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Telah melakukan penelitian yang dipublikasi pada bulan Maret tahun 2023 dengan judul sebagai berikut:

***Diversity and abundance of plankton community in Prigi and Tawang Bays, natural settlement habitats of Spiny Lobster larvae in East Java, Indonesia***

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: perizinan baru diperlukan jika penelitian memerlukan pengumpulan spesimen liar dari lapangan berupa ikan (hal 23 ; 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: pedoman tersebut hanya digunakan untuk hewan uji berupa ikan (Kelas: Chondrichthyes, Agnatha, dan Osteichthyes) dan Avertebrata (Kelas: Cephalopoda) (hal 13,14 ; terlampir).

Sehingga penelitian tersebut tidak perlu dilakukan ***Uji Ethical Clearance*** karena obyek penelitiannya berupa plankton (bukan ikan).

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

Mengetahui,  
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Surabaya, 27 April 2023

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

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## 5. Field Activities

### 5.1 Habitat and Population Considerations

Whether fishes are being collected live for investigations, preserved for study in a museum, or processed to obtain data needed for fisheries management, investigators should observe and pass on to students and employees a strict ethic of habitat conservation, and respectful and humane treatment of the animals in sampling, handling, and euthanasia (ASIH et al. 1987, 1988; AVMA 2013). Collecting should be conducted in a way that minimizes habitat disturbance and “excessive” mortality. The UFR Committee recognizes that currently no field collection techniques exist that will cause zero mortality events in the population being sampled. Research goals will generally dictate appropriate sampling methods. Given a set of alternative sampling methods and collecting gears, investigators can select the ones which cause the minimum levels of habitat disturbance and mortality in target and non-target fish populations. Gathering large series of animals from breeding aggregations should be avoided unless required to meet study objectives. Use of collecting techniques that damage habitat unnecessarily should also be avoided or performed to the minimum extent necessary to achieve study or sampling objectives. For example, trawling or other forms of dragged or towed gears is essential for documenting fish diversity or monitoring the health of fish populations; however, such gears can cause extensive disturbance to substrates, macrophytes, or other important structural elements of fish habitat. Sampling equipment and strategies can be designed to minimize incidental capture of non-target species. Collecting gears, such as gill nets deployed for nonlethal sampling, should be checked frequently to avoid unnecessary mortality. Regardless of the purpose of the experiment—whether to manipulate abundance or to study behavior, reproductive potential, or survivability—mortalities within the population and disturbance to habitat should be kept to the minimum amount that the investigator (along with the IACUC) determines to be acceptable.

The reader should note that some content in section 5 is not restricted to field activities but can extend to laboratory situations as well.

### 5.2 Field Collections

#### 5.2.1 Permits

Research with fishes frequently requires capturing wild specimens from the field, whether for field-based studies—such as data recording, marking, and relocation—or for laboratory studies of live or preserved specimens. Except when collecting in the open ocean (waters not under the jurisdiction of any particular country), the collection of fishes for all research purposes requires a scientific collector’s permit. Permits are issued by natural resource agencies of state, provincial, federal, and tribal entities in the United States and Canada (see section 3.4 Permits and Certificates). Permit applications generally request information about the research to be conducted, sampling methods, the areas to be sampled, and number and disposition of fish specimens to be collected. For a listing of state permitting agencies in the United States, as well

as other useful information about collection of fishes, see Walsh and Meador (1998). Collection of fishes on federal lands often requires a separate special use permit obtainable from the agency responsible for managing the land. The local, state, federal, and tribal authorities that issue collecting permits generally require collectors to notify them of the specific locations, dates, and proposed methods of sampling. Collection of fishes by federal personnel on private lands requires a permit approving access from the landowner.

### **5.2.2 Natural History Collections**

Systematists and taxonomists interested in conducting studies on preserved fishes should be aware of the wealth of specimens archived in natural history collections (see section 5.2.5 Museum Specimens and Other Preserved Specimens) before considering the removal of additional animals from the field. Repeated collections, however, are often warranted to provide information on temporal changes in the study population. The holdings of many ichthyological collections are accessible through database network portals such as Fishnet2 (<http://fishnet2.net/>) and the Global Biodiversity Information Facility (<http://www.gbif.org/>). Many state agencies and universities have accessible natural history collections. For listings of fish collections in the United States and Canada, see Leviton et al. (1985), Poss and Collette (1995), and Walsh and Meador (1998). A listing of institutional resource collections available internationally in herpetology and ichthyology, along with symbolic codes and citations, is made possible through ASIH (Sabaj Perez 2013, <http://www.asih.org/resources/standard-symbolic-codes-institutional-resource-collections-herpetology-ichthyology>).

