ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

Working with Fish

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and approved by the University of Melbourne's AECs.

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1. ASSOCIATED STANDARDS

There are currently no associated Standards.

However, work with other aquatic species, especially cephalopods, may require additional special requirements or considerations.

2. SUMMARY

2.1 Research on fish may be conducted in their natural environment as field work, or in a captive setting using purpose bred animals or wild-caught specimens. Fish that are collected, handled and released in the field require careful and considerate handling to limit the impacts of temporary capture and even greater care if they are being transported from their field site to a captive environment.

2.2 Specimens that are acquired from the wild for captivity face challenges associated with the stress of capture and transport, dietary transition to unfamiliar items and must rapidly adapt to an artificial environment. These events must be carefully managed to ensure there is minimal impact on their welfare and that stressors are minimized.

2.3 Many aquaculture-bred specimens face less stress associated with a tank environment, but transport and handling stress must still be minimised.

2.4 Fish maintained in captivity must be provided with appropriate, species-specific care and husbandry. Water quality is paramount to these animals and water quality parameters cannot be considered in isolation due to the interplay between water quality variables.

3. BENEFITS & RISKS

3.1 Field studies of fish allow investigators to view their role in their aquatic ecosystems and obtain information on their biology, diet, behaviour, breeding, social structure interaction with other species and longevity. This information may be used to improve the welfare of current or future captive colonies and to facilitate aquaculture, species management and conservation programs.

3.2 Maintaining fish for investigation allows comprehensive and long-term studies to be conducted, further increasing the available knowledge. Where possible, fish used for experimentation should be bred in captivity, thus reducing pressures placed on wild fish stock and fish health.

3.3 When in captivity, it is the investigator's responsibility to ensure fish are suitably housed, fed an appropriate, nutritious diet and provided with everything they need to thrive. Water quality testing should be routinely undertaken to ensure suitable water quality parameters are maintained at optimal levels. Substandard water quality or inadequate husbandry practices can lead to very serious health and animal welfare consequences for these animals with the potential consequence of death of a large number of fish in a short amount of time. This also includes the need to equip life support systems with an alarm system, wherever practical, to ensure staff can respond to emergency events.

4. PROCEDURE/PROTOCOL

4.1 Considerations for collection of fish

4.1.1 There are a minimum number of people to be present for all field based work as per the University of Melbourne fieldwork guidelines (http://safety.unimelb.edu.au). Required permits, notifications, certifications and other associated paperwork must be completed prior to undertaking collection of fish.

4.1.1.1 If the target species is venomous, poisonous or otherwise dangerous then investigators must consult with their faculty and University of Melbourne EH&S prior to undertaking field work. It is suggested that only personnel who have undertaken appropriate training in the handling of the target venomous, poisonous and dangerous species be permitted to catch them, and that an advanced certified first-aid officer (not participating) be present for the duration of the field trip with a first aid kit and oxygen kit (see 4.1.1).

4.1.2 Investigators should aim to conserve and protect the habitat they are working in by keeping disturbance of field sites to a minimum.

4.1.3 Where animals are to be removed from the wild, only the minimum number of animals required to produce scientifically valid results should be collected.

4.1.4 Special concern should be shown for species known to remain with their offspring or young during certain seasons (i.e. spawning season). Removal of individuals known to do this should be avoided during those seasons, unless it is strongly justified by scientific reasons.

4.1.5 The investigator must be knowledgeable of all regulations pertaining to the animals under study, and must obtain all necessary federal, state, and local permits for the proposed studies. Investigators working outside of Australia should ensure they comply with all wildlife regulations of the country in which the research is being performed. This includes compliance with the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) regulations where applicable.

4.1.6 Investigators should be aware of the potential zoonotic diseases associated with fish and EH&S.

4.1.7 Any bycatch of fish during collection must be handled as per laws, permits, animal welfare codes and any other legislation requirements. Researchers should be aware of these prior to the collection of any fish and make provisions for any bycatch, including treatment of any introduced species.

4.2 Methods of capture

4.2.1 *Trapping*

4.2.1.1 Traps should be selected to safely accommodate the target species and/or possible bycatch, and ideally be based on techniques from previous, relevant technical reports and/or publications. It must be of adequate dimensions to ensure fish can fit comfortably inside, and provide protection from the elements and predators once an animal is caught.

4.2.1.2 The interval between checks may be variable depending on species (including the target species or expected by-catch), weather, study objectives and type of trap. Traps of all varieties must be checked at least once daily, or more frequently in extreme weather conditions (heat or drought).

4.2.1.3 Traps should be removed when not in use between sampling periods and removed at the conclusion of the research. Where appropriate, nets should be set to allow a portion to remain out of the water to avoid inadvertent drowning of any non-target species (e.g. frogs).

4.2.2 *Direct capture methods*

4.2.2.1 Investigators should select a method of direct capture (e.g. hand netting or electro-fishing) that is appropriate to the size, type and environment of their target species. The capture technique

should pose the least amount of risk and distress to the animals and the safety of investigators.

4.3 Handling, grading and restraint

4.3.1 Investigators have a responsibility to determine and use the least amount of restraint necessary to perform their procedure in a humane manner, with minimal distress or suffering caused to the animal. In some cases, this may include the use of sedative or anaesthetic agents, especially for species which are venomous or capable of inflicting serious injuries on themselves or those handling them.

4.3.2 Fish should be fasted for an appropriate amount of time to prevent regurgitation or high amounts of waste production and provided with high water quality post restraint or handling.

4.3.3 Fish should be handled gently and as quickly as possible in order to minimise stress and damage to scales and slime coat.

4.3.4 Avoid handling fish in brightly lit areas or in direct sunlight. Where applicable shade can be provided which also elicits a calming effect in fish.

4.3.5 Fish should not be handled continuously out of the water for more than 30 seconds.

4.3.6 Handling, grading and restraint of fish can be performed under sedation with an aquatic animal anaesthetic (AQUI-S®) with the fewest possible number of fish selected for handling and restraint.

4.3.7 When handling fish there is the potential for personnel to be injured (i.e. bitten, stabbed). Some species of fish are venomous or poisonous and any wounds or even open skin contact may have potentially fatal consequences. Investigators should be familiar with the defensive strategies of their target species and others non-target species (bycatch) that may be encountered in the field.

4.3.8 Maintaining a barrier between the researcher and the animal may help to reduce the risk of injury. The use of traps that allow the animal to be visible to researcher prior to opening it is encouraged. For potentially hazardous fish, such as sharks or venomous species, chemical restraint is strongly advised and may be required.

4.3.9 Nitrile gloves should always be worn where practical to prevent damage to the delicate slime coat of fish. All equipment used should be smooth and prevent potential damage to the scales and slime coat of fish. This includes the use of sanitized non-knotted (smooth) nets, fish chutes, fish grading equipment, fish cradles or fish pumps.

4.3.10 Fish handlers should also be aware of the potential zoonotic diseases that can be transmitted from fish to humans and vice versa (i.e. *Mycobacterium, Erysipelothrix, various fungal infections*).

4.4 Procedures

4.4.1 Injections

Investigators must be familiar with the anatomy and vasculature of their target species prior to attempting injections. Injections should always be administered between the scales. Sites for injection vary depending on the location and species of fish. Listed below are general guidelines for injections in fish; however, due to species-specific variations, different approaches may be required. Aquatic veterinary, fish expert, or Animal Welfare Officer (AWO) advice should be sought in such cases.

4.4.2 Intramuscular injection

4.4.2.1 Intramuscular injection may be administered between the scales into the large dorsal epaxial and abdominal muscles taking care to not inject into the lateral line and ventral blood vessels.

4.4.3 Intracoelomic (Intraperitoneal) injections

4.4.3.1 Intracoelomic injections may be made into the coelomic cavity between the scales and taking care to avoid penetration of coelomic viscera.

4.4.3.2 Substances known to cause inflammation and irritation should not be injected into the coelomic cavity as they may cause intracoelomic adhesions.

