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Telah melaksanakan penelitian pada tahun 2017 dengan judul sebagai berikut :

Penambahan Asam Amino Taurin pada Pakan Buatan terhadap Peningkatan Pertumbuhan dan Sintasan Benih Ikan Kerapu Cantik (*Epinephelus fuscoguttatus* × *Epinephelus microdon*)

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari *American Fisheries Society* (AFS, 2014) yang berjudul *Guidelines for the Use of Fishes in Research* dan *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guidelines on the Care and Use of Fish in Research, teaching and Testing*. Sehingga penelitian tersebut tidak perlu dilakukan *Uji Ethical Clearence* karena ikan yang digunakan tidak disakiti sesuai dengan CCAC (2005) halaman 43 mengenai pemberian pakan pada ikan dan menghasilkan *out put* yang baik untuk akuakultur.

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

Surabaya, 19 Juni 2023

Dekan



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Guidelines for the Use of Fishes in Research

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Table of Contents

Use of Fishes in Research Committee, 2014	vii
Preface.....	ix
Acknowledgments.....	xi
Statement of Purpose	xiii
1. Introduction.....	1
2. General Considerations.....	3
2.1 Approval of Research Plans by IACUCs.....	3
2.2 Project Quality Assurance Plans and Standard Operating Procedures	4
2.3 Statistical Design.....	5
2.4 Mortality as an Experimental Endpoint	6
2.5 Fish Health Management: Control of Pathogens and Parasites	6
3. Statutory Requirements and Regulatory Bodies.....	9
3.1 International Regulations and Guidelines	9
3.2 Biosecurity	11
3.3 Federal, State, and Local Regulations.....	12
3.4 Permits and Certificates	14
4. Animal Welfare Considerations.....	17
4.1 General Considerations	17
4.2 Stress	17
4.2.1 Stages of Stress.....	18
4.2.2 Measuring and Avoiding Stress.....	18
4.3 Nociception and Pain	20
5. Field Activities.....	23
5.1 Habitat and Population Considerations.....	23
5.2 Field Collections	23
5.2.1 Permits.....	23
5.2.2 Natural History Collections.....	24

5.2.3 Representative Samples	24
5.2.4 Collection of Imperiled Species	25
5.2.5 Museum Specimens and Other Preserved Specimens	26
5.3 Live Capture Techniques and Equipment	28
5.4 Field Restraint of Fishes: Sedatives	28
5.4.1 Drugs Approved for Use on Fish.....	29
5.4.2 Low Regulatory Priority (LRP) Drugs	29
5.4.3 Investigational New Animal Drugs (INAD)	30
5.5 Dangerous Species and Specimens	30
5.6 Handling and Transport.....	31
5.7 Facilities for Temporary Holding and Maintenance	32
5.8 Field Acclimation.....	33
5.9 Collection of Blood and Other Tissues	34
6. Marking and Tagging.....	37
6.1 General Principles	37
6.2 External Tags and Marks.....	37
6.3 Internal Tags and Marks, and Biotelemetry.....	38
6.4 Genetic Markers	40
6.5 Stable Isotopes.....	41
6.6 Fatty Acids	42
7. Laboratory Activities	43
7.1 General Principles	43
7.2 Confinement, Isolation, and Quarantine	43
7.3 Acclimation to Laboratory Conditions.....	45
7.4 Facilities for Long-Term Housing of Fishes	45
7.5 Density of Animals.....	47
7.6 Feeds and Feeding.....	47
7.7 Water Quality	49
7.8 Water Recirculation Units.....	50
7.9 Effluents and Permits	51