### **5.2.3 Representative Samples**

Generally, the questions being explored and the study design itself dictate the number of specimens required for an investigation. Acquiring fishes for study generally involves the taking of a very small portion of the population or community present at a location. The general principle applied when sampling fishes is to take the fewest animals necessary to reliably address the hypothesis (see section 2.3 Statistical Design). The minimum number of fishes necessary to provide robust statistical results should inform the sampling protocol. Depending on the gear and methods, and the amount of handling required, high mortality rates may result. This is especially true in investigations involving fish eggs and early life stages. However, high levels of juvenile mortalities and rapid recoveries from population reductions are both characteristic events in the life histories of many fish species.

Sampling by using visual surveys alone is not always sufficient. This is the case in habitats that are structurally and biologically complex, where fish biodiversity data is necessary for their conservation and management. Small, cryptic fishes in coral-reef habitats, for example, are best collected by using small-scale sampling with ichthyocides; increased collection percentages of visually detected fish occurred with ichthyocide application (Smith-Vaniz et al. 2006; Ackerman and Bellwod (2000). The most commonly used ichthyocide is rotenone (see section 8.1



Euthanasia), a naturally occurring ketone from leguminous plants native to Southeast Asia and South America. The use of this chemical option has been diverse (McClay 2000); its use had been indicated with the threat of exotics (Rayner and Creese 2006). Robertson and Smith-Vaniz (2008) reviewed rotenone used by indigenous subsistence fishers and by fishery managers, as well as its toxicity and effects on other organisms. Rotenone anesthetizes and dispatches fishes by blocking the cellular uptake of oxygen (Singer and Ramsay 1994). A manual and SOP (<http://fisheries.org/shop/55061p>.) detail the proper use of rotenone (Finlayson et al. 2000; Finlayson et al. 2010), and numerous training courses are offered for fishery biologists and public agencies. Finlayson et al. (2010) recommended cautionary use of rotenone as a last resort due to potential harm to unintended targets. Investigation into alternative methods (Marking 2011) is prudent, as is the availability of taxonomic expertise (Walsh and Meador 1998) so to confirm the species present (see section 8.1 Euthanasia).

Sampling fish in contaminants studies is often inherent in biomonitoring of aquatic ecosystems because of capabilities of fish to accumulate environmental contaminants and to respond physiologically. Field procedures for sampling fish for chemical contaminants (Hughes et al. 2006; Schmitt et al. 1999) are useful, with protocols chosen according to study endpoints. A suite of documents and databases are available from USGS Biomonitoring of Environmental Status and Trends (BEST) program (<http://pubs.er.usgs.gov/publication/itr19990007>) and National Contaminant Biomonitoring Program (<http://www.cerc.usgs.gov/data/ncbp/ncbp.html>).

#### **5.2.4 Collection of Imperiled Species**

The term “imperiled species” applies not only to species officially listed as threatened or endangered by state or federal agencies but also to species that have been identified as candidates for such listings. The number of endangered, threatened, and vulnerable fish species in the southern United States has increased 125% between 1969 and 1989 (Warren et al. 2000). Investigators need to be aware of whether an aquatic habitat to be sampled supports imperiled species, as well as how to identify those species in the field (Warren and Burr 1994). Investigators can also determine if the habitats that support imperiled and nonimperiled species are considered areas of conservation concern and if species could be a focus of conservation concern (Jenkins et al. 2011). State wildlife action plans (Association of Fish and Wildlife Agencies 2007, <http://www.teaming.com/state-wildlife-action-plans-swaps>) and the network of U.S. Natural Heritage Programs (<http://www.natureserve.org/natureserve-network>) maintain listings of fishes (and other animal and plant species) of conservation concern. Lists of state-protected species may be obtained from offices that issue collection permits and from the Web sites of NatureServe (originally known as the Association of Biodiversity Information, <http://www.natureserve.org/visitLocal/index.jsp>) and the USFWS. The USFWS Endangered Species Program (<http://www.fws.gov/endangered/>) maintains lists of federally protected species. The list of federally threatened and endangered fishes may be also be searched on Web sites of the National Oceanic and Atmospheric Administration (NOAA, <http://www.noaa.gov/>)