4.4.4 Teleost (bony) fish blood sampling

4.4.4.1 The majority of healthy fish greater than 7.5cm in length can sustain the removal of 1ml/1kg of blood volume from their circulatory system. Volumes greater than 1ml/kg for non-lethal venipuncture sampling must be justified.

4.4.4.2 Haematocrit recovery of fish is temperature dependent and highly variable between species.

4.4.4.3 Typically blood circulating volume of healthy fish is 5% (compared to 10% in mammals). However due to their physiology they can sustain greater levels of total blood volume collection of up to 30% (compared to 10% in mammals).

4.4.4.4 The preferred site of non-lethal blood sampling in fish is the caudal vertebral vein or artery which is located along the ventral midline of the tail via either a ventral or lateral approach.

4.4.4.5 Needle gauge size varies with the size of the fish. The following guideline can be used: 22-26G can be used in smaller fish, 20-25G for medium-sized fish, and 18-22G for larger fish.

4.4.4.6 Care should be taken to apply pressure at the site of any venipuncture for 30-60 seconds after needle removal to give the blood a chance to clot at the site of collection. If bleeding occurs from the site after this time, gentle pressure should be applied until haemostasis can occur and no blood is noted.

4.4.4.7 Fish should be sedated during these procedures and gloves should be worn to prevent damage to both scales and the slime coat.

4.4.4.8 Ventral approach - Restrain the animal on its side on an appropriate surface (clean and smooth) or in its back in a fish cradle. Introduce the needle between the scales along the ventral midline near the base of the caudal peduncle and advance towards the ventral vertebrae. Once the needle has contacted the vertebral body, slightly withdraw the needle, and withdraw the plunger slightly until blood enters the hub. Slowly draw the sample out, allowing breaks in the suction for the vessel to refill. It may be necessary to slowly and gently rotate the needle and syringe if blood flow has ceased. Once the sample is acquired, remove the needle and place gentle pressure on the site for 30-60 seconds.

4.4.4.9 Lateral approach - Restrain the animal on its side on an appropriate surface (clean and smooth). Introduce the needle between the scales along the lateral aspect a few millimetres below the lateral line near the base of the caudal peduncle. Direct the needle towards the midline slightly below the ventral and gently withdraw the plunger slightly until blood enters the hub. Slowly draw the sample out, allowing breaks in the suction for the vessel to refill. It may be necessary to slowly and gently rotate the needle and syringe if blood flow has ceased. Once the sample has been collected, remove the needle and place gentle pressure on the site for 30-60 seconds.

4.4.4.10 For very small fish, a tail snip can be taken with blood collected in capillary tubes.

4.4.4.11 Blood collected from most species of fish should be immediately stored at 4°C to prevent deterioration. Depending on the parameters to be sampled and the species of fish the choice of anticoagulant may vary. Investigators should be aware of the ideal anti-coagulant agents and storage temperature required for their respective species of fish.

4.4.4.12 Large teleost fish may need to be sampled differently and investigators must demonstrate familiarity with venipuncture techniques, anatomy and vasculature of their target species prior to attempting blood collection. There are also EH&S implications with these types of fish that must be addressed by the investigators and their respective department(s).

4.4.5 *Non-Teleost fish and other species*

4.4.5.1 The preferred site of non-lethal blood sampling in non-teleost fish can vary. It is recommended to contact an aquatic veterinarian, fish expert, or the AWO for further and more detailed information.

4.4.6 Chemical restraint, Sedation and Anaesthesia

4.4.6.1 When working with a new anaesthetic protocol or species it is advisable to anesthetize a few animals and follow these animals through to full recovery to ensure drug dosages, concentrations and techniques are safe and provide sufficient anaesthetic depth for the intended procedures.

4.4.6.2 Where sedation is required for handling or other drugs are to be given, investigators must ensure they are familiar with the drug, dose, concentrations, mode of delivery, expected effects and potential side-effects of its administration.

4.4.6.3 Investigators and facility managers should be aware that AQUI-S® is the preferred anaesthetic and sedative agent of choice in most circumstances as it is the only licensed anaesthetic agent approved for use in fish by the Australian Pesticides and Veterinary Medicines Authority (APVMA). Others drugs or agents can be considered and used with justification and approval.

4.4.6.4 Further information can be sought from the AWO for specific anaesthetic protocols or drug information if required.

4.5 Acquisition of fish for laboratory research

4.5.1 Fish may be wild caught, obtained from existing captive collections and/or approved commercial suppliers.

4.5.2 Whenever possible, fish should be acquired from other captive collections or commercially approved aquatic suppliers rather than collected from the wild. Commercial suppliers should be able to provide a health certificate and check from an independent laboratory.

4.5.3 Information needed for the development of research protocols should aim to include: body weight (including normal range and any sexual dimorphism if known), size (e.g. Fork length - tip of the snout to the bifurcation of the tail fin), diet, longevity, behaviour, social structure, aggression, habitat and water quality requirements, any unusual aspects of biology, relevant known diseases or health issues and any other special needs.

4.6 Acquisition from the wild for captivity

4.6.1 Prior to initiating field research, investigators and their team, wherever possible, must be familiar with the target species and its response to disturbances, sensitivity to capture and restraint, and, if necessary, any known requirements for maintenance in aquatic captivity.

4.6.2 When planning an aquatic field expedition for the purpose of specimen collection, investigators and their team must develop a species-specific data sheet onto which data can be recorded. This is to ensure a clear plan is in place before any fish is captured, to reduce handling time and stress to the individual.

4.6.3 Field work applications must include a protocol with notes indicating environmental conditions suitable for safe capture from the native habitat. These may include: maximum and minimum temperatures, any weather events that might have an impact (e.g. storms), and maximum safe length of holding time prior to transport. Short term holding conditions or requirements should also include: transport container type, temperature management plan, water quality source and requirements (i.e. aerators), and an indication of whether fish must be individually housed. If grouped the maximum stocking density per container/bag or fish biomass per volume of water to be occupied by the fish should also be included in the application.

4.6.4 *Field procedures*

4.6.4.1 Following capture, an immediate and accurate record of body mass and length with an animal identification number (when practical) may be collected (i.e. via a fish measuring tube). The data collected should be clearly ordered with records able to identify the species, animal number, capture site (preferably GPS co-ordinates), time of capture and habitat type.

4.6.4.2 A brief examination should be conducted to assess for external parasites, such as *Ergasilids,* and to screen for obvious injuries or skin lesions that may reflect a known health issue, disease or pathogen. If abnormalities are found, then a decision must be made regarding the welfare of the animal and its ability to continue to survive in the wild. Investigators should refer to the intervention criteria sheet to assist with this decision making. If the animal is deemed unsuitable for study use but able to continue in the wild, then it should be released.

4.6.4.3 Fish held or enclosed in the field (i.e. aquatic enclosures) should be monitored carefully for natural behaviours (e.g. swimming, feeding). Water should be well oxygenated with appropriate water changes made and compatible for different species kept together. Enclosures should not be in direct sunlight to minimise temperature fluctuations. Aquatic enclosures should be designed and placed in such a way to provide protection from predators. Water quality parameters and depth of water should be checked regularly. If water quality parameters or depths are unable to be sufficiently maintained within the aquatic enclosure, fish must be released back into their environment. If held for over 24 hours, then sufficient food resources should be made available as appropriate to the species.

4.6.5 *Equipment care*

4.6.5.1 All equipment including boots, buckets, nets etc. should be cleaned at the site to remove debris, mud or dirt. After reducing the organic matter, they should initially be washed in soapy water to clean the remaining debris followed by soaking or spraying with an appropriate disinfectant. Disinfectants, such as F10sc®, bleach, and VirkonS®, require not only a clean surface to be effective, but also need the appropriate contact time and concentration to be

used. All equipment must be thoroughly rinsed prior to cleaning and use to ensure disinfectants have been completely removed. Further information on specific disinfectants can be obtained from the manufacturer or Material Safety Data Sheets (MSDS).

