



# UNIVERSITAS AIRLANGGA

## FAKULTAS PERIKANAN DAN KELAUTAN

Kampus C Mulyorejo Surabaya 60115 Telp. (031) 5911451 Fax (031) 5965741

Laman : <https://fpk.unair.ac.id>, e-mail : info@fpk.unair.ac.id

### SURAT KETERANGAN

Nomor: 1439/UN3.FPK/KP/2023

Yang bertanda tangan di bawah ini:

Nama : **Dr. Eng. Sapto Andriyono, S.Pi., MT.**  
NIP : 197909252008121002  
Pangkat/Golongan : Penata/III - C  
Jabatan : Wakil Dekan III

Dengan ini menerangkan bahwa:

Nama : **Dr. Endang Dewi Masithah, Ir., MP.**  
NIP : 196909121997022001  
Pangkat/Golongan : Pembina/IV - A  
Jabatan : Lektor Kepala

Telah melakukan penelitian yang dipublikasi pada tahun 2022 dengan judul sebagai berikut:  
***The addition of Spirulina platensis extract in feed on gill histopathology and survival rate of Osphronemus gouramy after infected with Aeromonas hydrophila***

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari:

1. *American Fisheries Society* (AFS, 2014) yang berjudul *Guideline for the Use of Fishes in Research* yang menyebutkan bahwa: Penggunaan mortalitas sebagai titik akhir dapat diterapkan jika sedikit atau tidak ada informasi yang berkaitan dengan tujuan penelitian yang tersedia pada spesies yang diminati atau variabel eksperimental yang diterapkan, (hal 6 ; terlampir), dan
2. *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guideline on the Care and Use of Fish in Research, Teaching and Testing* yang menyebutkan bahwa: ikan yang telah diketahui cara pembiakannya secara berkelanjutan dan juga jenis ikan komersil dinyatakan tidak memerlukan protokol (hal 16 ; terlampir).

Penelitian tersebut menggunakan hewan percobaan larva ikan gurami. Sehingga penelitian tersebut tidak perlu dilakukan ***Uji Ethical Clearence***.

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar** atas nama Dr. Endang Dewi Masithah, Ir., MP.

Surabaya, 27 April 2023

Wakil Dekan III FPK Unair

Dr. Eng. Sapto Andriyono, S.Pi., MT.  
NIP. 197909252008121002



Mengetahui,  
Dekan FPK Unair

Prof. Moch. Amin Alamsjah, Ir., M.Si., Ph.D.  
NIP. 197001161995031002

# **Guidelines for the Use of Fishes in Research**

Use of Fishes in Research Committee members:

J. A. Jenkins, Chair, H. L. Bart, Jr., J. D. Bowker, P. R. Bowser, J. R. MacMillan, J. G. Nickum, J. D. Rose, P. W. Sorensen, and G. W. Whitley on behalf of the American Fisheries Society; J. W. Rachlin and B. E. Warkentine on behalf of the American Institute of Fishery Research Biologists; and H. L. Bart on behalf of the American Society of Ichthyologists and Herpetologists

American Fisheries Society  
Bethesda, Maryland  
2014

A suggested citation format for this book follows.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland.

Cover art: Close-up photograph of Brown Trout, *Salmo trutta*, from the South Fork of the Cache la Poudre River, Colorado, taken by James Rose in 2010.

© Copyright 2014 by the American Fisheries Society

All rights reserved. Photocopying for internal or personal use, or for the internal or personal use of specific clients, is permitted by AFS provided that the appropriate fee is paid directly to Copyright Clearance Center (CCC), 222 Rosewood Drive, Danvers, Massachusetts 01923, USA; phone 978-750-8400. Request authorization to make multiple copies for classroom use from CCC. These permissions do not extend to electronic distribution or long-term storage of articles or to copying for resale, promotion, advertising, general distribution, or creation of new collective works. For such uses, permission or license must be obtained from AFS.

Printed in the United States of America on acid-free paper.