Fisheries Service (<http://www.nmfs.noaa.gov/>) Office of Protected Resources (<http://www.nmfs.noaa.gov/pr/>) Web site or NatureServe (<http://www.natureserve.org/>). A bulletin highlighting protected marine or anadromous fishes (<http://www.nmfs.noaa.gov/pr/species/fish/>) is also available from NOAA Fisheries Service. Lists of protected fishes in Canada, Mexico, and other foreign countries can be viewed online on the respective national Web sites (such as the Species at Risk Act Public Registry, [http://www.sararegistry.gc.ca/default\\_e.cfm](http://www.sararegistry.gc.ca/default_e.cfm) of the Canadian Wildlife Service), as well as via the “Foreign Species” report on the USFWS Endangered Species Program Web site ([http://ecos.fws.gov/tess\\_public/SpeciesReport.do?lead=10&listingType=L](http://ecos.fws.gov/tess_public/SpeciesReport.do?lead=10&listingType=L)). The International Union for Conservation of Nature and Natural Resources (IUCN) maintains The IUCN Red List of Threatened Species<sup>TM</sup>, version 2013.1 (<http://www.iucnredlist.org/>), providing global coverage of the conservation status of freshwater and marine fishes, as well as other plants and animals.

The collection of imperiled species is allowed only under special circumstances (e.g., conservation status surveys) and requires special permits. Only noninvasive handling techniques (handling that results in no harm whatsoever to the animal) are to be used. Examples can include blood and milt collection, and certain fin clipping and tagging methods. If the goal of the research is to collect an imperiled species for live study, or if incidental capture is anticipated as bycatch, then any collection methods that may be injurious (e.g., gill net catch without close monitoring) or lethal (e.g., ichthyocides) should be avoided.

Conservation efforts for imperiled fish species frequently involve translocations, either among natural localities or from nature to propagation facilities and then back to nature. The environmental laws governing translocations of imperiled fishes are complex and based on such matters as resource use, suitability and security of transplant sites, and the appropriateness of transplanted individuals among sites (i.e., sufficient numbers or freedom from disease; Minckley 1995). All translocation efforts must be conducted by the agency with authority and responsibility for the species and area in question and should not be attempted by unauthorized individuals.

### **5.2.5 Museum Specimens and Other Preserved Specimens**

The collection of fishes from natural populations for museum preservation is critical for (1) understanding basic biology and life history, (2) documenting and recording biodiversity, and (3) establishing reference collections essential for understanding evolutionary relationships and environmental effects (ASIH et al. 1987, 1988). Studies of ecosystem variation or delineation of new species frequently require collection of relatively large series (sufficient for computing statistics on counts and measurements) from multiple populations across geographic ranges (Hughes and McCormick 2006). Sampling natural fish populations for these purposes typically involves broad surveys and collection of specimens in proportion to their occurrence in natural

populations; moreover, such sampling may not be hypothesis-driven. Studies of molecular systematics typically involve very small numbers of specimens, or small amounts of tissue removed from study fishes. However, it is just as important in these studies as in general ecological surveys to deposit voucher specimens in natural history museums, where samples are maintained frozen or preserved in a fixative such as 95% alcohol (isopropanol) or 70% ethanol, for future reference (Wheeler 2003). Museum collections of fishes are also available for use in other types of research. Two important principles that should be followed in collecting fishes for museum preservation are (1) the numbers of specimens collected should be the minimum necessary to accomplish study goals, and (2) animals collected should serve a variety of studies. Precise notations containing specific field data (such as date, exact location, habitat type, etc.) should accompany each collection.

