



**SURAT KETERANGAN**

Nomor : 1666/UN3.FPK/KP.16/2023

Yang bertanda tangan di bawah ini :

Nama : **Prof. Moch. Amin Alamsjah, Ir., M.Si., Ph.D.**  
NIP : 197001161995031002  
Pangkat / Golongan : Pembina Utama Madya / IV/d  
Jabatan : Dekan

Dengan ini menerangkan bahwa :

Nama : **Dr. Woro Hastuti Satyantini, Ir. M.Si.**  
NIP : 196109071989032001  
Pangkat/Golongan : Lektor / III/c  
Jabatan : Wakil Dekan I

Telah melaksanakan penelitian pada tahun 2019 dengan judul sebagai berikut :

**The Effects of Laserpuncture on Gonadal Maturity and Sperm Quality of Male Striped Catfish (*Pangasianodon hypophthalmus*)**

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari *American Fisheries Society* (AFS, 2014) yang berjudul *Guidelines for the Use of Fishes in Research* dan *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guidelines on the Care and Use of Fish in Research, teaching and Testing*. Sehingga penelitian tersebut tidak perlu dilakukan *Uji Ethical Clearence* karena ikan yang digunakan tidak disakiti sesuai dengan CCAC (2005) Guidelines 64 halaman 46 menghasilkan *out put* yang baik untuk akuakultur.

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Lektor Kepala** atas nama Dr. Woro Hastuti Satyantini, Ir. M.Si.

Surabaya, 19 Juni 2023

Dekan



**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. Whitedge 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

## **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.

Each investigator and the IACUC should understand the conditions that minimize stress for the species in question. Extrapolation between taxa, however, must be avoided because differences exist among species (Schreck 2010). The factors and range of conditions appropriate for fishes typically will deviate substantially from those used for mammals. Assumptions and perceptions based on experiences with mammals, especially primates, must not be extrapolated to fishes; however, investigators should be aware of APHIS policy (i.e., Policy 11, USDA 2011, [http://www.aphis.usda.gov/animal\\_welfare/policy.php?policy=11](http://www.aphis.usda.gov/animal_welfare/policy.php?policy=11)).

#### **4.2.1 Stages of Stress**

Stress responses are elicited after a fish detects a threat. Recognizing and understanding the three stages of stress is important. Each warrants consideration in the design of animal care protocols:

- Stage 1. Primary stress responses vary among species but are characterized by immediate neuroendocrine responses including catecholamine and corticosteroid release and can be quantified by measuring blood hormones. Sometimes behavioral changes accompany these endocrine responses that help the animal cope with the stressor and, in and of themselves, have few consequences to health.
- Stage 2. The secondary stage of a stress response is characterized by changes in blood and tissue function evoked by the primary response. Secondary stress typically occurs within minutes of the primary response and is characterized by increased blood glucose and heart rate, diuresis, alteration of leukocyte count, altered osmolyte balance, and behavioral changes (see section 5.6 Handling and Transport). Although these responses can have short-term positive effects, many also are negative, so they should be avoided when possible. They can be evaluated through the study of extracted blood (see section 5.9 Collection of Blood and Other Tissues).
- Stage 3. Tertiary stress responses are associated with long-term exposure and negatively affect the well-being of the organism. Effects associated with tertiary stress include decreased growth, propensity to contract disease, and decreased reproductive function (Selye 1976; Schreck et al. 2001; Iwama et al. 2006; see sections 5.8 Field Acclimation and 7.3 Acclimation to Laboratory Conditions). The best way to avoid a tertiary stress response is to care for animals so as to minimize stress responses.

#### **4.2.2 Measuring and Avoiding Stress**

While the nature of stress is insidious, it also tends to be polymorphic, changing with time and taking different forms in different species at different stages in their lives. It is rarely feasible to measure changes in blood hormones to assess primary or secondary stress; therefore, investigators are advised to design experiments that avoid stress unless the purposes of the research require measurements of stress indicators. Important indicators of a lack of stress are persistence of normal behavioral activity and propensity to feed and grow. Careful experimental design and planning can ensure study results that are not confounded by unrecognized or



unmeasured stress. Unless the aim of the research is to establish optimal conditions for holding particular species of fish in captivity, such as captive propagation of endangered species, it is generally advisable for investigators to select species for experiments whose optimal holding conditions are known and can be recreated in the laboratory. Specific factors to consider include (1) choice of species, (2) history of the animals under study, (3) water chemistry, (4) water flow, (5) water temperature, (6) light conditions and cycles, (7) bottom substrate, (8) noise and other physical stimuli, (9) shelter, (10) stocking density, and (11) size of tank relative to body size and activity rate. Other variables, such as fish density or the presence or absence of tank covers, may be important. Species that are known as reliable laboratory models (e.g., Zebrafish or Japanese Medaka) or that are commonly used in fish culture (e.g., Channel Catfish *Ictalurus punctatus* or Rainbow Trout *Oncorhynchus mykiss*) might be selected whenever such a choice is compatible with research objectives.

