

The 2nd International Conference on Fisheries and Marine Science



Dr. Endang Dewi Masithah, Ir., MP.

AS A ORAL PRESENTER

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Universitas Airlangga

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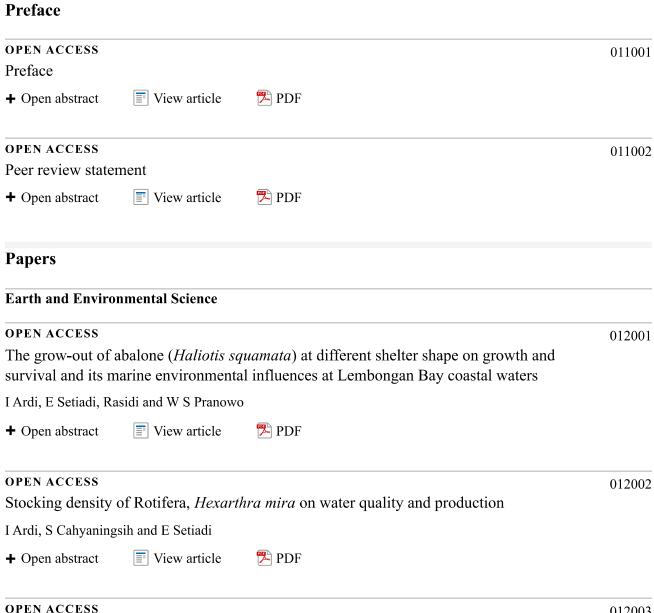
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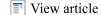


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Concentration of fermented tilapia feces suspension by decomposer bacteria as a feed to induce *moina macrocopa* sexual reproduction

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Abstract. Sexual induction of *M. macrocopa* can be induced by setting a density of feed concentration. To optimize the production of ephippia, sexual females must be given sufficient quality feed. Fermentation of tilapia fish from the results of preliminary studies showed a significant increase in protein concentration but fat concentration decreased. This research to determine the best concentration of fermented tilapia feces feed to produce ephippia *M. macrocopa*. This research is experimental by using a completely randomized design (CRD). This research consisted of 4 treatments of feed concentration is fermented feces suspension concentration of 33,30 mg/L (P1), 37,00 mg/L (P2) and 40,70 mg/L (P3) and control using rice bran suspension feed concentration of 37,00 mg/L(P0), with each using 5 replications. Induction sexual offspring is maintained at a density of 1000 ind/L for 6 days. During cultivation is calculated survival rate, *M. macrocopa* ephippia production, and some water quality parameters as support. Data were analyzed using ANOVA and Duncan test. This research showed that *M. macrocopa* cultivation using several concentrations of suspension feed of fermented tilapia feces at concentrations of 40.70 mg/L can induce sexual females and produce ephippia and showed the highest survival rate (1186 ± 26,45 grains/L) and 88,13%.

1. Introduction

M. macrocopa is a natural food for fish and shrimp larvae that are spread in freshwaters [1]. Increasing the price of cyst Artemia sp. making Moina sp. as an alternative natural feed for fish and shrimp larvae [2]. Moina sp. reproduces in two ways sexual and asexual (parthenogenesis) [3]. Sexual female Moina sp. does not reproduce by parthenogenesis so that if the egg is fertilized by a male. It will experience carapace thickening and ephippia are formed [4]. Maintenance of the population of Moina sp. with high density and adequate feeding can induce the production of male and female sexual offspring [5].

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Fish farming activities produce waste originating from feces and fish feed residues [6]. Preliminary research results show that tilapia feces contain protein (18,.19%), fat (1.46%), EPA (0.13%), DHA (0.20%), and amino acid histidine (0.26%) and arginine (0.78%).

Previous research has successfully cultivated *Moina* sp. with fecal fish fees which produce the highest fecundity [7] and populations with high densities of 1000 ind/L [8]. Tilapia feces can be used as direct feed if made in the form of suspension. To increase the solubility of tilapia fish, fermentation using decomposer bacteria can be done. This research tries to determine the effect of suspension concentration of fermented tilapia with decomposer bacteria on the induction of ephippia production from the sexual female *M. macrocopa*.

