



SURAT KETERANGAN

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

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Telah melaksanakan penelitian pada tahun 2017 dengan judul sebagai berikut :

The Identification of Plankton Tropical Status in the Wonokromo, Dadapan and Juanda Extreme Water Estuary.

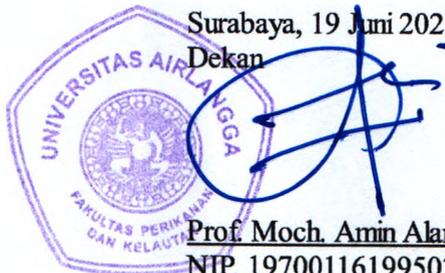
Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari *American Fisheries Society* (AFS, 2014) yang berjudul *Guidelines for the Use of Fishes in Research* dan *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guidelines on the Care and Use of Fish in Research, teaching and Testing*. Sehingga penelitian tersebut tidak perlu dilakukan *Uji Ethical Clearence* karena penelitiannya menggunakan mikroorganisme, tidak termasuk Vertebrata dan Cephalopoda, melainkan Avertebrata (CCAC, 2014 : Guideline-1, hal 14).

Artikel ini sudah published dan menghasilkan output yang sangat baik.

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



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Guidelines for the Use of Fishes in Research

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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.

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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

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5410 Grosvenor Lane, Suite 100
Bethesda, Maryland 20814
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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

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).

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.

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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.

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ISBN: 0-919087-43-4

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TABLE OF CONTENTS

A. PREFACE1

SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT3

B. INTRODUCTION13

1. Definition of Fish13
2. Rationale for Guidelines on the Care and Use of Fish13
3. Ethical Overview14
 - 3.1 Principles of the Three Rs14
4. Responsibilities15
 - 4.1 Responsibilities of investigators ..15
 - 4.2 Responsibilities of the animal care committee16
 - 4.3 Role of the veterinarian17
5. Government Regulations and Policies on the Use of Fish17
 - 5.1 International17
 - 5.2 Federal18
 - 5.3 First Nations20
 - 5.4 Provincial/territorial20
 - 5.5 Municipal20

C. AQUATIC FACILITIES21

1. Water Supply21
2. Water Quality21
3. Engineering and Design22
 - 3.1 Structural materials23
 - 3.2 Room ventilation and airflow in aquatic areas24
 - 3.3 Mechanical and electrical requirements25
 - 3.4 Lighting25

- 3.5 Redundancy in aquatic life support systems26

4. Types of Systems26

- 4.1 Flow-through systems27
- 4.2 Recirculation systems27
- 4.3 Static systems27
- 4.4 Mesocosms28

5. Fish Housing28

- 5.1 Fish well-being28
- 5.2 Tank/enclosure design28

D. FACILITY MANAGEMENT, OPERATION AND MAINTENANCE31

1. Security and Access31
2. General Maintenance of the Facility ..31
3. Environmental Monitoring and Control32
 - 3.1 Management of water quality ...33
 - 3.2 Temperature33
 - 3.3 Oxygen34
 - 3.4 Supersaturation34
 - 3.5 pH35
 - 3.6 Nitrogen compounds35
 - 3.7 Carbon dioxide36
 - 3.8 Salinity36
 - 3.9 Toxic agents37

E. CAPTURE, ACQUISITION, TRANSPORTATION AND QUARANTINE38

1. Capture of Wild Stock38
2. Killed Specimens38
3. Piscicidal Compounds38

4. Acquisition of Hatchery Fish	39
5. Transportation	39
6. Quarantine and Acclimation	40
6.1 Quarantine	40
6.2 Acclimation	41
F. HUSBANDRY	42
1. Record-keeping and Documentation	42
1.1 Standard Operating Procedures	42
1.2 General checklists	42
1.3 Assessment of fish well-being	42
2. Density and Carrying Capacity	42
3. Food, Feeding and Nutrition	43
3.1 Nutrition	43
3.2 Food and feeding	43
3.3 Feed quality and storage	43
3.4 Larval weaning	45
3.5 Use of medicated feeds	45
4. Broodstock and Breeding	46
4.1 Induction of spawning	46
3.3 Anesthesia	53
3.4 Surgical equipment	54
3.5 Incisions	54
3.6 Suture materials and techniques	54
3.7 Pathophysiology of surgery and wound healing in fishes	55
3.8 Postoperative care	55
4. Administration of Compounds and Devices by Various Routes	56
4.1 Branchial diffusion ("inhalation")	56
4.2 Oral	56
4.3 Injection	57
4.4 Implants, windows and bioreactors	57
5. Tagging and Marking	57
5.1 Tissue marking	58
5.2 Tagging	58
6. Collection of Body Fluids	58
7. Use of Infectious Disease Agents, Tumorigenic or Mutagenic Agents, and Toxic and Noxious Compounds	59
8. Endpoints and Criteria for Early Euthanasia	59
8.1 Recognition of "pain", "distress" and "stress"	59
8.2 Choosing an appropriate endpoint	60
9. Monitoring	62
10. Negative Reinforcement Modalities	62
11. Exercise to Exhaustion	62
12. Environmental Extremes	62
13. Genetically Modified Fish	62
I. EUTHANASIA	64
J. DISPOSITION OF FISH AFTER STUDY	65
G. HEALTH AND DISEASE CONTROL	47
1. Fish Health Program	47
1.1 Disease prevention	47
1.2 Disease diagnosis and identification of pathogens	47
1.3 Injuries and other disorders	48
H. EXPERIMENTAL PROCEDURES	50
1. Handling and Restraint	50
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 Fish65
2. Release of Fish to Wild65
3. Fish as Pets65
4. Transfer of Fish Between Facilities65
5. Disposal of Dead Fish65

K. REFERENCES66
----------------------------	------------

L. GLOSSARY73
--------------------------	------------

M. ABBREVIATIONS75
-------------------------------	------------

APPENDIX A RELEVANT GUIDELINES AND ORGANIZATIONS76
---	------------

APPENDIX B ZOO NOTIC DISEASE- TRANSMISSION OF FISH DISEASES TO MAN77
---	------------

APPENDIX C GUIDELINES FOR CONTAINMENT FACILITIES (FOR PATHOGEN STUDIES)79
--	------------

APPENDIX D WATER QUALITY CRITERIA FOR OPTIMUM FISH HEALTH – FOR COLDWATER, WARMWATER AND MARINE SPECIES OF FISH84
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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 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