2598/UN3.1.12/LL/2021/Incofims/213

Certificate

This certificate is granted to:

Dr. Endang Dewi Masithah, Ir., MP.

Title: Interprofessional collaboration for enhancing the aquatic ecosystem sectors

As Presenter in

"The 4th International Conference On Fisheries and Marine Sciences"

29 September 2021

Dean
Faculty of Fisheries and Marine
Universitas Airlangga

Prof. Ir. Mohammad Amin Alamsjah M. Si., Ph. D.
NIP.19700116199031002

Chairman of The Committe
Faculty of Fisheries and Marine
Universitas Airlangga

Dr. Veryl Hasan, S.Pi., M.P
NIP. 199002072018083101

ISSN: 1755-1315

IOP Conference Series:

Earth and Environmental Science

The open Access Journal for Conference Proceedings

iopscience.org/jpcs

Table of contents

Volume 1036

2022

Previous issue Next issue

The 4th International Conference on Fisheries and Marine Sciences (INCOFIMS 2021)
29/09/2021 - 29/09/2021 Online

Accepted papers received: 20 May 2022
Published online: 11 July 2022

Open all abstracts

Preface

The 4th International Conference on Fisheries and Marine Sciences (INCOFIMS 2021)

Monitoring the occurrence of *Zoea Syndrome* (ZS) in pacific white shrimp (*Litopenaeus vannamei*) larval from several hatcheries in East Java, Indonesia

P A Wiradana, M D Sani, R E Mawli, F N D Ashshoffa, I G Widhiantara and A T Mukti

+ Open abstract View article PDF

OPEN ACCESS 012004

Nanocalcium of *Pila ampullacea* Shell incorporated into Feed on Molting and Growth Performance of Crayfish *Cherax quadricarinatus*

F. B. A Jabbar, M. Ansar and Ardiansyah

+ Open abstract View article PDF

OPEN ACCESS 012005

Bacterial Viability of *Edwardsiella tarda* from Silver Rasbora (*Rasbora argyrotaenia*) after Infection with Immmersion Methods

N Husna, R Kusdarwati and M F Ulkhaq

+ Open abstract View article PDF

OPEN ACCESS 012006

The Use of Seaweed Flour (*Kappaphycus alvarezii*) as an Innovation in the Manufacture of High Fiber *Fettucine* Paste Products

D Pramestika, D Y Pujiastuti and Patmawati

+ Open abstract View article PDF

OPEN ACCESS 012007

Expression of heat shock protein 70 (hsp70) in liver and kidney organ of silver rasbora (*rasbora argyrotaenia*) exposed by sublethal organophosphate pesticides

A N Fadilah, L Sulmartiwi and L Lutfiyah

+ Open abstract View article PDF

OPEN ACCESS 012008

Quality Enhancement in Seaweed Dodol Using Edible Film Carrageenan Packaging

N A Savitri, E D Masithah and W Tjahjaningsih

+ Open abstract View article PDF

OPEN ACCESS 012009

Characteristics of fish crackers mackarel (*Euthynnus affinis*) with edible film surimi as packaging

Eka Saputra, Sapto Andriyono and Wahyu Isroni

+ Open abstract View article PDF

OPEN ACCESS 012010

Effectiveness of Liquid Smoke as a Source of Acetic Acid in Lowering Heavy Metals Levels in Blood Cockle (*Anadara granosa*)

F Swastawati, P H Riyadi, M Mulyono, A Nugraheni, M Muniroh and A N Hidayati

+ Open abstract View article PDF

OPEN ACCESS 012011

Molecular phylogenetic of silver barb *barbonymus gonionotus* (bleeker, 1849) (cypriniformes: cyprinidae) in Java, Indonesia

F S Valen, M S Widodo, R A Islamy, K P Wicaksono, Soemarno, L Insani and V Hasan

+ Open abstract View article PDF

OPEN ACCESS 012012

Application of modified starch on glass bioplastic based on carrageenan from *Eucheuma cottonii* on mechanic and biodegradation properties

T A Triani, M A Alamsjah and D Y Pujiastuti

+ Open abstract View article PDF

OPEN ACCESS 012013

Analysis of Blood Glucose Levels and The Development of Ectoparasite Infestation on Pacific White Shrimp (*Litopenaeus vannamei*) Which Were Given Crude Protein *Zoothamnium penaei* at High Stocking Densities

G Mahasri, W H Satyantini and A T Mukti

+ Open abstract View article PDF

OPEN ACCESS 012014

Mitigation and adaptation to climate change through sustainable mangrove management on the coast of Rembang Regency

H A Sutanto, I Susilowati, D D Iskandar and Waridin

+ Open abstract View article PDF

OPEN ACCESS 012015

Nursery tecnique of gouramy fish *(osphronemus goramy)* at instalasi perikanan budidaya, pasuruan, east java

