Canadian Council on Animal Care

guidelines on:

the care and use of fish in research, teaching and testing

This document, the CCAC *guidelines on: the care and use of fish in research, teaching and testing*, has been developed by the *ad hoc* subcommittee on fish of the Canadian Council on Animal Care (CCAC) Guidelines Committee.

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A. PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer review agency responsible for setting and maintaining standards for the care and use of animals used in research, teaching and testing throughout Canada. In addition to the *Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed., 1993 and vol. 2, 1984, which provide the general principles for the care and use of animals, the CCAC also publishes detailed guidelines on issues of current and emerging concerns. The CCAC *guidelines on: the care and use of fish in research, teaching and testing* is the seventh of this series. This document supersedes Chapter I - Fish, *Guide to the Care and Use of Experimental Animals*, vol. 2 (CCAC, 1984).

These guidelines aim to provide information for investigators, animal care committees, facility managers and animal care staff that will assist in improving both the care given to fishes and the manner in which experimental procedures are carried out.

The present document has drawn substantially from the work of organizations listed in Appendix A. Their contributions to the development of these guidelines are gratefully acknowledged.

The guidelines have been developed by the CCAC subcommittee on fish and were reviewed by a total of 69 experts. A preliminary first draft was agreed on by the subcommittee and circulated to experts in June 2002 (including representatives of the organizations listed in Appendix A), and a second draft was circulated for widespread comment in June 2003. A final review was carried out in August 2004 involving all individuals who had previously provided significant input to the development process. The development of these guidelines also involved consultation with the Canadian Association for Laboratory Animal Science (CALAS) and the Canadian Society of Zoologists (CSZ) through workshops held at annual meetings in Québec City (June 2003), Acadia University (May 2004), and Hamilton (June 2004). Consultations were also held at the Aquaculture Association of Canada and AquaNet annual meetings in Québec City (October 2004), and at the CCAC Workshop on the Fish Guidelines in Vancouver (April 2005).

The guidelines have been organized in a format that should facilitate easy access to relevant sections. Early sections provide an ethical overview relevant to the use of fishes in research, teaching and testing. This is followed

by a brief overview of regulations and responsibilities relevant to the care and use of fishes in science in Canada. The remainder of the document provides guidelines to assist in caring for fishes in laboratory facilities, followed by guidelines to help in the development and review of experimental protocols. An overview of the CCAC *guidelines on: the care and use of fish in research, teaching and testing* is provided through a summary of the guidelines listed in this document prior to the beginning of the main text.

The refinement of animal care and use guidelines is a continuous process. These guidelines are intended to provide assistance in the implementation of best practices, and should not be viewed as regulations. Where regulatory requirements are involved or where it is absolutely imperative to adhere to a particular guideline, the term *must* has been used.

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Section 3.3 Mechanical and electrical requirements, p. 25

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Guideline 75:

Fishes should be handled only when necessary, and the number of handling episodes should be minimized.

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Guideline 76:

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Guideline 77:

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Section 3.1 Surgical preparation and skin disinfection, p. 52

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Section 3.1 Surgical preparation and skin disinfection, p. 52

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Section 3.1 Surgical preparation and skin disinfection, p. 52

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Guideline 93:

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Section 3.8 Postoperative care, p. 55

Guideline 94:

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Guideline 104:

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Guideline 105:

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Genetically modified fishes must not be permitted to enter the food or feed chain unless they have undergone a thorough safety assessment and have received authorization for sale, manufacture and/or import as a food or feed by Health Canada and the Canadian Food Inspection Agency.

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I. EUTHANASIA

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lowed by a physical or chemical method to cause brain death.

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Guideline 113:

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Fishes should undergo health assessment before being transported between facilities. Appropriate regulatory approval and permits must be in place before any transfer.

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B. INTRODUCTION

The greatest challenge in providing *guidelines on: the care and use of fish* is the wide variety of fishes used in Canada and the diversity of their habits, behavior, life history, and environmental and husbandry requirements. In addition, the scientific information required to define the preferred conditions for fish well-being is limited. While considerable research has been conducted on culture strategies and environmental and water quality requirements, such studies have generally been aimed at determining conditions that optimize production in aquaculture systems, rather than improving the welfare of fishes, and have not usually addressed the difference between *tolerance* and *preference* (Fisher, 2000).

An important consideration in these guidelines is the naturally high mortality rates of juveniles in species whose ecological strategies include the generation of large numbers of progeny to ensure adequate survival in the wild. In addition, many experimental populations of species with usually high survival contain individuals that will not thrive to adulthood even under the best environmental conditions. In some situations, a population-based (or a group of study fish) approach to well-being may be appropriate, but individuals that are not likely to thrive should be euthanized as soon as they are identified.

Another consideration for these guidelines is the general acceptance by the public of the current killing methods used in harvesting wild fishes or in recreational angling. In general, the public appears to be willing to accept these killing methods for food production but not when fishes are used for research. These guidelines accept that for research, teaching, and testing use of any animal, including fishes, more emphasis will be placed on individual well-being than is generally accepted for the commercial harvesting or production of animals for food. It is recognized, however, that in some instances investigators may obtain fishes from people involved in commercial or recreational harvesting and have little influence over the capture methods.

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.

1. Definition of Fish

For the purpose of these guidelines, fishes are defined as all bony and cartilaginous fish genera (classes Chondrichthyes [cartilaginous fishes], Agnatha, and Osteichthyes [bony fishes]). Fish eggs, embryos or larvae that have not developed beyond exclusive reliance on their own yolk nutrients are not covered by these guidelines. Similarly, invertebrates (except cephalopods) are not covered under the CCAC system of surveillance, but institutions are encouraged to foster respect for these animals by ensuring that holding facilities and levels of husbandry meet standards equivalent to those used for fishes.

2. Rationale for Guidelines on the Care and Use of Fish

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 (DeTolla *et al.*, 1995; Fabacher & Little, 2000). 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 (FAWC, 1996; FSBI, 2002; Rose, 2002; Braithwaite & Huntingford, 2004). Nonetheless, fishes are capable of behavioral,

physiological and hormonal responses to stressors (including noxious stimuli) which can be detrimental to their well-being. These CCAC guidelines both support the leadership role that Canadians play in fish research, and ensure that the welfare of fishes is carefully considered during the use of fishes for research, teaching and testing, recognizing that better welfare will result in better science.

3. Ethical Overview

Guideline 1:

Fishes used in research, teaching and testing must be treated with the respect accorded to other vertebrate species.

The CCAC's surveillance system for animals used in research, teaching and testing is based on the principles of humane science, i.e. the Three Rs of Russell and Burch (Russell & Burch, 1959) - Reduction, Replacement and Refinement. For the CCAC, these principles are laid out in its *policy statement on: ethics of animal investigation* (CCAC, 1989). The *ethics of animal investigation* applies to all species covered by the CCAC system, i.e. all vertebrates and cephalopods.

In addition, the CCAC system takes a "moral stewardship" approach to the use of animals in science as explained in the CCAC Experimental Animal User Training Core Topics - Module 2, Ethics in Animal Experimentation (http://www.ccac.ca/en/CCAC_Programs/ETCC/Module02/toc.html).
The first guideline statement in the CCAC *guidelines on: institutional animal user training* (CCAC, 1999a) states, "Institutions must strive through their training programs to sustain an institutional culture of respect for animal life".

3.1 Principles of the Three Rs

According to the CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989), it is the responsibility of the local animal care committee (ACC) to ensure that fishes are used only if the investigator's best efforts to find a non-animal model have failed.

As for any other species covered by the CCAC system, 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 (Balls *et al*., 1995). 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. Heffner *et al.* (1996) and Festing *et al.* (2002) provide discussions on the appropriate treatment of samples and experimental units. Investigators are encouraged to consult with a statistician to develop study designs that have the appropriate statistical power to accomplish the research objectives (Nickum *et al.*, 2004).

The CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989) also requires adherence to the following principles:

- animals must be maintained in a manner that provides for their optimal health and well-being, 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 relevent protocol;
- expert opinion must attest to the potential value of studies with all animals, including fishes (e.g., scientific merit for research, see CCAC *policy statement on: the importance of independent scientific merit of animal based research projects* [CCAC, 2000a]; pedagogical value for teaching; and the appropriateness of the method to provide data for testing according to current regulatory requirements);
- 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 killed 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. There are two main ethical drivers for CCAC guidelines: to maximize animal well-being, and to minimize pain and/or distress. 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 behavioral 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.

4. Responsibilities

Descriptions of the responsibilities of investigators, animal care committees (ACCs) and veterinarians are provided here; however, more detailed information is given throughout these guidelines to assist both investigators and members of ACCs to meet their responsibilities.

4.1 Responsibilities of investigators

4.1.1 Protocols involving the use of fish

Guideline 2:

Projects involving the use of fishes for research, teaching or testing should be described within a protocol. Protocols should be approved by an animal care committee prior to the commencement of the work.

Investigators are responsible for obtaining ACC approval before beginning any animal-based work. For further details concerning the information that should be included in a protocol form to be submitted to an ACC, see CCAC *guidelines on: animal use protocol review* (CCAC, 1997a); and CCAC *policy statement on: terms of reference for animal care committees* (CCAC, 2000b) or most recent revisions. Investigators obtaining fishes from the wild or carrying out field studies should also consult the CCAC *guidelines on: the care and use of wildlife*, Section B 3.1.1.1 Protocols involving the use of wildlife (CCAC, 2003a).

When working outside of Canada, Canadian investigators are subject to the same guidelines that apply to work within Canada, as well as to the relevant legislation, regulations and guidelines pertaining to animal care in the country where the work is conducted. This also applies to collaborative research projects, whether the work is conducted in Canada or elsewhere (see CCAC *policy statement on: animal-based projects involving two or more institutions* [CCAC, 2003b]).

4.1.2 Studies and activities requiring protocols

4.1.2.1 Work requiring protocols and inclusion in animal use inventories

These guidelines provide recommendations for fishes when they are being used by investigators. Fishes should be treated humanely whether or not they are to be included in animal use protocols or inventories.

The following require protocols and inclusion in animal use inventories (i.e. CCAC Animal Use Data Form, see Reporting of Animal Use Data at www.ccac.ca/en/CCAC_Programs/Assessment/AUDFen.htm):

- fishes held live in confinement for any period of time (even hours) for research, display, teaching or testing;

- fishes lethally sampled in the field for research, teaching or non-routine testing purposes;

- fishes caught, sampled or otherwise manipulated and released in the field for research, teaching and testing purposes; and

- genetically modified fishes.

4.1.2.2 Work not requiring protocols or inclusion in animal use inventories

The following will not require protocols or inclusion in animal use inventories:

- fish eggs, embryos or larvae that have not developed beyond exclusive reliance on their own yolk nutrients;
- wild source or hatchery fishes that have not been assigned to research studies, and whose propagation is sufficiently understood to be considered routine;
- fishes being observed in the field that are not being handled or interfered with in any way;
- fishes being counted at installations such as counting fences and traps;
- fishes being lethally sampled under government or other regulatory mandate for established fish inspection procedures, abundance estimates, and other population parameters required for assessing stocks and for routine monitoring of contamination/toxin levels and disease; and
- fishes already killed in the course of established aquaculture industry or commercial fishing purposes.

Guideline 3:

Before working with fishes, investigators, technical staff and post-graduate students must be properly trained and have their competency evaluated.

According to CCAC *guidelines on: institutional animal user training* (CCAC, 1999a), investigators and students should complete the Core Components of the *Recommended Syllabus for an Institutional Animal User Training Program* (CCAC, 1999b) and should have completed the relevant hands-on training to meet the Syllabus requirements on the use of fish as a research animal. "Students" refers to post-graduate students; undergraduate students are expected to be supervised by a properly qualified individual. See the CCAC website (www.ccac.ca/en/CCAC_Programs/CCAC_Programs-ETC.htm) for further information on relevant courses for investigators using fish as a research animal. Animal users should receive refresher training on a five-year basis, and additional training should be given as needed in order to be able to carry out procedures competently.

Guideline 4:

Investigators are responsible for, and must comply with, occupational health and safety regulations regarding the protection of personnel from known or suspected physical and biological hazards.

As with any other laboratory, animal care facilities (including aquatic facilities) should have an occupational health and safety program. All personnel using the facility should be familiar with the requirements of relevant federal, provincial/territorial and municipal legislation. Chapter VIII of the CCAC *Guide to the Care of Experimental Animals* (CCAC, 1993a) provides additional details on occupational health and safety.

Guideline 5:

Investigators should be aware of the potential risks associated with zoonotic agents present in fishes.

A brief review of fish zoonotic agents is provided in Appendix B of this document.

4.2 Responsibilities of the animal care committee

The CCAC *Terms of Reference for Animal Care Committees* (CCAC, 2000b, or most recent version) should be consulted for detailed information on the roles and responsibilities of institutional ACCs. In particular, ACCs are responsible for reviewing all studies conducted by investigators belonging to their institution, whether the work is conducted in-house or elsewhere. ACCs should ensure that appropriate care will be provided for all animals at all stages of their life and under all experimental situations. ACCs are responsible for ensuring that there is appropriate management of the facilities housing the animals. In particular, ACCs should verify that there is a person clearly designated to be in charge of animal care and management of the facilities who should also be a member of the ACC. Additionally, members of the ACC should visit the animal facilities and areas in which animals are used on a regular

basis, in order to better understand the work being conducted within the institution.

ACCs are responsible for ensuring that veterinary care is available to all animals being used for experimental purposes within the institution.

4.3 Role of the veterinarian

The CCAC uses the CALAM/ACMAL Standards of Veterinary Care (CALAM/ACMAL, 2004) as the Canadian standards for the role and responsibilities of the veterinarian within an institution using animals for research, teaching or testing, and assesses participants in its program based on these standards. Veterinarians working at institutions with large populations of fishes are encouraged to have special training in fish health management in research, teaching or testing environments.

5. Government Regulations and Policies on the Use of Fish

Guideline 6:

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.

5.1 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)

- Signed and ratified by Canada in 1992, CBD sets out broad commitments to the protection of biological diversity. Categories of programs of work under CBD include Marine and Coastal Biodiversity and Freshwater Biodiversity. www.biodiv.org/convention

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

- CITES, in force since 1975, has 167 member countries (as of 2005), including Canada. Member countries ban commercial trade in endangered species and regulate and monitor trade in other species that might become endangered. CITES applies not only to live animals, but also to "Parts and parts thereof", which includes all types of biological samples (skin, hair, bones, blood, serum, etc.). www.cites.org

Code of Practice for Introduction and Transfer of Marine Organisms

- The International Council for the Exploration of the Sea (ICES) requests early notification of planned introductions which may affect joint water bodies, in order to carry out international review. www.ices.dk/reports/general/2004/ICESCOP2004.pdf

International Aquatic Animal Health Code for Finfish, Molluscs and Crustacea

- To facilitate international trade, l'Office International des Epizooties (OIE) uses this Code (updated every two years) which defines minimum health requirements to avoid the risk of spreading aquatic animal diseases. www.oie.int/eng/normes/en_acode.htm

Sanitary/Phytosanitary (SPS) Agreement

- This provides agreed-upon rules for General Agreement on Tariffs and Trade (GATT) and World Trade Organization (WTO) in the use of SPS measures in international trade. www.wto.org/english/tratop_e/sps_e/spsagr_e.htm

Canada-USA Free Trade Agreement (FTA) and North American Free Trade Agreement (NAFTA)

- These agreements provide SPS measures considered acceptable for trade between Canada,

USA and Mexico. www.nafta-sec-alena.org/DefaultSite/index_e.aspx?CategoryId=42

North American Commission (NAC) (Canada and USA) of the North Atlantic Salmon Conservation Organization (NASCO)

- The NAC establishes protocols for the introduction and transfer of salmonids on the Atlantic seaboard. www.nasco.int

International Joint Commission

- This Commission prevents and resolves disputes between US and Canada under the 1909 Boundary Waters Treaty. In particular, the Commission relies on applications for approval of projects affecting boundary and transboundary waters, and may regulate the operation of these projects. www.ijc.org/en/home/main_accueil.htm

Organisation for Economic Cooperation and Development (OECD)

- The OECD has developed test guidelines for human health and for environmental health. Investigators conducting studies to generate data to be submitted for regulatory acceptance should refer to Environment Canada or Health Canada to confirm compliance with the current OECD guidelines. www.oecd.org/home

5.2 Federal

Fisheries and Oceans Canada (DFO) is responsible for administering the Fisheries Act and the Oceans Act, and is a responsible minister under the Species at Risk Act (SARA) for aquatic organisms. DFO's program responsibilities include, but are not limited to, the management of commercial, recreational and aboriginal fisheries and fisheries habitat protection (including regulation of the movement and introduction of fish), and protection and recovery of aquatic species at risk. Certain pollution prevention sections (e.g., S36) of the Fisheries Act are administered by Environment Canada (for further information see Section H.7. Use of Infectious Disease Agents, Tumorigenic or Mutagenic Agents, and Toxic and Noxious Compounds). Although fisheries management is a federal responsibility, there are delegation agreements with a number of provinces regarding inland waters.

Fish cannot be caught or removed without a permit from the appropriate regulatory body. Investigators seeking to capture fishes for use in research, teaching or testing should contact their local DFO office to determine the proper licensing requirements for their respective region.

DFO also administers the Fish Health Protection Regulations (FHPR) under the federal Fisheries Act. The objective of the FHPR is to minimize the risk of introducing or spreading diseases of concern. A license is required in order to release live fish into any fish habitat, or to transfer live fish to any fish rearing facility. It is important that anyone seeking to import or transport fish contact DFO for advice on regulatory requirements.

