Module 12 – Care and Use of Fish

Objectives.

- to introduce the researcher to the proper acquisition, handling and care of fishes
- to review regulations in the acquisition of fishes
- to discuss the important environmental factors required to maintain healthy and non-stressed fishes

Introduction.

The use of fishes as experimental subjects has increased substantially over the past two decades. This increase in use is a result of the rapid development of the aquaculture industry, requirements for testing involving fishes as indicators of environmental change, and the use of fishes as a replacement for mammals in biomedical, pharmacological and genetic research. The trend toward the use of fishes as a replacement for studies that would previously have used mammals as experimental subjects is not discouraged. However, it must also be recognized that fishes have the capacity to perceive noxious stimuli. Noxious stimuli are those stimuli that are damaging or potentially damaging to normal tissue (e.g., mechanical pressure, extremes of temperature and corrosive chemicals). Whether or not fishes have the capacity to experience any of the adverse states usually associated with pain in mammals is subject to a great deal of debate in the scientific literature. Nonetheless, fishes are capable of behavioural, physiological and hormonal responses to stressors (including noxious stimuli) which can be detrimental to their well-being. These guidelines apply to fishes held in facilities for research, teaching and testing, as well as to fishes that are studied in their natural habitats.

Ethical Overview

As for any other vertebrate species used in teaching and research in Canada, investigators using fishes are required to use the most humane methods on the smallest number of animals necessary to obtain valid information. This requires the use of a sound research strategy, including: identification of key experiments that determine whether a particular line of enquiry is worth pursuing; use of pilot studies; staging of *in vitro* to *in vivo* experiments where possible; and implementation of staged increase in test stimuli where possible. The numbers and species of animals required depend on the questions to be explored.

Field studies, aquaculture studies and laboratory studies require different statistical designs; field studies and aquaculture production typically require the use of larger numbers of animals. The life stage of the fishes used in each study will also affect the numbers of animals needed. Studies of early life stages typically require large numbers of individuals. In all cases, studies should be designed to use the fewest animals necessary. Researchers are encouraged to consult with a statistician to develop study designs that have the appropriate statistical power to accomplish the research objectives. Use of fish in teaching and research in Canada also requires adherence to the following principles:

- animals must be maintained in a manner that provides for their optimal health and wellbeing, consistent with the demands imposed by the experimental protocol;
- animals must not be subjected to pain and/or distress that is avoidable and that is not required by the nature of the relevant protocol;
- expert opinion must attest to the potential value of studies with fishes;
- if pain or distress is a justified component of the study, the intensity and duration of pain/distress must be minimized; and an animal observed to be experiencing severe, intractable pain and/or distress should immediately be euthanized using an approved method of euthanasia.

Meeting the principles outlined above requires that fishes be accorded the same degree of care as other animals under the CCAC system. Any factor that disturbs the normal physiological balance of an animal has an effect on the studies being conducted, and therefore should be avoided or minimized for scientific as well as ethical reasons, unless the factor itself is the subject of investigation.

Fishes comprise a great number of species, each with specific anatomical, physiological and behavioural characteristics. Investigators and animal care staff should therefore acquaint themselves with the characteristics of the species proposed to ensure that appropriate facilities and husbandry procedures are in place prior to obtaining the animals.

Government Regulations and Policies on the Use of Fish

Anyone acquiring or transporting fishes, or conducting research on fishes, must be familiar with, and comply with, relevant international, federal and provincial/territorial legislation and policies governing the capture of fishes and/or their transfer from one water body or jurisdiction to another. It is important to verify current regulatory information with the regulatory agencies identified below to ensure compliance with current legal requirements.

International. There exist several international agreements, codes, and conventions that relate to the introductions and transfers of aquatic organisms. Requirements are typically incorporated through domestic legislation. Therefore, for activities occurring in or otherwise pertaining to Canada, verification with Canadian authorities and compliance with Canadian laws should ensure compliance with international standards. Some examples of international agreements, codes and conventions include:

- Convention on Biological Diversity (CBD)
- Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)
- Code of Practice for Introduction and Transfer of Marine Organisms
- International Aquatic Animal Health Code for Finfish, Molluscs and Crustacea
- Sanitary/Phytosanitary (SPS) Agreement
- Canada-USA Free Trade Agreement (FTA) and North American Free Trade Agreement (NAFTA)
- North American Commission (NAC) (Canada and USA) of the North Atlantic Salmon Conservation Organization (NASCO)
- International Joint Commission
- Organisation for Economic Cooperation and Development (OECD)

Re-introduction and release

In most instances, fishes used in research may not be released into the environment. However, in special cases such as telemetry studies involving the capture and release of wild fishes, appropriate permitting is required before capture and release is carried out through the I&T code of practice. Fishes involved in such studies may also require permanent marking or tagging if they have been subjected to treatment with anaesthetic agents or other drugs with withdrawal time requirements. In each province and territory, there are I&T Committees that evaluate requests to introduce or transfer fishes in accordance with the Fishery (General) Regulations. Regulations applied by the I&T Committees in each province/territory may differ. As a watershed may have a disease profile different from other watersheds, fishes can be moved only between watersheds of similar disease status and the permit must accompany the shipment (one for each shipment of live fish, fertilized or unfertilized sex products, or products of fish). I&T Committees also review the potential genetic and environmental impacts of such transfers.

Containment

There are several requirements for containment under various federal and provincial/territorial regulations. I&T Committees may issue transfer licenses with conditions listed (e.g., containment requirements). DFO and the Canadian Food Inspection Agency (CFIA) are currently developing containment guidelines for fish pathogens which will be implemented through regulations. In addition, information is being developed on containment requirements for research involving animal products of biotechnology including genetically modified fish.