4.6.5.2 Equipment should be checked prior to use to ensure it remains in good working order for the animal and animal handler safety. Any issues with equipment should be rectified prior to use and if needed replaced. Disposables (i.e. nitrile gloves) should also be checked and investigators should ensure there is sufficient quantity available before undertaking any action or procedures.

4.6.6 *Transport*

4.6.6.1 The specific type of transport container will depend on the size and species of fish. Small or medium sized fish (e.g. fish less than 30cm in length) can be transported in plastic bags within insulated containers (e.g. Cooler box or Esky®) that are modified to provide aeration. The exact number of individuals per container will depend on their sizes and species.

4.6.6.2 Transport containers selected must be suitable for fish transport. This includes selecting containers with surfaces that are easy to clean and disinfect as well as prevent injury to fish.

4.6.6.3 The transport container must not be left in direct sunlight, and investigators should aim to keep the temperature slightly cooler than the fish's usual environment. Temperature is crucial when transporting fish to avoid overheating and rapid reductions in water quality (i.e. dissolved oxygen) which can quickly become fatal. *Transporting fish in hot weather should be avoided*. Cooling methods can include air conditioning of vehicles and transport containers or cool packs inside transport containers. Ice should be avoided as a cooling method inside transport containers. Only compatible fish and fish species should be housed together. Aggressive or predatory fish should be housed separately.

4.6.6.4 As water quality is paramount to fish health, all reasonable precautions and steps must be undertaken to ensure water quality parameters remain within acceptable limits. This includes the use of appropriate water quality monitoring equipment (i.e. sensor, meters) and water quality enhancement equipment (i.e. aeration, filtration). Aeration can be provided by many methods, including battery powered air pumps and stones. For oxygen sensitive species consider aerating the water with oxygen.

4.6.6.5 Water can be sourced from the site of fish collection or externally sourced but must be of a suitable quality as outlined in the species-specific water quality parameters.

4.6.6.6 Using one or two strong plastic bags, fill 1/3 full of water (preferably using the water the fish came from). Place the sampled fish (typically 4-6 fish) in the water, remove the air, and then fill the bag with compressed oxygen. Ensure oxygen does not escape. Twist and double over the neck of the bags and securely tie using tape, cord, zip ties or rubber bands. Bags should then be packed in a watertight container, sealed and clearly labeled "Scientific Specimens – Perishable. Handle with care!" followed by the delivery address. Most specimens may be kept cool by using ice blocks next to the container housing the fish. Overnight transport is essential. When possible, avoid the feeding of fish prior to transport as fish are liable to empty their digestive tracts into the transport container which will can lead to a significant deterioration in water quality.

4.6.6.7 Where overnight stays at field sites or camps are required, it is the investigators responsibility to ensure suitable housing is available with an appropriate degree of climate control, aeration and water quality requirements. Water quality parameters should be checked and fish visually inspected whenever possible. If water quality parameters or conditions deteriorate, attempts should be made to improve the water quality (i.e. water turnover, aerators).

4.6.6.8 The time from first capture to placement into an approved facility should not exceed 24 hours. In exceptional circumstances (such as international or long-distance travel), this may be extended but ideally should not exceed 48 hours.

4.7 Acclimation

4.7.1 Transport and/or capture are highly stressful events for fish and great care must be taken to mitigate the negative effects that may occur. An acclimation period is necessary to allow the animals to adjust to their surroundings, which can include a change in diet, lighting, temperature water quality and housing.

4.7.2 Changes in water quality should be done slowly over time. Ideally fish transported in plastic bags of water should be secured to the new enclosure and left floating to allow the temperature to slowly equilibrate. Changes in water temperature of more than 2-3 °C can induce thermal shock in fish. Fish transported in eskies should be positioned within new enclosures and water should be slowly fed into the esky to allow the temperature to equilibrate.

4.7.3 Fish should not be used for procedures or investigations until they have had adequate time to acclimate. This is to ensure they have time to recover from stress and to avoid inaccurate results that may be altered by a stressed physiological state.

4.7.4 The time period required for acclimation will vary across the species, and wild-caught fish will require additional time to acclimate compared to those born in captivity. A recommended starting point for captive-bred animals or those previously acclimated and maintained in captivity is 7 days to adjust to the new facility before beginning experiments. Wild-caught animals may need to acclimate over 3-4 weeks, unless experience with the species suggests a different time period is required.

4.7.5 Prior to the use of a new aquatic system, sufficient time should be provided to enable the system to operate correctly and establish a biological filter. When starting a new aquatic system, investigators should test the system with fewer fish than what is estimated to be the carrying capacity of the system to ensure the aquatic system is operating correctly. Additional fish (up to carrying capacity) can be added later.

4.7.6 Daily monitoring by visual inspection, food intake, activity, and behaviour is essential for any new animal. Body weight or biomass can be measured prior to fish being transferred into their new enclosures. It is recommended to avoid further weight checks or fish movement for at least 2 weeks after placement into a new facility or enclosure.

4.7.7 Signs of acclimation in fish are demonstrated by normal behaviour, activity and feeding well. Fish will need to transition to their new diet slowly and where possible should receive the feed used by their previous facility. Wild-caught specimens may have special feed requirements which should be considered and available prior to capture.

4.7.8 Fish that are not feeding, are losing weight and/or appear to be visually unwell or abnormal over the first few weeks in the facility, may not be acclimating well. Additional interventions such as assisted feeding and increased monitoring may be required as per the Intervention Criteria Sheet (ICS). (See Appendix V).

4.7.9 The AWO can and should be utilised for additional advice if investigators have any concerns about the health and welfare of their fish.

4.7.10 Where a fish fails to acclimate to captivity as identified by the ICS, then humane killing may be required to ensure it does not suffer.

4.8 Biosecurity: Health checks and Quarantine

4.8.1 Any incoming animals, including those from commercial suppliers, should be subjected to careful inspection for potential health problems or pathogens prior to introduction to any existing laboratory colonies. A veterinarian with experience in fish and/or the AWO should be consulted if necessary.

4.8.2 Fish from other aquatic facilities should come with a pre-delivery health check certification. Wild caught fish and high-value or rare fish should be health checked (visual inspection, slime coat and gill examination) prior to entrance into the facility whenever possible. This should be undertaken on a selection of fish either at the time of stocking into the quarantine facility or 4-7 days after entrance to the facility which will allow fish to acclimatise prior to handling.

4.8.3 Ideally, fish should be in quarantine for at least one month. This is rarely practical in a research setting; however, it highlights the need for continued health monitoring and good hygiene where shorter periods are implemented. A minimum of 7 days quarantine is recommended if bringing new fish into a facility with an existing fish population.

4.8.4 Incoming fish should be treated as their own separate cohort for the purpose of quarantine. They should not be mixed with any other fish currently housed at a facility until an appropriate quarantine time has been served.

4.8.5 Quarantine housing should provide appropriate light cycles, lighting, temperature, water quality and diet.

4.8.6 An external examination for ectoparasites should be undertaken as part of the general health check (physical appearance, slime coat and gill examinations). If ectoparasites are detected, fish should be treated with an appropriately chosen ectoparasiticide.

4.8.7 All incoming animals should ideally be treated for endoparasites using an appropriate treatment that includes the treatment of tapeworms prior to transferring into the main facility holding areas.

4.8.8 Investigators and facility managers should be aware that many treatments are not licensed or approved for use in fish or have been in used in the species they are working with and are therefore off-label use. When in doubt, a small batch of fish should be selected for testing prior to use on the remainder of the cohort.

4.8.9 Specialist aquatic veterinarians, fish experts and the AWO can be contacted for advice on treatment regimens and should be approved prior to use.

4.8.10 Fish should be monitored closely in quarantine in case of failure to adapt to the captive diet, environment or due to underlying illness. The frequency of body weight measurements and fish health investigative techniques (gill and slime coat examinations) will need to be balanced with the potential stress that may be caused to the animal.

4.8.11 Quarantined animals should be cleaned, fed and handled last in animal care facility regimes to reduce the risk of disease transmission.