7.10 Dangerous Species and Specimens in Captivity	51
7.11 Restraint of Fishes: Sedatives and Related Chemicals.....	52
7.12 Surgical Procedures.....	53
7.13 Administration of Drugs, Biologics, and Other Chemicals	55
7.13.1 Drugs	55
7.13.2 Biologics and Other Chemicals	56
7.13.3 Chemical Facility Anti-Terrorism Standards (CFATS)	56
8. Final Disposition of Experimental Animals	59
8.1 Euthanasia	59
8.2 Storage or Return to Aquatic Habitat.....	60
9. Future Revisions	61
10. Literature Cited.....	63
Appendix.....	85
Brief Checklist for IACUC Readiness	85
List of Low Regulatory Priority Drugs and Consideration for Their Use	86
Appendix Table 1. Low regulatory priority aquaculture drugs, indications, and doses.	87
Appendix Table 2. OIE-notifiable causative disease agents for fish and amphibians.	88
Index of Terms and Acronyms.....	89
Note on Additional Readings	90

4. Animal Welfare Considerations

4.1 General Considerations

Research involving living animals, including fishes, must be based on experimental designs and animal care practices that can lead to scientifically valid results. Fishes are acutely sensitive to stress (e.g., Barton and Iwama 1991), and responses may include changes in behavior (e.g., Martins et al. 2012), reduced growth, changes in osmotic status, suppressed immune systems (with consequent disease onset), and altered reproductive capacity (Iwama et al. 2006; Schreck et al. 2001; Schreck 2010). Accordingly, unless the experimental objectives require actions or conditions designed to test responses to stress, fishes should be maintained, handled, and tested under conditions that will not create such responses. The Guidelines addresses the conduct of scientific research and focuses on established facts and the processes through which knowledge is developed. Research plans submitted to IACUCs should address animal care considerations, in addition to the details of research goals, objectives, and procedures. The extent to which IACUCs incorporate personal values concerning animal welfare into their institutional guidelines is determined within each institution.

4.2 Stress

The study of stress has focused on how animals have evolved physiological and behavioral mechanisms to address the challenges of changing environmental conditions and then to permit them to maintain homeostasis, or self-sustaining balance. The set of environmental variables (conditions) best suited for the well-being of each species typically encompasses a specific range for each factor and species (see section 5.7 Facilities for Temporary Holding and Maintenance), as stress responses are species-specific (Schreck 2010). Accordingly, when fishes are maintained within these ranges, a state of homeostatic balance is expected. Deviations from homeostasis characterize a stress response. While many definitions for stress have been proposed, we employ the definition of Schreck (2000) and Schreck et al. (2001): “a physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of insult.” When stressed, fish generally attempt to reestablish homeostasis via a process known as “allostasis regulation in which they adjust their physiological function to re-establish a dynamic balance” (Sterling and Eyer 1988). While allostasis is generally adaptive because it helps keep animals alive in the face of a short-term stressor(s), it can be maladaptive over the long term and have negative consequences on growth, reproduction, and immunological health (Schreck 2010). Accordingly, investigators need to understand those factors that might cause stress in their experimental animal(s), the potential consequences, and how stress might be avoided by optimizing experimental conditions.

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

4.2.1 Stages of Stress

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

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

4.2.2 Measuring and Avoiding Stress

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

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

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

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

Canadian Council on Animal Care



guidelines on:
the care and use of
fish in research,
teaching and
testing

This document, the CCAC *guidelines on: the care and use of fish in research, teaching and testing*, has been developed by the *ad hoc* subcommittee on fish of the Canadian Council on Animal Care (CCAC) Guidelines Committee.