Facilities conducting research involving fish pathogens are required to consult CFIA Biohazard Containment and Safety Division, to ensure appropriate levels of bio-containment are in place. Where this involves the holding of live aquatic animal hosts, pathogen challenges or treatment trials, DFO provides science advice to CFIA.

First Nations

The Aboriginal Communal Fishery Licenses Regulations (laws.justice.gc.ca/en/F-4/SOR-93-332/index.html) describe communal licenses for aboriginal communities for fishing and related activities. These regulations are under the Fisheries Act and are referenced in various provincial and territorial regulations.

Provincial/territorial

Provinces and territories also have legal requirements that may need to be met in relation to activities involving fish and aquatic ecosystems. Investigators who are uncertain of which agencies they are required to contact for appropriate regulations and permits should contact their provincial/territorial governments.

Municipal

Many municipal governments have regulations governing the holding and use of fish within municipal boundaries, as well as regulations pertaining to effluent and disposal of hazardous waste. Investigators must ensure that they adhere to appropriate municipal by-laws.

Aquatic Facilities

Aquatic facilities are complex systems that must be well designed to minimize stress to fishes, promote efficient operation of the facility, and ensure a safe working environment for personnel.

A healthy fish population is dependent on a stress-free environment, which in turn requires the best possible facilities and water system. Water provides the supporting medium for all aquatic species and serves two essential purposes: the provision of oxygen for all life processes, and dilution and removal of metabolic wastes. Both of these functions must be thoroughly addressed in any fish-holding facility. This section is primarily targeted to land-based holding systems such as the GLIER and Biology facilities. However, the provision of a stress-free environment is equally applicable to outdoor ponds as well.

Water Supply. Aquatic facilities should minimize pathogens in the water intake and prevent biofouling in water intake and distribution systems. Our facilities use municipal water but Detroit river water is also available in the Biology building from approx. May – October as the need arises. In the latter case the water quality may be quite variable due to the presence of land runoff, shipping waste (i.e. bilge water and fuel spills), or be subject to breaking waves or ice damage.

Water Quality. Water drawn from either the river or municipal source should be tested for, and treated to remove, contaminants and pathogens. A comprehensive analysis of water quality parameters (ions, pH, metals, etc.) should be conducted before a fish holding facility is used within a particular location or building to ensure that the water supply is suitable. This analysis is usually the responsibility of technician in charge of the facility. Water quality guidelines for the protection of aquatic life have been developed by the water quality task group of the Canadian Council of Ministers of the Environment (**www.ec.gc.ca/** CEQG-RCQE/English/Ceqg/Wa t e r /

default.cfm#dri) and may provide guidance on acceptable levels of contaminants. Depending on the reliability of the water source, further testing may be needed, for example on an annual basis. To protect fish from potential contaminants, other measures, such as a carbon filtering system or a reverse osmosis system, may be required where problems from the water source have been detected. Culligan water purification systems are available in both GLIER and the Biology building for the removal of unwanted chemical contaminants. Seasonal factors such as phyto- or zooplankton blooms, seasonal water mass turnovers and lake turnovers can have periodic effects (scales of hours, days or months) for river water, and these need to be anticipated.

Mesocosms. When dealing with large volumes of water (e.g., off-site ponds) to recreate aquatic ecosystems (mesocosms), it should be recognized that such sites may not operate in the same way as smaller tanks usually found in aquatic facilities. In particular, special considerations are needed depending on the material the mesocosm is made from (e.g., dug ponds and floating marine bag systems have very different requirements): water turnover rates may not be calculated the same way as smaller holding tanks; special requirements may be needed for cleaning and removal of debris and for catching the fish; and considerations need to be given to the impact of solar exposure (e.g., build up of algae). As for aquatic facilities such as those in GLIER and Biology, they are operated in a manner that does not negatively impact on the health and well-being of the animals.

Fish Housing

Aquatic environments must meet the established physical and behavioural requirements of the fishes in terms of shelter, social grouping, overhead cover and lighting. Many fishes have requirements for environmental conditions that will provide optimal welfare. Social behaviour and social influences on behaviour can be quite complex, and require researchers and animal care staff to have a good understanding of the species-specific requirements of the animals. Where the environmental requirements of fish are not well known, as far as possible the holding conditions should be used that approximate the source environment. Consideration should also be given to population densities, water flow rates or other physical features that may have an effect on social interactions. However, adding complexity to the aquatic environment should be balanced against the need to maintain a high standard of water quality.

Tanks

Several aspects of tank design need to be considered for housing of fish. They are:

- The shape, colour, depth and volume of tanks should be appropriate for the species and life stage being held.
- Tanks should have smooth, inert, sealed interior surfaces.
- Tanks should be self-cleaning, or adequate means for the regular cleaning of tanks should be incorporated into the design.
- Tanks should be equipped with a covering that prevents fishes from jumping from the tank, e.g., tank nets or rigid coverings.

Facility Management, Operation and Maintenance

Well managed aquatic facilities have a regularly scheduled preventive maintenance program for all life support systems, as well as an annual maintenance program for all equipment and for surfaces. The day-to-day operation of the facility, such as scheduled sanitation measures, feeding schedules, and environmental and fish health checks, are conducted by the animal care technician following standard operating procedures (SOPs) for management of the facilities.

Security and Access

Security and limited access to the fish holding facility requires the following considerations.