4.8.12 Before and after servicing the quarantine area as well as between individual cages, staff must thoroughly wash their hands in warm water and soap for at least 20 seconds, or use an alcohol or disinfectant based gel rub.

4.8.13 Separate equipment must be used in the quarantine area and must not be used in the main facility. This includes nets, automatic feeders, weight scales, water meters and cleaning equipment.

4.8.14 Fish acquired from the wild may not be permitted to be returned to the wild. Check specific collection permit guidelines for the disposal of target and non-target species.

4.9 Aquatic husbandry

4.9.1 The precise care requirements for fish are highly species-specific and must be tailored to the animal's ecology and habitat, behaviour, social structure of the species/taxonomic group and diet. The advice provided in this Standard should be considered a starting point for maintaining fish (unless specific species are referenced). It is not intended to supersede or provide expert guidance for individual species, but rather to specify the minimum considerations required to successfully maintain fish.

4.9.2 A guide must be prepared for each incoming species that outlines the water quality, husbandry and aquatic requirements to ensure facility staff and investigators are aware of their species-specific needs. The species care guide should be prepared in consultation with the current literature available and with further assistance from aquatic experts experienced in keeping the species, aquaculture or aquaria staff, investigators or faculty veterinarians with expertise in fish. The care guidelines should be made available for the AEC with the animal ethics application documents.

4.9.3 Individual tanks, aquatic enclosures (e.g. raceways) or water systems must have their own data monitoring sheet. Investigators or technicians should check the following type of information: water quality parameters (i.e. oxygen levels, pH, temperature, salinity, nitrogen), feeding protocol (including pellet size), fish housed (species name, number or bulk weight and if known the sex of the animal/s).

4.9.4 Animals in each enclosure, where feasible, should be individually identifiable, either by single housing or documentation of markings/patterns, chemical marking (i.e. calcein), tail clipping, external tags, or microchip implantation (shoulder region of the fish closest to the head).

4.9.5 ***Tank and Aquatic Enclosures***

4.9.5.1 The stocking density per tank will depend on the species with schooling fish being suited to group housing with solitary species housed singly or at appropriate densities to avoid aggression. Investigators should be aware there may be minimum or maximum stocking densities of fish that may increase aggression. Where pairs or groups of animals are housed together, then the care guides should note a maximum number of animals per aquatic enclosure and any special requirements for different sexes (sheet with pictures and/or defining characteristics).

4.9.5.2 Additionally, where possible, groups and types of fish should not be mixed (unless for grading or specifically approved experimental purposes).

4.9.5.3 The aquatic enclosure size will depend on the size and behaviour of the fish species to be maintained. The enclosure should be sufficient to maintain water quality parameters and optimal stocking densities.

4.9.5.4 Aquatic facility equipment and enclosure materials must be non-hazardous to fish and easy to clean and disinfect between cohorts of fish.

4.9.5.5 Size, design, shape, colour and behavioural enrichment should ideally mimic the natural environment of the fish. Enclosures should be sturdy, easy to clean and have lids or netting above the tanks to prevent fish from jumping out of enclosures.

4.9.5.6 Housing of dangerous species must have clear signage identifying the fish as venomous, poisonous or dangerous. The housing must be secure to avoid any potential escape, which may include the use of secured lids and double housing (an enclosure inside an enclosure) to prevent accidental contact from staff and investigators.

4.9.6 *Tank and Aquatic Enclosure Enrichment*

4.9.6.1 Furnishings for the enclosure will vary with the species being housed but may include a substrate at the bottom of the tank and ideally one hiding area or structure along with other items for environmental/behavioural enrichment. Rocks, logs, branches, small plants, ceramic tiles, PVC pipe or empty pots may all be used to provide both shelter and enrichment. Investigators should try and choose items that can be easily cleaned and disinfected.

4.9.7 *Water Quality*

4.9.7.1 All fish are poikilothermic and thus rely on their environmental temperature to support metabolic processes and must be housed within water quality suitable for the species of fish.

4.9.7.2 Fish should not be subjected to rapid changes to water quality (with the exception of dissolved oxygen increases). Rapid changes in water quality can cause unnecessary stress and compromise the immune systems of fish.

4.9.7.3 The water quality parameters of pH, temperature, dissolved oxygen, salinity, and nitrogen (nitrite, nitrate, ammonia) are the minimum parameters to be measured and maintained at acceptable limits.

4.9.7.4 Dissolved oxygen levels should be at least 90% to prevent hypoxia and investigators should avoid supersaturation of oxygen which can be fatal to fish.

4.9.7.5 There are many aquatic water quality guidelines available with species-specific guidelines for acceptable water parameters. If water quality parameters are unknown, then a similar species of fish water quality guidelines can be used.

4.9.7.6 Water quality measuring devices should be able to detect and react to changes in water quality levels before they become life-threatening.

4.9.7.7 Water quality parameter monitoring should be conducted in such a way as to allow water management strategies to be predictive rather than reactive.

4.9.7.8 Facilities should aim to maintain specific-pathogen-free water for their collections and experiments. This can be accomplished by pre-treatment of water via various methods including ultraviolet light treatment of incoming water sources.

4.9.8 *Lighting and Ultraviolet Light (Sunlight) access*

4.9.8.1 Fish should be provided with a day-night cycle appropriate to the time of year and their natural environment. This can be achieved using artificial lighting on a timer and/or using opaque coloured lids over tanks or enclosures.

4.9.8.2 A heating or cooling source must always be provided to enable optimal maintenance of water quality parameters.

4.9.8.3 Excessive light or UV exposure, by way of insufficient shade, can result in illness and disease, such as sun burn (darkening of the skin), skin tumours or cataracts problems or affect feeding and reproductive cycles.

4.9.9 *Diet and feeding*

4.9.9.1 Food for fish should only be acquired from reputable sources and preferably via commercial suppliers that offer a quality guarantee and is nutritionally appropriate to the species of fish. Any fresh produce offered should be of suitable commercial grade quality. All feed should be stored suitably to prevent disease and spoilage.

4.9.9.2 Investigators should be aware fish may need time to acclimatise to new food sources and pelleted foods. Therefore, ensure an adequate acclimation period is performed prior to the start of any experiments.

4.9.9.3 Fish should be fed at appropriate intervals (by hand or automatic feeders) with the appropriate size of feed (i.e. pellet size) and observed during feeding times. Fish should be feeding well and all fish should be able to access the feed. The amount of feed given at each feed should be measured and recorded with the approximate amount of feed eaten also recorded.

4.9.9.4 Investigators must outline a species-specific and appropriate feeding plan, feeding frequency, and feed type for each species and life stage of fish. If a species feeding requirements are unknown, then the feeding requirements of a similar species of fish can be outlined or investigators can propose (with supporting documentation) a novel feeding regime for fish. Most fish are fed daily with some species requiring feeding twice daily and others requiring feeding every other day.

4.9.9.5 Investigators should ensure fish are not overfed and that no excess food is left in tanks or enclosures. Excess food should be removed as soon as fish are finished feeding to prevent potentially detrimental deterioration in water quality.

4.9.9.6 Larval feeding and weaning are times of high mortality that can be worsened due to failure to feed or inappropriate feeding intervals and/or feed. Investigators should closely monitor larvae to ensure they are feeding appropriately. Increased monitoring frequency may be required and changes to the diet may be required to ensure larval feeding and weaning is successful.

4.9.9.7 The provision of live invertebrates, such as rotifers and *Artemia*, can be more suitable during the crucial stages of hatching, larval development, and weaning.

4.9.9.8 Those individuals which cannot be sufficiently weaned or refuse to feed may require humane euthanasia. The Animal Welfare Officers are available for further advice or guidance.

4.9.10 *Broodstock, breeding and spawning*

4.9.10.1 Wherever possible appropriate genetic management of broodstock should be used.

4.9.10.2 With all broodstock a strict disease and health control program should be implemented to ensure the healthy production of eggs and to prevent transmission of unwanted disease through genetics, water, or other sources.