Canada now has a National Code on Introductions and Transfers of Aquatic Organisms (I&T Code) (Fisheries and Oceans Canada, 2003) that includes all species of finfish, molluscs, crustaceans, echinoderms, and other invertebrates. The I&T Code is an agreement between federal, provincial and territorial governments (signed by the Canadian Council for Fisheries and Aquaculture Ministers in 2003) to conduct introduction and transfer approval processes in a stream-lined manner through I&T Committees established in each province and territory in Canada. The I&T Code process implements various federal and provincial regulations. Local DFO offices or the relevant provincial/territorial government should be contacted to determine the application and approval requirements for introductions of fish into fish habitat and/or transfers of fish into fish rearing facilities.

The Wild Animal and Plant Protection Regulations of International and Interprovincial Trade Act (WAPPRIITA) is the enabling legislation for CITES in Canada. The import or export of any animal (including fishes) on the CITES list requires a CITES permit from the Canadian Wildlife Service (CWS) of Environment Canada under WAPPRIITA and the appropriate import or export permit for the provincial or territorial agency responsible for wildlife.

WAPPRIITA also provides the authority to protect Canadian ecosystems from the introduction of listed harmful invasive species by requiring permits, and makes it an offence to transport an animal or plant from one province or territory to another, or

export from a province or territory, without the required provincial or territorial permits.

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) develops and maintains a national listing of Canadian species at risk, based on the best scientific evidence available (www.cosewic.gc.ca). COSEWIC consists of representatives from: the wildlife departments of all 13 Canadian provincial and territorial governments; federal departments and corporations concerned with wildlife, including CWS (which provides the Secretariat), Parks Canada, DFO and the Canadian Museum of Nature; and three non-governmental conservation organizations. It is the responsibility of the respective provincial and territorial jurisdictions where the species occurs to take whatever actions are appropriate to address the threats and limiting factors placing a species at risk. The federal government implements their responsibility through SARA which, upon legal listing, makes it illegal to kill or harm species designated as endangered or threatened.

Environment Canada and Health Canada are responsible for administering the New Substances Notification Regulations (NSNR) under CEPA, 1999. The purpose of the NSNR is to ensure that no new substance is introduced into the environment before an assessment has been conducted to determine potential risks to the environment or human health. New substances include live aquatic organisms that meet the CEPA 1999 definition of an "animate product of biotechnology" and can either be naturally occurring or genetically modified. Currently, notification is not required under the NSNR when the fish is to be used for research and development only and there is no release from the research facility of the living organism, the genetic material of the organism or material from the organism involved in toxicity. DFO has recently taken responsibility for administering the NSNR for aquatic organisms with novel traits. Investigators importing or using these organisms should contact the biotechnology office of DFO for regulatory advice. The New Substances Notification Branch of Environment Canada can provide more information on the regulatory requirements related to other aquatic new substances.

5.2.1 Reintroduction and release

In most instances, fishes 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 process. Fishes involved in such studies may also require permanent marking or tagging if they have been subjected to treatment with anesthetic 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 (see above section on National Code on Introductions and Transfers of Aquatic Organisms).

5.2.2 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 regarding containment requirements for research involving animate products of biotechnology including genetically modified fish under the NSNR are further outlined in Section H.13 Genetically Modified Fish.

Facilities conducting research involving fish pathogens are required to consult CFIA Biohazard Containment and Safety Division,

to ensure appropriate levels of biocontainment are in place (see Appendix C). Where this involves the holding of live aquatic animal hosts, pathogen challenges or treatment trials, DFO provides science advice to CFIA as stated in their Memorandum of Understanding on Biologics.

5.3 First Nations

The Aboriginal Communal Fishery Licenses Regulations (laws.justice.gc.ca/en/F-14/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.

5.4 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.

5.5 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.

C. AQUATIC FACILITIES

Guideline 7:

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

The most basic principle in the design of fish-holding facilities is that a healthy fish population is dependent on a stress-free environment, which in turn requires the best possible facilities and water system (Wedemeyer, 1996a). 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 system. Section C. Aquatic Facilities is primarily targeted to land-based holding systems, rather than to outdoor ponds or open water cages; however, the provision of a stress-free environment is equally applicable to these types of facilities.

1. Water Supply

Aquatic facilities should be designed to minimize pathogens in the water intake and may require methods of preventing biofouling in water intake and distribution systems. Water intakes located in bodies of water should be positioned in areas where they will not pick up debris, recycled wastewater or shipping waste (i.e. bilge water and fuel spills), or be subject to breaking waves or ice damage.

2. Water Quality

Guideline 8:

If fresh or sea water is drawn from an open body of water or a municipal source, it 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/testing facility is planned within a particular location or building to ensure that the water supply is suitable. This analysis is usually the responsibility of the engineer in charge of designing the facility, in consultation with a knowledgeable fish health professional. Laboratories capable of carrying out the initial comprehensive analysis of the proposed water supply can be found through the Canadian Association for Environmental Analytical Laboratories (CAEAL), www.caeal.ca. Water sources can be tested for a wide range of bacterial and chemical contaminants at most regional hospital laboratories, and some larger municipalities may also have water testing laboratories. 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/Water/default.cfm#dri) and may provide guidance on acceptable levels of contaminants. Appendix D, although drafted for on-going water quality evaluation, provides a list of the parameters which should be tested. Institutions that have commissioned the facility should ensure that this analysis has been carried out. Depending on the reliability of the water source, further testing may be needed, for example on an annual basis. The supply should also be evaluated to ensure that there is sufficient capacity, including periods of maximum demand or emergency situations. 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 (Huguenin & Colt, 2002).

Tests to determine the chemical composition and presence of contaminants/toxins will determine the treatment necessary to make the water suitable for use. Seasonal factors such as phyto- or zooplankton blooms, tidal cycles, seasonal water mass turnovers and lake turnovers can have periodic effects (scales of hours, days or months) for both sea water and fresh water, and these need to be anticipated.

Fresh water often requires treatment to remove chlorine or other disinfectants, reduce suspended sediments, release supersaturated gases, or eliminate pathogens (Fisher, 2000). With municipal water supplies, chlorine or chloramines must be removed; activated charcoal (for large volume systems) and sodium thiosulfate (for smaller systems) are commonly used for this purpose (see Hodson & Spry, 1985).

Another potential problem with municipal water is that the pipes through which it passes can release metals, which in relatively high concentrations can be toxic to fishes. The extent of release and toxicity will vary with water quality. There are generally no problems associated with black iron or plastic supply pipes from municipal sources. Galvanized iron piping, where used, may cause problems of zinc toxicity, and copper piping or copper and brass fittings may cause copper toxicity if levels exceed 5µg/L. In addition, problems with cadmium toxicity may be encountered if excessive amounts of solder have been used on copper fittings.

Well water does not require dechlorination, but it is often depleted of oxygen and may have high levels of metal ions or carbon dioxide, nitrogen and other gases. Other potential problems include the presence of ammonia and excessively high or low alkalinity (Stickney, 1994). If well water is to be used, a pressure drop test should be conducted to ensure that the supply is adequate and reliable. Water taken from a well has undergone filtration during percolation through the source geological formation, and water temperature may be fairly constant throughout the year. Depending on the system requirements, this can reduce system complexity and cost by reducing the need for some filtration, as well as sterilizing and heating equipment. The major problem with seawater wells is that site conditions and geology are often unfavourable, high flow rates can be difficult to obtain if wells are not carefully situated, and they can be subject to draw-down during periods of high usage. On the other hand, seawater wells have the advantage that they do not rely on pipe systems subject to storm and ice damage and blockage.

3. Engineering and Design

Guideline 9:

In designing and constructing aquatic facilities, assistance should be sought from people with experience in this field.

It is not possible in these guidelines to provide the engineering details required for aquatic facilities. However, several sources are available that provide information on the important engineering and design principles of the various types of aquatic systems, e.g., Stickney (1994), Pennell & Barton (1996), Ostrander (2000) and Huguenin & Colt (2002). The CCAC *guidelines on: laboratory animal facilities - characteristics, design and development* (CCAC, 2003c) should also be consulted for consideration of the general principles of facility design.

The design of the facility is critical, and significant input from investigators and operational personnel is required during the design and construction phase to ensure it meets the requirements of the fishes being held and the needs of the investigators and facility managers.

In aquatic facilities, it is particularly important to ensure that the floor is sufficiently strong to support the weight of the intended tanks plus water.

In addition to requirements for additional space, requirements for services (water, air, electrical power, etc.) usually increase during the life of a facility. Therefore, the flexibility to permit later retrofits should be included in the original design.

The facility design should ensure easy access to all systems for operation and maintenance, including cleaning. Supply lines, drain lines and other critical components should not be buried or otherwise made inaccessible.

Modular construction is desirable to allow for the easy removal of components with minimal disruption of operations.

Considerable care should be taken with all fittings and equipment on the suction side of pumps to prevent air leaks that will cause nitrogen supersaturation. Facilities should be designed to be able to

add in desaturation equipment when the potential for supersaturation exists.

Facilities should be designed with dedicated separate quarantine areas for the isolation of new stock as appropriate.

Conventional facilities should incorporate transfer areas, foot and hand cleaning stations, restricted access and other common sanitary measures to prevent the introduction and spread of aquatic animal pathogens of concern. Work requiring the containment of aquatic animal pathogens requires special design considerations (see Appendix C).

Water supply lines should be secure, protected from disruption and comply with local regulations. Where possible, water lines to and from aquatic holding tanks should consist of hard, permanently fixed pipes to prevent problems due to hose flattening, air locks, fouling, etc. This may not be possible in a research facility where flexibility of the facility is of paramount importance, but the use of hard plumbed lines before the main water pump are strongly recommended. All lines should be prominently labeled to prevent confusion and potentially lethal effects on fishes.

Water supply and drain lines should be designed to facilitate cleaning by simple, low technology methods.

Pressure gauges and flow meters should be installed at points throughout the system to monitor the condition of the lines and the performance of the pumps and filters. Performance should be monitored by flow, concentration of dissolved gases, temperature, pH, salinity, dissolved oxygen, etc.

All compressors providing gases to the system should have devices to remove moisture, and oil traps to prevent any oil that leaks from compressors from entering the fish tanks through the aeration system. Food-grade lubricants should be used wherever possible. Intakes to compressors should be located so that only clean air is used, free of engine exhaust, tobacco smoke or other airborn contaminants.

Main drains should be over-sized to handle transient large flows of water. Gutters should have covers that are flush with the floor and that permit water to drain quickly. Drains and gutters should be designed to self-clean under normal flow, and to permit the use of a 'cleaning pig' to remove any buildup of waste in the lines. Where feasible, drains on all tanks should have traps and easily accessible clean-out ports.

It is imperative to incorporate appropriate effluent treatment, following regulatory requirements for effluent, into the design of the facility. If the effluent is untreated, it should be discharged in a location that is remote from the system intake to minimize the chance that effluent disease agents will be re-circulated through the system. In addition, it is essential to take into account the potential impacts on wild aquatic organisms in water receiving the effluent. For the most part, discharge to a municipal sewer will provide adequate treatment of fish lab effluents. Calculations of dilutions should indicate that potentially noxious materials in fish lab effluents (e.g., disinfectants) will be diluted to non-toxic concentrations in sewers before the sewage reaches the sewage treatment plant.

When effluent treatment is required, an appropriate back-up system must be in place to ensure effluent treatment remains valid during times of power outages.

3.1 Structural materials

Guideline 10:

Construction materials for facilities housing fishes should be selected carefully for resistance to corrosion and water damage.

It is critical that suitable construction materials be chosen for an aquatic facility, including materials used for tanks, tank covers and stands, as well as plumbing, mechanical and electrical components. Surfaces should be sealed, smooth, easily cleaned and made of impervious material. Floors should be non-skid, especially when wet.

Porous materials, including wood, other organic material and materials prone to de-lamination should be avoided as they provide locations for some pathogens/parasites and can increase the organic matter load in tanks, leading to stressors

and disease problems. If these materials are used, they must be properly sealed with nontoxic, opaque sealants.

In particular, the use of wood should be eliminated in areas that are constantly wet (e.g., stands for tanks) to prevent the growth of toxigenic moulds and harbourage of pathogens. Wood is likely to rot and weaken in humid conditions.

Many materials dissolve or corrode in salt water. Selecting materials for marine applications is an engineering specialty and such expertise should be sought when building a marine facility.

Guideline 11:

Materials in aquatic facilities which are potentially toxic to fishes should be reduced to the minimum. Any toxic material should be listed, and the list must be available to staff.

Many common construction materials are toxic to fishes. Huguenin & Colt (2002) provide an excellent review of the biological constraints to the selection of construction materials. Materials may leach out or release specific ions, chemicals, or corrosion by-products from their surfaces. All metals should be sealed or be inert.

The application of metals in marine facilities requires consideration of the type of metals and their interactions with both sea and fresh water. The effects of that interaction and the influence of fish in the systems should also be considered. Aluminum, titanium, stainless steel, steel and cast iron are all commonly used in aquatic facilities, but each has its own unique characteristics which must be considered. Reviews of some of these characteristics can be found in Huguenin & Colt (2002).

If concrete structures are used, care must be taken to ensure the type of concrete is appropriate for its intended use. Marine grades of concrete should be used and should be sealed to prevent the incursion of salt, which can penetrate and weaken concrete and internal support materials such as steel rebar. Salt incursion is a major cause of failure in submerged concrete.

Pipes, fittings and valves should not contain copper, nickel, brass, zinc or galvanizing treatments as their use can result in toxic concentrations of heavy metals. Fisher (2000) provides concentration ranges generally regarded as safe for fish culture. Polyvinyl chloride (PVC) pipes and other materials must meet human drinking water standards and must be adequately flushed to eliminate acetone, methylethylketones and tetrahydrofurans that are released following gluing (DeTolla *et al.*, 1995). High-impact PVC can contain lead and other toxicants, and should be avoided in fish holding facilities.

Any silicone sealant must be labelled as being suitable for use in aquaria (or have this clearly stated in other information available for the product) as there is a high risk of toxicity from compounds added to regular sealants to control curing and mould growth. If silicone sealant is used, it must be allowed to cure to release any volatile toxins.

Less obvious sources of toxicity include paints, untreated fiberglass surfaces, insulating materials and wood preservatives (Huguenin & Colt, 2002). Toxicity can also be introduced into the water directly from the air through condensation or aerosols. Creosoted pilings can cause problems if located near water intakes. Overhead material (air ducts, pipes, paints, galvanized ducts, etc.) must be chosen carefully as condensation can cause them to drip toxic products into the water. Pressure treated wood must not be used inside due to its release of toxic vapours and condensates.

Because of the difficulty in identifying potential toxicants, it may be useful to test new facilities or additions with small groups of sentinel fish, such as trout, before subjecting the majority of the fishes to the new conditions.

3.2 Room ventilation and airflow in aquatic areas

Guideline 12:

Air handling systems should be engineered to ensure that aquatic areas are well ventilated and humidity is controlled, and to ensure that aerosol transfer between tanks and through the facilitity is minimized.

The science of air quality management in aquatic facilities is not highly developed. However, the airflow should be sufficient to ensure that surfaces dry properly. Excess humidity hastens

structural damage to tanks, room fixtures, etc. and encourages growth of pathogenic bacteria and fungi. The ventilation should ensure a comfortable working environment for personnel. Airflow directions should be designed to minimize the spread of potential aerosols and to allow for the safe handling of any dangerous substances.

Installation of any heating, ventilation and air conditioning (HVAC) system has the potential to interfere with the ventilation in the other areas of the building and requires experienced professional advice prior to and during installation.

3.3 Mechanical and electrical requirements

Guideline 13:

All electrical systems must be professionally installed to appropriate code standards (federal, provincial/territorial and municipal building codes) for operation in moist environments, and must include proper grounding and ground-fault interrupters on all circuits. Extension cords should be avoided, and electrical wires should be fixed safely, away from water and from personnel circulation areas.

Guideline 14:

Electrical components and equipment should be located outside the splash zone, and should be housed in moisture-proof enclosures. Electrical fixtures should be secured with gaskets to prevent incursion of water, and should be located above pipe runs.

Some equipment is designed to be submersible; however, any electrical component or equipment must have Canadian Standards Association (CSA) approval for its intended use. All ground fault interrupters should be tested on a frequent, regular basis.

Electric power and sea water are a dangerous combination due to the extreme corrosiveness and the high electrical conductivity of sea water. All electrical equipment must be properly checked for safe working condition before use in and around sea water. Care should be taken to avoid salt build-up on floors, and floors should be pitched to promote drainage and avoid puddles. Electrical equipment should not be placed under water pipes or tanks as condensation can cause water to drip on the equipment.

Guideline 15:

Machinery that produces noise and vibration should be isolated from areas housing fish.

Pumps and fans can be quite noisy and can cause vibration. Where possible, all such equipment should be located outside the immediate location where the fishes are housed, with sound and vibration attenuation. Reasonable access to machinery for servicing should be incorporated into the designs. Ideally, all servicing of equipment should be conducted outside the facility to minimize excessive noise, disturbance and contamination of the environment around the fishes (Popper, 2003; Smith *et al.*, 2004).

3.4 Lighting

Guideline 16:

Light should be phased on and off, and should incorporate wavelengths and intensities appropriate for the species where this is known. Where task lighting is needed for people working in the room, it should be restricted in its dispersion throughout the room or be placed at a lower level than the tank surface.

Fishes are easily startled when light switches are activated at night and may jump out of open tanks or incur injuries by bumping into the walls of the tank. Having lights on automated dimmer controls that allow the lights to be gradually brought up in intensity over several minutes is important (Stickney, 1994; DeTolla *et al.*, 1995). Lowering the daytime light intensity and having some residual light at night may eliminate the need for dimmer controls. Care should be taken to ensure that fish are not disturbed by nighttime security lighting entering through windows in the fish holding facilities.

Light influences, either directly or indirectly, almost all physiological and behavioural processes in fishes, including growth, development (e.g., rate of smoltification in salmon), and reproduction. The response of fishes to light is complex and light often acts synergistically with other environmental factors, such as temperature, making it difficult to predict the effect that

inappropriate lighting regimes can have on the fishes or the experimental results (Stickney, 1994). Also, while the influences of light are reasonably well understood in some species, e.g., salmonids (Pennell & Barton, 1996), in many others little is known about how light affects behavior and physiology.