Library of Congress Control Number 2014943876  
ISBN 978-1-934874-39-4

American Fisheries Society Web site address: [www.fisheries.org](http://www.fisheries.org)

American Fisheries Society  
5410 Grosvenor Lane, Suite 100  
Bethesda, Maryland 20814  
USA

## Table of Contents

Use of Fishes in Research Committee, 2014 .....	vii
Preface.....	ix
Acknowledgments.....	xi
Statement of Purpose .....	xiii
1. Introduction.....	1
2. General Considerations.....	3
2.1 Approval of Research Plans by IACUCs .....	3
2.2 Project Quality Assurance Plans and Standard Operating Procedures .....	4
2.3 Statistical Design.....	5
2.4 Mortality as an Experimental Endpoint .....	6
2.5 Fish Health Management: Control of Pathogens and Parasites .....	6
3. Statutory Requirements and Regulatory Bodies.....	9
3.1 International Regulations and Guidelines .....	9
3.2 Biosecurity .....	11
3.3 Federal, State, and Local Regulations.....	12
3.4 Permits and Certificates .....	14
4. Animal Welfare Considerations.....	17
4.1 General Considerations .....	17
4.2 Stress .....	17
4.2.1 Stages of Stress.....	18
4.2.2 Measuring and Avoiding Stress.....	18
4.3 Nociception and Pain .....	20
5. Field Activities.....	23
5.1 Habitat and Population Considerations.....	23
5.2 Field Collections .....	23
5.2.1 Permits.....	23
5.2.2 Natural History Collections.....	24

5.2.3 Representative Samples .....	24
5.2.4 Collection of Imperiled Species .....	25
5.2.5 Museum Specimens and Other Preserved Specimens .....	26
5.3 Live Capture Techniques and Equipment .....	28
5.4 Field Restraint of Fishes: Sedatives .....	28
5.4.1 Drugs Approved for Use on Fish .....	29
5.4.2 Low Regulatory Priority (LRP) Drugs .....	29
5.4.3 Investigational New Animal Drugs (INAD) .....	30
5.5 Dangerous Species and Specimens .....	30
5.6 Handling and Transport .....	31
5.7 Facilities for Temporary Holding and Maintenance .....	32
5.8 Field Acclimation .....	33
5.9 Collection of Blood and Other Tissues .....	34
6. Marking and Tagging .....	37
6.1 General Principles .....	37
6.2 External Tags and Marks .....	37
6.3 Internal Tags and Marks, and Biotelemetry .....	38
6.4 Genetic Markers .....	40
6.5 Stable Isotopes .....	41
6.6 Fatty Acids .....	42
7. Laboratory Activities .....	43
7.1 General Principles .....	43
7.2 Confinement, Isolation, and Quarantine .....	43
7.3 Acclimation to Laboratory Conditions .....	45
7.4 Facilities for Long-Term Housing of Fishes .....	45
7.5 Density of Animals .....	47
7.6 Feeds and Feeding .....	47
7.7 Water Quality .....	49
7.8 Water Recirculation Units .....	50
7.9 Effluents and Permits .....	51

7.10 Dangerous Species and Specimens in Captivity .....	51
7.11 Restraint of Fishes: Sedatives and Related Chemicals.....	52
7.12 Surgical Procedures.....	53
7.13 Administration of Drugs, Biologics, and Other Chemicals .....	55
7.13.1 Drugs .....	55
7.13.2 Biologics and Other Chemicals .....	56
7.13.3 Chemical Facility Anti-Terrorism Standards (CFATS) .....	56
8. Final Disposition of Experimental Animals .....	59
8.1 Euthanasia .....	59
8.2 Storage or Return to Aquatic Habitat.....	60
9. Future Revisions .....	61
10. Literature Cited .....	63
Appendix.....	85
Brief Checklist for IACUC Readiness .....	85
List of Low Regulatory Priority Drugs and Consideration for Their Use .....	86
Appendix Table 1. Low regulatory priority aquaculture drugs, indications, and doses. ....	87
Appendix Table 2. OIE-notifiable causative disease agents for fish and amphibians. ....	88
Index of Terms and Acronyms.....	89
Note on Additional Readings .....	90

Studies of early life stages may require very large numbers of individuals. In all cases, studies should be designed to use the fewest animals necessary to reliably answer the questions posed. The use of adequate numbers to establish variance and to ensure reliability is essential so as to prevent needless repetition of the study (ASIH et al. 1987, 1988). A true “replicate” is the smallest experimental unit to which a treatment can be applied independently. Pseudoreplication can result from wrongly treating multiple samples from one experimental unit as multiple experimental units or from using experimental units that are not statistically independent (Heffner et al. 1996). Statistical power analysis can improve designs of experiments (Peterman 1990). Conducting statistical power analyses ensures the development of study designs that have the appropriate statistical power to accomplish research objectives.