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TABLE OF CONTENTS

A. PREFACE	1
SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT	3
B. INTRODUCTION	13
1. Definition of Fish	13
2. Rationale for Guidelines on the Care and Use of Fish	13
3. Ethical Overview	14
3.1 Principles of the Three Rs	14
4. Responsibilities	15
4.1 Responsibilities of investigators ..	15
4.2 Responsibilities of the animal care committee	16
4.3 Role of the veterinarian	17
5. Government Regulations and Policies on the Use of Fish	17
5.1 International	17
5.2 Federal	18
5.3 First Nations	20
5.4 Provincial/territorial	20
5.5 Municipal	20
C. AQUATIC FACILITIES	21
1. Water Supply	21
2. Water Quality	21
3. Engineering and Design	22
3.1 Structural materials	23
3.2 Room ventilation and airflow in aquatic areas	24
3.3 Mechanical and electrical requirements	25
3.4 Lighting	25
3.5 Redundancy in aquatic life support systems	26
4. Types of Systems	26
4.1 Flow-through systems	27
4.2 Recirculation systems	27
4.3 Static systems	27
4.4 Mesocosms	28
5. Fish Housing	28
5.1 Fish well-being	28
5.2 Tank/enclosure design	28
D. FACILITY MANAGEMENT, OPERATION AND MAINTENANCE	31
1. Security and Access	31
2. General Maintenance of the Facility ..	31
3. Environmental Monitoring and Control	32
3.1 Management of water quality ..	33
3.2 Temperature	33
3.3 Oxygen	34
3.4 Supersaturation	34
3.5 pH	35
3.6 Nitrogen compounds	35
3.7 Carbon dioxide	36
3.8 Salinity	36
3.9 Toxic agents	37
E. CAPTURE, ACQUISITION, TRANSPORTATION AND QUARANTINE	38
1. Capture of Wild Stock	38
2. Killed Specimens	38
3. Piscicidal Compounds	38

4. Acquisition of Hatchery Fish	39	3.3 Anesthesia	53
5. Transportation	39	3.4 Surgical equipment	54
6. Quarantine and Acclimation	40	3.5 Incisions	54
6.1 Quarantine	40	3.6 Suture materials and techniques ..	54
6.2 Acclimation	41	3.7 Pathophysiology of surgery and wound healing in fishes	55
F. HUSBANDRY	42	3.8 Postoperative care	55
1. Record-keeping and Documentation ..	42	4. Administration of Compounds and Devices by Various Routes	56
1.1 Standard Operating Procedures ..	42	4.1 Branchial diffusion ("inhalation")	56
1.2 General checklists	42	4.2 Oral	56
1.3 Assessment of fish well-being ...	42	4.3 Injection	57
2. Density and Carrying Capacity	42	4.4 Implants, windows and bioreactors	57
3. Food, Feeding and Nutrition	43	5. Tagging and Marking	57
3.1 Nutrition	43	5.1 Tissue marking	58
3.2 Food and feeding	43	5.2 Tagging	58
3.3 Feed quality and storage	43	6. Collection of Body Fluids	58
3.4 Larval weaning	45	7. Use of Infectious Disease Agents, Tumorigenic or Mutagenic Agents, and Toxic and Noxious Compounds ..	59
3.5 Use of medicated feeds	45	8. Endpoints and Criteria for Early Euthanasia	59
4. Broodstock and Breeding	46	8.1 Recognition of "pain", "distress" and "stress"	59
4.1 Induction of spawning	46	8.2 Choosing an appropriate endpoint	60
G. HEALTH AND DISEASE CONTROL	47	9. Monitoring	62
1. Fish Health Program	47	10. Negative Reinforcement Modalities ..	62
1.1 Disease prevention	47	11. Exercise to Exhaustion	62
1.2 Disease diagnosis and identification of pathogens	47	12. Environmental Extremes	62
1.3 Injuries and other disorders	48	13. Genetically Modified Fish	62
H. EXPERIMENTAL PROCEDURES .	50	I. EUTHANASIA	64
1. Handling and Restraint	50	J. DISPOSITION OF FISH AFTER STUDY	65
1.1 Restraint of dangerous species ..	51		
2. Restricted Environments	51		
3. Surgery	51		
3.1 Surgical preparation and skin disinfection	52		
3.2 Water quality during surgery ...	53		