4.9.10.3 Breeding activities: Investigators should be aware that environmental factors such as temperature, day length, habitat/tank design, nutritional content of feed and stocking density are critical to reproductive success.

4.9.10.4 Where feasible, any spawning activity should be recorded with the date, location, and water quality parameters recorded on the tank/aquatic enclosure.

4.9.10.5 Induction of spawning using injectable hormones can be used and is used in commercial fisheries operations. Researchers should carefully choose the induction method and technique. Scientific or government literature, fish experts, and aquatic veterinarians should be consulted to ensure methods and doses are consistent with established practices.

4.9.10.6 If hormones are used, withdrawal times for food producing species must be observed.

4.9.10.7 Investigators are to be warned that induction of spawning may result in increases in fish mortality and disease.

4.9.10.8 All persons working with chemicals for the induction of spawning should be aware of the EH&S hazards associated with working with hormonal chemicals. Staff should contact their department and EH&S managers for further information.

4.9.10.9 Thorough records should be kept when eggs are laid and when/if these offspring hatch on the monitoring sheet. If the reproductive activity is part of a breeding program, it is essential to record when breeding pairs or groups are placed together and/or mating is observed. Any overt displays of courtship behaviour should also be noted.

4.9.11 *Fish eggs and embryos*

4.9.11.1 Fish eggs and embryos are to be accorded the same basic welfare conditions and standards as adult or sub-adult fish.

4.9.11.2 Water quality, choice of diet, biosecurity measures (including preventative treatments) and appropriate provision of aquatic facilities is paramount to fish health and welfare in hatchery and larval stages of fish.

4.9.11.3 Genetic selection of broodstock and appropriate care of broodstock prior to spawning is also of high importance to ensure diseases are not passed from broodstock to their progeny.

4.9.12 *Cleaning*

4.9.12.1 Tanks, equipment and enclosures should be disinfected between uses.

4.9.12.2 A disinfectant capable of destroying bacteria, viruses, fungi and parasite eggs should be selected for use in the facility. All surfaces should be thoroughly rinsed and allowed to dry fully before their next use.

4.9.12.3 Commercially available disinfectants should always be made up according to the manufacturer's instructions and allowed adequate contact time based on their concentration. Personal protective equipment should be used as per OH&S guidelines.

4.9.12.4 Most disinfecting agents have reduced efficacy if applied onto surfaces or equipment with organic material (i.e. faeces, urates, and urine or food matter) so surfaces and equipment should first be cleaned with scourers or sponges to reduce the build-up of organic matter.

4.9.12.5 Any equipment and enrichment should be cleaned and disinfected as needed or between uses.

4.10 Humane Killing of Fish

4.10.1.1 Humane killing of fish must be undertaken persons trained and deemed competent by the (AWO), animal facility manager, or the animal ethic committees.

4.10.1.2 Ideally euthanasia of fish should be a two-step process with the first step being sufficient anaesthesia (at least to the point at which fish lose their equilibrium) followed by another physical or chemical method.

4.10.1.3 The use of acceptable physical techniques of euthanasia following anaesthesia are pithing to the brain and brainstem, decapitation at the cervical vertebrae, or exsanguination via severance of the vessels to the gills by severing the gill arches.

4.10.1.4 The sole use of chemical methods within the water to euthanize fish is acceptable if adequate monitoring time is performed to ensure death. It is recommended that fish are immersed for at least 2 hours after being declared dead. Death can be declared in a fish that has not shown any signs of respiratory effort or opercula movements for at least 10 minutes.

4.10.1.5 Physical methods of killing (iki jimi or priesting), should be used secondary to anaesthesia.

4.10.1.6 Only in extreme circumstances may physical methods be used without anaesthetic. These are cases where animals are in such a state of distress that the time required to administer an anaesthetic would result in greater and more prolonged distress levels.

4.10.1.7 Methods of euthanasia that are unacceptable without prior anaesthesia (or explicit exemption from the animal ethic committee) are exsanguination, pithing, and decapitation.

4.10.1.8 The use of concussive forces (i.e. priesting), in conjunction with pithing, exsanguination or decapitation is acceptable with reservation, and requires specific justification and approval from the AEC. Persons performing this technique must be deemed appropriately competent by the AEC or the AWO.

4.10.1.9 Unacceptable methods of euthanasia are the use of hypothermia (freezing), hypoxia or electrocution.

4.10.1.10 Any equipment must be in good working order and checked prior to performing euthanasia. All equipment should be cleaned and disinfected as needed or between uses.

4.10.1.11 Any investigator or person involved in the euthanasia of fish is to be well informed about the pharmacological and physiological impacts of their proposed method of euthanasia.

5. MONITORING & INTERVENTION

5.1 Captive Aquatic Monitoring Sheets

The following sheets will be required:

A- Species-specific care guide (per project)- See Section 4.9

B- Fish assignment list (per facility or per room) –Appendix II

C- Tank card –Appendix I and Section 5.1.1

D- Water quality monitoring sheet (per enclosure) –Appendix IV

E- Fish monitoring sheet (per individual animal or animals in one cage) –Appendix III

F- Intervention Criteria Sheet (per project) –Appendix V

5.1.1 Tank or aquatic enclosure cards

5.1.1.1 Each tank or aquatic enclosure should be labelled with a waterproof tank card containing information pertaining to the study and the animals. It should display the investigators initials, Animal Ethics ID, tank/enclosure number, total fish in the aquatic enclosure or tank, date of acquisition, place of origin (i.e. location for wild caught specimens, name of supplier, bred in-house), the Latin name and common name of the species, sex of the animals (if known or appropriate), initial size (individual or bulk weight) on entry, diet required, feeding frequency and day of next feeding. (See Appendix I for example)

5.1.2 Record keeping and monitoring sheets

5.1.2.1 One central assignment list must be maintained per fish facility or room that identifies total number (or bulk weight with approximate number) of fish, their species, date of entry, place of origin and date of exit. (See Appendix II)

5.1.2.2 Each tank should have their own fish monitoring sheet that notes the date and time of checks and observations of the animal. Weight or bulk weights, health checks, any abnormal findings, and procedures performed should be recorded here.

5.1.2.3 Water quality parameters (i.e. dissolved oxygen, pH, temperature, salinity) for each aquatic system or enclosure should also be recorded on a monitoring sheet or lab book at least every week. Minimum parameters should include dissolved oxygen, temperature, ammonia and pH levels. Water loggers should have their data downloaded and backed up appropriately a minimum of once a week.

5.1.2.4 All entries must be initialled by the person undertaking the check. (See Appendix III for example). These may be kept in a folder or log book within the room.

5.1.3 Feeding

5.1.3.1 The diet and feeding frequency for the species should be noted on the tank card for easy reference, with full details available in the species care notes.

5.1.3.2 The amount and type of food offered (e.g. volume, brine shrimp) should be recorded, along with approximately how much was consumed, and feeding activity observed on the monitoring sheet.

5.1.4 Weighing and Measurements

5.1.4.1 Fish should ideally be weighed (bulk or individual) prior to transport and arrival to the facility. Digital scales with 1-2 decimal places should be used to weigh fish, with small kitchen scales being suitable to most species under 100g. Larger commercial scales can be used for larger fish. Note: For ease of handling and accuracy during the weighing and measuring of fish, a cradle or chute can be used

5.1.4.2 The frequency of weighing fish will vary depending on the project; however, keep in mind weighing fish will always cause some level of stress and may affect research outcomes.

5.1.4.3 Weighing should be undertaken efficiently and quickly to reduce the amount of time the fish has been removed from the water and always handled with gloves to prevent injury to scales and the slime coating. This also protects the handler from fish slime which can contain infectious agents.

5.1.4.4 Sufficient chemical restraint (i.e. AQUI-S®) and aeration/oxygenation should be employed to minimise stress.

5.1.4.5 After weight checks and chemical restraint, fish must be observed until they have recovered and resumed normal swimming and behaviour.

5.1.4.6 Any individual fish that are removed from a group of fish to be weighed, must recover fully before being returned to their tank of origin.