- Accesses to fish facilities are restricted to those personnel required for maintenance of the facility and the care of the fishes, and those using the facilities for experiments or teaching.
- A security system must be in place that is appropriate for an aquatic system.
- Personnel should access fish facilities only when necessary.
- Movement through the facility should be from the cleanest areas to the dirtiest areas.
- Facility-specific clothing and footwear should be worn in the entry area, and personnel should wash their hands when they enter and leave the facility.

General Maintenance of the Facility

All engineering specifications and drawings of the facility are available to the technicians in charge of running the facility, as well as all operating manuals for special equipment such as pumps, chillers and computer control systems.

Aquatic facilities must have written maintenance schedules developed specifically for the facility(i.e., GLIER and Biology Building). A documented preventive maintenance program is required for all life support systems. All electrical equipment, life support equipment and air and filtration systems should be checked and serviced at regular intervals. Checklists should be available to ensure the duties have been completed and that there is a record of all service.

Facilities must be kept clean and orderly. Tanks should be disinfected before and after every experiment.

The staff responsible for operating an aquatic facility in GLIER and Biology must have specialized knowledge, experience and training for proper function, operation and maintenance of the water system.

Staff are available for animal care and facility management and maintenance 365 days a year for both routine and emergency needs. Trained staff are on call or accessible 24 hours a day, 7 days a week.

Environmental Monitoring and Control

An environmental monitoring system is essential for aquatic facilities. Water management involves a great many mechanical and electrical components, the malfunction of which can quickly result in stressful and possibly lethal consequences for fishes. The GLIER and Biology facilities have simple systems where unexpected environmental changes are unlikely to occur. In GLIER and Biology routine monitoring occurs daily by visual system and animal checks, and limited testing is done with hand-held equipment such as a thermometer, dissolved oxygen (DO) meter and a pH meter.

Water quality parameters should be monitored at an appropriate frequency for the facility, and should allow predictive management of water quality, rather than only reactive management of crises in water quality. Parameters that need to be measured and the frequency of measurement vary greatly, depending on whether the system is an open or a recirculation system. At a minimum, environmental monitoring systems should provide information on water flow or oxygen saturation and water temperature.

Water quality must be monitored and maintained within acceptable parameters for the species being held. The most common water quality factors known to affect fishes are temperature, dissolved oxygen, pH, suspended solids/sediment, carbon dioxide, ammonia, nitrite, nitrate and chlorine. For some aquatic species copper levels need to be controlled. These must be monitored on a regular basis, especially in closed recirculation systems. The definition of acceptable range is complicated by the fact that appropriate conditions are not well-defined for many species and the requirements of individual species may vary between different life stages (e.g., larvae, juveniles and adults) or according to physiological status (e.g., spawning, feeding and previous history of exposure). Water quality is the most important factor in maintaining the well-being of fishes and in reducing stress and the risk of disease. Fishes show varying degrees of flexibility to changing water quality conditions. Some degree of acclimation may be necessary when transferring fishes and this should be carried out over as long a period as possible.

Temperature

Fishes should not be subjected to rapid changes in temperature, particularly to rapid increases in temperature. Fishes are ectothermic, meaning that their body temperature is similar to that of their environment. Therefore, water temperature is a very important water quality parameter and highly species specific. All vital functions are influenced by body temperature and the rates of these functions increase or decrease according to the surrounding water temperature. All species of fishes have a specific temperature range in which they function normally and maintain good health. The temperature tolerance ranges of fishes vary greatly, both among species and life history stages, and fishes are often referred to as cold (0 to 10ºC), cool (10 to 20ºC) or warm (20 to 30ºC) water species, depending on the thermal regime to which they are naturally adapted. Most fish species can tolerate a range of temperatures, although each fish species has its own Standard Environmental Temperature. The term "rapid changes" is very species specific. Even within the same species it varies, as it is dependent on the temperature of the water in relation to the thermal maximum for the fish. This is different between summer and winter. As a general rule of thumb, temperature changes should not exceed more than 2ºC per 24-hour period.

The term "acclimation" is used widely to describe any "adaptation" to changed circumstances. However, it must be understood that true acclimation of fishes to a new temperature is a process that involves production of new variants of many metabolic enzymes, changes in lipid types and actual cellular restructuring. One to two weeks acclimation may be fine for salmon at 10°C, but at 5°C acclimation is likely to take longer. It will vary widely among species as to rate and scope; some species simply lack the ability to acclimate to the new temperature.

Oxygen

Fishes should be kept in water with an adequate concentration of oxygen. In most species, water O2 saturation levels should be above 90%, although some species thrive at lower O2 concentration. In general, cold water fishes have lower tolerance for low oxygen levels than warm water species of fish. Oxygen concentration will vary according to temperature, atmospheric pressure and salinity. As the temperature increases, the water's capacity to carry oxygen decreases; in addition, the fishes' demand for oxygen increases due to an increase in metabolic rate. A variety of other factors can dictate the amount of oxygen required by a fish. The congregation of fish at the tanks' water inlet or gasping behaviour at the surface is an indication of insufficient oxygen. In some instances, low O2 levels can be remedied by aeration, reducing the stocking density and decreased feeding. Balancing these variables is essential to prevent low O2 levels. Airstones can be used to improve the aeration of the water; however, placement and type of stone should be chosen so as not to disrupt the self-cleaning action of the tank. Where necessary, supplementary oxygenation of tank water should be provided.