2. Materials and methods

This study was conducted in the Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga and Balai Riset dan Standardisasi Industri Surabaya, Surabaya, East Java, Indonesia.

2.1 Materials

The materials used in the research are: *M. macrocopa*, freshwater, dolomite, tilapia feces suspension, molasses, EM-4 agriculture, rice bran suspension, and detergent.

2.2 Research methods

This research uses an experimental method. The design used is a Completely Randomized Design (CRD) with 4 treatments and 5 replications, are:

P0: Feeding of M. macrocopa using suspension rice bran (control) concentration of 37.00mg/ L

P1: Feeding of M.macrocopa using suspension of fermented tilapia feces concentration of 33.30 mg/L

P2: Feeding of *M.macrocopa* using suspension of fermented tilapia feces concentration of 37.00 mg/L

P3: Feeding of M.macrocopa using suspension of fermented tilapia feces concentration of 40.70 mg/ L

2.3 Provision of inoculants and culture of M. macrocopa

M. macrocopa used in this study was obtained from waters in the Surabaya region, then cultivated individually (one *Moina*/20 mL) in several generations to obtain species that have the best growth and production performance of offspring. Furthermore, *Moina* is cultured with bran suspension feed for 2 months at a density of 20 / L volume of 10 L water. The cultivated Moina offspring now become inoculants in this study with the same initial density [3].

2.4 Research parameters

The main parameters in this research are the survival rate of the broodstock, the amount of ephippia production. The ephippia calculation is performed on the fifth to the seventh day of each treatment. Ephippia was taken based on maintenance jars for each treatment. The percentage of survival of the broodstock is calculated using the formula [9] as follows:

Survival Rate =
$$\frac{N_t}{N_0} \times 100\%$$

Supporting parameters in this research are measurements of water quality in *M. macrocopa* maintenance media, which include pH, DO, temperature, and alkalinity. Observation of water quality is carried out every day in the morning and evening.

2.5 Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) to determine the effect of treatment and continued with the DUNCAN test to determine and determine the treatment with the best results.

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3. Results and discussion

3.1 Results

ANOVA test results showed that the cultivation *M. macrocopa* using several concentrations of fermented tilapia suspension feces showed an influence on total ephippia production and ephippia per brood (P<0.05) (Table 1). The highest total production of ephippia *M. macrocopa* (3015 grains/L) and the highest production of ephippia per brood was (3.36 grains/brood) produced from aquaculture using rice bran suspension (control) feed.

Table 1. Ephippia Production of *M. macrocopa*

Treatment	Ephippia Production (Ind/L)		
	Total Ephippia \pm SD	Ephippia per Brood \pm SD	
Control	$3015^a \pm 57.88$	$3.36^a \pm 0.09$	
P1 (33.30 mg/L)	$438^d \pm 35.60$	$0.52^{\rm d}\pm0.05$	
P2 (37.00 mg/L)	$817^{c} \pm 53.36$	$0.95^{\circ} \pm 0.07$	
P3 (40.70 mg/L)	$1186^{b} \pm 26.45$	$1.35^{b} \pm 0.05$	

Note: Different superscripts in the same column show significant differences (P<0.05).

The graph of the ANOVA test results showed that the cultivation of *M. macrocopa* using several concentrations of fermented tilapia suspension feces showed an influence on the daily ephippia production (P<0.05) (Figure 1).

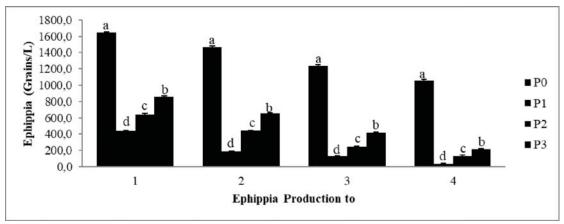


Figure 1. Daily Ephippia Production of M. macrocopa

ANOVA test results showed that *M. macrocopa* cultivation using several concentrations of fermented tilapia suspension did not affect the survival rate (P>0.05) (Table 2). The survival rate of *M. macrocopa* on the density of 1000 ind/L with fermented tilapia suspension feces was 83.93-89.73%. Water quality during the maintenance of *M. macrocopa* that still supports life sustainability (Table 3).