5.1.4.7 Biomass calculations can be used instead of bulk weight where appropriate.

5.1.5 Observations

5.1.5.1 Prior to handling fish for weighing and other procedures, the investigator or carer must first observe them in their enclosure.

5.1.5.2 Fish should be assessed for their behaviour and activity levels, physical appearance, social interactions and respiratory effort by watching the operculum and mouth. When stimulated prior to handling, their response should be noted in case it is abnormal (i.e. a typically fast-moving species of fish is suddenly slow or vice-versa).

5.1.6 Temperature

5.1.6.1 The temperature of each aquatic enclosure and/or system should be checked daily or once per week at a minimum. Water monitoring devices and loggers should have their recordings downloaded at least weekly and their information electronically backed up regularly.

5.1.6.2 A recording of the tank or aquatic enclosure temperature as well as the water inlet or water source should be taken to ensure incoming water is of a suitable temperature, and that any water heating or cooling systems are functioning.

5.1.6.3 The use of a 24-hour water monitor logger is suggested to track the temperature and the data recorded electronically backed up regularly.

5.1.7 Reproductive activity

5.1.7.1 Any spawning activity should be recorded with the date, location, and water quality parameters recorded on the tank/aquatic enclosure card.

5.1.7.2 Thorough records should be kept when eggs are laid and when/if these offspring hatch on the monitoring sheet. If the reproductive activity is part of a breeding program, it is essential to record when breeding pairs or groups are placed together and/or mating is observed. Any overt displays of courtship behaviour should also be noted.

5.2 Intervention points

5.2.1 The ICS should provide guidance to investigators when abnormalities are noted in the health or behaviour of fish maintained as part of their project.

5.2.2 Where any moderate signs listed on the ICS are identified, investigators should complete the Troubleshooting Checklist and implement increased twice daily visual monitoring of fish. Water quality should also be checked by the facility and water samples taken for potential further testing

5.2.2.1 If water quality is suspected to be of concern, then water quality should also be monitored daily and recorded.

5.2.3 If one or more severe signs from the ICS are noted, then the AWO must be contacted immediately.

5.2.3.1 If not already implemented, commence once daily visual inspections and an immediate water quality check, with water samples taken for potential further testing.

5.2.3.2 Following a discussion, the AWO may instruct the investigator to monitor the fish more frequently, send water quality samples to an external lab for further investigation, or, if appropriate, humanely euthanize moribund fish for immediate necropsy and testing and/or send moribund fish to an appropriate pathology facility for further testing.

5.2.4 A single missed feed is acceptable provided the fish are otherwise bright and active. If, however, fish do not eat for 2 feeds then additional steps for monitoring and care must be undertaken.

5.2.5 Investigators who are concerned about the health or welfare of fish in their care at any time must contact the AWO or AFM to seek advice as soon as the concern arises.

5.3 Unexpected adverse event and pathology collection

5.3.1 Where an animal suffers from an unexpected adverse event, that is not anticipated, and the animal has pain, distress or dies of a cause other than humane killing, the body must be preserved appropriately until a necropsy is performed. The AFM and AWO should also be contacted immediately and correct preservation instructions will be given. An adverse event that may be considered to be

anticipated may include; a fish escaping from its housing, a fish that is bullied by other fish, fish trapped by housing furnishings, or natural mortality due to known phenomenon (i.e. weaning).

5.3.2 Where humane killing is required, investigators should refer to the following section 4.10 of this Standard. Humane killing must only be performed by trained and competent individuals.

5.3.2.1 A necropsy must be performed on any animal whose illness or death constitutes an unexpected adverse event. The body of an animal found deceased or humanely killed as a consequence of an unexpected adverse event must be refrigerated and the necropsy performed in a timely manner to provide for accurate and reliable results. A full necropsy report as well as any relevant photographs and external Laboratory results should be submitted to the AWO alongside the adverse event report.

5.3.2.2 Competency to conduct a necropsy should be a listed skill on the AEC application, with investigators noting if they need 'Training' or are 'Competent'. Training for this procedure can be provided by the AWO or a competent investigator if required.

5.3.2.3 Dead fish should not be frozen. Freshly dead fish can temporarily be placed onto damp paper towels and into durable, thick, sealed plastic bags and refrigerated or placed onto ice-filled insulated containers or coolers. Fish should be examined within 12 hours by trained personnel or sent to the appropriate pathology lab.

5.3.2.4 Do not store or keep dead fish in water or place into freezers. Due to the rapid decomposition and delicate nature of fish tissues freezing fish or leaving them in water will accelerate their decomposition and render tissues unsuitable for pathological testing.

5.3.3 If dead fish cannot be examined within 12-24 hours then preservation methods must be performed and recorded by a trained investigator, ideally with photos taken throughout the necropsy.

5.3.3.1 In fish smaller than 3cm, the whole body should be placed in 10% neutral-buffered formalin (1:10 – tissue to formalin), or if unavailable ethanol (75%-95% mixture). Dead fish from 4cm - 10cm to in length can have their operculum removed and an incision made into the coelomic cavity from the vent to gill arches and placed into an appropriate tissue fixative. Larger fish will need a full necropsy performed by a trained investigator or under the guidance of the AWO. Keep dead fish in separate containers.

5.3.3.2 The use of an appropriate fixative ensures tissues are preserved sufficiently for follow up pathology testing if required. Formalin is a suitable method for most specimens. There are many other fixatives that can be used for the preservation of fish tissues which can be used if the facility has access including Davidson's fixative or Bouin's fixative. Investigators are encouraged to contact their contracted pathology lab or histologist to determine their ideal fixative based on their experimental requirements.

5.3.3.3 Information that should be included in pathology submissions or adverse events include: general observations, environmental, husbandry or system changes, treatments, water source, diet, recent introduction of new fish or water sources, clinical signs, date mortality began and estimated mortality rate, stocking density, type of facility or production system, water quality parameters, species of fish, size, any suspected or recently diagnosed disease, named investigator with contact details (phone number and email address) and date of pathology submission.

5.3.4 In cases of adverse events, where high numbers of fish are affected or dead (i.e. entire tanks or aquatic systems), ideally 6-10 fish should be selected for necropsy and/or preservation for pathology and further diagnostic testing.

5.3.5 Water quality should also be tested in-house and water samples collected for further laboratory testing.

5.4 Medications for use in Fish

5.4.1 All medications, drugs and treatments must be recorded onto monitoring sheets and logbooks with the appropriate withdrawal times (if any) recorded.

5.4.2 It is important to note that most drugs and treatments used in fish are considered "off-label" as these products are often not registered for use in finfish species. Toxicological and pharmacokinetic studies

are not always available in fish and those that have been studied are unlikely to have been performed in the particular species of interest. It is accepted amongst finfish veterinarians that extrapolation of doses may be used as a guide across the group under veterinary advice and if required veterinary prescription.

5.4.2.1 It is highly recommended to test any drugs, whether prescription or non-prescription, on a small batch of fish as a precaution in case of adverse drug reactions.

5.4.2.2 Withdrawal times for food-producing animals must be strictly followed. Consultation with the AWO may be required if there is minimal advice in the literature or conflicting advice.

5.4.3 Water quality parameters may change during fish treatments or inadvertently create adverse events during or after fish treatments. Investigators should endeavour to take reasonable precautions and seek further information on treatments selected for fish use to ensure that during treatment, water quality parameters remain compatible and within appropriate fish husbandry requirements.

5.4.3.1 Failure to be aware of the potential water quality side effects or adverse water quality events may compromise fish welfare, reduce drug efficacy and can result in death or fish-kill events (i.e. formalin reduces oxygen levels).

5.4.4 Separate treatment tanks or enclosures should be used for fish treatments or drug administration with sufficient aeration or oxygenation as needed. If not, after fish have been treated, water quality must be returned to appropriate levels and the aquatic system adequately flushed to remove drugs and treatment chemicals from the aquatic system.