pH

Water pH should be maintained at a stable and optimal level as changes in pH may influence other water quality parameters. The pH of water will vary greatly according to its source and composition. In addition to the influence of naturally occurring minerals (e.g. contact with silicates will lower the pH, whereas water flowing through carbonate rock will have a higher pH), industrial pollution will alter pH levels of water supplies. A number of factors can influence pH, including the addition of sulphur dioxides and nitrogen oxides, sewage and agricultural run off, compounds that are added or become introduced to the water, and CO2 from the atmosphere and from the respiration of the fishes. Most fishes can adapt to a wide range of pH, provided that any pH change is gradual. The majority of freshwater species live in waters with pH values ranging from pH 6 to 8. Outside the range of pH 6 to 9, freshwater fishes become stressed, grow slowly and are prone to infectious diseases. The optimal pH range for maintaining freshwater fishes is between 6.5 and 7.5. Changes in pH, especially when becoming more acidic, can be limited by carrying out partial water changes (i.e. 10% over a two-week period). An alternate but less effective method is the addition of sodium bicarbonate to add carbonate buffering capacity. Most importantly, water pH and ammonia levels are closely related, which is especially relevant in recirculation systems. Ammonia is much less toxic at lower pH levels. For this reason, it is advisable to maintain the systems at the lowest pH suitable for the species.

Nitrogen compounds

Free ammonia and nitrite are toxic to fishes and their accumulation must be avoided. Ammonia toxicity is extremely pH dependent, and therefore, control of pH and feed management are critical in minimizing ammonia accumulation. One of the main excretory products of fishes is ammonia which is discharged into the water through the gills and urinary tract. Dissolved urea, as well as particulate wastes (feed and faeces), are converted to inorganic compounds such as ammonia and phosphate. Other sources of ammonia may be from contamination of water by organic compounds such as antibiotics, paint fumes, fumes from ammonia-based cleaning compounds and insecticides. In aqueous solution, ammonia is present in two forms: ionized (NH_4^+), and non-ionized or "free" (NH_3). The term ammonia refers to the sum of the total concentration of the ammonium ion (NH_4^+) plus the concentration of free ammonia (NH_3). NH_3 and NH_4^+ are in constant equilibrium. It is much more important to know the concentration of non-ionized ammonia, as only the non-ionized form is toxic to fishes. Other measures of minimizing ammonia include increased flushing rate, biofiltration (i.e. filtering the water through a matrix of nitrifying bacteria to convert ammonia to nitrite and then immediately to nitrate), fish density or temperature reductions, or use of ammonia-absorbent compounds in fresh water.

Media such as zeolite, crushed oyster shell, sand, dolomite, and pea gravel, or synthetic substances (e.g., styrofoam beads, plastic rings or fibre filters) are good adsorbents. In general, the larger the surface area to volume ratio of the filter, the better the filter, bearing in mind that an effective filter needs to optimize the balance between water flow and surface area. However, small particles such as sand can clog the system and therefore sand-based filters are more difficult to maintain.

Carbon dioxide

Carbon dioxide is produced by fishes during respiration and dissolves in water to form carbonic acid, thus lowering the pH and increasing the potential for hypercapnea. Although high CO_2 concentration can be fatal to fishes, in general this is not likely to be a problem in aquatic facilities, provided there is adequate ventilation. However, when recirculation technology is applied to high density fish culture systems, CO_2 may become the major limiting factor.

Salinity

Salinity changes are inherently stressful for fishes, and should be conducted slowly and with attention to the physical status of the fishes. Salinity requirements of fishes vary according to whether they are marine or freshwater in origin. Some species are able to tolerate a wide range of salinity; others can tolerate only narrow ranges of salinity. In others, salinity tolerance may vary according to life stage (e.g., Atlantic salmon). Maintaining fishes at a suboptimal salinity can result in osmoregulatory stress, impaired growth rates and reduced disease resistance. However, it should be noted that rapid, dramatic, short-term salinity shifts are sometimes used as therapeutic treatments.

Toxic agents

When there is reason to believe hazardous materials or infectious agents have accidentally entered the water system, that system should be isolated and tested. A host of infectious and toxic agents exist that are potentially harmful to fishes. Susceptibility to both chronic and acute toxicants varies with species, life stage, acclimation conditions, and other environmental conditions (e.g., temperature, water hardness, etc.). Common problems encountered in water systems include toxicity from chlorine and other additives, copper from copper pipes, and gas supersaturation. Systems that rely on ambient fresh water may undergo seasonal increases in bacterial burden and the presence of pathogenic bacteria often originating in sewage. Other problems can result from the use of fly sprays, paints, solvents, etc. For most toxic agents, there is often no local expertise in regulatory or university departments to recognize when such problems exist, to perform the analyses needed to verify and quantify the problem, or to recommend or implement solutions in a timely fashion. When a toxic agent is known to have entered the system, there are rarely defined solutions, other than to "flush the system". The laboratory personnel are most likely to be able to deduce what the problem is (e.g., copper from a new source of copper pipe); however, other experts should be consulted for advice or help, including veterinarians, fish health experts, regulatory personnel or analytical laboratory personnel.

Chemical products should be safely stored away from the aquatic housing area and the water supply. As many paints, insecticides, cleaners, fixatives, adhesives, caulking agents or solvents are toxic, great care should be taken in their use around aquatic facilities.

Occupational health and safety guidelines and other regulations (e.g., dangerous goods regulations) must be followed when storing chemicals.

Capture, Acquisition, Transportation and Quarantine

Capture of Wild Stock

Wild fishes should be captured, transported and handled in a manner that ensures minimal morbidity and mortality. Research goals will generally dictate the appropriate sampling method; however, investigators should select the method that has the least impact on the fishes and on the local ecosystem.

The stress of collecting, handling and transporting fishes from the wild can make them susceptible to disease. Modifications to capture techniques may be required to improve survivability of the fishes. In particular, electro-fishing is a stressful procedure, leading to acute physiological disturbances, increased susceptibility to predation and significant effects on other taxa in the vicinity. Less stressful procedures should be used whenever possible.