Jamal Asfani and Lailatul Lutfiyah

+ Open abstract View article PDF

OPEN ACCESS 012016

Acute Toxicity of domestic landfill leachate to carp fish (*Cyprinus carpio*)

H C Pratiwi, B S Rahardja and W Tjahjaningsih

+ Open abstract View article PDF

OPEN ACCESS 012017

Quality and Halal Certification of Micro and Small Enterprises Fishery Products in Sidoarjo, East Java, Indonesia

Sukoso, P T Al Huda, H Muyasyaroh, Y A D Susanti and L H Adila

+ Open abstract View article PDF

OPEN ACCESS 012018

Quality improvement formulation of salak chili paste paste based on curly red chili (*Capsicum annuum var. hailux*) and pondoh salak (*Salacca edulis reinw var. semeru*) using sensory evaluation, activity evaluation of bioactive compounds and microbiological evaluation

Joni Kusnadi, Arinta Julia Buwana Saputra, Siti Narsito Wulan and Estri Laras Arumingtyas

+ Open abstract View article PDF

OPEN ACCESS 012019

Broodstock rearing techniques brown-marbled grouper (*epinephelus fuscoguttatus*) at balai perikanan budidaya air payau (BPBAP) situbondo, east java

Dimas Khoironi Nur Septian, Darmawan Setia Budi, Suciyono and Lailatul Lutfiyah

+ Open abstract View article PDF

OPEN ACCESS 012020

The effect of barnacles powder as a source of protein feed substitution against the digestibility of crude protein and crude fiber on male rabbits rex

B Agustono, F Maulana, S H Warsito and M A Al-Arif

+ Open abstract View article PDF

OPEN ACCESS 012021

Diet and feeding strategy of the common silver-biddy, *Gerres oyena* (Forsskål 1775) in the seagrass beds of Karang Congkak Island, Kepulauan Seribu National Park

A K Putri, M F Nazal and C P H Simanjuntak

+ Open abstract View article PDF

OPEN ACCESS 012022

Sensory and Chemical Properties of Long Jawed Mackerel (*Rastrelliger kanagurta* L.) Fish Balls with Addition of Canna (*Canna edulis* Kerr.) Starch Concentration as a Filler

Novita Herdiana, A Sapta Z, Susilawati and Silaturahmi Widaputri

+ Open abstract View article PDF

OPEN ACCESS 012023

Comparison Effects Of Seaweed Concentrations On Total Bacteria And Yeast Kombucha *Gracilaria Verrucosa* During The Production Process

Wahyu Andrianto, Sudarno, Rahayu kusdarwati and dan Dwitha nirmala

+ Open abstract View article PDF

OPEN ACCESS 012031

Sublethal Toxicity Insecticide Organochlorine Endosulfan on The Value of Blood Sugar Levels and The Level of Life in Tilapia (*Oreochromis niloticus*) Seeds

S Nurhadi, E D Mashitah and dan L A Sari

+ Open abstract View article PDF

OPEN ACCESS 012032

Chitosan from snapper fish scale waste (*Lutjanus* spp.) for edible coating

A Florencia, S E D Putra and Y P Mukti

+ Open abstract View article PDF

OPEN ACCESS 012033

Application of modified starch on plastic bag bioplastic based on carrageenan from *Eucheuma cottonii* on mechanic and biodegradation properties

R A Sofianto, M A Alamsjah and D Y Pujiastuti

+ Open abstract View article PDF

OPEN ACCESS 012034

Characterization of Tilapia collagen-loaded chitosan nanofibers synthesized by electrospinning method for wound dressing

C A M Rani, A Safira, M Suryadiningrat, F Fikri, D K Wardhana and M T E Purnama

+ Open abstract View article PDF

OPEN ACCESS 012035

Determining of water bioremediation dosage in recirculating water system for Cantik Grouper (*Epinephelus fuscoguttatus* × *Epinephelus microdon*) nurseries

B Astari, S Ismi and K Mahardika

+ Open abstract View article PDF

OPEN ACCESS 012036

The Effect of Laserpuncture on Accelerate Gonadal Maturity of Female Striped Catfish (*Pangasianodon hypophthalmus*)

M Patmadevi, A T Mukti, A S Mubarak and S D Astuti

+ Open abstract View article PDF

OPEN ACCESS 012037

Nutritional Fact of Traditional Cracker (Amplang and Getas) from Bangka Belitung

Arief Rubiana Basarah, Patmawati, Muhamad Amin, Ahmad Fahrul Syarif, Mu'alimah Hudatwi and Sapto Andriyono

+ Open abstract View article PDF

OPEN ACCESS 012038

Characterizations of Milkfish Sauce on Amino Acid Content with Variations in Addition of Salt and Pineapple Extract Concentration

N. P. Listyaningrum, R. Lailatussifa and T. R. Andayani

+ Open abstract View article PDF

OPEN ACCESS 012039

Polysaccharide isolation of Brown seaweed: *Sargassum* sp and its photoprotection activity