5.4.4.1 Investigators and animal facility managers should be aware that many treatment chemicals, drugs and medications can affect the bio-filtration systems. Appropriate monitoring and measures should be taken to maintain and monitor the bio-filtration system(s) post-treatments or post-medications to ensure they are working at optimum levels. This may include the monitoring of nitrogenous wastes such as ammonia concentrations or nitrite levels.

6. ADDITIONAL INFORMATION

6.1 Where investigators are looking to acquire a species of finfish they are not familiar with or which has limited published data to support husbandry decisions, then they are advised to contact the AWO to discuss alternative, reputable sources of information and expertise. This may include aquatic veterinarians and finfish specialists, finfish societies, academics or other references.

6.2 There is a huge diversity of species within the finfish group along with a myriad of laboratory and field research situations. This has led to the frequent use of the word ‘should’ in the preparation of this Standard, as the information may not be available to provide specific information and techniques that can be applied to multiple species, field sites or projects. We recognise that context is important when developing protocols for each project, but above all else the aim of the Standard is to ensure optimal welfare standards for finfish are maintained across all species, disciplines and research projects.

7. ENFORCEABLE REQUIREMENTS

7.1 Completion of a species-specific husbandry guide for review by the AEC, prior to acquiring fish.

7.2 Provision of an adequate aquaculture or aquatic or tank system to house fish.

7.3 Provision of an appropriate diet with nutritional supplements as needed.

7.4 Use of a water quality monitoring sheet or use of recordings from water quality loggers and an intervention sheet for experimental projects as documented in the approved ethics application.

7.5 Water quality and fish health monitoring is completed by trained personnel as listed in the approved ethics document.

7.6 Adherence to monitoring protocols and monitoring frequency and the approved ethics application.

7.7 Any welfare and health issues are attended to promptly by reporting to the appropriate personnel (see Section 5.1) and are immediately recorded on the daily/routine monitoring sheet or interventions sheets.

7.8 Necropsies to be performed as soon as possible (fish tissues degrade very quickly) for any unexpected adverse events, for any animal to be used for pathology sampling, or any animal that dies of a cause other than humane killing. Necropsy records must be kept (this may include photos and/or samples) and forwarded to the AWO as part of the Unexpected Adverse Event report.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

9. UNEXPECTED ADVERSE INCIDENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise investigators of the appropriate response.

10. GLOSSARY

Scientific Term	Lay Description
Aquaculture	The process of cultivating freshwater and saltwater aquatic animal or plant populations under controlled conditions
Coelomic cavity	The body cavity or space than runs the length of a vertebrate in an animal (fish)
Grading	A commonly used process of sorting individual fish into groups of similar size to improve fish welfare and production
Gill arches	A series of bony "loops" present in fish, which support the gills
Ergasilids	Genus of copepod crustaceans occurring in both the ocean and fresh water, often called gill lice or gill maggots
Fish biomass	The total weight of a fish species in a given area
Fish bulk weight	The estimated total weight of fish within a tank or enclosure
Fish-kill events	The death of a large number of fish or other aquatic animals over a short period of time and often within a defined geographic area
Fish measuring tube	A smooth tube (typically plastic or metal) used to facilitate measuring of fish
Hypoxia	A deficiency in the amount of oxygen reaching the tissues
Moribund	At the point of death
Operculum	The hard, bony flap covering and protecting the gills
Peduncle	The narrow part of the body to which the tail attaches
Slime Coating	The slimy external coating of a fish (muco-proteinaceous coating) that forms one of the fish's main defenses against infection and disease
Standard length	Tip of the snout to the last vertebrae
Stocking density	Number of fish or bulk weight of fish per volume of water (cubic metres)
Supersaturation	The state of a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances

Tapeworm	Flat, segmented worms that live in the intestines of some animals
Teleost fish	A group of bony fishes including most living species
Water monitor logger	Equipment that can be installed or used inside an aquatic system to electronically monitor water quality parameters
Water Quality Parameters	Describes the condition of the water, including chemical, physical, and biological characteristics, usually with respect to its suitability for a specific activity (aquaculture or aquariums).
Wt.	Abbreviation for weight
AQUI-S	An aquatic anaesthetic
Iki jimi	A euthanasia technique that involves the insertion of a spike quickly and directly into the hindbrain
Priesting	A euthanasia technique that involves quickly killing fish by using a tool to deliver a quick sharp blow to the head and brain

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Alaska Department of Game and Fish Pathology Laboratory website. Accessed 21/7/2016 http://www.adfg.alaska.gov/index.cfm?adfg=fishingpathologylab.main
- Boyd, C.E., (1982). Water Quality Management for Pond Fish Culture, Research and Development Series No. 22. Link Accessed 21/7/2016 from http://www.dpi.nsw.gov.au/fishing/aquaculture/publications/water-quality-management/monitoring-ammonia
- Boyd, C.E. and Tucker, C.S. (1998). Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, USA.
- Canadian Council on Animal Care Guidelines on the Care and Use of Fish in Research, Teaching, and Testing. Accessed 28/07/2016 from http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf
- Handlinger, J., Collection and Submission of Samples for Investigation of Diseases of Fin Fish. Kings Meadow Tasmania. Accessed 21/7/2016 from https://view.officeapps.live.com/op/view.aspx?src=http%3A%2F%2Fwww.scahls.org.au%2FProcedures%2FDocuments%2FAQANZSDP%2FFinFish_Sampling.doc
- Mumford, S. L., USFWS, Olympia Fish Health Center, Olympia Washington USA. Accessed 21/7/2016 from https://www.fws.gov/policy/aquatichandbook/Volume_2/Chapter13Histology.pdf

The following resources may provide additional or supplementary information:

- Canadian Council on Animal Care Guidelines on the Care and Use of Fish in Research, Teaching, and Testing http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf
- New South Wales Water Quality Management for Aquaculture http://www.dpi.nsw.gov.au/fishing/aquaculture/publications/water-quality-management

APPENDIX I: Tank or Aquatic Enclosure template

NB. Where multiple choices are listed, circle the appropriate option.

TANK/AQUATIC ENCLOSURE CARD		
Investigator: (initials)		Ethics ID:
Tank/Aquatic enclosure number:	Total animals (bulk weight):	Animal ID(s): *As appropriate*
Date of entry: DD/MM/YY		Species: *Genus name* (Common name)
Sex: M/ F/ Unknown - *As appropriate*		Weight (bulk weight) on entry:
Place of origin: internal/ wild / external		Frequency of feeding: Daily/ weekly / fortnightly / monthly
Diet:		Days of feeding: M Tu W Th F Sa Su
Water Quality Testing Protocol: *Water loggers, test kits, external laboratories, water probes etc., Frequency of testing, and water quality parameters to be tested*		Treatment(s) or Medication(s) Information: *Dose, length of time, type, frequency, withdrawal time etc.*

APPENDIX II: Fish assignment list (per facility or per room)

Date of entry	Animal ID or Group ID	Species	Investigator (initials)	Ethics ID	Date of exit

APPENDIX III: Fish Monitoring Sheet

Monitoring sheet for (Ethics Application #):

Principal Investigator: **Contact details (BH):** **Emergency contact details (AH):**

Secondary contact person: **Contact details:**

Tanks or animal number(s) covered by this sheet: **Species:**

Frequency of monitoring: Daily/ Weekly **Frequency of feeding:** **Feeding schedule:** M Tu W Th F Sa Su

Intervention: See Intervention Criteria Sheet attached.