## **2.4 Mortality as an Experimental Endpoint**

In laboratory studies, experimental endpoints, other than death of the experimental subjects, should be developed unless mortality is required by the study protocol. The use of mortality as an endpoint is appropriate when one or both of the following criteria are met: (1) Little or no information pertaining to research objectives is available on the species of interest or the experimental variable being imposed (e.g., short-term, limited mortality studies may be used to develop experimental limits for subsequent sublethal studies), and (2) mortality data are required, or at least preferred, by a sponsoring agency to provide a basis for criteria development as part of a regulatory process. Studies that require mortality endpoints include, but are not limited to, those concerning the effects of pathogens and parasites, toxicological research, and physiological tolerance.

## **2.5 Fish Health Management: Control of Pathogens and Parasites**

In laboratory studies involving fishes, healthy subjects are prerequisites for reliable data (Jenkins 2011a), unless an infectious disease is part of the experimental protocol. Fish used in research must be free of any notable microbial presence that could indicate a diseased condition. Fish free from infectious fish pathogens generally will be satisfactory; however, an unrecognized disease condition, even at chronic or nonlethal levels, can seriously confound research results (Lawrence et al. 2012). The source of fish used in research will, in general, influence their health status. Fish raised in captivity have a level of health oversight that will not occur in wild-caught fish. When inquiring about the health status of fish at a culture facility, the researcher can request specific information including any available fish health inspection reports. When fish are brought into a laboratory setting from the wild, the researcher should expect that microorganisms are present. If no disease symptoms are apparent, this is no guarantee that these wild-caught fish are free from problematic disease organisms. Once those fish are in a laboratory setting, the culture conditions and associated stressors will be very different from those in the natural environment, whereby an active disease event can develop. Many laboratories will administer formalin baths to newly arrived fish during an acclimation period (see section [7.3 Acclimation to Laboratory Conditions](#)). The goal is to eliminate external protozoa and monogeneans from the

## **4. Animal Welfare Considerations**

### **4.1 General Considerations**

Research involving living animals, including fishes, must be based on experimental designs and animal care practices that can lead to scientifically valid results. Fishes are acutely sensitive to stress (e.g., Barton and Iwama 1991), and responses may include changes in behavior (e.g., Martins et al. 2012), reduced growth, changes in osmotic status, suppressed immune systems (with consequent disease onset), and altered reproductive capacity (Iwama et al. 2006; Schreck et al. 2001; Schreck 2010). Accordingly, unless the experimental objectives require actions or conditions designed to test responses to stress, fishes should be maintained, handled, and tested under conditions that will not create such responses. The Guidelines addresses the conduct of scientific research and focuses on established facts and the processes through which knowledge is developed. Research plans submitted to IACUCs should address animal care considerations, in addition to the details of research goals, objectives, and procedures. The extent to which IACUCs incorporate personal values concerning animal welfare into their institutional guidelines is determined within each institution.

### **4.2 Stress**

The study of stress has focused on how animals have evolved physiological and behavioral mechanisms to address the challenges of changing environmental conditions and then to permit them to maintain homeostasis, or self-sustaining balance. The set of environmental variables (conditions) best suited for the well-being of each species typically encompasses a specific range for each factor and species (see section 5.7 Facilities for Temporary Holding and Maintenance), as stress responses are species-specific (Schreck 2010). Accordingly, when fishes are maintained within these ranges, a state of homeostatic balance is expected. Deviations from homeostasis characterize a stress response. While many definitions for stress have been proposed, we employ the definition of Schreck (2000) and Schreck et al. (2001): “a physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of insult.” When stressed, fish generally attempt to reestablish homeostasis via a process known as “allostasis regulation in which they adjust their physiological function to re-establish a dynamic balance” (Sterling and Eyer 1988). While allostasis is generally adaptive because it helps keep animals alive in the face of a short-term stressor(s), it can be maladaptive over the long term and have negative consequences on growth, reproduction, and immunological health (Schreck 2010). Accordingly, investigators need to understand those factors that might cause stress in their experimental animal(s), the potential consequences, and how stress might be avoided by optimizing experimental conditions.



