Are any of the following to be used?** *If so, please give details.*

7a **Surgical procedures:** No
Details: X

Who will be conducting the surgery? X

7b **Anaesthetics:** No
Reason for use: X

Drugs: X **Dose rates:** X

Routes of administration: X

Duration: X

Who will be inducing/monitoring anaesthesia?: X

7c **Analgesics:** No
Reason for use: X

Drugs: X **Dose rates:** X

Routes of administration: X

Duration: X

7d **Neuromuscular blockers:** No
Note: Neuromuscular blockers must not be used without general anaesthesia or surgical procedure to eliminate sensory awareness.
Reason for use: X

Drugs: X **Dose rates:** X

Routes of administration: X

Duration: X

What steps will be taken to avoid and to monitor pain? X

8 **Is blood to be collected as part of your proposal research?** No *(Please delete one)*
If Yes, please provide the following details:

8a **The volume of blood to be collected?**
X

8b **The frequency of blood collection**
X

8c **The period over which blood will be collected**
X

8d **The route by which blood will be collected**
X

8e **The technique by which blood will be collected (including whether fish will be anaesthetised and how they will be restrained)**

8f **The methods of animal monitoring and frequency with which these methods will be implemented**

8g **The experience of the operator relevant to the type of fish to be used and the blood collection procedures to be undertaken**

9 **Have any of these animals been used previously for research?** No *(Please delete one*)
If so, please give details of the animals, the previous work and why they are being used again.

10 **Source of fish:**
The native fish hatchery at the Narrandera Fisheries Centre

11 **Animal transport, housing and nutrition:**
Fish will be transported to the study site via aerated fish boxes containing water from the experimental site. At the conclusion of the experiment, any surviving fish will be housed in flow through troughs in the native fish hatchery that are specifically designed to house larvae for extended periods of time. The troughs are 5m long by 30cm wide with a depth of 20cm. River water will be provided on a continuous flow through and fish will be monitored twice a day. Water quality will be monitored on a daily basis. Fish will be fed sufficient amounts of artificially reared artemia three times a week when held.

12 **Animal care and monitoring including provision of emergency care:**
Fish are retained for the minimum time required to collect data and ensure that they are not adversely affected by the electrofishing treatments before being returned to the hatchery.

13 **Method of disposal of animals at completion of the experiment:**
Any dead animals may be inspected for signs of injury post mortem. Fish that survive will be returned to the hatchery and used in stocking activities.

14 **Are there any aspects of the proposal that raise special ethical considerations?** No *If so, please detail:*
Staff will be using well-established methods to conduct these trials, we merely seek to confirm that current practices are safe for fish.

15	**Does the proposal present a health or mortality risk to any non-target species (including wildlife species) or people?** *If so, please give details and steps taken to reduce these risks:* The use of electricity poses a potential threat to staff members. Staff have been well trained to sufficiently minimise potential risks.	Yes

16	**What will be the maximum time an individual animal is held?** 4 Days to investigate post-experimental injuries. After this time, any surviving fish will be released into hatchery ponds.

Please note that as a condition of this application, you will need to report on any effects of the research on non-target species (including wildlife species). If the fishing method(s) cause the death of any non-target vertebrate, these deaths will need to be reported, giving location (description and grid reference), species (scientific name if possible) and date Noted

Application by each Investigator and Animal Care staff member for an Animal Research Authority

I have read this application, understand the aims of the project and I am aware of the responsibilities imposed on me under the Animal Research Act 1985, the Animal Research Regulation 1995 and the Code of Practice for the Care and Use of Animals for Scientific Purposes.

Name	Work Address	Signature	Date
Dr lee Baumgartner	Narrandera Fisheries Centre		
Mr Tim MCGarry	Narrandera Fisheries Centre		
Mr Ian Wooden	Narrandera Fisheries Centre		
Mr Martin Asmus	Narrandera Fisheries Centre		
Mr Jamie Hutchison	Narrandera Fisheries Centre		
Mr Mick Bettanin	Narrandera Fisheries Centre		
Mr Jarrod McPherson	Narrandera Fisheries Centre		
Ms Prue McGuffie	Narrandera Fisheries Centre		
Ms Frances Cory	Narrandera Fisheries Centre		
Mr Garry McLean	Narrandera Fisheries Centre		
Mr Lachie Jess	Narrandera Fisheries Centre		

Application by Other Research Collaborators (outside NSW DPI)

I have read this application, understand the aims of the project and I am aware of the responsibilities imposed on me under the Animal Research Act 1985, the Animal Research Regulation 1995 and the Code of Practice for the Care and Use of Animals for Scientific Purposes.