				Observations (Tick any that apply): **Add specific signs appropriate to study**									**Comments/Food consumed/Procedures performed/Actions taken (include fate):**	**Initials:**
Date:	**Time:**	**Animal ID or Group:**	**Body weight or Bio-mass**	**Food offered**	**Normal**	**Water looks normal**	**Abnormal lesions or observations**	**Abnormal behaviour**	**Abnormal response to stimulation**	**Abnormal activity or swimming/ paralysis**	**Abnormal respiration**	**Other signs (describe)**		

APPENDIX IV: Tank or Aquatic Enclosure Water Quality Monitoring Sheet

Water Quality monitoring sheet for (Ethics Application #):

Principal Investigator:

Tank or Aquatic Enclosure Number:

Species:

Temperature range:

pH range:

Dissolved Oxygen range:

Salinity range:

Total Ammonia-Nitrogen range (see Table 1 below):

Frequency of monitoring of water quality parameters:

Date:	**Time:**	**Frequency**	**Temperature (°C)**	**pH**	**Dissolved Oxygen levels (mg/L)**	**Oxygen Saturation (%)**	**Salinity (ppm)**	**Total Ammonia-Nitrogen (mg/L)**
21/3/16	*13:00*	*Daily*	*19*	*7*	*6.0*	*95%*	*2ppm*	*0.0*

TABLE 1: Percentage of total ammonia-nitrogen (TAN) in the toxic unionised form NH3 at different temperature and pH values. *From Boyd (1982) "Water quality management for pond fish culture".*

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

Example: To obtain the concentration of NH_3: water at pH 8.4, 28°C and 2 mg/l of TAN (TAN has already been determined by sampling) contains: 15% of 2 mg/l
2 mg/l x 15.0 (from chart) ¸ 100 = 0.3 mg/l of NH_3

Intervention Criteria Sheet for fish

Intervention Criteria	
Criteria are based on the severity of signs, as classified by the table below:	
Observations (see Severity Table below)	**Action required**
No/Mild signs	Once daily visual observation unless project specifies more frequent checks are required (e.g. for larvae)
1 or more "Moderate" signs	Visual inspection twice daily. Complete "Troubleshooting Checklist".
1 "Severe" sign	Consult AWO immediately and seek advice
2 or more "Severe" signs (Criteria 1 to 5)	Euthanasia

Please amend table to account for species-specific differences

	Severity Table		
	No or mild	**Moderate**	**Severe**
	Non-specific sign(s):		
1. Appearance	Alert with normal posture, swimming or movement. Normal operculum movement.	Slightly abnormal posture and swimming behaviour. Minimal or excessive movement within enclosure or excessive time in one spot. Mild operculum flare.	Dull with obvious abnormal posture and swimming behaviour. Moderate to severe operculum flare and/or gasping.
2. Body condition *Species-specific*	In good body condition with belly present	Reduced body condition, no belly present on fish.	Poor body condition; no belly and abnormally thin appearance to fish.
3. Body weight or Biomass	Body weight or biomass stable or increasing.	Body weight or biomass reduced by 5-15%.	Body weight or biomass decreased by 15-20%.
4. Behaviour *See Table 2*	Normal behaviour, responds to stimulus (no sign of distress or abnormalities)	Subdued but responsive, decreased interaction with feeding or with stimulus	Minimally responsive or unresponsive to feeding, stimulus and provocation
5. Integument (Scales, fins and skin) *See Table 2*	No lesions; integument is clean, scales and slime coat are normal in colouration and appearance for species; Fins look healthy and normal	Small lesions present. Integument is dull or changes in slime coat. Abnormal or darker colour of skin with possible changes or damage to scales or fins.	Ulcers, bleeding or other lesions, scales falling off or abnormal in appearance. Fins and/or slime coat looks abnormal in appearance or structure.
	Intervention criteria: Specific conditions or abnormal clinical sign(s):		
6. Not eating*	Eating all or most of food offered; Does not eat for one feed.	Food intake reduced by 50% or does not eat for 2 consecutive feeds or 24 hours.	Not interested in food at all. Does not eat for 3 feeds or more than 48 hours.
7. Swimming or Movement* *See Table 2*	Normal swimming and movement and normal position in the water column	Faster or slower swimming and movement and/or position in the water column	Abnormal swimming and movement and/or unable to obtain fully upright position
8. Injection site reactions	No visible reaction	Mild to moderate swelling (for IM injections)	Site of injection sloughing scales, bleeding or has discharge. Significant swelling of the site.
9. Other signs	Seek AFM/AWO advice re: appropriate action or euthanise if animal is in severe pain or distress		

***6. Not eating** Fish who have been eating well and either gradually eat less, or suddenly refuse food should be monitored carefully. A single missed feed or reduced appetite is not generally cause for alarm if the fish are otherwise bright and alert, however if the problem persists then monitoring should increase and intervention is warranted. If the majority of a tank or enclosure is affected, then the AWO and AFM must be notified.

Common reasons for not eating, or eating less:

- Low temperatures or water quality parameters not species appropriate
- Normal variation in appetite
- Seasonal or hormonal cues
- Inappropriate food offered (unlikely if animal has been eating the food previously without issue)
- Underlying illness
- Other husbandry issues or equipment malfunction (i.e. auto-feeders jamming)

ACTION REQUIRED

1. Check all water quality parameters. Complete Troubleshooting Checklist and make corrections as necessary. If an issue is identified, then allow the animal 24 hours to readjust to the changes. If water quality parameters are abnormal then ensure it is safe and appropriate to feed fish again.
2. If fish are group-housed, label the enclosure with a yellow "Health Monitoring" card. The relevant monitoring sheet(s) should be started and if required the fish quarantined from the water supply.
3. If after 3 days fish have not shown signs of improvement, then the AWO should be contacted for further advice and veterinary care as needed.

***7. Swimming or Movement, Integument or Behaviour** If fish are unwell, these are key and important external indicators for investigators and staff.

Table 2: Evaluation of Clinical Signs for Fishes Involved in Research or Testing

Taken from the Canadian Council on Animal Care Guidelines on the Care and Use of Fish in Research, Teaching, and Testing

Physical Appearance	normal/abnormal
	eye condition
	fin and skin condition (Turnball *et al.*, 1998)
	mucus production
	colour change (usually a darkening associated with disease or bilateral blindness)
Measurable Clinical Signs	feed consumption
	respiratory rate and operculum flaring
	posture in water column, i.e. the individual's position in the water (upright, upside down, tilted, etc.)
Unprovoked Behaviour	position in the water column (e.g., crowding near the inlet or outlet pipe, shoaling, etc.)
	social interactions • direct attack, domination of choice tank locations, schooling • social isolation, i.e. fish either socially isolated or choosing to isolate themselves from the group • not responsive to external stimulation
	hyperactivity/hypoactivity (Juell, 1995; Holm *et al.*, 1998) • movement (abnormal movements such as flashing or scraping the body) (Furevik *et al.*, 1993) • unexpected jumping or escape behaviour
Provoked Behaviour	feeding activity

	threat response
	avoidance reaction to mechanical prod or movement
	avoidance reaction to light beam

ACTION REQUIRED

1. Check all water quality parameters. Complete Troubleshooting Checklist and make corrections as necessary. If an issue is identified, then allow the animal 24 hours to readjust to the changes. If water quality parameters are abnormal then ensure it is safe and appropriate to feed fish.
2. Collect a sample of water and place into freezer.
3. Ensure all equipment is working appropriately, including water filtration systems.
4. If fish are group-housed place label the enclosure with a yellow “Health Monitoring” card. The relevant monitoring sheet(s) should be started and if required the fish quarantined from the water supply.
5. If after 3 days fish have not shown signs of improvement, then the AWO should be contacted for further advice and veterinary care as needed.

Troubleshooting Checklist

1. Is the enclosure size appropriate for the size, stocking density and species of the fish?

2. What is the overall appearance of the water and enclosure (i.e. increased turbidity, algae)?

3. Is the temperature appropriate? Check the temperature.

4. Is the dissolved oxygen content sufficient? Check the dissolved oxygen.

5. Is the pH suitable? Check the pH levels.

6. Are the nitrogen (nitrites and nitrates) levels within acceptable limits? Check both nitrates and nitrites.

7. Is the water flow or turnover adequate for the system?

8. Have any new animals been added to the enclosure? Have any new fish come to the facility?

9. How have the fish been feeding? When and what did they last eat? Was this a new or familiar diet?

10. Are there any noticeable physical changes or abnormalities on the fish?

11. Has fish behaviour or movement/swimming changed at all (i.e. slow, gasping)?

12. Where are the fish positioned in the tank or enclosure? Are they by the water inlet source or the surface etc.?