The natural history of the species, in particular the normal swimming depth, can provide clues to help meet the species preferences for the wavelength and intensity of light. Some species of fishes reportedly experience adverse effects from some wavelengths of light. The output of fluorescent lights can be diminished by using dummy bulbs to reduce light levels. Security and night lighting should not create visible shadows at night in fish tanks, as this can impact natural biorhythms. Screening or other remedies should be used to protect the fishes' natural photoperiod.

3.5 Redundancy in aquatic life support systems

Guideline 17:

All aquatic facilities should have an emergency contingency capacity, capable of maintaining aerated and filtered water and assuring the continuation of life support.

Generators or other emergency power sources should be available to support vital functions during power shortages and should be tested regularly. Plans for longer-term power outages must also be in place.

Considerable planning is necessary to anticipate the problems that develop in any aquatic facility, and to develop systems and strategies to minimize the consequences of failures. The strategies employed will depend on the size and type of facility. Knowledge of the length of time that the water quality is maintained in the event of a power failure is necessary, in order to evaluate the level of redundancy required. In addition, this will be dependent on the staff response time in the event of an emergency. As a minimum, back-up life support must be available for the length of time it would require for staff to rectify the problem or to euthanize the fishes if necessary.

Guideline 18:

Critical systems, including pumps, should be duplicated to ensure that failures cause only minimal interruptions in service.

Main system water pumps, filters and other essential life support components should have back up so that they may be replaced without affecting the supply of water or operation of the system. It is often better to use spare equipment regularly to ensure its operational efficiency. If water lines are critical, they should be duplicated.

Depending on the complexity of the system and the survival time of the fishes within the system in the event of an emergency, facilities may rely on observation or alarm systems for alerting staff to changes in critical parameters (see Section D.3. Environmental Monitoring and Control).

Once the monitoring system detects a failure, the cause(s) should be corrected as soon as possible.

4. Types of Systems

The choice of system (i.e. flow-through, recirculation or static tanks) should take into consideration the types of studies which are intended to be carried out in the system.

It is recognized that some research will require establishment of simulated ecosystems. This should be justifiable and have direct benefits to research.

Aquatic systems are either static or flowing. Flowing systems can be either recirculation or flow-through systems. Flow-through or single-pass systems are ones in which water continuously enters, flows over and exits the areas occupied by fishes. Recirculation systems are systems where a proportion of the water exiting the system is recycled after it receives treatment to restore its quality. An intermediate type of system involves the constant addition of new water along with the re-use of some percentage of the water in the system (Fisher, 2000).

The engineering requirements for flow-through and recirculating systems differ substantially because the influent (source water) and effluent

water quality requirements vary (Fisher, 2000; Huguenin & Colt, 2002). Fisher (2000) provides a flow chart of the elements involved in the two types of systems.

Guideline 19:

An adequate water supply of suitable quality should be provided for the fish at all times.

Water flow within a system should be sufficient to remove suspended solids and wastes and to ensure that water quality parameters are maintained within acceptable levels. Water flow also should be appropriate to enable fishes to swim correctly and to maintain normal behavior.

For static tank systems, debris should be siphoned off on a regular basis, in order to maintain water quality parameters within the predetermined range for species-specific requirements. In addition, water should be regularly removed and replaced to avoid the build up of nitrogenous material, which can become toxic.

4.1 Flow-through systems

Flow-through systems offer many advantages for fishes used for research or testing, and are less complex to build and operate than recirculating systems (Fisher, 2000). However, they require a continuous supply of large quantities of water of a consistently high quality. Potential sources of water include municipal supplies, wells (either fresh or sea water) or natural bodies of water. Caution should be exercised in use of municipal supplies as the presence of chlorine can kill fishes in culture. Section C.2. Water Quality, discusses water quality and the parameters which are important to measure and control irrespective of the type of system.

Systems pumped directly from the water source (e.g., ocean, lake or river), rather than from a well, present additional challenges for ensuring stable water quality. Depending on the season and tidal cycles, there may be problems such as temperature fluctuations, organic/biotic content, corrosion/fouling of supply system, presence of contaminants, salinity fluctuations, pathogens and disease, toxins from algal blooms, etc.

Effluent from flow-through systems may require treatment before release to ensure that biological and chemical waste does not exceed environmental standards and that potential disease organisms are not released (Ackefors *et al.*, 1994; Fisher, 2000). It is expected that all municipal, provincial and federal waste disposal standards will be met.

4.2 Recirculation systems

Water management in closed systems is complex. Pennell & Barton (1996) and Huguenin & Colt (2002) provide useful reviews and bibliographies of water re-use systems; however, since recirculation technology is changing rapidly, system managers need to stay up-to-date on innovations.

Recirculating systems involve the complete or partial re-use of water by circulating it through a treatment system that reduces biological and chemical waste loads to levels comparable to the influent water (Fisher, 2000). Recirculation systems use a series of processing stages that provide some or all of the following: 1) separation of large suspended solids (uneaten food and faeces); 2) fine filtration; 3) processing to reduce suspended solids, chemical oxygen demand and dissolved organic carbon, and to re-oxygenate the water; 4) removal of dissolved organic materials (foam fractionation); 5) disinfection; 6) biological filtration/nitrification/denitrification; and 7) toxicant removal with activated charcoal (Fisher, 2000). While stages 1 to 7 are ideal, it should be noted that some partial re-use systems do not (and cannot) use all these treatment or processing stages.

Filters, particularly for marine systems, should be as large as necessary to maintain appropriate water quality, even up to the same volume as the holding tanks, depending on stocking density and species. Regular changing of charcoal filters is required before saturation occurs to ensure that toxicants are not released back into the water.

4.3 Static systems

Static systems are systems in which no new water is continuously added from outside or recirculation sources. For this reason, these systems are sometimes called 'bath systems'. Static systems are often challenging to maintain, have very low inherent biological carrying capacity and are prone to water quality changes.

To assure water quality is maintained in static systems, the following measures should be taken:

- acceptable limits for water quality parameters should be pre-defined;
- water quality parameters and animal condition must be frequently monitored;
- regular partial volume water changes should be used to improve water quality, along with devices which create water movement such as corner filters, undergravel filters, hang filters and internal impeller or jet pumps;
- establishment of beneficial bacterial communities should be encouraged by housing a small number of fish initially to mature the water. These bacterial communities should be preserved where possible by rinsing filters in seasoned water, rather than in chlorinated tap water which can kill the bacteria;
- regular tank cleaning and vacuuming to decrease bioburden from food and faeces; and
- the use of low stocking densities of aquatic organisms, as the nitrogen loading of static systems is rapid and the denitrification potential is low.

4.4 Mesocosms

When dealing with large volumes of water (usually 50,000L and above) to recreate aquatic ecosystems (mesocosms), it should be recognized that they 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). It is therefore important that these systems be operated by individuals with the relevant experience. As for any other aquatic facility, they should be operated in a manner that does not negatively impact on the health and well-being of the animals. Therefore, the general guidelines concerning the use of fishes for research and testing also apply to mesocosms.

5. Fish Housing

5.1 Fish well-being

Guideline 20:

Aquatic environments should be designed to meet the established physical and behavioral 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, and where feasible, these should be included in the design of aquatic environments for fishes. Social behavior and social influences on behavior can be quite complex, and require investigators and animal care staff to have a good understanding of the species-specific requirements of the animals. For example, many species of flatfish and eel do best in long-term holding when provided with burrow type environments. In aggressive species, the provision of vertical barriers or shelters may eliminate or curb aggressive behavior.

Where the environmental requirements of fish are not well known, as far as possible the holding conditions should be designed to approximate the source environment. Long-term holding of such species should be considered experimental rather than routine, and should be conducted with increased oversight and monitoring of indicators of well-being such as feed intake and growth.

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.

5.2 Tank/enclosure design

Guideline 21:

The shape, colour, depth and volume of tanks should be appropriate for the species and life stage being held.

Tank design should take into account species preferences. For some larger species, particularly bottom dwellers, the surface area of the tank is more important than the volume or depth of water. However, fishes that occupy the entire water column depend more on the volume of water in the tank. Fishes that maintain their position by orienting themselves to a directional current or flow require oval or round tanks, while species that need considerable space for swimming, such as the common carp, prefer an oblong tank. The depth of the tank can also be important as some species will not feed in shallow tanks. Smaller species, such as zebra fish are commonly housed in rectangular glass aquariums.

The natural habitat and behavior of the fishes should be considered in selecting the appropriate colour of the tank. For example, fishes that are adapted to camouflage with the bottom of the tank require tank colours within their natural range of experience in the wild.

Most species adapt well to fiberglass (FRP), plastic, metal or concrete tanks or raceways, and many species can be reared without difficulty in cages or net-pens with mesh bottoms (Stickney, 1994). Concrete is an excellent material for fish tanks, but should have an appropriate lining which protects the fish from leachants and prevents water from penetrating the concrete. Wood should not be used as a tank material in contact with system water as it is a porous material that may contain toxic elements (in particular, pressure treatment or glue in plywood), is subject to rot, and requires the use of sealants, which can be toxic. Vinyl tanks are only suitable for temporary holding, as the plasticizers can be toxic and vinyl often contains contaminants. Glass is also suitable for tanks, but as with other clear materials, particular consideration should be given to the effect of external stimulation of the fishes. For example, some fishes are stressed if placed in glass tanks with no substrate on the bottom. Nonetheless, glass tanks are particularly useful for studies where good visibility is essential (e.g., studies with a behavioral component or toxicology studies).

Newly manufactured tanks require a conditioning/depuration period to flush out solvents (Schreck & Moyle, 1990).

Guideline 22:

Tanks should have smooth, inert, sealed interior surfaces.

The need for a smooth, inert, sealed surface to facilitate tank cleaning should be balanced with the preferences of the fish for a more complex environment. For example, in some experiments, investigators may use gravel at the bottom of the tank to replicate the natural environment, and this can be removed to permit thorough cleaning of the tank and substrate.

Guideline 23:

Tanks should be self-cleaning, or adequate means for the regular cleaning of tanks should be incorporated into the design.

Good water conditions should be maintained, as retention of wastes in fish tanks promotes the proliferation of pathogenic bacteria, protozoa and fungi, and leads to oxygen depletion. The accumulation of ammonia is a prime issue in determining the frequency of cleaning necessary, as un-ionized ammonia is extremely toxic to fishes (See Timmons *et al.*, 2001, particularly Chapter 5; and Section D.3.1 Management of water quality).

The turnover time of water in a holding tank is important from the perspective of tank hygiene. The recommended turnover time is a function of species, stocking density, social behavior, dissolved gas levels in influent water, the tank configuration and feeding frequency. Tank turnover times should also consider rates of oxygen consumption and ammonia production. Sprague (1969) provided a nomograph to estimate 90 to 99% molecular replacement times based on flow rate and tank volume. Simple arithmetic division of tank volume by water inflow rate does not yield replacement time; replacement time is considerably longer (Sprague, 1969). Turnover times should ensure that water quality is maintained. Where increasing the water flow is not possible, aeration of the water and regular cleaning of the holding tank should be performed.

Guideline 24:

Tanks should be equipped with a covering that prevents fishes from jumping from the tank, e.g., tank nets or rigid coverings.

Tank lids also serve as an important barrier against the introduction of any foreign objects, animals or chemicals. The height between the water surface and lid should be such that it minimizes the risk of damage to the fish if they jump.

The ability to visually assess all fishes in tanks and incoming water flows to each tank is essential. Where tanks are covered, visual access should be easily attained through removal or partial removal of the lid. Lids may also be constructed of materials such as plexiglass or clear acrylic which permit visual observation and are suitable for contact with aquatic animals.

Tank supports must be properly designed, strong, sturdy and durable, given the considerable weight of water and the potential catastophic effects of any collapse on staff, animals and the building. Transfer of weight to the floor structure should be taken into account.

D. FACILITY MANAGEMENT, OPERATION AND MAINTENANCE

Well managed aquatic facilities will have a regularly scheduled preventive maintenance program for all life support systems, as well as an annual maintenance program for all other 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, should be conducted in a standard fashion. The development of standard operating procedures (SOPs) for management of facilities is strongly encouraged to ensure consistency (CCAC, 2000b).

1. Security and Access

Guideline 25:

Access to fish facilities should be designed to minimize traffic through the area. Access should be 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 should be in place that is appropriate for an aquatic system. Environments with high humidity and salt in the air can cause problems for card access and keypad security systems.

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 supplied in the entry area, and personnel should wash their hands as soon as they enter the facility.

2. General Maintenance of the Facility

Guideline 26:

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

Inventories of necessary spare parts for all essential facility components should be maintained.

Guideline 27:

Aquatic facilities must have written maintenance schedules developed specifically for the facility.

A documented preventive maintenance program is required for all life support systems.

Both routine maintenance and equipment overhaul and replacement should occur while the equipment is still operating normally. 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 (Shepherd & Bromage, 1988).

Guideline 28:

Facilities should be kept in a clean and orderly manner. Tanks should be disinfected before and after every experiment.

Guideline 29:

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

Guideline 30:

Sufficient numbers of staff must be available for animal care and facility management and maintenance 365 days a year for both routine and emergency needs.

The inherent nature of aquatic facilities requires that there be constant maintenance, repair and upgrading. Trained competent staff need to be on call and accessible 24 hours a day, 7 days a week. In particular, facilities must have a method for assuring a rapid emergency response by staff outside of normal operating times.

3. Environmental Monitoring and Control

Guideline 31:

An environmental monitoring system is essential for aquatic facilities and should be designed to suit the water management system.

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.

Many facilities have simple systems where unexpected environmental changes are unlikely to occur. These facilities should not need to establish costly water monitoring systems for a rare event, and in these situations monitoring through regular visits by custodial staff is usually sufficient. For small scale aquatic facilities, routine monitoring may simply consist of daily visual system and animal checks, and limited testing with hand-held equipment such as a thermometer, dissolved oxygen (DO) meter and a pH meter.

Large sophisticated systems, however, will require extensive, generally computer-based, monitor and control systems with redundant and fail-safe modes and automated emergency contact systems. Remote monitoring of water quality can reduce the number of visits per day into holding rooms, which is particularly relevant for quarantine rooms and during experiments.

Guideline 32:

Water quality monitoring systems should be able to detect and react to changes in water quality before they become life-threatening to animals housed in the system.

Early detection of physiologically significant fluctuations in environmental conditions is important through automated or repeated manual testing. Environmental change is more likely to occur in the following circumstances: water quality is known to fluctuate based on previous testing; previous occurrences of compromised fish in studies with similar holding conditions; or when large complex systems depend on many continual water quality adjustments (e.g., recirculated systems for large numbers of fish). It is critical that all malfunctions be immediately recognized by staff, or that staff are alerted by automated systems, so that corrective action can be taken. Remote monitoring of water quality can reduce the number of visits per day into holding rooms, which is particularly relevant for quarantine rooms and during physiologically-sensitive experiments.

Guideline 33:

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, and whether it is a seawater or freshwater system (Fisher, 2000; Huguenin & Colt, 2002). At a minimum, environmental monitoring systems should provide information on water flow or oxygen saturation and water temperature. Examples for frequency of testing are listed in Appendix D; however, these are suggested measurements only. For example, while there may be no need to measure nitrites/nitrate in a high volume flow-through system (depending on the source of the water), such measurements are critical with recirculation systems. Parameters measured should also be relevant to the health of the species housed in the system, and taken at a frequency that allows adjustments to be made well in advance of catastrophic morbidity and mortality (Wedemeyer, 1996a). In addition, the ability to conduct rapid tests is important when a change in the water quality is suspected.

In general, recirculation systems should be monitored for a larger number of parameters including, but not restricted to, dissolved oxygen, temperature, salinity (marine systems), pH, ammonia, nitrite, nitrate and total dissolved solids (Fisher, 2000).

When water quality analysis is done, it should be at a time reflecting greatest demand on the system (usually after feeding) to identify potential problems.

Guideline 34:

Good water quality measuring equipment should be available, regularly calibrated and well maintained. Records of water quality should be maintained and should be retrievable for retrospective analysis in the event of problems.

3.1 Management of water quality

Guideline 35:

Water quality must be monitored and maintained within acceptable parameters for the species being held.

Water quality, in the context of fish holding systems, refers to all the factors—physical, chemical and biological—that influence the well-being of the animals. The term "quality" implies that no factor should exceed concentrations likely to be toxic in the context of the facility or study, or fail to remain within the species-specific range for life-sustaining factors (Ackefors *et al*., 1994).

In experiments, it is important that water quality parameters remain relatively constant throughout the initial holding period and the experimental period (except, of course, for the variables that are being manipulated experimentally).

The most common water quality factors known to affect fishes are temperature, dissolved oxygen, pH, suspended solids/sediment, carbon dioxide, nitrogen supersaturation, ammonia, nitrite, nitrate (Wedemeyer, 1996a; Kreiberg, 2000) and chlorine. These must be monitored on a regular basis; close monitoring is particularly important 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.

Preferences and tolerances for some species of fishes are known. For other species, preferences may be unknown, and may require pilot studies to determine the appropriate conditions. These pilot studies should be overseen by the veterinarian or experienced fish health expert and the ACC.

3.2 Temperature

Guideline 36:

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. In peak summer temperatures, a change of 5ºC may be too much, whereas in the middle of winter when the ambient temperature is low, adjustment may be easier. As a general rule of thumb, temperature changes should not exceed more than 2ºC per 24-hour period.

Changes in ambient temperature for fishes are much more significant to many vital functions

than in the case of land animals. Susceptibility to diseases, parasites, and toxicants is greatly affected by temperature. The further the temperature shifts in either direction from the optimal range, the greater the potential for stress and disease.