Table 2. *M. macrocopa* Survival rate

Treatment	Survival Rate (%) \pm SD		
Control	$89.73^a \pm 2.01$		
P1 (33.30 mg/L)	$83.93^{\circ} \pm 1.84$		

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P2 (37.00 mg/L)	$85.67^{bc} \pm 1.70$
P3 (40.70 mg/L)	$88.13^{ab} \pm 2.08$

Note: Different superscripts in the same column show significant differences (P<0.05).

Table 3. *M. macrocopa* Maintenance ParameterWater Quality

Parameter	Value Range	Optimal Conditions
pН	8.19 – 8.25	7.0 – 8.0 (Miah <i>et al.</i> , 2013)
DO (ppm)	5.13 – 5.49	> 3.50 (Miah <i>et al.</i> , 2013)
Temperature (°C)	28.28 – 28.64	25 – 31 (Tan and Wang, 2010)
Alkalinity (mg/L)	24.00 – 31.00	> 50 (Tan and Wang, 2010)

3.2 Discussion

Induction of M. macrocopa sexual females in Cladosera has been developed using induction factors which include water quality, population density [10] as well as quantity and quality of feed [11]. The induction of M. macrocopa sexual female production in this research was carried out by setting the density of 660 ind/L by giving 37.00 mg/L concentration of rice bran suspension feed, as in previous studies that the cultivation of M. macrocopa with rice bran concentration of 37.00-64.40 mg/L produces females with the highest ephippia production (3052 \pm 199 grains/L) [12].

The induced child is then reared at a density of 1000 ind/L and fed with fermented tilapia suspension. [10] stated that the saplings of *Moina* sp. maintained at a density of 1000 ind/L can induce ephippia production, as in research *M. branchiata* which are cultivated with densities of 750-1000 ind/L produce 70% sexual females and cultivation with child densities of 1000 ind *Moina*/L, using rice bran suspension feed concentration of 37.00-64.4 mg/L produces the highest ephippia production as many as 2102±120 grains/L.

Cultivation using fermented tilapia suspension fermented tilapia concentration of 40.70 mg/L produced the highest ephippia production (1186 \pm 26.45 grains/L) compared to 33.30 mg/L concentration (438 \pm 35.60 grains/L), but the ephippia production is still lower than the cultivation using bran suspension feed (3015 \pm 57.88 grains / L), this is presumably due to the higher protein content in fermented tilapia suspension at 72.82%.

High protein concentration is a limiting factor in ephippia production. High protein content limits ephippia production [13], but high concentrations of fatty acids can produce ephippia [14]. The success of *M. macrocopa* in producing ephippia requires the role of omega-3 fatty acids in the form of EPA and DHA, where these needs begin in the period of previtellogenesis to the process of ovulation [14]. The availability of EPA and DHA has the potential to increase the process of gametogenesis because omega-3 fatty acids have an impact on egg production during reproduction [15]. High protein in fermented tilapia suspension (72.82%) resulted in lower ephippia production (438-1186 grains/L) compared to rice bran suspension feed (20.66%) which resulted in ephippia of (3015 grains/L).

The production of ephippia *M.macrocopa* can also be influenced by amino acids in the form of histidine and arginine. The amino acid histidine in tilapia feces was (0.26%) and arginine was (0.87%), while the amino acid histidine in rice bran suspension was (1.61%) and arginine (3.82%). The low amino acid in tilapia suspension stool feed could potentially support the production of ephippia *M. macrocopa* compared to rice bran suspension feed, but the high protein content in

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fermented tilapia suspension was (72.82%) compared to rice bran suspension feed (20.66%) thought to be able to limit the production of *M. marocopa* ephippia.

