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Telah melakukan penelitian yang dipublikasi pada bulan Februari tahun 2020 dengan judul sebagai berikut:

Provision Study of Lactobacillus spp. and Barley Straw Against Dynamics of DO, pH and Plankton Abundance

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: untuk memenuhi kaidah biosecurity bahan penelitian yang bersifat patogen telah diatur penggunaannya, selain itu juga menyebutkan bahwa penelitian dalam kondisi laboratorium baru mengatur tentang hewan percobaan berupa ikan hidup (hal 11,12,43 ; 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 hidup (Kelas: Chondrichthyes, Agnatha, dan Osteichthyes) dan Avertebrata (Kelas: Cephalopoda) (hal 13,14 ; terlampir).

Sedangkan dalam penelitian tersebut tidak menggunakan bahan penelitian yang bersifat patogen, juga tidak menggunakan ikan hidup tetapi menggunakan mikroorganisme (bakteri dan plankton) sebagai bahan penelitiannya. 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

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Guidelines for the Use of Fishes in Research

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landing fish, is excluded; however, responsibilities for operating a fishery and for the welfare of the fishes contained therein are not excluded (Defra 2006). The Centre for Environment, Fisheries and Aquaculture Science (Cefas, <http://www.cefas.defra.gov.uk/>), an executive agency of Defra, works with scientific entities to deliver science-based recommendations, conducts research, and facilitates international relationships. An additional resource is the Fish Veterinary Society (FVS, <http://www.fishvetsociety.org.uk/>), a forum for veterinary surgeons, fish health professionals, and veterinary students. The FVS promotes fish care and health management for multiple settings, including those for ornamentals.

In Australia, the Department of Agriculture (<http://www.daff.gov.au/>) plays an important role in promoting the biological, economic, and social sustainability of Australian fisheries and provides a multitude of services and resources for the fisheries communities, including biosecurity concerns of importing and exporting countries. The Fisheries Research and Development Corporation (FRDC, <http://frdc.com.au/research/Pages/default.aspx>) is a partnership between the government and the fishing and aquaculture industries for Australian research partners. The Australian Fisheries Management Authority (AFMA, <http://www.afma.gov.au/>) establishes research priorities (<http://afma.gov.au/resource-centre/research/research/>), the legislation and policy for which were defined mostly in the 1990s (<http://www.afma.gov.au/about-us/legislation-and-policy/>).

In Canada, the Canadian Council on Animal Care (CCAC, <http://ccac.ca/>) oversees the ethical use of animals in science. Governed by a council of representatives from more than 20 national organizations, the CCAC is a “quasi-regulatory” body that sets standards through guideline documents and policy statements on animal use in science. The CCAC guidelines on the care and use of fish (CCAC 2005, <http://ccac.ca/Documents/Standards/Guidelines/Fish.pdf>) were developed in response to the increased use of fishes as experimental subjects. The Canadian Food Inspection Agency (CFIA, <http://www.inspection.gc.ca>) and Fisheries and Oceans Canada (<http://www.dfo-mpo.gc.ca>) are responsible for developing containment guidelines for fish pathogens.

3.2 Biosecurity

Biosecurity can be defined in many ways (Falk et al. 2011); it is the process of taking precautions to minimize the risk of introduction and spread of infectious organisms into or among populations. The FAO defines biosecurity as a strategic and integrated approach that encompasses the policy and regulatory frameworks that analyze and manage risks in the sectors of food safety, animal life and health, and plant life and health, including associated environmental risk. FAO declared that “biosecurity covers the introduction of plant pests, animal pests and diseases, and zoonoses, the introduction and release of genetically modified organisms (GMOs) and their products, and the introduction and management of invasive alien species and genotypes” (FAO 2013, <http://www.fao.org/biosecurity/>). Disease occurrence is

dependent on the health of the animals, the condition of the environment, and the presence of a pathogen at levels sufficient to negatively affect health (see section 7.2 Confinement, Isolation, and Quarantine). The term “biosecurity” is also relevant to environmental biodiversity. Generally, regulations, appropriate permits (see section 3.4 Permits and Certificates), and other specific concerns regarding biosecurity within a country are addressed within the guideline documents mentioned herein. New Zealand, by virtue of its unique geographic isolation and economic agricultural base, has a plethora of legislation dealing with biosecurity issues (Biosecurity Act 1993).