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.

Mr John Batt, Dalhousie University  
Dr Kristina Bennett-Steward, Bioniche  
Mr Cyr Couturier, Memorial University  
Dr Larry Hammell, University of Prince Edward Island  
Dr Chris Harvey-Clark, University of British Columbia (Chair)  
Mr Henrik Kreiberg, Fisheries and Oceans Canada  
Dr George Iwama, Acadia University  
Dr Santosh Lall, National Research Council  
Dr Matt Litvak, University of New Brunswick at St John  
Dr Don Rainnie, University of Prince Edward Island  
Dr Don Stevens, University of Guelph  
Dr Jim Wright, University of Calgary  
Dr Gilly Griffin, Canadian Council on Animal Care

In addition, the CCAC is grateful to former members of CCAC Council: Ms Susan Waddy, Fisheries and Oceans Canada; Dr Jack Miller, University of Western Ontario; and Dr Choong Foong, Dalhousie University; and to Dr David Noakes, University of Guelph who provided considerable assistance in preliminary phases of this project. CCAC thanks the many individuals, organizations and associations that provided comments on earlier drafts of this guidelines document. In particular, thanks are extended to representatives of Fisheries and Oceans Canada, Environment Canada, the Canadian Aquaculture Institute, the Canadian Food Inspection Agency and the Canadian Society of Zoologists.

© Canadian Council on Animal Care, 2005

ISBN: 0-919087-43-4

Canadian Council on Animal Care  
1510-130 Albert Street  
Ottawa ON CANADA  
K1P 5G4

<http://www.ccac.ca>

## TABLE OF CONTENTS

<b>A. PREFACE</b> .....	<b>1</b>		
<b>SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT</b> .....	<b>3</b>		
<b>B. INTRODUCTION</b> .....	<b>13</b>		
1. Definition of Fish .....	13		
2. Rationale for Guidelines on the Care and Use of Fish .....	13		
3. Ethical Overview .....	14		
3.1 Principles of the Three Rs .....	14		
4. Responsibilities .....	15		
4.1 Responsibilities of investigators ..	15		
4.2 Responsibilities of the animal care committee .....	16		
4.3 Role of the veterinarian .....	17		
5. Government Regulations and Policies on the Use of Fish .....	17		
5.1 International .....	17		
5.2 Federal .....	18		
5.3 First Nations .....	20		
5.4 Provincial/territorial .....	20		
5.5 Municipal .....	20		
<b>C. AQUATIC FACILITIES</b> .....	<b>21</b>		
1. Water Supply .....	21		
2. Water Quality .....	21		
3. Engineering and Design .....	22		
3.1 Structural materials .....	23		
3.2 Room ventilation and airflow in aquatic areas .....	24		
3.3 Mechanical and electrical requirements .....	25		
3.4 Lighting .....	25		
3.5 Redundancy in aquatic life support systems .....	26		
4. Types of Systems .....	26		
4.1 Flow-through systems .....	27		
4.2 Recirculation systems .....	27		
4.3 Static systems .....	27		
4.4 Mesocosms .....	28		
5. Fish Housing .....	28		
5.1 Fish well-being .....	28		
5.2 Tank/enclosure design .....	28		
<b>D. FACILITY MANAGEMENT, OPERATION AND MAINTENANCE</b> .....	<b>31</b>		
1. Security and Access .....	31		
2. General Maintenance of the Facility ..	31		
3. Environmental Monitoring and Control .....	32		
3.1 Management of water quality ..	33		
3.2 Temperature .....	33		
3.3 Oxygen .....	34		
3.4 Supersaturation .....	34		
3.5 pH .....	35		
3.6 Nitrogen compounds .....	35		
3.7 Carbon dioxide .....	36		
3.8 Salinity .....	36		
3.9 Toxic agents .....	37		
<b>E. CAPTURE, ACQUISITION, TRANSPORTATION AND QUARANTINE</b> .....	<b>38</b>		
1. Capture of Wild Stock .....	38		
2. Killed Specimens .....	38		
3. Piscicidal Compounds .....	38		