Acquisition of Hatchery Fish

Fishes may come from hatcheries with defined health status and preferably known genetic history. In the interests of obtaining high quality research animals, high quality fishes should be sourced from reputable fish suppliers. Where possible, site visits of the hatcheries should be carried out, in order to provide quality assurance of their processes and practices.

Transportation

High survival rates should be obtained even when fishes are transported long distances. Success in transporting fishes requires preventing the physiological problems which can be caused when relatively large numbers of fishes are held in relatively small volumes of water.

The major challenge in transporting fishes is the maintenance of appropriate water quality. A life support system must be provided that will prevent adverse water quality changes and meet the physiological requirements of the fishes. Some of the critical elements required for fish transportation are:

- The transportation container should be well insulated to minimize temperature changes during transport; in some cases heat or refrigeration may be required to ensure temperature is maintained within the appropriate range for the species.
- All containers should have opaque lids to minimize water slop and loss of fishes, and to reduce light levels within.
- Whenever possible before transport, fishes should be fasted for 12 to 48 hours, depending on species, age and water temperature, to ensure an empty gut and minimize nitrogenous waste and water pollution, and to conserve metabolic energy.
- It is common practice to cool the water temperature to reduce fish activity and metabolism during transport.
- Whenever possible, water testing instruments, such as dissolved oxygen meters, should be used throughout the transport.
- For long hauls when fish densities are very high, it may be necessary to remove nitrogenous products using circulation and filtration.
- Small quantities of fishes can be transported in plastic or polyethylene bags under an atmosphere of pure oxygen.

Sedation of fishes prior to and during transport may be useful in reducing sensory awareness, and hence mitigating the stress of transport. The level of sedation should be sufficiently light to allow the fishes to maintain equilibrium, swimming and breathing.
The choice of anaesthetic agent for sedation is important, as some anaesthetics which are effective in the rapid induction of deep anaesthesia (e.g., TMS and 2-phenoxy ethanol) have an excitatory effect during initial absorption, which defeats the purpose of calming the fishes. Metomidate is the most appropriate choice for sedation during transport.

Quarantine and Acclimation

After transport and before use in experiments, fishes should be acclimated to laboratory conditions during a period of quarantine and acclimation. A combined approach for acclimation and quarantine should be used as far as possible so that both are accomplished simultaneously

As far as possible, fish from various sources should not be mixed.

Quarantine

Quarantine areas should be subject to extra vigilance in monitoring fish and good record keeping to detect and respond to any health problems in quarantined fish.

The purpose of quarantine after receipt of shipments of fish is to isolate those fish from the main populations in the facility to permit observation and testing until such time as the newly arrived fish are determined to be healthy and free from communicable disease. Thereafter, these fish can be integrated into the populations of the facility.

The duration of quarantine should be appropriate to assure the health of the fishes. Quarantine areas should be managed according to rigorous infectious agent control practices. Particular vigilance should be paid to practices such as effluent disinfection, footbaths, hand washing stations, dedicated accessories (such as nets) and hand implements, and clean to dirty traffic flow in the quarantine area, in order to avoid the potential transfer of pathogens to the main areas of the facility.

Acclimation

On arrival in a facility, new fishes should be handled as little as possible, and care should be taken to prevent thermal shock. For practical purposes, thermal shock may be defined as an abrupt change in temperature of more than 2 or 3°C. If fishes have been transported in plastic bags, the bags should be floated in the receiving tank until the temperature has equilibrated. Ideally, fishes transported in tanks should be adapted to their new environment by slowly transferring water from the new system into the transport tank. When fishes arrive in poor quality water, where the stress of staying in the poor water exceeds the physiologic impact of the transition to good quality water, the fishes must be removed immediately. Acclimation involves ensuring a gradual adjustment of the living conditions for the fishes.

Husbandry

Record-keeping and Documentation

Detailed Standard Operating Procedures should be developed for the maintenance and care of all fishes and for sanitation of tanks, rooms and equipment. Researchers should follow a facility Standard Operating Procedures (SOPs) manual for acceptable fish husbandry practices and standards. In particular, SOPs should be available to ensure that tanks are properly disinfected and kept clean between experiments.

Assessment of fish well-being

Basic physical and behavioral parameters indicative of well-being in fishes should be monitored daily and written records should be maintained. Any perturbation of these parameters should be investigated and the causes identified and corrected. It is important to be aware of an animal's state of well-being, both before and during the conduct of any studies. The objective evaluation of key behavioral and physical variables should help in the detection of abnormalities, whether related to environmental or husbandry factors, or to the effects of experimental procedures themselves. The assessment of well-being in fishes is challenging because their responses to adverse conditions are not always displayed, as occurs in mammals, and because significant observational restrictions are imposed by the rearing environment itself.

Density and Carrying Capacity

Each species should be housed at a density that ensures the well-being of the fish while meeting experimental parameters. However, in some cases, the ideal environment for the care and use of fish in research, teaching and testing, for a given species will have to be developed using performance-based criteria such as growth rate. Established maximum densities should not be exceeded. The number of fish that can be carried in a given water supply is extremely variable and depends on the species, water temperature, pathogen load, dissolved oxygen level, metabolic rate of the fish, feeding rate, and how fast the water is being exchanged. It is important to recognize that there are profound effects of both maximal and minimal densities; below certain densities territorial behaviour may increase (for example, in salmonids housed below minimal densities, feeding is diminished). To prevent problems in feeding due to territoriality and aggression when dissimilar sized fish are housed together, the fish should be graded periodically to ensure similar sizes within groups.