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. This process will generally have advanced substantively after 24 hours at the new temperature, but may require as much as 6 to 8 weeks for completion. The duration of acclimation is a temperature-dependent process. At lower temperatures, the actual change in physiological processes will occur at a slower rate. 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. Hochachka & Somero (1971) provide a comprehensive overview of this process.

3.3 Oxygen

Guideline 37:

Fishes should be kept in water with an adequate concentration of oxygen.

In most species, water O_2 saturation levels should be above 90%, although some species thrive at lower O_2 concentration. Some species (air-breathing fishes) have evolved methods of extracting oxygen from air, and a subset of these fishes will drown if not allowed access to air, underlining the importance of understanding the specific requirements for the species of fish to be used. 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; for example, the age, health and activity rate of the fish, as well as any handling procedures.

The congregation of fish at the tanks' water inlet or gasping behavior at the surface is an indication of insufficient oxygen.

In some instances, low O_2 levels can be remedied by aeration, reducing the stocking density and decreased feeding. Balancing these variables is essential to prevent low O_2 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. Better quality airstones produce smaller bubbles with less agitation of the water column and are superior in oxygen transfer. Where necessary, supplementary oxygenation of tank water should be provided.

3.4 Supersaturation

Guideline 38:

Aquatic systems are susceptible to acute or chronic supersaturation. Individuals responsible for operating aquatic systems should understand the causes of gas supersaturation and how to mitigate potential problems.

Supersaturation of water is a possibility in any fish holding system; therefore, fish care staff should be aware of the acute signs of supersaturation, both in the fish and within the facility. Supersaturation is a condition where the total gas pressure in a body of water exceeds the barometric pressure in the overlying atmosphere. It may arise due to an excess of any one or more gases present in the water, and is best measured by devices which assess the sum of all partial gas pressure in water (e.g., saturometers). Supersaturation is discussed in detail by Colt (1984, 1986).

Water can become supersaturated with dissolved gases under a variety of conditions, including when water is heated in a closed vessel or experiences a pressure change, or when gas (including air) is compressed into water. Water may be supersaturated in a source location which can further complicate the situation. Although oxygen and carbon dioxide can become problematic when supersaturated, dissolved nitrogen is perhaps the most dangerous. It is advisable that

total gas levels in fish holding tanks be monitored on a regular basis.

Gas bubble disease can occur when fishes are exposed to supersaturated water; fishes absorb the gas and it is then released from suspension in body fluids, forming internal bubbles (Speare, 1998a). Sub-clinical supersaturation can be a chronic stressor for fishes; therefore, using acute gas bubble disease as an endpoint for tolerable levels is inadequate. The biological response to gas supersaturation varies with species, life stage, water quality, and the animal's depth in the water column (Colt & Orwicz, 1991). Affected fishes may show a variety of signs, including bubbles of gas under the skin, between fin rays, in fin tips and in gill tissue. Gas bubble formation in capillary beds causes ischaemia and tissue necrosis, and bubbles in the circulatory system, including in the heart and brain, cause rapid death.

Supersaturation can be reduced in a number of ways. The preferred methods of addressing supersaturation involve passing the water through trickle columns packed with surface area rich media such as pea gravel or bio-rings, vacuum degassing or O_2 injection. Other methods include vigorously breaking up the water to allow for the escape of excess gases into the atmosphere or the use of spray bars.

Facilities should have the ability to rapidly check the dissolved gas saturation levels in the event of acute morbidity/mortality incidents. It is critical that staff understand the causes of supersaturation and the means to mitigate the problem. Several good references are available on the topic, e.g., Colt & Orwicz (1991), Pennell & McLean (1996) and Huguenin & Colt (2002).

3.5 pH

Guideline 39:

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 CO_2 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 range for saltwater fishes is narrower: pH 7.5 to 8.5. The optimal pH range for maintaining freshwater fishes is between 6.5 and 7.5. The optimal pH range for maintaining marine fishes in natural seawater is between 8.0 and 8.5. The pH range for maintaining marine fishes in synthetic seawater is 7.5 to 8.5. Although seawater has a high buffering capacity, marine animals produce acidic waste, reducing the pH value of the water over time. The fall 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. It is also important to consider the optimal pH for growth of bacteria within the biological filters in recirculation systems.

The pH of the water has a considerable effect on other important parameters of water quality. 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.

3.6 Nitrogen compounds

Guideline 40:

Free ammonia and nitrite are toxic to fishes and their accumulation must be avoided.

Ammonia toxicity is extremely pH (H^+) 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 un-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 un-ionized ammonia, as only the un-ionized (non-ionized) form is toxic to fishes. For a review of allowable limits of ammonia for fish, see US Environmental Protection Agency (1999).

Avoiding ammonia accumulation, in particular within recirculation systems, is very difficult. However, more important than the accumulation of nitrogenous products (ammonia and nitrite) is the balance of these products in relation to the pH of the water (Ip *et al.*, 2001). The physiological effects of ammonia and nitrite toxicity have been reviewed by Speare (1998b). 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.

Aside from control of pH and good feed management practices, other measures are usually an essential element of recirculation systems. Biological filters provide the substrate necessary for colonization by bacteria (for example *Nitrosomonas* and *Nitrobacter*) that oxidize ammonia. The substrate can be media such as zeolite, crushed oyster shell, sand, dolomite, pea gravel, or synthetic substances (e.g., styrofoam beads, plastic rings or fiber filters). 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.

Nitrifying bacteria are sensitive to sudden pH changes and do not generally grow well outside the range of pH 7.2 to 8.5. Their growth can also be affected by chemicals used for disease treatment, as well as by sudden changes in temperature.

Ammonia can be stripped from fresh water using an ion exchange apparatus. This involves passing the water through a bed of zeolite crystals to trap the ammonia which is subsequently released as gas.

3.7 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.

3.8 Salinity

Guideline 41:

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 treatements.

3.9 Toxic agents

Guideline 42:

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 vary with species, life stage, acclimation conditions, and other environmental conditions (e.g., temperature, water hardness, etc.) (Barton, 1996).

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 or seawater 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.

Guideline 43:

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.

E. CAPTURE, ACQUISITION, TRANSPORTATION AND QUARANTINE

Irrespective of the source of fish, investigators should ensure that the relevant regulations for capture and acquisition are followed (see Section B.5. Government Regulations and Policies on the Use of Fish).

1. Capture of Wild Stock

Whether fishes are being collected for live study, preserved for study in a museum, or being processed to obtain data needed for fisheries management field studies, investigators should observe and pass on to students and employees a strict ethic of habitat conservation and respectful treatment of the animals. 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.

Guideline 44:

Wild fishes should be captured, transported and handled in a manner that ensures minimal morbidity and mortality.

Investigators should be aware that 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, electrofishing is a stressful procedure, leading to acute physiological disturbances, increased susceptibility to predation (Schreck *et al*., 1976) and significant effects on other taxa in the vicinity (Bisson, 1976). Less stressful procedures should be used whenever possible.

The largest number of fishes used in research are obtained from private or government hatcheries, but other genera of wild or exotic fishes may be required. Where fishes are to be taken from the wild, all necessary collecting permits and authorizations must be obtained before starting the project. Local fisheries/conservation officers must also be notified.

Guideline 45:

Where exotic fishes are obtained from aquarium suppliers or collection sources, local, provincial/territorial and federal authorities should be consulted to determine the risk of escape, accidental introduction, exotic diseases and other detrimental outcomes, and how to minimize these risks.

Investigators should be aware of the list of native species that are endangered, threatened or vulnerable. Lists of protected fishes in Canada can be found at: www.speciesatrisk.gc.ca/default_e.cfm.

2. Killed Specimens

The numbers of fishes collected should be the minimum number required to accomplish the study, and each animal collected should serve as many types of study as possible. The most humane euthanasia techniques should be used, bearing in mind the health and safety of the personnel (see Section I. Euthanasia). Fish should be euthanized prior to immersion in formalin or other preservatives.

3. Piscicidal Compounds

Guideline 46:

Alternatives to the use of piscicidal compounds should be sought, such as anesthetic agents with minimal environmental and non-target species impacts.

On rare occasions, it may be necessary to use piscicidal compounds in field situations to capture dead specimens. If piscicidal compounds are to be used, an impact analysis should be carried out prior to use to determine the potential local effects (e.g., by-catch).

4. Acquisition of Hatchery Fish

Guideline 47:

Fishes should come from hatcheries with defined health status and preferably known genetic history. Hatcheries should be encouraged to develop husbandry and management practices consistent with those used in the production of other laboratory animals.

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. Institutions are encouraged to develop a list of reputable fish suppliers.

5. 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 (Wedemeyer, 1996a; Wedemeyer, 1996b). 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.

Shepherd & Bromage (1988), FAWC (1996) and Wedemeyer (1996a; 1996b) provide detailed information on fish transportation.

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 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.

- Once loaded, and at regular intervals during transport, the behavior of the fishes, transport tank temperature and oxygen levels should be checked to ensure there are no problems. On arrival, particular care should be taken to check water temperature to ensure that fishes are not exposed to temperature shock during transfer.

- For transport of large fish shipments or where more than minimal distances are being traveled, it is advisable to use fish transport vehicles that are constructed to supply high quality oxygenated water and have on-board monitoring of life support systems.

- Auxiliary aeration or oxygenation systems should be installed to ensure oxygen saturation and serve as backup should a failure occur with the water circulation system.

- 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. These bags should be transported in a cooler to maintain water temperature as close as possible to the fishes' initial/starting temperature. Small bags of fishes are likely to heat up quickly, and fishes could become thermally stressed.

- All tanks and pipes in the transportation systems should be disinfected between shipments, followed by thorough soaking and rinsing to remove all traces of potentially lethal disinfectants.

Any stressful event, such as handling or transportation, causes a rapid increase in adrenaline. Adrenaline causes temporary changes in gill permeability. In freshwater, this results in dilution of blood by excessive entry of water, and vice-versa in seawater. Blood levels of important electrolytes are pushed out of the normal ranges for as much as 24 hours following a brief stress such as dip netting (Wedemeyer, 1972). For these reasons, brackish water (i.e. water with an osmotic pressure similar to blood) has been recommended as an effective tool for the transport of a number of fish species (Kreiberg, 2000).

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 (Wedemeyer, 1996b). Studies have shown that the initial crowding stage during capture and transportation is the most stressful for fishes. Therefore, fishes should be sedated prior to transportation (Kreiberg, 1992). 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 (Kreiberg, 2000). Metomidate is the most appropriate choice for sedation during transport.

6. Quarantine and Acclimation

Guideline 48:

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 (see Section 6.1 Quarantine).

Guideline 49:

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

Some of the factors that influence the responses of fishes are genetic differences, age, growth rate, environmental and nutritional history, and exposure to biogenic compounds. It is important to document the strain used in each experiment and to use the same strain throughout an experiment. For these reasons, investigators need to ensure that fishes to be used for experimental purposes are obtained from a reputable supplier with good health management, and that new arrivals are carefully screened and quarantined, and are healthy before entering the main population.

6.1 Quarantine

Guideline 50:

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.

Quarantine can also be used to isolate populations of fish that become sick some time after entry into the facility. Quarantine is primarily a measure to ensure that fish are isolated and sanitary measures are put in place to ensure there is no escape of viable pathogens or their hosts from the facility and into the surrounding waters, or transfer of pathogens to other animals in the facility. Information specifically for containment of marine and freshwater organisms used in disease studies or incidentally infected with transmissible diseases is given in Appendix C.

Ideally, quarantine should involve isolation of fishes being studied or held for different purposes in separate areas. However, for facilities with only one room, plastic sheeting may be used to wall off a quarantine tank to prevent splash or aerosol transmission. The water supply should be separate so that water from quarantined tanks is not circulated to other tanks, and effluent should also be separate when there is a need to treat the water, prior to recirculation or release. In addition, rigorous SOPs for disinfection should be in place.

Guideline 51:

The duration of quarantine should be appropriate to assure the health of the fishes.

In instances in which the fish are received from a health certified source (such as a salmonid hatchery that is in compliance with DFO's Fish Health Protection Regulations), the duration of the quarantine period may be decreased depending on the health status, but quarantine is highly advisable for any fish introduction regardless of the source. In other instances, such as receipt of wild fishes captured in the field, a longer duration of quarantine is more appropriate. This is especially important if the newly acquired fishes will be added to healthy existing populations (DeTolla *et al.*, 1995). Minimum quarantine time should be established based on the holding temperature, source of fishes, and the anticipated timeframe for expression of the pathogens of concern. New stock should undergo routine health screening, including necropsy examination, if they are to be mixed with existing stocks.

Guideline 52:

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.

Newly arrived fishes may bring pathogenic organisms with them, either in an active or carrier state, to which the resident populations have not been exposed. As a result of the stress of handling and crowding in tanks, the fishes' immune system may be depressed and a disease outbreak may occur. The danger of introduction of new pathogens is especially relevant if fishes from wild populations with unknown disease histories are used. Prior to bringing wild fishes into the lab, fish health professionals should be consulted for advice on appropriate prophylactic disinfection procedures to be used, in order to determine appropriate treatments.

6.2 Acclimation

On arrival in a facility, new fishes should be handled as little as possible, and care should be taken to prevent thermal shock (Wedemeyer, 1996a). 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. See Section D.3.2 Temperature, for notes about changing the water temperature.

Acclimation involves ensuring a gradual adjustment of the living conditions for the fishes. In general, fishes brought into a facility should be allowed to adjust to their new environment (including water quality, temperature, illumination and diet). This period should also ensure that any problems related to the stress of transport (e.g., anorexia, unanticipated morbidity and mortality) have been resolved.

Fishes should be gradually reintroduced to feeding during acclimation. It is common for newly transported fishes to refuse to feed, particularly when a new feed is introduced. Where possible, samples of the feed previously used by the original supplier should be obtained to permit transition to the new feed source by blending. Return to feeding is a good indication that the fish are acclimating successfully.

As a last resort, larger fishes such as recently acquired wild-captured fishes can be sedated and force fed to initiate digestive processes and encourage return to feeding.

F. HUSBANDRY

Good fish husbandry requires attention to detail and the rigorous and consistent performance of routine chores. The importance of a high standard of husbandry cannot be over emphasized and should be regularly reinforced.

1. Record-keeping and Documentation

1.1 Standard Operating Procedures

Guideline 53

Detailed Standard Operating Procedures should be developed for the maintenance and care of all fishes and for sanitation of tanks, rooms and equipment.

Each facility should prepare a facility Standard Operating Procedures (SOPs) manual for acceptable fish husbandry practices and standards. The manual should be reviewed and updated regularly, and the ACC and facility management should ensure that users follow the SOPs. In particular, SOPs should be developed to ensure that tanks are properly disinfected and kept clean between experiments.

1.2 General checklists

Guideline 54:

Checklists should be used for each group of fish so that records are maintained of all cleaning, maintenance and experimental procedures.

As a minimum, the following records should be maintained on all captive fishes.

1. On the tank or holding area:

- source of fish and date of arrival;
- species and sex (if identifiable);
- estimated age and weight;
- name of principal investigator and list of emergency contacts;
- animal-use protocol number, including expiration date;
- transfer history of fish (i.e. where they have been housed within the facility);
- number of fish in tank;
- daily records of husbandry (including feeding schedule), maintenance, experimental procedures and water quality parameters as required (see Appendix D); and
- morbidity/mortality.

2. Readily accessible in the facility records:

- history of the fish, including disease and ongoing health status;
- documentation of regulatory compliance; and
- copy of animal-use protocol.

1.3 Assessment of fish well-being

Guideline 55:

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/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.

2. Density and Carrying Capacity

Guideline 56:

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

maintaining 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 behavior may increase (for example, in salmonids housed below minimal densities, feeding is diminished). Wedemeyer (1996a) has reviewed the physiological responses of fish to crowding.

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.

3. Food, Feeding and Nutrition

Most species display daily and seasonal feeding rhythms, and may be specialized to feed on specific types of food (Groot, 1996; Madrid *et al.*, 2001). 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 (Goddard, 1996; Alanärä *et al.*, 2001).

3.1 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 and amino acids, lipid and fatty acids, vitamins and minerals. Deficiency of these nutrients can reduce growth rate and feed consumption, and lead to diseases (NRC, 1993; Conklin, 2000). As fishes are ectothermic, their metabolic rate is determined by the water temperature. Therefore, feeding rates and quantities need to take temperature into consideration (Alanärä *et al.*, 2001; Kestemont & Baras, 2001).

3.2 Food and feeding

Guideline 57:

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.

If fish are to be introduced into the food or feed chain (see Section J. Disposition of Fish After Study), the fish feed must be in compliance with the Feeds Act and Regulations (laws.justice.gc.ca/en/F-9).

Guideline 58:

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.

3.3 Feed quality and storage

Guideline 59:

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 (Goddard, 1996). 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.

Feed can be frozen to extend its shelf life. This is an option when relatively low amounts of feed are required for a specific research project. However, certain micronutrients such as B complex vitamins are degraded by freezing and thawing, and therefore supplements may be required.

Oxidative rancidity is one of the most serious changes than can occur in stored feed (Wedemeyer, 1996a; O'Keefe, 2000). In the absence of antioxidant protection, lipids rich in polyunsaturated fatty acids, including the essential fatty acids, are highly susceptible to autooxidation, which produces harmful breakdown products that include free radicals (Hardy & Roley, 2000). The pathological effects of feeding oxidized oils are summarized by Tacon (1992).

Under no circumstances should mouldy feed be used, as it is highly toxic (Wedemeyer, 1996a).

Guideline 60:

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.

Guideline 61:

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.

When automated feeders are used, the equipment should be regularly serviced and the rate of intake of the fish checked as frequently as possible.

The practice of feeding involves determining the proper size and appropriate properties of the food for the species (e.g., bottom feeders are fed more easily with sinking pellets). The ration size needs to be determined, as well as the feeding frequency, the preferred time for feeding, and the most efficient means of distributing the feed. When new feed is introduced, it should be mixed with the accepted feed until the transition is made between the two feeds.