4. Acquisition of Hatchery Fish . . . . .	39	3.3 Anesthesia . . . . .	53
5. Transportation . . . . .	39	3.4 Surgical equipment . . . . .	54
6. Quarantine and Acclimation . . . . .	40	3.5 Incisions . . . . .	54
6.1 Quarantine . . . . .	40	3.6 Suture materials and techniques . .	54
6.2 Acclimation . . . . .	41	3.7 Pathophysiology of surgery and wound healing in fishes . . . .	55
<b>F. HUSBANDRY . . . . .</b>	<b>42</b>	3.8 Postoperative care . . . . .	55
1. Record-keeping and Documentation . .	42	4. Administration of Compounds and Devices by Various Routes . . . . .	56
1.1 Standard Operating Procedures .	42	4.1 Branchial diffusion ("inhalation") . . . . .	56
1.2 General checklists . . . . .	42	4.2 Oral . . . . .	56
1.3 Assessment of fish well-being . .	42	4.3 Injection . . . . .	57
2. Density and Carrying Capacity . . . . .	42	4.4 Implants, windows and bioreactors . . . . .	57
3. Food, Feeding and Nutrition . . . . .	43	5. Tagging and Marking . . . . .	57
3.1 Nutrition . . . . .	43	5.1 Tissue marking . . . . .	58
3.2 Food and feeding . . . . .	43	5.2 Tagging . . . . .	58
3.3 Feed quality and storage . . . . .	43	6. Collection of Body Fluids . . . . .	58
3.4 Larval weaning . . . . .	45	7. Use of Infectious Disease Agents, Tumorigenic or Mutagenic Agents, and Toxic and Noxious Compounds . .	59
3.5 Use of medicated feeds . . . . .	45	8. Endpoints and Criteria for Early Euthanasia . . . . .	59
4. Broodstock and Breeding . . . . .	46	8.1 Recognition of "pain", "distress" and "stress" . . . . .	59
4.1 Induction of spawning . . . . .	46	8.2 Choosing an appropriate endpoint . . . . .	60
<b>G. HEALTH AND DISEASE CONTROL . . . . .</b>	<b>47</b>	9. Monitoring . . . . .	62
1. Fish Health Program . . . . .	47	10. Negative Reinforcement Modalities . .	62
1.1 Disease prevention . . . . .	47	11. Exercise to Exhaustion . . . . .	62
1.2 Disease diagnosis and identification of pathogens . . . . .	47	12. Environmental Extremes . . . . .	62
1.3 Injuries and other disorders . . . .	48	13. Genetically Modified Fish . . . . .	62
<b>H. EXPERIMENTAL PROCEDURES . .</b>	<b>50</b>	<b>I. EUTHANASIA . . . . .</b>	<b>64</b>
1. Handling and Restraint . . . . .	50	<b>J. DISPOSITION OF FISH AFTER STUDY . . . . .</b>	<b>65</b>
1.1 Restraint of dangerous species . .	51		
2. Restricted Environments . . . . .	51		
3. Surgery . . . . .	51		
3.1 Surgical preparation and skin disinfection . . . . .	52		
3.2 Water quality during surgery . . .	53		

1. Consumption of Fish .....	.65
2. Release of Fish to Wild .....	.65
3. Fish as Pets .....	.65
4. Transfer of Fish Between Facilities .....	.65
5. Disposal of Dead Fish .....	.65
<b>K. REFERENCES .....</b>	<b>.66</b>
<b>L. GLOSSARY .....</b>	<b>.73</b>
<b>M. ABBREVIATIONS .....</b>	<b>.75</b>
<b>APPENDIX A RELEVANT GUIDELINES AND ORGANIZATIONS .....</b>	<b>.76</b>

<b>APPENDIX B ZOOBOTIC DISEASE- TRANSMISSION OF FISH DISEASES TO MAN .....</b>	<b>.77</b>
--	------------

<b>APPENDIX C GUIDELINES FOR CONTAINMENT FACILITIES (FOR PATHOGEN STUDIES) .....</b>	<b>.79</b>
--	------------

<b>APPENDIX D WATER QUALITY CRITERIA FOR OPTIMUM FISH HEALTH – FOR COLDWATER, WARMWATER AND MARINE SPECIES OF FISH .....</b>	<b>.84</b>
--	------------

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



## **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, 2<sup>nd</sup> 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.

## 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](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 relevant 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 informa-

tion 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](http://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](http://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