In addition to the aforementioned factors that are associated with long-term maintenance, additional considerations apply when fishes are handled or subjected to various experimental manipulations.

- Handling should be minimized. Merely catching fish in nets can induce release of stress hormones, such as cortisol, within one minute. Fishes should be given time to recover from handling prior to use in experiments. The amount of recovery time needed may vary with species and conditions; therefore, preliminary tests would help to establish the appropriate recovery period.
- Effects of stressors can be reduced through the use of sedatives or by adding environmental salts to the holding water to reduce osmotic and related stress. (Note that marine fishes, due to their osmoregulatory requirements, can be an exception.) The specific salts and concentrations will vary depending on each fish species and environmental conditions. Sedatives themselves, however, can evoke physiological stress responses (Trushenski et al. 2012a), so they should be employed cautiously and in accordance with established guidelines.
- Environmental conditions from which fish originated, or are held, should not be changed rapidly. This is especially true for temperature conditions. An instantaneous change of 2°C in water temperature generally is not lethal, but it can cause detectable stress responses. Tolerable changes depend on the species, the life history stage, previous thermal history, and the initial holding conditions. Effects due to previous thermal history have been detected for as long as a month posttreatment. Rapid, substantial changes in water quality also should be avoided (see section 7.7 Water Quality).
- Fish densities should be appropriate. Fish which live in shoals should be kept as groups but not in such large groups that they are crowded and compete for food and space or degrade water quality.

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> .....	1	3.5 Redundancy in aquatic life support systems .....	26
<b>SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT</b> .....	3	<b>4. Types of Systems</b> .....	26
<b>B. INTRODUCTION</b> .....	13	4.1 Flow-through systems .....	27
1. Definition of Fish .....	13	4.2 Recirculation systems .....	27
2. Rationale for Guidelines on the Care and Use of Fish .....	13	4.3 Static systems .....	27
3. Ethical Overview .....	14	4.4 Mesocosms .....	28
3.1 Principles of the Three Rs .....	14	<b>5. Fish Housing</b> .....	28
4. Responsibilities .....	15	5.1 Fish well-being .....	28
4.1 Responsibilities of investigators ..	15	5.2 Tank/enclosure design .....	28
4.2 Responsibilities of the animal care committee .....	16	<b>D. FACILITY MANAGEMENT, OPERATION AND MAINTENANCE</b> .....	31
4.3 Role of the veterinarian .....	17	1. Security and Access .....	31
5. Government Regulations and Policies on the Use of Fish .....	17	2. General Maintenance of the Facility ..	31
5.1 International .....	17	3. Environmental Monitoring and Control .....	32
5.2 Federal .....	18	3.1 Management of water quality ..	33
5.3 First Nations .....	20	3.2 Temperature .....	33
5.4 Provincial/territorial .....	20	3.3 Oxygen .....	34
5.5 Municipal .....	20	3.4 Supersaturation .....	34
<b>C. AQUATIC FACILITIES</b> .....	21	3.5 pH .....	35
1. Water Supply .....	21	3.6 Nitrogen compounds .....	35
2. Water Quality .....	21	3.7 Carbon dioxide .....	36
3. Engineering and Design .....	22	3.8 Salinity .....	36
3.1 Structural materials .....	23	3.9 Toxic agents .....	37
3.2 Room ventilation and airflow in aquatic areas .....	24	<b>E. CAPTURE, ACQUISITION, TRANSPORTATION AND QUARANTINE</b> .....	38
3.3 Mechanical and electrical requirements .....	25	1. Capture of Wild Stock .....	38
3.4 Lighting .....	25	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	<b>APPENDIX B</b>
2. Release of Fish to Wild .....65	<b>ZOONOTIC DISEASE-</b>
3. Fish as Pets .....65	<b>TRANSMISSION OF FISH</b>
4. Transfer of Fish Between Facilities .....65	<b>DISEASES TO MAN .....77</b>
5. Disposal of Dead Fish .....65	
<b>K. REFERENCES .....66</b>	<b>APPENDIX C</b>
	<b>GUIDELINES FOR CONTAINMENT</b>
<b>L. GLOSSARY .....73</b>	<b>FACILITIES (FOR PATHOGEN</b>
	<b>STUDIES) .....79</b>
<b>M. ABBREVIATIONS .....75</b>	<b>APPENDIX D</b>
<b>APPENDIX A</b>	<b>WATER QUALITY CRITERIA FOR</b>
<b>RELEVANT GUIDELINES</b>	<b>OPTIMUM FISH HEALTH – FOR</b>
<b>AND ORGANIZATIONS .....76</b>	<b>COLDWATER, WARMWATER AND</b>
	<b>MARINE SPECIES OF FISH ....84</b>

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, nutri-

tion, 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.