Feeding techniques for captive fishes have the general aim of encouraging rapid consumption, thus increasing feed ingestion, preventing leaching of water-soluble nutrients, and reducing wastage. Not only is a suitable diet important to obtain a good feeding response, but the culture environment also influences the feeding response. For example, temperatures at the low and high end of the tolerance range inhibit feeding, as do stressful conditions such as low oxygen levels and the development of social hierarchies within the population (Kestemont & Baras, 2001).

Feed or feces retention in the environment is a particular concern in situations of overfeeding, and especially in recirculation systems. The influence of feed quality and quantity on water quality should be addressed in the study design.

Fish must not be overfed, except in experiments where fish are fed *ad libitum*. When fish are fed *ad libitum*, however, they should be monitored and excess feed should be removed soon after the feeding period. 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 (De Silva & Anderson, 1995; Carter *et al.*, 2001). 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 (see Section 3.4 Larval weaning, for the exception to this rule).

Feeding and fish size-sorting practices should be optimized to ensure all fish have the opportunity to feed. In the event of prolonged feed refusal, alternative plans should be in place, including consultation with a feed manufacturer, a fish nutritionist or a veterinarian. The primary modes of feed detection by fish are through olfaction and sight, but the taste and texture of the feed is the key factor that determines whether the feed will be swallowed or rejected. When changing feed sizes, a mixture should be fed for a week to allow fish to make the adjustment. Certain feeding stimulants added to fish feeds enhance palatability and feed acceptability.

In some cases, for instance where wild fish have been brought into captivity, pelleted rations may not be recognised as food. In addition, many small aquarium species, as well as the larval stage of many larger marine fish species, are either unable or reluctant to feed on prepared feeds. It may also be necessary to feed live prey for studies on fish foraging, and for short-term holding of fish from the wild. While live feeds (principally rotifer and brine shrimp) can give excellent results, in most cases, provision of live foods requires culture of the prey item in addition to the fish. The ethical cost of feeding live feed (in particular live fish) to fish must be considered, in addition to other potential disadvantages, such as the possibility of variable nutritional planes or the potential for introduction of disease. Additional information, including a general overview of nutrient requirements can be found in other publications (e.g., NRC, 1993; Conklin, 2000; Halver & Hardy, 2001).

Studies involving food restriction should undergo careful consideration. The CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989) states that for "experiments requiring withholding of food and water for periods incompatible with the species-specific physiological needs, such experiments should have no detrimental effect on the health of the animal". In general, fish should not be permitted to lose more than 15% of body weight during periods of food restriction (Home Office, 2003). As with any other studies, the ACC is responsible for approving the endpoint of the proposed study, in consultation with the investigator and veterinarian or fish health specialist. It should be noted that for fish that have naturally ceased to feed (e.g., spawning salmon), it is not necessary to attempt to feed.

3.4 Larval weaning

It is recognized that early life stages of many species have high natural mortality (see Section B. Introduction). Failure to begin feeding or to acquire sufficient food has often been suggested as a major cause of early mortality (Robin & Gatesoupe, 1999).

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 (Noakes & Godin, 1988). 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 (Watanabe & Kiron, 1994). For all fish species, the change from endogenous feeding to exogenous feeding, and again when weaning from a live diet, are critical periods where large numbers of fish may die. In general, this is not a welfare concern, but individuals judged unlikely to thrive should be euthanized.

The timing of first feeding and availability of suitable prey is critical. Altricial young, which are not highly developed prior to first feeding, require a higher concentration of food to compensate for their underdeveloped gut and rapid gut transit time. Precocial young, which are more developed at the first feeding stage, are able to feed more efficiently on scattered individual prey items (Noakes & Godin, 1988).

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.

3.5 Use of medicated feeds

Guideline 62:

Medicated feeds must only be used under veterinary prescription and supervision.

Medicated feeds may be used to treat clinical conditions, such as bacterial infections, or to investigate models of disease control. Medicated feeds must only be used under veterinary prescription with accompanying caution for withdrawal times, particularly in the case of antibiotics. The use of medicated feeds for therapeutic reasons should be conducted under veterinary supervision and with due care to the development of antibiotic resistant bacteria. Rational antibiotic selection should be the result of clinical judgement and the use of antimicrobial sensitivity testing. As far as possible, only agents approved for use in fishes should be used.

Medicated feed may be less palatable and the fish may refuse to eat it. It is important when starting medicated feeds to monitor the feeding behaviour of the fish and to be prepared to use adjunct measures, such as gradual mixing with non-medicated feed, to encourage consumption. Use of diet palatability agents for medicated feeds (e.g., coating the diets with krill or marine fish hydrolysate) may be considered to induce feeding. Records should be maintained of the duration of the treatment and its effects.

In recirculation systems, the use of chemotherapeutic agents should be carefully considered due to the possible detrimental effects of these compounds on nitrifying biofiltration flora.

4. Broodstock and Breeding

Guideline 63:

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.

Guideline 64:

Where possible, rational genetic management of broodstock should be used. For broodstock, 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.

4.1 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.

G. HEALTH AND DISEASE CONTROL

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.

1. Fish Health Program

Healthy fish are pre-requisites for reliable data (Jenkins, 2000). Fish used for research should be free of any notable disease agents that could lead to a diseased condition (unless it is part of the experimental protocol).

If a disease condition is part of the experimental design, the potential effects of the pathogen or parasite on the research results should be predictable, or constitute a variable that is being tested through the research protocol.

Guideline 65:

All facilities must have a fish health monitoring program.

Institutions housing fish for research, teaching and testing should have access to expertise in fish health, and preferably to a veterinarian with aquatic medicine experience and training. This individual can assist in the development of SOPs to limit the introduction of disease into the facility, and should be available for consultation on matters relating to the health of the fish (see Section B.4.3 Role of the veterinarian).

1.1 Disease prevention

Guideline 66:

Strategic measures for disease prevention should include: 1) a formal written agreement with a fish health professional (usually a veterinarian) responsible for the management of morbidity and mortality problems at the facility; 2) a program for the detection and management of disease conditions and water quality problems related to physiological stress; 3) strategic application of disease control measures, such as quarantine, immunization, and prophylactic treatments; and 4) a system of regular monitoring and reporting for health assessment purposes.

Stress, nutritional problems, water quality problems, disease outbreaks from infectious causes, cannibalism and predation can all cause major problems in captive fishes (Wedemeyer, 1996a). Methods for early detection of emerging health problems should be implemented to facilitate mitigation and restoration of a level of health compatible with the study objectives. Good health management is required in studies using live fish because study results can be adversely affected or compromised by suboptimal health or disease events.

Many diseases in fish holding systems can be prevented through good husbandry, starting with stock that have been pre-screened for infectious disease agents, subjecting incoming animals to a period of isolation, and low level handling to minimize stress. This may also involve quarantine where aquatic animals "downstream" of the holding facility are considered to be susceptible to any escaped pathogens. Appropriate holding conditions should also be maintained as outlined elsewhere in these guidelines.

1.2 Disease diagnosis and identification of pathogens

Guideline 67:

A health management program should focus on early diagnosis and identification of the causal agents, stressors and mechanisms so that correct control measures can be initiated.

Disease management protocols should include a reliable system for the detection and reporting of clinical signs, and criteria to distinguish between

acceptable and unusual levels of mortality. Isolation and rapid removal of dead fish will help reduce the spread of disease.

If unexpected losses of fish occur, staff should immediately take water, food and fish samples for later analysis should they be required. Samples of affected fish should be retained for diagnostic purposes. It is advisable to develop an SOP with the assistance of an experienced fish health or veterinary clinician to standardize sample collection methods.

With active clinical problems, consultation with a clinical veterinarian is preferred. Where possible, live untreated fish with and without symptoms should be provided to diagnostic laboratories as this is far more valuable than the provision of dead specimens.

Guideline 68:

Fish health management programs should strive to identify both clinical and subclinical/ adventitious pathogens which may occur as a result of experimental stressors.

The presence of infectious diseases in an experimental population, even one not showing clinical disease, may lead to results that are difficult to interpret due to the potential confounding variables caused by sub-clinical disease. The scientific validity and reproducibility of experiments made in a morbid or sub-clinically affected population is questionable. The application of treatments may also be an experimental variable.

Guideline 69:

Particular attention should be paid to monitoring fishes following any potentially stressful event.

All fishes in the facility should be monitored on a daily basis. However, fishes undergoing stressful procedures have an increased risk of developing opportunistic infections, therefore additional care should be taken in observing the fishes for one to five days following a potentially stressful event. Reluctance to eat, unusual behavior, discoloration of the integument and lesions are signs of possible developing health problems.

Physical damage is one type of stress, but more common stressors are new introductions, crowding, handling, transportation, and degradation of water quality (such as sublethal changes in temperature, reduced dissolved oxygen, and increased ammonia) (Wedemeyer, 1996a; Reddy & Leatherland, 1998; Speare, 1998b). Such stress can suppress the immune response, allowing disease organisms to proliferate. However, stress can also lead to mortality in the absence of infectious disease agents.

1.3 Injuries and other disorders

1.3.1 Handling injuries

Guideline 70:

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 (Speare, 1998b). 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.

Information on handling and restraint is given in Section H.1. Handling and Restraint.

1.3.2 Behavioral interactions causing injury

Guideline 71:

Health management measures should be used to ensure that behavioral interactions with negative consequences such as aggression are avoided.

Various steps, such as size-sorting fish, adjusting density or providing visual sight barriers, can be employed to minimize aggressive encounters.

Some fish exhibit territorial behavior, which can lead to wounds (Speare, 1998b). Social interactions and density have an effect on behavioural interactions (Speare, 1998b) and can lead to compromised behaviors, for instance the suppression of feeding.

1.3.3 Feed-related disorders

Nutrition can also influence the health of fish by causing nutrient deficiencies, imbalances or toxicoses, or by introducing infective agents (see Section F.3. Food, Feeding and Nutrition).

1.3.4 Toxicities resulting from use of chemotherapeutants and environmental toxins

Guideline 72:

A Standard Operating Procedure should be established for any standard treatments, and include the definition of endpoints should fish be adversely affected.

Treatment of fish should be carried out in consultation with a veterinarian. SOPs for standard treatments should also be developed in consultation with the veterinarian or fish health professional.

Medications are usually delivered to groups of fish rather than to individual sick fish, which puts more animals at risk of unexpected effects of the treatment. As far as possible, fish that need to be treated should be isolated until the treatment is completed. When bath treatments are administered, there should be close observation and maintenance of water quality, as this is a major source of problems. In cases where the anticipated effects are unknown, a small number of fish should be tested before application to the group as a whole.

H. EXPERIMENTAL PROCEDURES

1. Handling and Restraint

Guideline 73:

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.

Guideline 74:

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.

Guideline 75:

Fishes should be handled only when necessary, and the number of handling episodes should be minimized.

Even routine handling procedures may cause morbidity and mortality, if carried out by personnel who have not been adequately trained (see Section G.1.3.1 Handling injuries).

Nearly all fishes held in the laboratory have to be physically handled. Handling and disturbance appear to be stressful events for fishes, although they can be conditioned to the handling (Kreiberg, 2000). Appropriate handling equipment, preferably a bucket (or alternatively knot-free-nets) and sanitizable tables, should be used to minimize damage to fishes during handling. Measurements of body condition, such as weight and length, which involve hands-on manipulation should be conducted quickly and in a manner that is minimally stressful. Procedures that involve more than momentary restraint or require that large numbers of fish be handled should be conducted under sedation, unless the fish have been conditioned to the handling. In the long term, the effects of stress can include loss of appetite, inhibition of growth, impaired reproductive success and impaired immune response (Reddy & Leatherland, 1998). Depending on the species and the frequency and intensity of the stressor involved in handling, it may take from a few hours to several days to resume normal feeding.

Where fish are to be handled repeatedly, a suitable period of recovery should be permitted between handling procedures. Recovery from stress can be prolonged. Repeated handling may require an increased level of monitoring, and the stress may be alleviated by the use of previously proven sedation (see Kreiberg, 2000).

Guideline 76:

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.

Fishes are highly reliant on the integrity of their mucus and epidermal body covering as a barrier to osmotic stress and infectious agents. As the skin is relatively delicate in many species, anesthetics and sedatives are frequently used to prevent external damage during procedures which would not require anesthesia in other species. Polymeric water additives such as poly-vinyl-pyrrolidone (PVP) have been found useful in transport and handling of fish (Carmichael &

Tomasso, 1988; Wedemeyer, 1996b). In general, such compounds are considered to bond temporarily to exposed tissue, serving as a short-term replacement for shed mucus and restoring the protection that mucus provides.

Guideline 77:

Restraint and handling of fishes should be carried out in a manner to minimize visual stimulation. Where feasible, fishes should be protected from direct light and rapid changes in lighting while being restrained.

Exclusion of light, wholly or in part, has been recommended as a practice to reduce stress in fish undergoing handling (Wedemeyer, 1985; Hubbs *et al.*, 1988)

Manual restraint may be a practical means of performing rapid, minimally stressful procedures, but requires skilled and careful handlers. Many fishes are sensitive to visual stimuli, especially light, so handling in a dimly lit area may help lessen handling stress.

Guideline 78:

In general, fishes should not be kept in air continuously for more than 30 seconds.

In general, the length of time fishes are held out of water should be minimized, and should not exceed 30 seconds (Ferguson & Tufts, 1992); however, some species such as eels and catfish can tolerate longer periods out of water. The damaging effects of even brief periods out of water on gill epithelial tissue in some fish species has been described; therefore, when out of water the gill lamellae should be kept moist.

Large fishes such as broodstock are less responsive out of water if their head is covered with a damp cloth or foam rubber, which also preserves moisture in the gill region.

1.1 Restraint of dangerous species

Guideline 79:

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.

Prior consultation with colleagues experienced in working with the species and review of any relevant literature is important (CCAC, 2003a; Nickum *et al.*, 2004).

2. Restricted Environments

Guideline 80:

Every effort should be made to provide fishes held in restricted environments with as non-stressful 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.

3. Surgery

For a thorough review of fish surgical techniques, see Johnson (2000). CCAC *Guide to the Care and Use of Experimental Animals*, vol. 1, Chapter IX Standards for Experimental Animal Surgery (CCAC, 1993b) should also be consulted for general guidance, bearing in mind the difference in environmental conditions necessary for the successful conduct of surgery on fishes.

3.1 Surgical preparation and skin disinfection

Guideline 81:

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.

Guideline 82:

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 anesthetic and surgical time, and contribute to a faster recovery of the animal.

Guideline 83:

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

The removal of mucus and disruption of scales that occurs during surgical preparation is generally thought to devitalize tissue and render the area more subject to attack by saprophytic agents, particularly fungal and bacterial invasion. A variety of molecules with antibacterial properties are found in the mucus and outer epidermal layers of fish, as are phagocytic and mononuclear cells. Beyond the gentle removal of grossly visible dirt and debris, preparation should be limited in scope (Wagner *et al.*, 1999).

The type of aggressive surgical scrub procedure used in mammalian surgery to render a site "surgically clean" is not generally used in fish surgery. There are also species differences, with species such as sharks having tough resistant skin, while others such as catfish have delicate, relatively unscaled skin. Dilute aqueous-based povidone-iodine solutions appear to be well tolerated by many species and can be irrigated over an area before draping and incision. Quaternary ammonium compounds and alcohol may be irritating and toxic when applied to the skin in some species, and should be avoided.

Although creating surgically clean skin (defined in mammals as having fewer than 10,000 bacteria per gram tissue) is problematic in fish, the provision of sterile occlusive drapes will help to maintain a sterile to surgically clean operating field. Sterile plastic food wrap (saran wrap) is preferable to fabric drapes, as the latter is prone to absorption of water and introduction of bacteria in the water. Surgical incisions can be made in sterile fashion by cutting directly through the drape into underlying tissue.

Guideline 84:

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.

There is a lack of published information on the effect of adventitious bacteria on the healing of surgical wounds in fish; however, it is reasonable to take precautions to minimize bacterial contamination and colonisation of wounds and body cavities by using sterile techniques where feasible.

Instruments may be gas sterilised or autoclaved, or where this is not possible, cold sterilisation for 10 minutes using benzalkonium chloride or related cold sterilants could be used, although this technique is unlikely to be sporicidal. These latter agents are tissue toxic and the instruments should be rinsed thoroughly in sterile water before being used on tissue. In multiple surgeries, two sets of instruments should be rotated through a cold sterilizing solution. A hot bead sterilizer may also be used and is the most practical method for many lab situations. Many surgical disinfectants, such as alcohol and glutaraldehyde, cause devitalisation or excessive mucus production when applied to fish skin and should be avoided.

3.2 Water quality during surgery

Guideline 85:

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

Water used to irrigate fish gills during prolonged anesthetic procedures should be circulated and treated to maintain proper anesthetic levels, oxygen, temperature, pH and salinity, and to remove particulates. As water temperature can be affected by room air temperature and use of surgical lights, it should be carefully monitored and controlled. Water quality can also be affected by the production of mucus, urine or feces during surgery, and should be changed or renewed accordingly.

In some instances, the addition of conditioning solutions to the water may be justified, particularly for the replacement of electrolytes following trauma or stress. Proprietary mixtures containing electrolytes, artificial film materials based on polyvinylpyrrolidine and oxidative scavenger molecules are used to lessen stress and morbidity following surgery; however, their efficacy has not been scientifically proven.

3.3 Anesthesia

Guideline 86:

Anesthetics 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.

Anesthesia is generally defined as a state caused by an applied external agent, resulting in depression of the nervous system, leading to loss of sensation and motor function.

The use of anesthetics 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 anesthetics 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 (Iwama *et al.*, 1988; Davis, 1992; Iwama, 1992). Anesthetic overdoses are also used routinely as an effective and humane means of euthanizing fishes.