Food, Feeding and Nutrition

Although fishes brought in from the wild generally prefer live feed to formulated feed, most learn to feed effectively on pellets and show remarkable flexibility in their ability to ingest and digest formulated feeds. The acceptance of feed depends upon chemical, nutritional and physical characteristics of ingredients selected for feed formulation as well as feed processing. The structure and function of their digestive systems influences the patterns of food intake and digestive efficiency; meal sizes and feeding frequencies should be set accordingly.

Nutrition. Nutritionally balanced diets and appropriate feeding regimes are critical in ensuring that fishes remain healthy. Commercially manufactured fish feeds contain nutrients and energy sources essential for growth, reproduction and health. Essential nutrients include protein, amino acids, lipids, fatty acids, vitamins and minerals. Deficiency of these nutrients can reduce growth rate and feed consumption and lead to diseases. As fishes are ectothermic, their metabolic rate is determined by the water temperature. Therefore, feeding rates and quantities need to take temperature into consideration.

Food and feeding. Fish feed should be purchased from sources that manufacture feed according to standards employed in the feed industry for fish and other domestic animals, and according to published nutrient requirements for the species, if available.

Feed bags should be labeled with date of manufacture and guaranteed analysis information. Small aliquots of feed should be retained for independent testing when large feed lots are received.

Feed should be stored in dedicated areas that are dark, temperature and humidity controlled, and pest-free to ensure its nutritional quality. Feed for immediate use and feed in feeders should be similarly protected. Feed used for daily feeding should be kept in sealed-top containers to protect it from humidity and light, and frequently replaced with feed from storage. All feeds, whether moist, semi-moist or dry, are susceptible to degradation with time. Moist feeds containing minced raw fish or ensilaged fish should be fed within a few hours or frozen. Dry feeds should be stored at temperatures $< 20°C$ and humidity $<$ 75%. High humidity increases susceptibility to mould, and high temperatures destroy certain vitamins and enhance the degradation of lipids. Vitamins in feeds can also be destroyed by oxygen, ultraviolet light and lipid peroxidation.

Fishes must be fed at appropriate intervals and with a nutritionally adequate, properly sized feed. Optimal feeding techniques are essential for good health and well-being, and to prevent the fouling of water with uneaten feed.

Whether fishes are fed manually or automatically, they should be observed regularly to determine whether they are responding as expected, and whether the ration is sufficient or overfeeding is occurring.

Most fish can survive for long periods without feed and, in most instances, lack of food for a few days will not be overly distressful. Overfeeding, on the other hand, causes serious problems because of its effects on water quality and the stimulation of potentially harmful bacterial and fungal growth.

Larval weaning

It is recognized that early life stages of many species have high natural mortality. Failure to begin feeding or to acquire sufficient food has often been suggested as a major cause of early mortality. The switch to an exogenous feed supply is a crucial stage for fish, particularly for marine fish. This transition is the major feature used to define the end of the embryonic period. In general, highly fecund fish producing small eggs, for example cod, haddock and flounder, are small at hatching, the yolk sac stage is shorter, and they are more difficult to rear on artificial diets.

The most crucial factor in the weaning process is the provision of live invertebrates, such as rotifers and *Artemia* of the preferred size. Generally these food organisms are enriched with limiting nutrients (e.g., essential fatty acids and amino acids) to provide larval growth and survival. Young fish, if deprived of exogenous food, will reach a point of no return when the effects of food deprivation become manifest as irreversible starvation.

Broodstock and Breeding

Holding systems and environmental conditions for broodstock should be appropriate for the species. Particular attention should be paid to the importance of environmental cues for the maintenance (or manipulation) of endogenous reproductive rhythms. Environmental factors, such as temperature, photic environment, habitat/tank design, nutrition, holding density and species mix, are critical to reproductive success.

Where possible, rational genetic management of brood stock should be used. For brood stock, a strict disease and health control program should be implemented with veterinary advice to ensure the production of healthy progeny and prevention of disease transfer through water sources, fish or eggs.

Induction of spawning

The induction of spawning through administration of injectable hormones is a common practice in broodstock management. The literature should be consulted to ensure doses and regimes are consistent with established standard methods. Treated broodstock fish should be retained for the necessary withdrawal times if they are to be killed for food. Induction of spawning may result in morbidity and mortality due to retention of ova and other unforeseen effects. Personnel working with chemically induced spawning fish should be aware of the complications associated with hormone treatment.

Health and Disease Control

Fish Health Program

All facilities must have a fish health monitoring program. Under conditions of confinement in an artificial environment, fish are sensitive to variations in water quality, nutrition, presence of pathogen(s) and management practices of the facility. The expression of disease, whether infectious or non-infectious, cannot be considered in isolation from any of these factors. Microorganisms are distributed throughout any aquatic environment; however, their presence may only be obvious under sub-optimal environmental conditions.

Injuries and other disorders

Handling injuries

Handling procedures should be carried out only by competent individuals using techniques that minimize the potential for injury. Efforts should be made to minimize morbidity and mortality caused by osmoregulatory compromise, systemic acidosis, and opportunistic infections of damaged skin that can result from handling and traumatic injuries. Traumatic injuries can result from handling procedures or abrasions from contact with tanks and equipment, other fish or predators. Malfunctioning equipment or inexperienced fish handlers can turn routine procedures into events that cause disease outbreaks. Some factors that can increase the risks to fishes during handling include:

- malfunctioning or improper equipment;
- inexperienced fish handlers;

• dry or abrasive surfaces which fish will contact
during handling, such as measuring boards, balances, etc.
• warmer water temperatures;
• prolonged handling times; and
• repetition of procedures on the same individual.