Name	Work Address	Signature	Date

Declaration by Principal Investigator

I hereby apply for an Animal Research Authority in respect to the above application. I am aware of the responsibilities imposed on me under the Animal Research Act 1985, the Animal Research Regulation 1995 and the Code of Practice for the Care and Use of Animals for Scientific Purposes. I declare that the qualifications and experience of investigators and other operators involved in this project are appropriate to the procedures to be performed. I undertake to submit annual and final returns stating the number and fate of animals used and the condition of surviving animals and their fate at completion of the work.

Signed:..... Date .....

Approval by 'Research Leader' or Director

Signed:..... Date .....

10.3 Example of an annual renewal for an animal research authority

ACEC Ref:	03/09

Project Title:	Impacts of electrofishing on larval native fish

Principal Investigator:	Dr Lee Baumgartner

Is this application a major three yearly renewal:	Yes *(Please delete one)*
If Yes, you must also complete a 'New Application for an Animal Research Authority'	

Application for **ANNUAL RENEWAL of** Animal Research Authority
[Animal Research Act 1985 and Regulation 1995]

Please note

1 To reduce the risk of delayed approvals, send protocols to reach the Executive Officer at least two (2) weeks before the scheduled ACEC meeting at which you want your proposal considered. This will allow distribution to members and discussion of clarifications/modifications with you before the meeting. If further clarification is needed you may be asked to attend the meeting. ACEC meetings will normally be held in March, June, September and December.

2 Send to: The Executive Officer
Animal Care and Ethics Committee
Port Stephens Fisheries Institute
Taylors Beach Road
TAYLORS BEACH NSW 2316
Tel: (02) 4982 1232
Fax: (02) 4982 1107

GUIDELINES FOR APPLICATIONS TO ANIMAL CARE AND ETHICS COMMITTEE
For **NEW** applications the ACEC requires clear justification that the work needs to be done. The Committee also requires that the researchers propose to use the minimum number of animals to produce a scientifically satisfactory result.
For **RENEWALS** the ACEC requires evidence that management can adequately service the research with adequate housing, feed and staffing.

Office Use:
Received: Considered: Amended:
Approved: Authority Issued:

Date Project Commenced:	Nov 2003	**Expected Date of Completion:**	Ongoing

Please provide a brief summary of the need for the Project: (maximum 100 words)
Should summarise the need described in original application

Electrofishing is a major sampling tool used by freshwater researchers worldwide to monitor and assess freshwater fish assemblages. However, researchers have poorly investigated the long and short-term effects of electrofishing on both target and accidentally caught species. Both the NSW DPI Animal Care and Ethics and national electrofishing working group have expressed an interest in determining the impacts of electrofishing on both Australian native fish and aquatic mammals that may also be affected as by-catch. The project aims to assess any potential effects of electrofishing using larval fish produced by the native fish hatchery at the Narrandera Fisheries Centre and also fish obtained from the Murrumbidgee River. By continuously improving our fishing methods, we will subsequently improve the quality of field data that is collected whilst reducing any potential impacts on target and non-target species. Therefore, the project aims to improve existing sampling techniques so that NSW DPI freshwater research staff remain at the forefront of such technology.

Please provide a brief summary of results, conclusions and implications of the research to date: (maximum 200 words)

Work undertaken in 2003/04 determined that boat electrofishing has no impact on Murray cod larvae. Experiments were conducted in a larval rearing pond at the Narrandera Fisheries Centre. Murray cod larvae, Murray cod eggs and juvenile Golden perch were subjected to electric fields of 0-0.1 Volts per square centimetre, 0.1–0.5 V/cm^2, 0.5-1.0 V/cm^2 and 0 V/cm^2 (control). No Murray cod larvae died as a result of exposure to field of this magnitude. Although some Murray cod eggs did not hatch, there was no difference between the hatching rates of treatment groups or control groups. In addition, no golden perch died as a result of the electrofishing. It is important to note that all golden perch were fully immobilised by the electrofisher and all of them recovered.