Specimens collected for museum deposition should be preserved in a manner that maximizes their utility for study and minimizes the need for additional collecting. Formalin fixation is the standard practice used to ensure long-term preservation quality of fish specimens. The preferred method for archival storage is direct immersion in a 10% formalin (3.7% formaldehyde) solution, followed by transfer to alcohol (70% ethanol, un-denatured preferred) for long-term preservation and storage, as with voucher specimens. Chemicals are often added to formalin to buffer the solution or to preserve color (e.g., Ionol) (Fink et al. 1979). Although formalin is the fixative of choice for vertebrate tissues, other fixatives are sometimes used for specialized study purposes such as histology (Bouin's or Gilson's fluid) and electron microscopy (glutaraldehyde) (Luna 1992, 1992; Presnell et al. 1997; Clark 1981). Fixation by these methods typically involves small pieces of tissue dissected from specimens that may be sacrificed by means other than immersion in formalin. Carcasses for long-term archiving as voucher specimens should be fixed in formalin and later transferred to alcohol. Euthanizing fish prior to immersion in formalin should be practiced, provided that the sedative does not cause effects detrimental to the objectives of the research. A variety of chemicals, such as tricaine methanesulfonate (MS-222), may be used to anesthetize or euthanize fishes (see section 7.11 Restraint of Fishes: Sedatives and Related Chemicals). When study interests demand that specimens be fixed without prior treatment with sedatives, the specimens can be numbed in ice water, or for small fishes, immersed directly in liquid nitrogen (see section 8.1 Euthanasia).

Portions of animal specimens, including sperm, ova, embryos, tissues, and serum, are sometimes tissue banked. For example, the National Animal Germplasm Program ([http://nrnc.ars.usda.gov/A-GRIN/main\\_webpage/ars?record\\_source=US](http://nrnc.ars.usda.gov/A-GRIN/main_webpage/ars?record_source=US)) acquires and preserves genetic resources to secure biological diversity for population reconstitution or genomic studies. The San Diego Zoo Institute for Conservation Research (<http://www.sandiegozooglobal.org/ICR/purpose>) uses stored genetic resources in multiple technologies. Various iterations of specimen banking for retrospective analyses occur globally

for a multitude of investigations, including environmental monitoring, genetics research, and systematics. Fish tissue (liver and muscle) has been collected for the long-term storage of a variety of environmental specimens by the National Institute of Standards and Technology (NIST, <http://www.nist.gov/index.html>) through the National Biomonitoring Specimen Bank (Wise and Koster 1995; Becker and Wise 2006).

### **5.3 Live Capture Techniques and Equipment**

The choice of a sampling method should be dictated by worker safety, research objectives, seasonal considerations, and the habitat type to be sampled. Capture techniques should prevent or minimize injury and stress (see section 4.2 Stress) (McMichael et al. 1998; Henry and Grizzle 2003; Henry et al. 2003). Live wells or tanks should be provided if fishes are to be kept for more than the time needed to collect essential metrics. Care should be taken to avoid accidental capture of nontarget species and to ensure release of incidentally collected individuals with minimal or no injury (ASIH et al. 1987, 1988). Species that may be dangerous to workers due to size or species-characteristic behavior or capabilities require additional precautions (see sections 5.5 Dangerous Species and Specimens and 7.10 Dangerous Species and Specimens in Captivity).

Several studies have shown electrofishing to be among the most effective techniques for obtaining fish assemblage data in freshwater habitats (Yoder and Smith 1998). Electrofishing can be performed by wading methods or boat-mounted methods. Appropriate electrofishing protocols should consider the sampling purpose and physical constraints of the environment (e.g., conductivity, water depth, and presence of obstructions), as well as use of gear and techniques that minimize potential for electrofishing injury to fishes (Snyder 2003; Dean and Temple 2011). Alternative sampling methods, such as seining, gill or trammel nets, trawls, cast nets, lift or push nets, rigid traps (e.g., minnow traps, slat traps), hoop nets, fyke nets, weirs, or angling, can be just as injurious to fishes if not conducted properly. The sampling methods chosen should allow for efficient capture of the species and sizes of fish needed to address research objectives while minimizing injury and mortality of collected fishes and non-target organisms. Multiple sampling gears may be required for the collection of a broad range of fish sizes or species or if diverse habitats are covered. Passive capture methods, such as set nets and traps, should be checked frequently enough to prevent unnecessary mortality of both target and non-target species. Nets and traps should be carefully positioned, anchored, and flagged and then removed at the cessation of sampling to avoid “ghost fishing” (lost or abandoned fishing gear that continues to kill fish and other sea life). Bonar et al. (2009) and Zale et al. (2013) provide additional information concerning standard sampling methods for fishes in freshwater environments, as well as the efficiency and specificity of various collecting gears.

### **5.4 Field Restraint of Fishes: Sedatives**

Prolonged restraint that causes physiological stress should be avoided. In some cases, use of a sedative or anesthetic agent to minimize stress may be advisable. Although the terms

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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# *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