1. Consumption of Fish65	APPENDIX B
2. Release of Fish to Wild65	ZOONOTIC DISEASE-
3. Fish as Pets65	TRANSMISSION OF FISH
4. Transfer of Fish Between Facilities65	DISEASES TO MAN77
5. Disposal of Dead Fish65	
K. REFERENCES66	APPENDIX C
	GUIDELINES FOR CONTAINMENT
L. GLOSSARY73	FACILITIES (FOR PATHOGEN
	STUDIES)79
M. ABBREVIATIONS75	APPENDIX D
APPENDIX A	WATER QUALITY CRITERIA FOR
RELEVANT GUIDELINES	OPTIMUM FISH HEALTH – FOR
AND ORGANIZATIONS76	COLDWATER, WARMWATER AND
	MARINE SPECIES OF FISH84

maintaining a given species will have to be developed using performance-based criteria such as growth rate. Established maximum densities should not be exceeded.

The number of fish that can be carried in a given water supply is extremely variable and depends on the species, water temperature, pathogen load, dissolved oxygen level, metabolic rate of the fish, feeding rate, and how fast the water is being exchanged.

It is important to recognize that there are profound effects of both maximal and minimal densities; below certain densities territorial behavior may increase (for example, in salmonids housed below minimal densities, feeding is diminished). Wedemeyer (1996a) has reviewed the physiological responses of fish to crowding.

To prevent problems in feeding due to territoriality and aggression when dissimilar sized fish are housed together, the fish should be graded periodically to ensure similar sizes within groups.

3. Food, Feeding and Nutrition

Most species display daily and seasonal feeding rhythms, and may be specialized to feed on specific types of food (Groot, 1996; Madrid *et al.*, 2001). Although fishes brought in from the wild generally prefer live feed to formulated feed, most learn to feed effectively on pellets and show remarkable flexibility in their ability to ingest and digest formulated feeds. The acceptance of feed depends upon chemical, nutritional and physical characteristics of ingredients selected for feed formulation as well as feed processing. The structure and function of their digestive systems influences the patterns of food intake and digestive efficiency; meal sizes and feeding frequencies should be set accordingly (Goddard, 1996; Alanära *et al.*, 2001).

3.1 Nutrition

Nutritionally balanced diets and appropriate feeding regimes are critical in ensuring that fishes remain healthy. Commercially manufactured fish feeds contain nutrients and energy sources essential for growth, reproduction and health. Essential nutrients include protein and amino acids, lipid and fatty acids, vitamins and miner-

als. Deficiency of these nutrients can reduce growth rate and feed consumption, and lead to diseases (NRC, 1993; Conklin, 2000). As fishes are ectothermic, their metabolic rate is determined by the water temperature. Therefore, feeding rates and quantities need to take temperature into consideration (Alanära *et al.*, 2001; Kestemont & Baras, 2001).

3.2 Food and feeding

Guideline 57:

Fish feed should be purchased from sources that manufacture feed according to standards employed in the feed industry for fish and other domestic animals, and according to published nutrient requirements for the species, if available.

If fish are to be introduced into the food or feed chain (see Section J. Disposition of Fish After Study), the fish feed must be in compliance with the Feeds Act and Regulations (laws.justice.gc.ca/en/F-9).

Guideline 58:

Feed bags should be labeled with date of manufacture and guaranteed analysis information. Small aliquots of feed should be retained for independent testing when large feed lots are received.

3.3 Feed quality and storage

Guideline 59:

Feed should be stored in dedicated areas that are dark, temperature and humidity controlled, and pest-free to ensure its nutritional quality. Feed for immediate use and feed in feeders should be similarly protected. Feed used for daily feeding should be kept in sealed-top containers to protect it from humidity and light, and frequently replaced with feed from storage.

All feeds, whether moist, semi-moist or dry, are susceptible to degradation with time. Moist feeds containing minced raw fish or ensilaged fish should be fed within a few hours or frozen (Goddard, 1996). Dry feeds should be stored at temperatures < 20°C and humidity < 75%. High humidity increases susceptibility to mould, and high temperatures destroy certain vitamins and