No work was undertaken in 2008/09 as no funding was available to continue the assessments. Researchers plan to prepare a report on the initial results as these have implications for monitoring programs.

Titles of relevant publications:
Please provide a brief list of publications that are relevant to this research or may assist the committee for further information.

No publications have been produced as part of this project.

Please indicate any minor changes to the original methods, eg. differences in collection methods, changes in animal care staff etc *(You only need to complete this section if this is <u>not</u> a major three yearly renewal, because if it is, you need to fill in a new application form as well as this Annual Renewal form)*

No changes in methods are proposed and it is uncertain whether any further work will be completed this year.

Please report any wildlife species (eg. platypus, water rats, birds, turtles etc) affected (eg. stunned, captured, released, drowned etc) by the fishing methods used during this study.
Please provide a brief report detailing location (description and grid reference), effect, species (scientific name if possible) and date:

N/A, no work was undertaken this year.

Animal Use in Proposal

Species *(Use common & scientific names)*	Requested last year	Requested this year	Actually used last year	Unintentional mortality[1]	Euthanased[2] last year	Total[3] last year
Murray cod *Maccullochella peelii* (larvae)	1500	1000	0	0	0	0
Murray cod *Maccullochella peelii* (eggs)	500	500	0	0	0	0
Murray cod *Maccullochella peelii* (Juveniles)	0	1000	0	0	0	0
Silver perch *Bidyanus bidyanus* (larvae)	1000	1000	0	0	0	0
Silver perch Bidyanus bidyanus (juveniles)	1500	1000	0	0	0	0
Golden perch *Macquaria ambigua* (larvae)	1000	1000	0	0	0	0
Golden perch Macquaria ambigua (juveniles)	1500	1000	0	0	0	0

[1] Died as an unintentional result of research – if numbers are 'significant' please explain why and indicate what steps are being taken to minimise such losses in the future

[2] Deliberately euthanased (eg. for samples or analyses)

[3] Total of animals that died (ie. [1] plus [2])

Last year – period covered by your existing authority
This year – period for the next 12 months

Renewal Application by each Investigator and Animal Care staff member for an Animal Research Authority

I have read this renewal application, understand the aims of the project and I am aware of the responsibilities imposed on me under the Animal Research Act 1985 and the Animal Research Regulation 1990 including the Code of Practice.

Name	Work Address	Signature	Date
Dr lee Baumgartner	Narrandera Fisheries Centre		
Mr Tim MCGarry	Narrandera Fisheries Centre		
Mr Ian Wooden	Narrandera Fisheries Centre		
Mr Martin Asmus	Narrandera Fisheries Centre		
Mr Jamie Hutchison	Narrandera Fisheries Centre		
Mr Mick Bettanin	Narrandera Fisheries Centre		
Mr Jarrod McPherson	Narrandera Fisheries Centre		
Ms Prue McGuffie	Narrandera Fisheries Centre		
Ms Frances Cory	Narrandera Fisheries Centre		
Mr Garry McLean	Narrandera Fisheries Centre		
Mr Lachie Jess	Narrandera Fisheries Centre		

Renewal Application by Other Research Collaborators (outside NSW DPI)

I have read this renewal application, understand the aims of the project and I am aware of the responsibilities imposed on me under the Animal Research Act 1985 and the Animal Research Regulation 1990 including the Code of Practice.

Name	Work Address	Signature	Date

Declaration by Principal Investigator

I hereby apply for a Renewal for another year of an Animal Research Authority in respect to the above application. I am aware of the responsibilities imposed on me under the Animal Research Act 1985 and the Animal Research Regulation 1990 including the Code of Practice. I declare that the qualifications and experience of investigators and other operators involved in this project are appropriate to the procedures to be performed. I undertake to submit annual and final returns stating the number and fate of animals used and the condition of surviving animals and their fate at completion of the work. I certify that the aims and objectives of the project are unchanged, the source, housing, diet and health care of the animals is unchanged, the procedures to be undertaken on the animals are unchanged, the ultimate fate of the animal is unchanged. I have reported and will continue to report significant deteriorations in the animals' health and welfare to the Animal Care and Ethics Committee. The numbers and fates of animals used in the past year and proposed for the next year are detailed on Page 3.