Information on the characteristics of the major anesthetics used on fishes, essential parameters for their application, including optimum and lethal doses, as well as induction and recovery times is provided by Iwama *et al*. in *Anesthetics*, a supplement to these guidelines available on the CCAC website (www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Guidelis.htm). Possible physiological effects and cautionary notes are also given. Additional information is available in Iwama & Ackerman (1994).

Guideline 87:

Anesthetics 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 anesthetic 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 anesthetic agents not approved for veterinary use in Canada.

Where anesthetics are to be used in the field, the CCAC *guidelines on: the care and use of wildlife* should be consulted for advice, in particular for recommendations concerning drug residues (CCAC, 2003a).

Where anaesthesia will be of longer duration, a recirculation technique which ensures continuous delivery of oxygenated water and anesthetic to the gills should be employed (Iwama & Ishimatsu, 1994).

Guideline 88:

Regardless of the application, anesthetics should be tested on a small sample of fish, as the effect of an anesthetic can vary with local water conditions, as well as the species, life stage, and size of the fish.

The reaction of any fish being considered for surgery to the proposed anesthetic should be well understood. Trials with healthy fish are recommended to ensure proper dosage and to accurately calculate the time to reach the necessary anesthetic plane (Johnson, 2000).

Guideline 89:

Personnel working with anesthetic agents in fish must be adequately trained and protected with personal protective equipment.

Many of the anesthetics in use have the potential to cause harm to humans if they are misused.

3.4 Surgical equipment

An overview of the equipment used in fish surgery is provided by Brattelid & Smith (2000) and Johnson (2000).

3.5 Incisions

Guideline 90:

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 (Johnson, 2000).

Abdominal incisions may be made on the ventral midline or lateral to this region. Midline incisions have been shown to minimize behavioral changes in trout (Wagner & Stevens, 2000). Ventral midline approaches may intrude on blood vessels and do not invade tissues such as muscle, but the incision is likely to come in contact with the substrate in the tank, which may cause damage to the incision site. Lateral approaches avoid this problem, but invade muscle and may result in accidental puncture of underlying organs.

Hemorrhage encountered during surgery must be controlled by direct pressure using swabs, by ligation or by cautery. Gel foam can also be used. Cauterization should be used sparingly and with care as it devitalizes tissue and predisposes it to wound infection and breakdown.

3.6 Suture materials and techniques

Guideline 91:

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.

Internal layers and organs can be closed using synthetic absorbable materials. Layers such as skin, that will be exposed to water and bacteria, should be closed with non-wicking, inert suture materials, such as monofilament nylon (non-absorbable) or polydioxanone (absorbable).

Cyanoacrylate surgical adhesives may have application in certain types of surgery on aquatic species; however, these materials have three major disadvantages in that they only bond dry tissues, lack good holding strength in maintaining wounds closed in wet environments, and tend to promote inflammatory responses at the site to which they are applied (Harvey-Clark, 2002).

Summerfelt & Smith (1990) provide instructions on suture technique. Suture patterns that

give good tissue apposition and maximum safety, such as simple interrupted and horizontal mattress, are recommended. These techniques have been further examined by Wagner *et al*. (2000).

3.7 Pathophysiology of surgery and wound healing in fishes

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.

The basic physiology and histological response to surgical wound healing of fishes has been characterised (Wagner *et al.*, 1999). Factors that affect wound healing include:

- water quality (hardness, levels of salt and other osmotically active compounds, and water temperature), which regulates immune response and tissue metabolism rate;
- presence of tank mates, cannibalism of surgical wound area by conspecifics, and exclusion of animals with postoperative morbidity during competition associated with feeding;
- nutritional plane before surgery, good nitrogen balance, anorexia after surgery (stress response), and speed of return to normal feeding and other behaviours;
- hormonal status, especially smoltification in salmonids;
- changes in electrolyte balance due to open wounds and passive loss of water (marine fish) and of electrolytes (freshwater fish) to the surrounding water;
- integrity of mucus layers and biofilms on fish; and
- presence/absence of opportunistic bacterial and fungal pathogens in water during and after surgery.

3.8 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 (Vecino *et al.*, 1992; Rodriguezmoldes *et al.*, 1993; Zaccone *et al.*, 1994; Balm & Pottinger, 1995), and the response of goldfish to analgesia has been shown to be similar to that of a rat (Jansen & Green, 1970). 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 (Verheijen & Buwalda, 1988).

Guideline 92:

In laboratory or applicable field situations, fish must receive careful attention and monitoring following surgery.

Although recovering fish may appear to be normal, there may be prolonged metabolic effects following the stress of anaesthesia and surgery. In situations where monitoring is not possible, pilot scale evaluations of procedures should be considered. Where possible, fish should be allowed to recover from anesthesia until able to resume normal behavior. As anesthesia itself causes prolonged stress, careful procedures for recovery are vital, for example, a quiet, well aerated, possibly darkened tank will facilitate recovery.

Fish require extra attention in the postoperative recovery period. A number of common complications may occur, including wound dehiscence and infection, osmotic imbalances related to surgical incisions, and anorexia. Transient postsurgical shock is a common problem in fish and includes problems with oxygen debt, catabolic processes, fluid and electrolyte loss, and hormonal imbalance. It is important to keep recovery water clean.

Guideline 93:

Fish should be held in a manner that reduces or eliminates intraspecific interactions in

tanks, and meets appropriate living conditions for the species.

The return of the fish to the pre-surgery tank, including the presence of tank mates, has to be considered. The benefits of companion fish (social interaction, schooling fish and feeding activity stimulation) have to be weighed against the disadvantages (predation, competition for feed and biting of sutures).

Well oxygenated water, low lighting, shelter areas for recovering fish, and the use of water conditioning agents to improve the buffering ability of the water and to supply lost electrolytes may help speed recovery.

Guideline 94:

The costs and benefits of the use of prophylactic antibiotics post surgery should be carefully considered.

The indiscriminate use of antibiotics is not recommended because of the possibility of encouraging resistant strains of bacteria. In particular, antibiotics should not be administered to fishes in the wild following tagging or other minor surgical procedures.

Where surgical conditions cannot be made aseptic, the early administration of broad-spectrum antibiotics of low toxicity to fish, chosen based on knowledge of opportunistic flora in the specific aquatic environment and on culture and sensitivity results in infected animals, may be appropriate (see CVMA, 2000).

A review of antibiotic properties and doses in fishes can be found in Stoskopf (1993). However, a veterinarian should be consulted to determine the appropriate treatment.

Guideline 95:

Social factors, such as size differences, ability to feed or exclude other fish from feed, and agonistic behavior, should be considered in experimental design and when maintaining social groups of recovering fish.

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

4. Administration of Compounds and Devices by Various Routes

Morton *et al.* (2001) should be consulted for guidance on best practices for the administration of substances. Although principally focussed on mammals, there are recommendations for fishes and a useful checklist to consult when planning procedures. As with any procedure, administration of compounds should be carried out by competent individuals under expert supervision, preferably a veterinarian.

4.1 Branchial diffusion ("inhalation")

The most prevalent route of exposure of fish for chemical agents is via the gills. Fish gills have a large surface area due to a series of lamellae protruding from the surface. The epithelium of the lamellae is extremely thin and designed to facilitate the diffusion of respiratory gases. In addition to gas transfer, fish gills also permit uptake of other molecules. Diffusion or uptake efficiency of chemicals by the gills depends primarily on their hydrophobicity and molecular size (Black, 2000a).

4.2 Oral

Guideline 96:

If a treatment compound is to be administered orally, the volume dose rate should not exceed 1% body weight (1 mL/100 g).

Fishes may be force-fed liquids and semi-solid solutions using flexible rubber tubing and a syringe. Force-feeding is useful for the delivery of stable isotope-labelled compounds and other test substances. In some species, light anaesthesia is necessary to prevent struggling and vomiting. In others, such as some sharks, brief restraint and the use of a rigid speculum permits safe passage of the tube.

Regurgitation may occur in some species after force-feeding. Fish should be carefully observed,

particularly following resuscitation, to ensure that the administered agent is retained. However, many fish have a J- or U-shaped stomach or pyloric flexure that prevents the introduced substance from being regurgitated, providing the tube is inserted into the stomach past this flexure. In general, no more than 1% body weight should be administered orally in a single dose, although many species have highly distensible stomachs and can tolerate larger percentages.

Electronic transmitters may be inserted via the oral route into the stomach using a hollow plastic tube with a central blunt trocar to push the transmitter into the stomach.

4.3 Injection

Guideline 97:

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. Details of injection techniques, suggested needle sizes, and injection volumes are available, e.g., Summerfelt & Smith (1990), Stoskopf (1993) and Black (2000b).

Chemicals to be injected should be dissolved directly in sterile physiological saline. However, hydrophobic chemicals should be dissolved in very small quantities of co-solvent (e.g., ethanol, methanol, or dimethyl sulfoxide [DMSO]) prior to dilution in saline. For chemicals that are not soluble or stable at neutral pH, the pH of the injection solution may be adjusted with an acid or base (Perry & Reid, 1994).

Final injection volumes should be as small as possible to minimize physiological disturbances to the fish. In addition, control fish (vehicle and/or sham injected) should be part of the experimental protocol to correct for any effects of the injection procedure or the vehicle.

4.4 Implants, windows and bioreactors

Guideline 98:

Implanted materials should be biocompatible and aseptic, and should be implanted using sterile techniques.

Bioabsorbable pellet implants of bioactive compounds in absorbable and nonabsorbable matrix vehicles are available from commercial sources or can be custom fabricated. These can be surgically implanted in the peritoneal cavity or implanted with a trocar introducer into muscle masses. Osmotic minipumps can be implanted in a similar fashion as can transmitters and telemetry units. Windows to visualize visceral changes, such as splenic size change during blood loss, have been successfully used (Yamamoto *et al.*, 1985).

5. Tagging and Marking

Tagging and marking techniques are used in both field and laboratory studies. For field studies, general principles are outlined in the CCAC *guidelines on: the care and use of wildlife* (CCAC, 2003a). As well, *Concerted Action for Tagging of Fishes* (www.hafro.is/catag/) provides detailed information on current best practices for tagging and telemetry in field research.

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.

Guideline 99:

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.

The following criteria should be applied as far as possible:

- marking should be quick and easy to apply;

- marking code (numbers or colors) should be readily distinguishable;
- markings should persist on animals until all research objectives are fulfilled;
- animals should experience no long-term adverse effects on health, behaviour, longevity or social life;
- accurate records of the marking procedure should be kept;
- marking of animals in the wild must comply with federal, provincial/territorial and other agency regulations; and
- marking must allow for seasonal changes and growth of juvenile animals.

In choosing an acceptable marking technique, the investigator should consider the nature and duration of restraint, the amount of tissue to be removed or damaged, the potential for pain and/or distress, and the risk of infection. When invasive procedures of more than short duration are proposed, or when species that are prone to damaging themselves during handling are concerned, anesthesia or sedation should be used.

Fish biologists use a variety of internal and external tags, ranging from fin clips to subcutaneous and abdominal transponders. No single tag or marking approach is suitable for all fishes or applications. Marks can range in longevity from days to decades. Some tags cause virtually no adverse reaction, such as visual implant tags, while others cause considerable cutaneous damage (Johnson, 2000).

5.1 Tissue marking

Guideline 100:

Marking techniques which cause significant tissue injury, such as branding, tattooing or clipping important fins, should only be used if evidence is provided to an animal care committee indicating that alternative methods cannot achieve the desired result.

Any marking should be evaluated for its potential effects on health and behavior.

5.2 Tagging

The methodology for tagging fish has been well described (Neilsen, 1992). Tagging operations may involve stress and injury, both from handling the fish and the wound caused by application of the tag. Therefore, the effects of the marking on fish behavior and health should be considered (DeTolla *et al.,* 1995). Where these are unknown, a pilot study should be run, using a few fish in the laboratory. The size, shape and placement of tags should allow normal behaviour, and should not cause entanglement in aquatic cover. Brightly colored tags may compromise an animal's camouflage or possibly act as a predator attractant in the wild. If fish are released, they must be in good health, able to function in the environment, and be released only within their native range of distribution (DeTolla *et al.*, 1995).

5.2.1 Genetic markers

The current use of genetic technology in the identification of fish stocks entails the removal of blood or tissue for identification.

5.2.2 Internal tags and marks

Information concerning the use of implanted wire tags, passive integrated transponder (PIT) tags, otolith marks and natural parasites to identify fish is reviewed by Parker *et al.* (1990). Morton *et al.* (2003) and the CCAC *guidelines on: the care and use of wildlife* (CCAC, 2003a) provide general principles to be considered in the implantation of telemetry devices. In particular the effect of the weight, shape and size of the device on the physiology and behaviour of the fish must be considered.

6. Collection of Body Fluids

A review by Black (2000b) provides details of techniques used to collect blood, urine, feces, and sperm from fish. In general when blood is collected, sample size is recommended to be up to 1ml/kg body weight, although fish can sustain higher percentages of blood volume removal. The fish must be permitted to recover their haematocrit level prior to subsequent blood collection. Hematocrit recovery times are tempera-

ture dependent and highly variable between species.

Guideline 101:

Sedation or anesthesia should be used to restrain fish for collection or cannulation purposes. It is important to realize that both restraint and anesthesia 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.

Longer term cannulation for repeated samples in conscious fish can be achieved in many teleosts larger than 150 grams by cannulating the dorsal aorta (Schreck & Moyle, 1990; Black, 2000b).

The use of indwelling catheters for urine collection in teleosts has been described by Schreck & Moyle (1990) and Black (2000b).

7. Use of Infectious Disease Agents, Tumorigenic or Mutagenic Agents, and Toxic and Noxious Compounds

As noted in Section B.5. Government Regulations and Policies on the Use of Fish, regulations and guidelines are issued under Section 36 (5) of the Fisheries Act of Canada. The regulations for the pulp and paper industry, and for the metal mining industry, specify monthly acute lethality testing with rainbow trout on all effluent streams, and the frequency of these tests. These regulations also specify Environmental Effects Monitoring programs which require twice yearly sublethal toxicity tests with fish, invertebrates and plants. Guidelines for the petroleum refining industry and potato processing industry also specify similar acute lethality tests with rainbow trout. Environment Canada conducts its own audit testing for these regulations and guidelines. For the Fisheries Act and all the regulations (Pulp and Paper, Metal Mining, Potato Processing, etc.) see laws.justice.gc.ca/en/F-14/index.html. Environment Canada's Method Development & Applications Section has published a series of peer-reviewed Biological Test Methods (for acute lethal, and sublethal tests) covering fish, plants, bacteria, and invertebrates, developed under the guidance of the Inter Governmental Environmental Toxicology Group (IGETG). These published methods, found at www.etc-cte.ec.gc.ca/organization/spd_e.html, describe the care, disease prevention and use of fish in testing in detail. Several provinces have similar fish testing requirements for industrial wastewater discharges, and these specify the use of the Environment Canada Biological Test Methods for this testing.

For protocols involving infectious disease agents, tumorigenic or mutagenic agents, or toxic and noxious compounds, it is of particular importance that protocols should include the use of the earliest endpoints that meet the scientific goals of the study; see CCAC *guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (CCAC, 1998).

8. Endpoints and Criteria for Early Euthanasia

8.1 Recognition of "pain", "distress" and "stress"

Guideline 102:

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 behavior may be causes of distress. Fishes respond to noxious stimuli with altered behavioral, 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 (Sneddon *et al.*, 2003). 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.

Although fishes lack some of the structures associated with pain perception in mammals (e.g.,

well-developed cortex and neospinothalamic tract), there exists evidence that fishes respond in a similar manner to noxious stimuli, learn to avoid "unpleasant" experiences and respond with an amelioration of pain response after treatment with morphine (Jansen & Green, 1970). Fishes also react to aversive stimuli with a full scale of endocrine and metabolic responses. Changes in corticosteroid and catecholamine levels, as well as increases in plasma glucose and lactic acid as demonstrated in some fish species, are generally recognized to be indicators of acute stress.

8.2 Choosing an appropriate endpoint

Guideline 103:

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.

Whenever live animals are used for research, teaching and testing, investigators have an ethical obligation to minimize any pain or distress experienced. The importance of identifying the scientific objectives for a study, and ensuring that these reflect a clear understanding of the mechanisms being studied and the consequences to the animals used, is underlined in the CCAC *guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (CCAC, 1998).

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. As discussed earlier, this can be a challenge in many fish species. One way to address this challenge is to draw up a checklist of anticipated and potential clinical signs. Each study may require its own checklist, depending on the protocol. Both 'population' clinical signs (such as feed intake) and 'individual' clinical signs (such as skin lesions) should be present on the check list. Information accompanying the checklist should include instructions concerning what actions should be taken when abnormalities are observed (including who to contact) and instructions about additional procedures to be taken if a fish is euthanized.

Guideline 104:

When conducting research with defined, early pre-lethal endpoints, a list of parameters should be established to permit an objective assessment of health status.

Establishing a checklist or scoring sheet may involve a literature review and compilation of documented clinical signs. In some cases, it may be necessary to carry out a pilot study in order to obtain accurate information on clinical signs. It is the responsibility of the investigator, working with the ACC and veterinarian, to decide when a clinical sign is a reliable predictor of an event (e.g., death) and what margin of error is acceptable. This is generally undertaken in consultation with the veterinarian before approval for the endpoint to be used as part of the protocol review process. Often it will be necessary to use a combination of clinical signs to make decisions to terminate an experiment or euthanize an experimental animal.

Recognizing the great physiological and behavioral diversity in fishes, the following parameters are given as suggestions for evaluation of clinical signs for fishes involved in research or testing. Other useful references include Goede & Barton (1990).

If the morbidity and clinical signs are known, then the parameters at which point an intervention (likely euthanasia) becomes essential can be defined; for instance, anorexia of "x" days duration alone or in combination with certain other clinical signs (e.g., more than 10 parasites per fish in a parasite infection study) or a certain number of days post-infection (e.g., parasite load number times number of days infected).