Feed-related disorders

Nutrition can also influence the health of fish by causing nutrient deficiencies, imbalances or toxicoses, or by introducing infective agents

Handling and Restraint

Fishes should be fasted prior to handling. Defecation and vomiting are responses made by fishes in the interest of conserving metabolic energy stores when exposed to acute stress. Digestion requires body energy; when there is immediate danger, animals get rid of undigested food so as to maximize the energy available to flee, fight or recover from injuries. Fasting fishes ensures that digestion does not consume energy during handling, and that the fishes are left with energy stores to assist in recovery. Additionally, fasting somewhat reduces ammonia output from the fishes and lessens the risk of bath contamination from gut contents. Gut emptying times are longer for larger fishes and colder temperatures. High quality water for procedures and recovery should be provided so that any gut emptying that does occur does not cause welfare problems for the fishes; in particular, this strategy should be used so that any diagnostic or therapeutic intervention is not delayed.

- Personnel involved in handling fishes should undergo training in methods to ensure their expertise and to minimize injury and morbidity to fishes in their care.
- Fishes should be handled only when necessary, and the number of handling episodes should be minimized.
- Fishes should be handled in a fashion that minimizes damage to their mucus-skin barrier.

Prolonged physical restraint of unsedated fishes should be avoided as damage to skin and mucous membranes may result, as well as myopathy. This is particularly true for salmonid species; more sedentary fishes appear to be less stressed by physical restraint.

Restraint of dangerous species

Those who work with dangerous species must be trained and competent to do so. Appropriate emergency items (e.g., antivenom, an appropriate first aid kit, etc.) must be on hand. In general, dangerous species will be encountered only under field conditions; however, the recommendations are equally applicable to the laboratory situation. Dangerous species should be handled in a manner that is safe both for the investigator and for the animal being handled. Procedures should minimize the amount of handling time required and reduce or eliminate contact between the handler and animal. Investigators should never work alone when handling dangerous species. A second person, knowledgeable in the capture and handling techniques and emergency measures, should be present at all times.

Restricted Environments

Every effort should be made to provide fishes held in restricted environments with as nonstressful an environment as possible, within the constraints of the experimental design. Fish are frequently kept in physically restricted environments, such as metabolic chambers, swim tunnels and calorimeters, for long periods. These fish should be accustomed to the restricted environment before the study, and should be kept in such environments for the shortest duration possible. Fish that fail to thrive in these environments should be removed.

Surgery

Surgery should be performed by individuals with appropriate training. Surgery in fish can be complex and intricate. Anyone attempting any invasive surgery should be properly trained in surgical aseptic technique, or should obtain the services of a veterinary surgeon. Fish surgery should normally be covered under the institutional veterinary care program.

Before surgery is attempted on living animals that are expected to recover, suture and surgical techniques should be practiced on inanimate materials or dead specimens until competency is attained. Practice using cadavers and non-survival trials can be useful in training investigators. Appropriate training and practice will help to minimize anaesthetic and surgical time, and contribute to a faster recovery of the animal.

Surgical sites should be prepared in a fashion that minimizes tissue damage and contamination of wound areas.

Attention should be paid to the use of asepsis, disinfection and the use of sterile instruments to minimize wound contamination and maximize the healing response. Instruments should be cleaned and sterilized between surgical procedures and if inadvertently contaminated.

During prolonged surgery, water quality should be maintained at a high level, with minimal bacterial and organic burden. Water for anaesthesia should be from the same source as the tank water to minimize shock caused by differences in temperature, pH, electrolytes, etc.

Anaesthesia

Anaesthetics should be used in experiments where there is expected to be noxious stimuli, and in experiments entailing extensive handling or manipulation with a reasonable expectation of trauma and physiological insult to the fish. The use of anaesthetics facilitates work with fishes and is required for invasive studies such as surgical preparations for physiological studies, where the fish must be held immobile for extended periods of time. Sedation is also used for the manipulation of animals during procedures such as transport, grading or vaccination. Although the use of anaesthetics is primarily for holding fishes immobile while being handled, it is also used to lower the level of stress associated with such procedures and may alleviate pain.

Anaesthetic overdoses are also used routinely as an effective and humane means of euthanizing fishes.

Anaesthetics should be chosen on the basis of their documented ability to provide predictable results, including immobilization, analgesia and rapid induction and recovery, while allowing for a wide margin of safety for the animals and the operators. The investigator should ensure that the anaesthetic selected has no toxic side-effects for the fish or the handler, is biodegradable and can be cleared from the fish, and has no persisting physiological, immunological or behavioural effects. Investigators should also be aware that currently only TMS (MS-222) and metomidate are registered for veterinary use with fish in Canada. Investigators are individually responsible for the use of anaesthetic agents not approved for veterinary use in Canada.

Personnel working with anaesthetic agents in fish must be adequately trained and protected with personal protective equipment. Many of the anaesthetics in use have the potential to cause harm to humans if they are misused.

Surgical equipment

Any incisions should avoid the lateral line and should follow the longitudinal axis of the fish. Some fish scales may need to be removed following the skin preparation. Scales should be removed individually by pulling in a posterior direction to minimize damage. Only the scales necessary to create the incision should be removed as the scales provide protection and stability to the wound area. The epidermis and peritoneum layers are easily torn, but the overall skin is tough due to a layer of dense collagen in most species. Practice is necessary to obtain the optimal scalpel pressure to achieve a clean incision and hence achieve rapid healing.

In general, strong, inert, non-hygroscopic monofilament suture material and atraumatic needles should be used for closure of incisions in fish skin.