Various circumstances hold biosecurity as a concern with regard to working with fish. These include state, regional, national, and international transfers of fish and fish products, aquaculture production, and the ornamental industry. Implementation of biosecurity at the global level and organizations working therein were delineated by Scarfe (2003). Biosecurity is a dynamic discipline because of advances in diagnostic technologies and knowledge about epidemiology and pathogenesis. When considering cryopreserved gametes and early life stages of aquatic species, biosecurity practices enable artificial spawning methods to deliver genetics safely. Issues involve disease transmission, introduction of exotic species, genetic consequences for target species, and genetic consequences for ecosystems (Tiersch and Jenkins 2003). Microorganisms (see section 5.9 Collection of Blood and Other Tissues) in archival samples can jeopardize valuable germplasm resources by lowering cell quality (Jenkins 2011b). Pathogen control strategies are a concern for small fish models used as biomedical models, especially Zebrafish *Brachydanio rerio* (also known as zebra danio *Danio rerio*), Japanese Medaka *Orzias latipes*, and species of the genus *Xiphophorus* (Lawrence et al. 2012). Contemporary international and national regulatory frameworks, treaties, partnerships, and agreements addressing the transfer of aquatic animals and aquaculture products can be adapted as mechanisms for the oversight of unique temporal and geographic biosecurity issues inherent with the circumstance. Some countries may already have regulations, which are likely aligned with OIE recommendations and EU requirements, for the activities of mammalian artificial insemination industries. Additionally, core institutional biosecurity tenants may be necessary in achieving compliance with international regulations.

3.3 Federal, State, and Local Regulations

In the United States, federal authority for the use of animals in research is found primarily in two agencies, the U.S. Department of Health and Human Services (HHS; <http://www.hhs.gov/>) and the U.S. Department of Agriculture (USDA; <http://www.usda.gov/wps/portal/usda/usdahome>). If endangered, threatened, or candidate species for listing are involved, the U.S. Department of the Interior (<http://www.fws.gov/endangered/>) or the U.S. Department of Commerce (<http://www.nmfs.noaa.gov/pr/>) has additional authorities. Authority for each Department is found in specific Acts of Congress. Legislative mandate for the Public Health Service (PHS; <http://www.usphs.gov/>) policy for use of animals in research is provided by the Health Research

7. Laboratory Activities

7.1 General Principles

Working with live fishes under laboratory conditions requires attention to many details concerning the requirements for, and limits of tolerance of, the particular species under study. Acceptable physical facilities and an adequate supply of water with good quality must be provided, even if the fishes are to be held for only short periods of time. Although fish may tolerate marginal facilities and conditions for a few hours or even several days, holding them under less than optimal conditions will affect the results of the research. Standards for humane treatment of animals must also be maintained, regardless of the length of time that the fishes are held.

The reader should note that some content of section 7 is not restricted to laboratory activities, but may be applicable to field situations, as well.

7.2 Confinement, Isolation, and Quarantine

Prior to bringing fishes into a laboratory, facilities and plans should be in place to ensure that the fish cannot escape, especially species not native to the watershed, and that the introduced fishes can be isolated physically from fishes already present. Each holding unit should have its own set of nets and other equipment. Facilities and equipment used for previous studies should be disinfected prior to use in new studies, typically with a chlorinated disinfectant or another disinfectant such as Virkon[®] Aquatic (www.wchemical.com/). If the introduced fishes may carry disease agents, especially pathogens or parasites that are not endemic to the area, quarantine-level facilities should be used. The level of quarantine required will vary with the seriousness of the known or suspected disease agent (see section 2.5 Fish Health Management: Control of Pathogens and Parasites).