The amino acid histidine and arginine in feed can reduce the production of ephippia in Cladocera [13]. The amino acid histidine influences protein synthesis [16], while the amino acid arginine influences the reproduction of *Moina* sp.[17].

Feed concentration also affects the availability of nutrients (protein, fat and amino acids) *Moina* sp.[18]. Different treatment of fermented tilapia feces feed concentration, is thought to cause the availability of nutrients (protein and fat) for the production of ephippia *M.macrocopa*, this is consistent with the statement [8] that different nutritional values will have different effects on population development and ephippia production. High concentrations of feed can increase the availability of nutrients (protein, fat and amino acids) that can affect the body's metabolism thereby increasing the fecundity or production of ephippia [18], this is according to research [19], that Cladosera uses 68% of the energy produced by its metabolism to reproduce. According to [20] that, low concentrations of feed can reduce nutrients in feed, thus affecting the regulation of stress in Cladosera and can affect the reproductive model.

M. macrocopa began producing ephippia on the fourth day of maintenance. Production of ephippia on the fifth day and subsequently decreased, this was thought to decrease the availability of nutrients (protein and fat) in feed for the reproduction of *M. macrocopa*. The production of ephippia in aquaculture uses fermented tilapia suspension feed fermented on the first day (1200-2363 grains/L), the second day ephippia production (527-1807 grains/L), ephippia production on the third day (353-1150 grains/L), and the production of ephippia on the fourth day is (110-610 grains/L), while the production of ephippia in cultivation uses rice bran suspension feed on the first day (4590 grains/L), the second day ephippia production is (4083 grains/L), ephippia production on the third day (3450 grains/L), and ephippia production on the fourth day (2953 grains/L). According to [21] that a large nutritional deficiency in feed can cause decreased egg production produced by the ovaries.

M. macrocopa cultivation in research with fermented tilapia suspension feed with a concentration of 40.70 mg/L has a higher survival rate, but lower than rice bran suspension feed, this is because the rice bran suspension contains lower protein from tilapia suspension fermented which is thought to support the survival of *M. macrocopa*, due to the growth of *Moina* sp. supported by appropriate feed nutrition to accelerate growth [22].

Cultivation of *M. macrocopa* density of 1000 ind/L fed fermented tilapia suspension fermented fish with different concentrations had water quality values, temperatures ranged from 28.28 to 28.64°C, alkalinity ranged from 24-30 mg/L, DO ranged from 5.13-5.49 ppm, and the pH of water ranges from 8.19 to 8.25 which still supports the survival of *M. macrocopa* [23; 24]. The increase in pH value during the study was caused by the media added with dolomite CaMg(CO3)2. The presence of calcium (Ca) in the media reacts with H+ which causes the pH to increase [25].

4. Conclusion

The conclusion from the research of suspension concentration of fermented tilapia that has been fermented with decomposer bacteria, namely the daily production of ephippia, ephippia per brood and the highest total ephippia occurred in the cultivation of *M. macrocopa* concentration of 40.70 mg/L of 215.3-857.3 grains/L, 1.35±0.05 ind/L and 1186±26.45 ind/L

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Telah melakukan penelitian yang dipublikasi pada tahun 2020 dengan judul sebagai berikut: Concentration of fermented tilapia feces suspension by decomposer bacteria as a feed to induce Moina macrocopa sexual reproduction

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: penelitian dalam kondisi laboratorium baru mengatur tentang hewan percobaan berupa ikan hidup, untuk hewan percobaan berupa zooplankton tidak termasuk (hal 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 (Kelas: Chondrichthyes, Agnatha, dan Osteichthyes) dan Avertebrata (Kelas: Cephalopoda) (hal 13,14; terlampir).

Sedangkan dalam penelitian tersebut menggunakan zooplankton (*Moina macrocopa*) sebagai hewan percobaan. 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.

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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 wellbeing, 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 relevent 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