Footnote: If you are unable to certify all of the above please delete the relevant clause(s) and attach an explanation.

Signed:..... Date .....

10.4 Example of an animal research authority certificate

Names of Applicants:	Location of Research:	Conditions of Authority:
STEWART FIELDER LUKE CHEVIOT TBA	Freshwater and marine finfish will be sampled from a range of riverine and impoundment systems throughout NSW. Australian bass will be sampled from Northern, Central and Southern zones of the population.	That all blood collected by non-DPI staff is from previously euthanased fish.

are authorised by

Industry & Investment NSW (Primary Industries)

to conduct the following type of research

SURVEY FOR THE PRESENCE OF NODAVIRUS IN WILD FINFISH POPULATIONS IN NEW SOUTH WALES
ACEC REF 07/02 – PSFC

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

PRIMARY INDUSTRIES (FISHERIES)
ANIMAL CARE AND ETHICS COMMITTEE

This authority remains in force from

10 JUNE 2009 to 10 JUNE 2010

unless suspended, cancelled or surrendered.
(Major three yearly review due in 2010)

JO PICKLES
EXECUTIVE OFFICER

GEOFF ALLAN
CHAIR

23 June 2009

10.5 Example of an animal research authority final report

FINAL REPORT: ACEC Proposal 93/4 – Port Stephens
PRINCIPAL INVESTIGATOR: Geoff Allan
TITLE: Silver perch digestibility studies

Aims of project
To investigate the potential for increasing marketing opportunities for Australian oilseeds, grain legumes and other agricultural products by using them to partially or totally replace fish meal and other protein sources in aquaculture diets. Specifically, this will involve the measurement of digestibility coefficients for oilseeds, grain legumes and other promising protein sources. Secondly, the evaluation of methods of increasing the digestibility of alternative protein sources to fishmeal will be investigated. This data will be used to design and formulate aquaculture diets for silver perch in which some or all of the fishmeal can be replaced.

Number of animals used – approximately 13 500
Digestibility experiments were conducted in a laboratory that housed 27 digestibility and 9 spare tanks. Generally, for each experiment between 10 –15 juvenile silver perch were stocked into each tank (36 X 15 = 540 fish/exp). Since the inception of this proposal, approximately 25 digestibility experiments have been performed, accounting for approximately 13 500 animals.

Fate of animals used
The condition of fish in each experiment was closely monitored. Survival, weight gain and feed intake were also assessed in addition to digestibility as a measure of a diets nutritional adequacy. Fish which became moribund during experiments were removed as soon as they were identified and immediately killed by administering an overdose (>60 mg L^{-1}) of ethyl-p-aminobenzoate (benzocaine) in a freshwater bath. A representative sample of 10-15 fish from the stocked population was killed at the beginning and end of each experiment for subsequent whole carcass composition if required.

Condition of surviving animals
Surviving animals were returned to the holding facilities at PSFC and kept separate from the unused population of fish. After a short period of observation, these fish were transported and released into enclosed freshwater dams (e.g. Tocal Agricultural College).

Outcome of study
The partial or full replacement of fishmeal in aquaculture diets remains a priority for the majority of aquaculture species in Australia and throughout the world. We have evaluated the digestibility of over 60 different ingredients with potential for use in silver perch aquaculture diets. Some of the ingredients with the most potential include lupins, field peas and other grain legumes, oilseeds such as peanut meal, cereal grains such as wheat and several rendered animal meals. We have also investigated and developed cost effective methods of improving the digestibility of some ingredients through additional processing such as dehulling, protein concentration, steam conditioning and extrusion. These digestibility coefficients have been fundamental in formulating diets for silver perch which contain little if any fishmeal and which have reduced the costs of feeding. The outcomes of this research has been extensively reported in target scientific journals such as Aquaculture and Aquaculture Research as well as in non-scientific publications and newsletters such as NSW Fisheries Magazine, Aquafeed International Magazine and final reports to funding agencies such as the Fisheries Research & Development Corporation.