The CCAC *guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (CCAC, 1998) includes a useful discussion on clinical signs to establish pre-lethal endpoints for toxicology studies, clinical observations specific to fishes, and suggestions for quantifying pain and distress in finfish.

Table 1: Evaluation of Clinical Signs for Fishes Involved in Research or 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
	posture in water column, i.e. the individual's position in the water (upright, upside down, tilted, etc.)
Unprovoked Behavior	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 behavior
Provoked Behavior	feeding activity
	threat response
	avoidance reaction to mechanical prod
	avoidance reaction to light beam

Guideline 105:

In any study where there is expected morbidity and mortality, the criteria for early euthanasia should be clearly defined.

The use of fish in toxicological research and toxicity testing is well established. Lethal endpoint tests may be required by regulatory agencies, for example in the assay of environmental toxicant mixtures and in fish vaccine efficacy tests. Such tests have the potential to cause pain and/or distress in fishes; therefore where feasible, the development of pre-lethal endpoints in such tests is encouraged.

Regulatory agencies should be contacted during the development of the study protocol to agree on endpoint parameters. In general where lethal endpoints are required, studies should make provision for the humane killing of animals expected to die before the next scheduled observation.

Fishes should not be held indefinitely in a facility without a protocol describing their future use, as well as endpoints for aging fish and fish in poor condition (see Section J. Disposition of Fish After Study).

9. Monitoring

Guideline 106:

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.

If the timeframe for morbidity is unknown, a pilot study should be conducted under veterinary and ACC oversight to determine the most critical period for observation of the fish during the study.

10. Negative Reinforcement Modalities

Guideline 107:

Pilot studies and literature searches should be used to establish the least invasive method of obtaining a consistent response when using negative reinforcement modalities in fishes.

Certain studies may entail the application of noxious substances, alarm substance addition to tank water, or the application of electrical shock to induce swimming or fear/avoidance responses in fishes. The guiding principle of ethical research in this instance is to avoid excessive or unavoidable stress where possible, and to use procedures that minimize distress. For instance, when designing an experiment, a preference test with an avoidable negative stimulus should be substituted for an unavoidable negative reinforcement schedule.

11. Exercise to Exhaustion

Guideline 108:

Studies involving the forced swimming of fishes to the point of exhaustion, often in conjunction with negative reinforcement, should be conducted with strict adherence to guiding principles of minimization of distress of animals. Fishes used in exercise to exhaustion studies should be monitored continuously.

Recovery of fishes after exercise to exhaustion may entail special holding and handling arrangements, such as segregation from normal conspecifics and provision of low current environments.

12. Environmental Extremes

Guideline 109:

Studies involving the exposure of fishes to environmental extremes should select the earliest endpoint possible.

In some instances, fish may be subjected to extreme heat or cold in their holding environment as part of an experimental design. This type of study would be regarded as highly invasive in most vertebrate species.

When proposing studies involving exposure to extreme environments, the definition of endpoints becomes very important and should be carefully defined to reduce: 1) the use of animals in future studies; and 2) distress for fish involved in ongoing studies.

If behavioral endpoints are proposed, it is important that the behavior be observed and recorded in a repeatable fashion using rigorous methodology.

Animals undergoing environmental extremes are likely to experience a range of after-effects, including immunological and physiological changes, which may preclude their use as normal animal models in subsequent studies.

13. Genetically Modified Fish

The local ACC should ensure that investigators have complied with applicable regulatory requirements before approval of a protocol involving an aquatic organism that is an animate product of biotechnology (e.g., genetically modified fish). Like many other countries, the federal government of Canada has developed regulations, policies and guidelines applicable to research involving animate products of biotechnology in order that the potential for adverse effects on the environment and human health may be assessed. Schedule XIX of the New Substances Notification Regulations (NSNR) under the Canadian Environmental Protection

Act, 1999, specifies the information that must be provided 120 days in advance of the import or manufacture of an aquatic organism that is an animate product of biotechnology. These regulatory requirements apply to research and development organisms unless specified containment criteria are met (i.e. no release into the environment of the organism, genetic material of the organism or material from the organism involved in toxicity). Organisms determined to have, or suspected of having, potential adverse effects on the environment or human health may be controlled as necessary, including by prohibiting or imposing conditions on their import or manufacture.

Investigators involved in importation, creation or use of aquatic organisms with novel traits should contact the biotechnology office of DFO for more information on the NSNR requirements. The New Substances Notification Branch of Environment Canada can provide more information on the NSNR requirements related to other aquatic new substances (telephone: 1-800-567-1999 [toll-free in Canada] or 819-953-7156 [outside Canada]; facsimile: 819-953-7155; email nsn-infoline@ec.gc.ca).

Guideline 110:

Genetically modified fishes may have changes in physiology and anatomy as the result of their genetic alteration, and should be closely monitored.

A review of transgenic techniques is provided in Chen *et al*. (1996), Jowett (1999), Linney *et al*. (1999), Fan & Collodi (2002), Lu *et al*. (2002), Maclean *et al*. (2002), and Rocha *et al*. (2004). In addition, the CCAC *guidelines on: transgenic animals* (CCAC, 1997b), and future revisions, should be consulted.

GM fish may have different metabolic and environmental requirements compared to non-GM fish. The normative tables generated for non-transgenic fish cannot be automatically applied to transgenic fish (Stevens *et al*., 1998).

Guideline 111:

Genetically modified fishes must not be permitted to enter the food or feed chain unless they have undergone a thorough safety assessment and have received authorization for sale, manufacture and/or import as a food or feed by Health Canada and the Canadian Food Inspection Agency.

I. EUTHANASIA

Guideline 112:

Where feasible, the euthanasia of fishes should consist of a two-step process, with initial anesthesia 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 anesthesia; the exception being when animals are in extreme distress and the time taken in preparation of anesthesia 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.

Kreiberg (2000) provides discussion on acceptable methods of euthanasia in fishes. The onus remains on the fish handler to be well informed about the pharmacology and physiological impacts of a proposed method of euthanasia. Robb & Kestin (2002) and Lines *et al.* (2003) provide further information on humane methods of euthanasia for fishes.

Guideline 113:

If a physical technique of euthanasia is used when killing fishes, it should entail the physical destruction of brain tissue by pithing or crushing the brain.

Use of hypothermia (including putting fish on ice) before euthanasia should be avoided. Because many species of fishes continue to have brain activity in the face of advanced cerebral and systemic hypoxia, physical euthanasia techniques such as decapitation alone should be avoided (Flight & Verheijen, 1993). It is therefore desirable to physically destroy, freeze, or pith the brain in fishes that have been euthanised using a primary physical technique such as blow to the head.

The American Veterinary Medical Association (AVMA), in their recommendations on methods of euthanasia for lower vertebrates such as reptiles, no longer support the use of hypothermia/freezing as a technique because of concerns about the induction of pain during ice crystal formation (AVMA, 2001).

Exsanguination under anesthesia is also an acceptable method of euthanasia.

Various forms of electrocution have been used commercially, including prolonged exposure to AC and DC voltage; however, these methods may be associated with spinal fractures and muscle damage.

When large numbers of fish need to be euthanized, the use of immersion anesthetic at lethal dose in the holding tank is acceptable.

J. DISPOSITION OF FISH AFTER STUDY

1. Consumption of Fish

Guideline 114:

Fishes destined for food and subjected to sedation or anesthesia should be held for the designated withdrawal time before being killed.

In studies involving aquaculture fish species, it may be acceptable to release such fishes for human food, providing the fishes have not been treated with any unlicensed compounds and the advice of a veterinarian has been sought.

2. Release of Fish to Wild

Guideline 115:

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.

3. Fish as Pets

Some institutions release healthy research fishes (not GM fishes) that are commonly accepted pet or companion species to individuals with the knowledge and ability to provide adequate care. No GM fish may be removed from research facilities to private premises.

If fishes are to be released to the care of an individual as companion animals, the institution should develop an appropriate policy describing the conditions that need to be fulfilled before their release.

4. Transfer of Fish Between Facilities

Guideline 116:

Fishes should undergo health assessment before being transported between facilities. 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.

5. Disposal of Dead Fish

Fish must be disposed of according to acceptable federal, provincial/territorial and municipal regulations for the disposal of biological materials.

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L. GLOSSARY

Acclimation — a persisting physiological, biochemical or morphological change within an individual animal during its life as a result of a prolonged exposure to an environmental condition such as a high or low temperature; generally, the changes are reversible

Adaptation — the observation that the physiology, biochemistry and morphology of any animal is usually very well matched to the environment that the animal lives in; these features have been shaped through evolution by natural selection over many generations, and involve irreversible changes in the genetic material

Analgesia — decrease in response to noxious stimuli

Anesthesia — a state caused by an external agent, resulting in depression of the nervous system, leading to loss of sensation and motor function

Asepsis — absence of living germs, free from septic and poisonous putrefactive products

Atraumatic needle — a needle with a suture permanently attached

Distress — a state of excessive stress in which the animal is unable to make the necessary adaptations to stressor(s)

Ecosystem — a complex of the plant and animal communities within an area, along with the nonliving components of the environment and the interactions among these

Ectothermic — an animal that assumes the temperature of its surroundings

Euthanasia — literally, a good death; rapid loss of consciousness and death, with no pain or distress accompanying the procedure

Exsanguination — a procedure causing extensive loss of blood due to internal or external hemorrhage

Fish — one or more individuals of one species

Fishes — individuals of more than one species

Fomites — non-living objects that can carry disease organisms (e.g., feeders, mops, etc.)

Hygroscopic — readily absorbs water

Hypothermia — lower than normal body temperature

Integument — the natural outer covering of an animal; the skin

Lamellae — area of the gills where the exchange of gases and waste products occurs

Morbidity — visible manifestation of a diseased state

Mortality — loss of life; death

Myopathy — muscle damage resulting from anaerobic muscle function; predisposition may be due to improper capture procedures

Noxious stimuli — those stimuli that are damaging or potentially damaging to normal tissue

Pain (in fish) — fish pain is a response to a noxious stimulus that results in a change in behaviour or physiology and the same noxious stimulus would be painful to humans (a working definition)

Progeny — offspring

Protocol — a written description of a study or activity that includes details of the goals, the use of animals, the procedures that are to be followed and the personnel involved; the purpose of the protocol is to ensure the quality and integrity of the study or activity

Quarantine — the segregation or isolation of animals from all others to prevent the spread of disease

Regurgitation — passive return of food or fluid to the mouth from the stomach

Salmonidae — the family of fishes that includes salmon (*Oncorhynchus spp*. and *Salmo salar*), trout (*Salvelinus spp*., *Salmo spp*.) and char (*Salvelinus alpinus*); whitefish (*Coregonus spp*., *Prosopium spp*., *Stenodus leucichthys*); and grayling (*Thymallus arcticus*). Also used informally as the name of the subfamily which includes salmon, trout and char

SOP — Standard Operating Procedure; written documents specifying procedures for routine activities that must be followed to ensure the quality and integrity of the study

Standard Environmental Temperature — the temperature for optimum growth of a species of fish

Supersaturation — a condition where the total gas pressure in a body of water exceeds the barometric pressure in the overlying atmosphere

Telemetry — the use of devices to transmit information to a distant station where it is recorded; commonly used in field studies to monitor animals in order to answer questions about their physiology, behavior, habitat use, survival and movements

Welfare — a term used to describe the quality of life that an animal is experiencing

Well-being — a state or condition of physical and psychological harmony between the organism and its surroundings. Good health and manifestation of normal behavioral repertoire are the most commonly used indicators of an animal's well-being

Withdrawal time — the length of time between when an animal is given a drug and the prescribed time period for clearance of residues of that product

Zoonotic — relating to the transmission of a disease from a non-human species to humans

M. ABBREVIATIONS

ACC — Animal Care Committee

CAEAL — Canadian Association for Environmental Analytical Laboratories

CALAM — Canadian Association of Laboratory Animal Medicine

CCFAM — Canadian Council for Fisheries and Aquaculture Ministries

CFIA — Canadian Food Inspection Agency

CITES — Convention on International Trade in Endangered Species of Wild Fauna and Flora

COSEWIC — Committee on the Status of Endangered Wildlife in Canada

CSA — Canadian Standards Association

CSZ — Canadian Society of Zoologists

CWS — Canadian Wildlife Service

DFO — Fisheries and Oceans Canada

FAWC — Farm Animal Welfare Council

FHPR — Fish Health Protection Regulations

FRP — Fiberglass reinforced plastic

FTA — Free Trade Agreement

GATT — General Agreement on Tariffs and Trade

GFI — Ground fault interrupter

HVAC — Heat, ventilation and air conditioning

ICES — International Council for the Exploration of the Seas

IV — Intravenous

IP — Intraperitoneal

NAC — North American Commission

NAFTA — North American Free Trade Agreement

NASCO — North Atlantic Salmon Conservation Organization

OECD — Organization for Economic Cooperation and Development

OIE — Office International des Epizooties

SOPs — Standard Operating Procedures

SPS — Sanitary/Phytosanitary

TMS — Tricaine methane sulphonate

WAPPRIITA — Wild Animal and Plant Protection Regulations of International and Interprovincial Trade Act

WTO — World Trade Organization

APPENDIX A
RELEVANT GUIDELINES AND ORGANIZATIONS

American Fisheries Society (AFS)
www.fisheries.org

American Society of Ichthyologists and Herpetologists (ASIH)
www.asih.org

AquaNet www.aquanet.ca

Canadian Aquaculture Institute, PEI
www.upei.ca/~cai/

Canadian Association of Aquaculture Veterinarians

Canadian Association for Environmental Analytical Laboratories (CAEAL)
www.caeal.ca

Canadian Council for Fisheries and Aquaculture Ministries (CCFAM)
www.aquaculture.ca/English/CAIA_CCFAM.html

Canadian Food Inspection Agency (CFIA)
www.inspection.gc.ca

Committee on the Status of Endangered Wildlife in Canada (COSEWIC)
www.cosewic.gc.ca

Canadian Society of Zoologists (CSZ)
www.csz-scz.ca/jpellerin/csz/

Canadian Veterinary Medical Association (CVMA) canadianveterinarians.net

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)
www.cites.org

Environment Canada (EC) (and Intergovernmental Environmental Toxicology Group)
www.ec.gc.ca

European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123) - *Appendix A: Guidelines for accomodation and care of animals* — Species-specific provisions for fish
www.coe.int/T/E/Legal_affairs/Legal_cooperation/Biological_safety%2C_use_of_animals/Laboratory_animals/A_texts_docs.asp#TopOfPage

Farm Animal Welfare Council (FAWC)
www.fawc.org.uk

Fish Health Protection Regulations (FHPR)
laws.justice.gc.ca/en/F-14/C.R.C.-c.812

Fisheries and Oceans Canada (DFO)
www.dfo-mpo.gc.ca

Fisheries Society of the British Isles (FSBI)
www.le.ac.uk/biology/fsbi

Health Canada (HC)
www.hc-sc.gc.ca

Office International des Epizooties (OIE)
www.oie.int

APPENDIX B
ZOONOTIC DISEASE – TRANSMISSION OF FISH DISEASES TO MAN

Zoonoses, also called zoonotic diseases, are animal diseases that may be transmitted to humans. Piscine zoonotic diseases have been extensively reviewed elsewhere (Nemetz & Shotts, 1993) and will only be briefly summarized here. In general, transmission of fishborn zoonoses is relatively rare, and the vast majority of these organisms produce self-limited bouts of gastroenteritis, usually secondary to consumption of undercooked fishes, or localized wound infections, usually due to contamination of cuts or abrasions while handling either live fishes or fish tissues. However, a few of the more virulent organisms have the ability to produce systemic infections in humans and, in very rare instances, death (Nemetz & Shotts, 1993). Although zoonotic diseases are rare in healthy individuals, the risk is markedly increased in people with depressed immunity (e.g., patients with AIDS, organ transplant recipients receiving immunosuppressive drugs).

Diseases of fishes can be induced by a variety of bacteria, rickettsiae, roundworms (nematodes), cestodes (tapeworms), flukes (trematodes), protozoa, viruses, and fungi (Nemetz & Shotts, 1993; Fryer & Bartholomew, 1996). The type of infestation is dependent upon a myriad of factors, including the species, the supplier, the geographic origin, and the diet of the fish; other important issues are water quality and salinity.

The majority of important zoonotic pathogens of fishes are gram negative bacteria. In North America, members of the genus *Vibrio* are probably the most important bacterial pathogens of marine and estuarine fishes (some species also infect freshwater fishes); members of *Vibrio* species (*spp*.) are also important potential sources of zoonotic disease. Other gram negative bacteria causing fishborn zoonoses include *Plesiomonas shigelloides*, *Aeromonas spp*., *Escherichia coli*, *Salmonella spp*., *Klebsiella spp*., *Edwardsiella spp*., *Yersinia ruckeri*, and *Leptospira icterohaemorrhagica* (Nemetz & Shotts, 1993). Efficacious fish vaccines are currently available to protect fishes against some of these gram negative organisms (Fryer & Bartholomew, 1996).

Pathogenic gram positive bacterial infection in fishes is less common. Gram positive bacteria that can cause zoonoses include *Streptococcus spp*., *Erysipelothrix rhusiopathiae*, *Mycobacterium spp*., *Clostridium spp*., and *Staphylococcus spp*. Several cases of cellulitis caused by accidental inoculation with *Streptococcus iniae*, a fish pathogen associated with menigoencephalitis in tilapia, have been reported in patients in Canada that had injured their hands while handling tilapia (Weinstein *et al*., 1997). *Erysipelothrix* and *Mycobacterium* also usually cause a localized skin infection but, under some circumstances, can cause a disseminated infection (i.e. septicemia). In contrast, skin contact with *Clostridium* and *Staphylococcus* does not usually cause an infection in humans, but rather produce disease when contaminated fishes containing their bacterial toxins are consumed.