The skin of most teleost fishes consists of epidermis with scales, dermis and hypodermis; all layers are closely associated with one another and with underlying muscle, peritoneum and other layers. Single interrupted sutures to appose all layers are sufficient as epithelial cells migrate rapidly to cover an incision, protecting the fish from the effects of the aquatic environment. Closure of individual layers is recommended only for very large fish.

In general, fish skin heals faster than mammalian skin; however, fibrous proliferation can be slow and varies with temperature. The speed with which the fibrous proliferation closes the internal wound and provides sufficient strength to replace the sutures is temperature dependent, and should be considered when selecting appropriate suture material.

Postoperative care

Little is known about the effect of analgesic drugs on fishes. However, investigators are encouraged to use post-operative analgesia where appropriate as suitable analgesic agents become available. Fishes do appear to produce opioid substances in response to pain and fear, similar to higher animals, i.e. Substance P, enkephalins and ß-endorphins, and the response of goldfish to analgesia has been shown to be similar to that of a rat. The response of carp (*Cyprinus carpio*) to electric shock, to the presence of alarm substance chemicals in water, and to hook and line fishing indicates that reactions to repeated shocks is graded, non-reflexive, and similar to that in mammals.

Recovery tanks should be designed to promote smooth recovery with reduced risk of long-term effects from anaesthesia. Considerations for suitable recovery tanks include opportunity to observe subjects, good quality uncontaminated water (with removal of excreted anaesthetic), avoidance of environmental stimulation, consistent temperature, and decreased exposure to other compromised fish which may be a source of infectious disease agents.

Injection

Care should be taken during injection to introduce the needle in spaces between the scales. Intramuscular injections may be made into the large dorsal epaxial and abdominal muscles, taking care to avoid the lateral line and ventral blood vessels. Intraperitoneal (IP) injections should avoid penetrating abdominal viscera as substances that cause inflammation may lead to adhesion formation. The most useful routes for injection in fish are intravascular, intraperitoneal and intramuscular.

Tagging and Marking

Tagging and marking techniques are used in both field and laboratory studies. When choosing a marking method, primary consideration should be given to methodologies that are not invasive, do not require recapture for identification, and will remain visible for the duration of the study. Where possible, investigators are encouraged to use natural features as marks, rather than removing or damaging tissues or attaching auxiliary markers.

Investigators must aim to minimize any adverse effects of marking and tagging procedures on the behaviour, physiology or survival of individual study animals. Where such effects are unknown, a pilot study should be implemented.

Collection of Body Fluids

Sedation or anaesthesia should be used to restrain fish for collection or cannulation purposes. It is important to realize that both restraint and anaesthesia may alter physiological parameters such as serum glucose and various hormone levels.

Blood collection should only be undertaken by trained personnel using sterile equipment. Blood may be collected by a number of routes including the ventral tail vessels and dorsal aorta, and by cardiac puncture

Endpoints and Criteria for Early Euthanasia

Investigators should eliminate, mitigate or minimize potential pain and distress whenever feasible and consistent with good scientific practice.

Fishes have the potential to experience pain, and manipulations that provoke stress or avoidance/escape behaviour may be causes of distress. Fishes respond to noxious stimuli with altered behavioural, physiological and hormonal parameters. In general, the greater the intensity of stimuli, the greater the deviation from normal. In addition, the pattern of response to nociception generally corresponds to the pattern seen in more highly evolved vertebrates. Nonetheless, the recognition and evaluation of pain and/or distress in fishes is not easy. Many fish species are prey animals and are genetically predisposed not to exhibit signs of injury or pain.

A defined endpoint should be established for studies which involve potential pain and/or distress to the animal. A pilot study should be used to identify clinical signs to be used as the endpoint and to establish appropriate monitoring of the animals. Selection of appropriate endpoints that meet the scientific goals but minimize the adverse effects for the animals, requires the ability to identify signs of "pain" and/or "distress" for fishes.

Monitoring

Depending on the study and the time of morbidity, monitoring should be done at least daily. Frequency of monitoring should allow for the timely removal of fish before severe morbidity occurs. Frequency of monitoring should be increased where mortality is expected to be high.

Euthanasia

Where feasible, the euthanasia of fishes should consist of a two-step process, with initial anaesthesia to the point of loss of equilibrium, followed by a physical or chemical method to cause brain death. Physical techniques such as percussive stunning and gill-cut methods, commonly used in commercial aquaculture, should be used secondary to anaesthesia; the exception being when animals are in extreme distress and the time taken in preparation of anaesthesia would result in prolonged distress. Use of lethal levels of central nervous system depressants, such as buffered TMS, are the preferred method of euthanasia. Alternatively, a stunning blow to the head performed by an experienced person is also acceptable if followed by pithing or cervical dislocation. Use of carbon dioxide is not an acceptable method of euthanasia, nor is suffocation by draining the tank or removing the fish from water.

Disposition of fish after study

In general, research fishes that have been kept in captive environments must not be released into the wild. Release into the wild is only permissible under appropriate licence under the Fisheries (General) Regulations or similar provincial/territorial regulations. Before being transported between facilities, fishes should undergo health assessment.

Appropriate regulatory approval and permits must be in place before any transfer. The transfer of unhealthy fishes between facilities should be avoided, other than when requested by a veterinarian for the purposes of clinical investigation and diagnosis.

Disposal of Dead Fish

Fish must be disposed of according to acceptable federal, provincial/territorial and municipal regulations for the disposal of biological materials. The ACC has developed SOPs for fish disposal and they are available from technicians at both GLIER and the Biology facility.