10.6 Primary Industries (Fisheries) ACEC Terms of Reference

The Primary Industries (Fisheries) Animal Care and Ethics Committee (ACEC) is responsible for ensuring that all animal care and use for research and education by authority holders and supply of animals by animal suppliers within I&I NSW and NSW Livestock Health and Pest Authorities (LHPAs) complies with the *Animal Research Act 1985* and the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NHMRC, 2004).

Responsibilities

ACECs are responsible for:

(i) approving guidelines for the care of animals that are bred, held and used for scientific purposes on behalf of I&I NSW and LHPAs;

(ii) monitoring the acquisition, transportation, production, housing, care, use and fate of animals;

(iii) recommending to I&I NSW and LHPAs, any measures needed to ensure that the standards of the Code are maintained;

(iv) examining and approving, approving subject to modification, or rejecting written proposals relevant to the use of animals for scientific purposes. The Committee may approve only those studies for which animals are essential and justified and which conform to the requirements of the Code, taking into consideration factors including ethics, the impact on the animal(s) and the anticipated scientific or educational value;

(v) withdrawing approval for any project or authorising the treatment or euthanasia of any animal;

(vi) examining and commenting on all plans and policies of and LHPAs which may affect the welfare of animals used for scientific purposes;

(vii) maintaining a record of proposals, projects, minutes of meetings and interim and final reports;

(viii) complying with the reporting requirements of I&I NSW and LHPAs and the Code (as specified in clause 2.2.40).

Membership

- The Director, Research Operations of I&I NSW Primary Industries will appoint people to the ACEC, at least one and preferably two in each of the following categories.

Category A A person with qualifications in veterinary science or with similar expertise.

Category B An animal researcher or person with substantial recent experience in the use of animals in scientific or teaching activities.

Category C A person with demonstrable commitment to, and established experience in, furthering the welfare of animals and who is not involved in the care and use of animals for scientific purposes. This person should be selected on the basis of active membership of, and nomination by, an animal welfare organisation.

Category D An independent person who does not currently and has not previously conducted scientific or teaching activities using animals.

- Before appointment, all members of the ACEC should acknowledge in writing their acceptance of the terms of reference and operating procedures of the Committee and the provisions for confidentiality outlined in section 56 of the *Animal Research Act.*
- Terms of appointment are normally for four years after which time positions in categories A, C and D will be advertised. Incumbents are eligible to reapply.

10.7 Primary Industries (Fisheries) ACEC Grievance Procedures

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

Procedures have been developed to handle the following:

a) complaints from independent people who are not associated with research;

b) complaints by staff of Primary Industries or NSW Rural Lands Protection Boards;

c) disputes between investigators and Primary Industries ACECs;

d) serious disagreements between members of an ACEC; and

e) disagreements between an ACEC and management of I&I NSW Primary Industries institutes and research stations.

Types a) and b) are to be referred to the relevant ACEC 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 Operations 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 ACEC who is obliged to raise the matter with the ACEC as soon as possible to allow the complaint to be resolved quickly.

Types c), d) and e) may need referral to the Director, Research Operations, I&I NSW Primary Industries. 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 DRO. The DRO may investigate procedural matters, may request the ACEC to re-examine the matter or refer new information to the ACEC for consideration but may not overrule a decision of the ACEC made on the basis of correct procedure in compliance with the legislation.

Where necessary, the DRO may request the ACEC to seek further technical advice or may appoint an independent mediator to assist resolution of such matters.

All complaints are to be treated confidentially.

10.8 Primary Industries (Fisheries) ACEC Terms of Appointment

The following Terms of Appointment have been prepared for the Primary Industries (Fisheries) ACEC:

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

2 Nominations for vacant positions will be advertised through Primary Industries 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 in the Australian and New Zealand Association of Animal Societies Newsletter (or other appropriate publication). Positions for Community representatives will be advertised in the local newspaper serving the Cronulla area (St George and Sutherland Shire Leader).

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

4 Animal researcher categories filled by nomination by Primary Industries Executive for period of three years.

5 Chair position should be for a period of four years.

6 Chair position may be filled by representative for any category provided the nomination is endorsed by Primary Industries Executive.

7 The final approval of the appointment of all positions is made by the Animal Research Review Panel. This approval is based on a recommendation from the Chairperson of the ACEC and if appropriate the Director, Research Operations of I&I NSW Primary Industries after the submission of a detailed curriculum vitae especially on relevant experience of the candidate.

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