A number of viruses and fungi have been identified in fishes (Fryer & Bartholomew, 1996). However, no documented case of human infection by either fish viruses or fish fungi have been reported (Nemetz & Shotts, 1993). Although a number of fish parasites including both worms and protozoa can infect humans, such infections are almost invariably due to consumption of undercooked fish and are exceedingly rare in North America (Nemetz & Shotts, 1993).

Finally, some marine organisms produce toxins that can cause illness and death in humans. Ciguatera and scombroid are examples of food poisonings that can result from eating carnivorous tropical marine fishes. Ciguatoxin is believed to be produced by a marine dinoflagellate that adheres to reef plants and then is consumed by herbivorous fishes; these fishes are subsequently consumed by carnivorous fishes, thus concentrating the ciguatoxin in their tissues. Patients with ciguatera poisoning usually dis-

play gastrointestinal and neurological symptoms. The mechanism of scombroid toxicity is unclear, but is associated with human consumption of marine fishes from the family Scombroidae (e.g., tuna, bonito, mackerel, skipjack, etc.). Scombroid often produces allergic symptoms in patients (Nemetz & Shotts, 1993).

Although all of these fishborn zoonoses are rare, prompt and accurate diagnosis expedites appropriate treatment. Because they are so rare, most physicians, even those specializing in the treatment of infectious diseases, have little or no experience diagnosing them. Thus, it is important for people working with fishes to also be aware of the existence of fishborn zoonoses and, if being evaluated by a physician, to mention that they have occupational exposure to fishes. With the advent of the use of fishes as a source of implantable biomaterials, in xenotransplantation, or as bioreactors for large scale production of human proteins, the possibility of emerging xenozoonoses transmitted from these sources should be considered. At the present time, disease agents recognized as a hazard in other xenotransplantation models, such as mammalian endogenous retroviruses, are not a recognized threat from fish tissues; however, the knowledge of this area is in its infancy.

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APPENDIX C
GUIDELINES FOR CONTAINMENT FACILITIES (FOR PATHOGEN STUDIES)

Although there are currently no national standards developed and approved by federal agencies that are specific for aquatic biocontainment systems, the Canadian Food Inspection Agency (CFIA) and Fisheries and Oceans Canada (DFO) are working on developing guidelines that cover both veterinary biologics and aquatic animal live-holding, in view of meeting several objectives, including genetically modified (GM) organisms and fish with novel traits concerns, and introductions and transfers concerns (e.g., escapee impacts from ecological, spawning and trophic competition). The standards will bring much needed transparency, clarity, consistency and objectivity for both the handlers of aquatic animals and their pathogens for experimental or commercial development purposes, and the inspectors. For aquatic animal pathogen laboratories conducting *in vitro* work, such as diagnostic laboratories, all physical containment and operational practices for the appropriate containment level must be followed as per the existing *Containment Standards for Veterinary Facilities* (Agriculture and Agri-Food Canada, 1996). These Standards can be accessed at: www.inspection.gc.ca/english/sci/lab/convet/convete.shtml. This Appendix is therefore intended to provide additional guidance to investigators and Animal Care Committees (ACCs) working with aquatic animals and/or their pathogens until federal agency guidelines are developed. The approach taken in these CCAC guidelines reflects current best practices. Facilities engaged in studies requiring biocontainment are subject to inspection by provincial and federal authorities. CFIA's Biohazard Containment and Safety division (www.inspection.gc.ca/english/sci/bio/bioe.shtml) is the point of contact for any queries or construction plans regarding laboratories conducting *in vitro* work and for any containment questions related to *in vitro* use of imported veterinary biologics. DFO's Aquatic Animal Health Office is the point of contact for any containment questions related to live aquatic animal transfers or *in vivo* infection trials.

There are some prescriptive standards provided in the *Fish Health Protection Regulations: Manual of Compliance* (Fisheries and Oceans Canada, 1984) concerning importation and movement of salmonid fish, their eggs and tissues.

Principles of containment described within this Appendix also extend to research involving GM fish. DFO is the Canadian lead agency developing requirements and containment standards for live-holding of aquatic organisms with novel traits (e.g., GM fish). DFO's biotechnology office is the point of contact for any containment questions related to aquatic organisms with novel traits, and DFO's Aquaculture Management Directorate for containment questions related to live aquatic animal transfers.

1. Aquatic Biocontainment Laboratory Physical Plant

The categorization of aquatic pathogens to Animal Biosafety Levels I to IV (established for terrestrial systems) is problematic. Most agents are currently considered as Biosafety Level I or II (See *Laboratory Biosafety Guidelines* [Health Canada, 2004] at www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/). The criteria used to assess the level of risk are subjective, e.g., the lack of viral envelope in the case of some viral pathogens.

In aquatic systems, surface contamination, contamination of fomites such as nets and tanks, and aerosol-born transmission are all possible sources of accidental transfer of aquatic pathogens. Biocontainment in aquatic systems relies to a large extent on common sense hygiene measures such as fomite control and disinfection, use of tank-specific hardware, external clothing dedicated to biocontainment facilities, hand washing and the use of disinfectant footbaths.

Guideline A:

Facilities conducting research, teaching or testing on fish using aquatic animal pathogens must do so with the knowledge and permission of local DFO, CFIA and appropriate provincial/territorial authorities.

Guideline B:

Facilities used for aquatic animal pathogen research must be properly contained and physically separate from other holding rooms and facility functions, such as the holding and rearing of production fish or holding of broodstock fish. Effluent must be rendered noninfectious before being returned to the environment.

The location and physical attributes of a containment facility must prevent accidental release during flooding, storms or other natural disasters. In order to ensure adequate containment, facilities should be built on dry land and sited above historic flood levels. If these conditions cannot be met, then projects should be small enough to permit all animals to be moved safely to an alternate site, or destroyed, within a realistic time-frame permitted by natural disaster warnings. Containment facilities are particularly sensitive to entry of predators and pests, and the design of the entire facility must address this risk. In particular, drain systems, portals for personnel and equipment access, and heating, ventilation and air conditioning (HVAC) systems are vulnerable.

Consideration must be given to maintenance and other required access to mechanical and accessory systems. Where possible, these systems should be accessible without entry to the containment area from outside of the facility.

Guideline C:

Materials and surfaces used for facility construction should be durable, nonporous and easily sanitized using surface cleaning and potent surface disinfectants that have proven efficacy. Wood, porous materials and unsealed concrete should not be used for biocontainment facilities.

Materials and surfaces used in any fish facility should be easily sanitized. However, in containment facilities, surfaces may be subjected to more rigorous decontamination procedures, and therefore should be tested to ensure that they can withstand stronger or more repetitious chemical treatments.

Guideline D:

Containment rooms should be ventilated to permit drying conditions and even mixing of air, but prevent aerosol-born pathogens from escaping via air movement or condensation on surfaces or clothing.

Temperatures should ensure that wall, floor and ceiling surfaces dry rapidly, as most aquatic pathogens survive longer in a wet environment. The use of tank covers ensures that humidity levels are maintained at a reasonable level within the room and also reduce the risk of splash transfer of pathogens between tanks or onto the floor.

Guideline E:

All pathogen control processes must have fail-safe backup.

In the event of a failure in any automated effluent disinfection system, there should be adequate containment to prevent untreated water from leaving the facility, as well as an emergency alert to ensure that the responsible authorities respond as quickly as possible to rectify the situation. Automated systems should be programmed to measure residual disinfectant concentrations and ensure these fall within pre-set parameters for pathogen inactivation. These parameters should be tested prior to bringing in high risk research animals and/or their pathogens, by bacteriological or virological tests of 'spiked' effluent. All effluent from containment systems should be routed through hard plumbing to effluent treatment tanks which ensure adequate contact time for decontamination of effluent. In the event of a system failure, inflow systems should be engineered to shut off, preventing overflow of the system.

Any release of effluent to the environment must meet local regulations, and ensure that viable pathogens are not released. In general, this will require local municipal sewage authority approval and/or an environmental impact assessment if the effluent is to be released to the local waterways. Disinfected products, whether solid or liquid, should be neutralized prior to

release from the containment facility because they can be toxic to fishes and other aquatic resources.

1.1 Facility entrance

Guideline F:

Facilities should have secured entry systems, be clearly signed and have means of logging who enters and at what time.

Traffic within the facility should be minimized, with entry permitted on an 'as needed' basis only. The entry should be locked and accessible solely to authorized personnel. There should be a clothing transfer area immediately adjacent to the entry area where outside clothing can be exchanged or covered by containment facility-specific outerwear (coveralls, footwear and gloves).

Guideline G:

Foot baths and hand wash stations should be available at the entry and exit points of the containment facility.

Personnel must use footbaths and hand wash stations each time they enter or exit the containment facility.

1.2 Rooms

Guideline H:

Individual rooms should have door and wall seals.

Individual rooms should have raised dams across the doors and floors, with waterproof seals running along the walls to a depth sufficient to hold all water within the containment room should it leak or spill from the holding tanks or water supply reservoirs.

Guideline I:

Room surfaces, piping, tanks and water transfer systems in rooms should be designed for complete access and sterilization between studies, and must be designed to prevent back flow from animal holding tanks and effluent handling systems.

All pipes should be hard-plumbed with removable access points for cleaning and quality control culture following studies. Fail-safe plumbing systems should be used that prevent tanks from self-draining in the event of loss of water supply.

Guideline J:

Rooms should be engineered so that in the event of catastrophic failure, such as tank leakage, all untreated effluent is prevented from escape.

Floor drains should be routed to a holding reservoir that can process all water held within the facility.

Guideline K:

Room surfaces should be smooth, impervious and able to be disinfected readily.

Floor surfaces should be smooth, sealed and nonporous, and corners should be coved. Walls and bare fixtures should be disinfectable, as must all materials used for handling or likely to come in contact with the experimental animals.

Guideline L:

Electrical fixtures should be ground fault interrupted, gasketed, sanitizable and provided with emergency back-up power.

Wall switches should be sealed and waterproof to allow disinfection. Ceiling lamp fixtures should be gasketed, waterproof and sanitizable. Ceilings should be smooth, impervious and sanitizable. Electrical outlets should, ideally, be positioned well above floor level, and above water supply lines.

Guideline M:

Exposed piping should be secured to wall and floor surfaces with smooth sanitizable brackets, and should be accessible for cleaning.

Piping should have access points for cleaning. Valves should be accessible from within and outside individual rooms to regulate flows in the event of emergencies. Where walls are penetrated for pipe runs, they should be caulked or securely sealed and gasketed to prevent harbouring pathogens. Any drain holes that go direct to municipal drainage (not recommended) should be protected with sealed standing pipes to pre-

vent untreated effluent escape. Methods to reduce condensation should be employed.

Guideline N:

Equipment for cleaning and sanitation, dry-moist feed storage bins and equipment used in the room (such as nets) should be room-specific.

Nets and other equipment should be tank-specific, in order to minimize transfer of pathogens between tanks. There should be immersion disinfection buckets for the regular sanitation of room-specific equipment. The regular changing of disinfection solutions used in such rooms should be scheduled by an SOP, and the efficacy of such methods should be assessed with a regular validation procedure. Appropriate concentration and type of disinfectant to achieve 100% kill of pathogens in the environment should be used.

1.3 Water flow and tanks

Guideline O:

All tanks must be enclosed with tightly fitting, removable covers to prevent water, aquatic animal or pathogen escape.

Tank lid perforations for airstones, standpipes, etc. should be gasketed. Tank stands should be constructed of rigid, smooth, impervious material such as aluminum, stainless steel or fiberglass. For saltwater systems, the tanks and tank stands should be salt water corrosion proof. The use of porous or organic materials, such as concrete is inadvisable. Any porous materials, such as airstones, should be disposed of and replaced by new airstones between experiments, or should be disinfected between use.

Guideline P:

All effluent water must be collected and held in treatment tanks for a recommended disinfectant contact time, and the effluent from these tanks must be regularly monitored for effective level of disinfection.

The disinfection tank should undergo regular testing for integrity, as should the disinfectant injection system. The tank should have an automated disinfectant system which is monitored by an alarm system in case of failure. In the event of any failure in the containment system, automated alarms to pagers or another means of immediately contacting emergency personnel should be in place. Recirculation systems in aquatic biocontainment laboratories represent a special challenge because of the potential to contain and harbour pathogens, particularly in concentration components of the system such as biofilters. Such systems should be designed with the capacity to isolate, remove and disinfect all system components including tanks, pipes, biofilters and other ancillary equipment without disrupting animals in the system.

Guideline Q:

Spill kits to contain and disinfect spills of pathogen-contaminated water should be in place in all rooms and areas where spills are a possibility. Designation and training of personnel in the use of spill-kits must be maintained.

When a spill occurs, it should be physically contained using absorbent material, and the infectious agent destroyed using an effective disinfectant for a recognized contact period; after which, the spill can be cleaned up.

1.4 Facility wear

Guideline R:

Facility-specific clothing and footwear worn in the containment area must be kept in the area.

Street clothing should be kept outside the facility in separate locker areas away from the containment area. Facility-specific footwear should be donned to enter the outer areas of containment facilities such as hallways. At the room level, a complete outerwear change with footwear change is needed. Within individual rooms, personnel should wash their hands before leaving the room and when leaving the outer area of the containment facility.

Facility clothing, tools, etc. should be regularly disinfected.

1.5 Disposal of materials

Guideline S:

Any contaminated material, including clothing, fomites and carcasses, should be secure-

ly bagged and autoclaved or incinerated before cleaning or disposal.

Clothing which requires cleaning should be disinfected prior to removal from the facility for cleaning, unless laundering facilities are available within the containment zone and are proven effective against the pathogens.

Alternative means of sterile disposal of carcasses and other contaminated biological wastes includes incineration or autoclaving and rendering.

2. Operation of an Aquatic Biocontainment Facility

The following aspects must be followed in order to ensure the proper functioning of a biocontainment facility:

- only authorized personnel should be permitted entry;
- there must be entrance procedures for maintenance staff;
- if aquatic zoonotic agents are in use (e.g., *Streptococcus iniae* or *Mycobacterium marinum*), a health and medical surveillance program must be in place to protect personnel from infection;
- personnel must be trained in all infectious, chemical and physical hazards likely to be encountered, and must demonstrate competence; such training must be documented;
- facility-specific SOPs must be developed and followed to ensure consistent practices in all areas, such as entry and exit procedures, traffic flow from clean to dirty, disinfection and garment change on entry and exit;
- staff must understand the physical structure, plumbing and air handling systems in the facility and how these work, and adhere to strict containment protocols (i.e. not wedge doors open, etc.);
- emergency procedures and equipment to deal with loss of containment, fire, etc. must be in place, posted and understood by all workers; and
- all spills, loss of containment and accidents must be reported and investigated.

References:

Agriculture and Agri-Food Canada (AAFC) (1996) *Containment Standards for Veterinary Facilities*. 71pp. Ottawa ON: Agriculture and Agri-Food Canada. Available at www.inspection.gc.ca/english/sci/lab/convet/convete.shtml

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Health Canada (2004) *Laboratory Biosafety Guidelines*, 3rd ed. 113pp. Ottawa ON: Health Canada. Available at www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/

APPENDIX D
WATER QUALITY CRITERIA FOR OPTIMUM FISH HEALTH – FOR COLDWATER, WARMWATER AND MARINE SPECIES OF FISH

(mg/L except for pH, temperature and salinity)

Characteristics	Coldwater	Warmwater	Marine	Monitoring frequency		Comments
				Recirculation	Open Flow Through	
Temperature	9 to 15°C	20 to 32°C	Species-specific, range too broad to state definitively	daily	daily	
Oxygen	7 to saturation	5 to saturation	5.5 to saturation	at least daily	daily	Oxygen may need to be checked more frequently if other values change or if fish are in high density situations (above 15 kg/m^3)
pH	6.5 to 8	7.5 to 9	7.5 to 8.5	daily	weekly	pH may need to be checked more frequently if other values change or if fish are in high density situations (above 15 kg/m^3)
Ammonia (un-ionized)	0 to 0.0125	0 to 0.02	0 to 0.0125	twice a week	monthly (unless high density)	If system has a biofilter should be checked daily during start-up
Nitrate	0 to 3.0	0 to 3.0	species-specific	twice a week	monthly (unless high density)	If system has a biofilter should be checked daily during start-up

Characteristics	Coldwater	Warmwater	Marine	Monitoring frequency		Comments
				Recirculation	Open Flow Through	
Nitrite	0 to 0.2	0 to 0.1	0 to 0.2	twice a week	monthly (unless high density)	If system has a biofilter should be checked daily during start-up
Chlorine	0 to 0.01	0 to 0.01	not applicable	annually	daily (if using municipal water)	Chlorine should be checked daily if taken from municipal water sources which use chlorine as a disinfectant
Total hardness ($CaCO_3$)	20 to 450	50 to 450	>125 mg/L	twice a week	twice a week	Total hardness is a measure of calcium and magnesium but may contain other hardness producing minerals; changes in total hardness can relate to changes in total alkalinity and pH
Total alkalinity ($CaCO_3$)	10 to 450	50 to 450	>150 mg/L	twice a week	twice a week	Alkalinity should be monitored as the processes of recirculation cause a reduction in alkalinity and may reduce pH
Nitrogen (gas saturation)	<100%	<100%	<100%	weekly	weekly	Values are for adult fish and may be less in early life stages; should also be checked during any suspected fish health problems
Salinity	0.1 to 3.0g/L	0.1 to 3.0g/L	28 to 35ppt	weekly	weekly	

Adapted from Plumb (1999) and Fisher (2000)

References:

Fisher J.P. (2000) Facilities and husbandry (large fish models). In: *The Laboratory Fish*. (ed. G.K. Ostrander), pp. 13-39. San Diego CA: Academic Press.

Plumb J.A. (1999) Principles of health maintenance. In: H*ealth Maintenance and Principal Microbial Diseases of Cultured Fishes*. pp. 1-23. Ames: Iowa State University.