Individual fish with suspected ill health should be quarantined from the others so as to negate the potential for spread of potential disease agents. Such fish should be evaluated by an individual with expertise in fish diseases (fish pathologist or veterinarian), and the proper therapeutant should be applied as directed. Providing guidance for the treatment of specific diseases is beyond the scope of this document. The investigator is strongly urged to establish a working relationship with individuals with expertise in fish health with whom they may consult.

Experimentation with nonindigenous fishes, transgenic fishes, or other genetically modified fishes is a special situation that requires additional precautions to preclude their escape. Permitting with site visits by state wildlife agencies may be required for holding nonindigenous species (see section 3.4 Permits and Certificates). The specific barriers may be similar to those used to prevent the escape of disease agents but must be developed to fit the physical characteristics of the laboratory or experimental facility. The USDA has developed

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, 2nd ed., 1993 and vol. 2, 1984, which provide the general principles for the care and use of animals, the CCAC also publishes detailed guidelines on issues of current and emerging concerns. The CCAC *guidelines on: the care and use of fish in research, teaching and testing* is the seventh of this series. This document supersedes Chapter I - Fish, *Guide to the Care and Use of Experimental Animals*, vol. 2 (CCAC, 1984).

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

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

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

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

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

this document prior to the beginning of the main text.

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

B. INTRODUCTION

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

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

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

These guidelines apply to fishes held in facilities for research, teaching and testing, as well as to fishes that are studied in their natural habitats.

1. Definition of Fish

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

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

The use of fishes as experimental subjects has increased substantially over the past two decades. This increase in use is a result of the rapid development of the aquaculture industry, requirements for testing involving fishes as indicators of environmental change, and the use of fishes as a replacement for mammals in biomedical, pharmacological and genetic research (DeTolla *et al.*, 1995; Fabacher & Little, 2000). The trend toward the use of fishes as a replacement for studies that would previously have used mammals as experimental subjects is not discouraged. However, it must also be recognized that fishes have the capacity to perceive noxious stimuli. Noxious stimuli are those stimuli that are damaging or potentially damaging to normal tissue (e.g., mechanical pressure, extremes of temperature and corrosive chemicals). Whether or not fishes have the capacity to experience any of the adverse states usually associated with pain in mammals is subject to a great deal of debate in the scientific literature (FAWC, 1996; FSBI, 2002; Rose, 2002; Braithwaite & Huntingford, 2004). Nonetheless, fishes are capable of behavioral,

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

3. Ethical Overview

Guideline 1:

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

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

In addition, the CCAC system takes a "moral stewardship" approach to the use of animals in science as explained in the CCAC Experimental Animal User Training Core Topics - Module 2, Ethics in Animal Experimentation (http://www.ccac.ca/en/CCAC_Programs/ETCC/Module02/toc.html).

The first guideline statement in the CCAC *guidelines on: institutional animal user training* (CCAC, 1999a) states, "Institutions must strive through their training programs to sustain an institutional culture of respect for animal life".

3.1 Principles of the Three Rs

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

As for any other species covered by the CCAC system, investigators using fishes are required to use the most humane methods on the smallest

number of animals necessary to obtain valid information. This requires the use of a sound research strategy, including: identification of key experiments that determine whether a particular line of enquiry is worth pursuing; use of pilot studies; staging of *in vitro* to *in vivo* experiments where possible; and implementation of staged increase in test stimuli where possible (Balls *et al.*, 1995). The numbers and species of animals required depend on the questions to be explored. Field studies, aquaculture studies and laboratory studies require different statistical designs; field studies and aquaculture production typically require the use of larger numbers of animals. The life stage of the fishes used in each study will also affect the numbers of animals needed. Studies of early life stages typically require large numbers of individuals. In all cases, studies should be designed to use the fewest animals necessary. Heffner *et al.* (1996) and Festing *et al.* (2002) provide discussions on the appropriate treatment of samples and experimental units. Investigators are encouraged to consult with a statistician to develop study designs that have the appropriate statistical power to accomplish the research objectives (Nickum *et al.*, 2004).

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

- animals must be maintained in a manner that provides for their optimal health and well-being, consistent with the demands imposed by the experimental protocol;
- animals must not be subjected to pain and/or distress that is avoidable and that is not required by the nature of the 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