M A P Panjaitan, R D Kasitowati, N Putri, A Yamindago, R Asmara, D Aliviyanti and D C Pratiwi

+ Open abstract View article PDF

OPEN ACCESS 012040

Proximate Analysis of Fisheries Dried Products from Mamuju Traditional Market Post-Earthquake Disaster

Eka Saputra, Wahyu Isroni, Arief Rubiana Basarah and Sapto Andriyono

+ Open abstract View article PDF

OPEN ACCESS 012041

The Intensive Program of Hatchery Aquaculture Performance of Gourami (Osphronemus gouramy) in IPB Pandaan

V B Pribawastuti and S H Samara

+ Open abstract View article PDF

OPEN ACCESS 012042

The effect of different maceration solutions towards characteristic gelatin from bone of common carp (*Cyprinus carpio*)

E Ristyanti, J Triastuti and E Saputra

+ Open abstract View article PDF

OPEN ACCESS 012043

Growth pattern and abundance of *Varuna Literatta* (Fabricius, 1798) in the new industrial area in the north coast of Gresik, East Java

F Fariedah, M S Widodo and R Yuwanita

+ Open abstract View article PDF

OPEN ACCESS 012044

The Effect of Rumen's Cattle Waste as Alternative Nutrient Sources for *Thalasiossira* sp Aquaculture

M B Santanumurti, M I Ogara, L Santoso and S Hudaidah

+ Open abstract View article PDF

OPEN ACCESS 012045

Inhibitory Rate of Chitosan Against *Vibrio harveyi* use in vitro method

Haryagita, R Kusdarwati and H Suprapto

+ Open abstract View article PDF

OPEN ACCESS 012046

Measurement of Indonesian Marine Health Index to Assess The Health of The Coastal Ecosystem of Tuban, East Java

M I Joesidawati and S Suwarsih

+ Open abstract View article PDF

OPEN ACCESS 012047

Physical characteristic and antioxidant activities of liquid bath soap with substitution of β-carotene crude extract from *Gracilaria* sp.

M M Ircham, A S Mubarak and E Saputra

+ Open abstract View article PDF

OPEN ACCESS 012048

Cultivation of Phytase-Producing Bacteria as Probiotic Candidate on Molasses and Tempe-Processing Waste

Zaid Al Gifari, Ihza Agistna, Khairil Anwar, Anwar Rosyidi, Muhamad Ali and Muhamad Amin

+ Open abstract View article PDF

OPEN ACCESS 012049

Effectiveness of striped catfish (*Pangasianodon hypophthalmus*) cultivation in aquaponic system with three different plant against ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3)

S Andriani, N N Dewi and B S Rahardja

+ Open abstract View article PDF

OPEN ACCESS 012050

The effect of different feed on Ctenopoma (*Ctenopoma acutirostre*) production through survival rate and growth

M B Santanumurti, L Alfarisi, L Santoso and S Hudaidah

+ Open abstract View article PDF

OPEN ACCESS 012051

Potential of sappan wood extract as a feed additive on the feed conversion on organic matter digestibility on the buck exposed to heat stress

B Agustono, I Rahmaniyah, F Fikri and R A Prastiya

+ Open abstract View article PDF

OPEN ACCESS 012052

Reproductive ability of endemic species of Lake Ajamaru *Melanotaenia ajamaruensis* Allen & Cross, 1980 on spawning at various sex ratio

D S Said, N Mayasari and dan T Kadarini

+ Open abstract View article PDF

OPEN ACCESS 012053

Diversity of holothuroidea by spicule type in the intertidal zone of Sepanjang Beach, Gunungkidul, Yogyakarta

N Yunita, A Amalia, F A Ponkiyawati, L N Janah, L U Khasanah, M B Gavintri, Z Nurahmah, F B R Khalallia and R Epilurahman

+ Open abstract View article PDF

OPEN ACCESS 012054

Length-weight relationships and condition factors of fifteen fish species from Pabean Bay, West Java, Indonesia

L S Syafei, D Sudinno and I N Y Parawangsa

+ Open abstract View article PDF

OPEN ACCESS 012055

Study on the potential of sea urchin *Tripneustes gratilla* (Linnaeus, 1758) as a bioindicator dangerous plastic pollution in environment of gunungkidul beach Yogyakarta

S D Pratita, M B Gavintri, A N Rizkyta, L U Khasanah, F A Ponkiyawati and B Retnoaji

+ Open abstract View article PDF

OPEN ACCESS 012056

Opportunities and its problem for the development of Tilapia aquaculture business in Banjar Regency, South Kalimantan

N Nurlaili and H Hikmah

+ Open abstract View article PDF

OPEN ACCESS 012057

The dominance and proportions of plankton in Pacific white shrimp (*Litopenaeus vannamei*) ponds cultivated with the intensive system in Bulukumba Regency, South Sulawesi, Indonesia

M D Sani, P A Wiradana, A Y Maharani, R E Mawli and A T Mukti

+ Open abstract View article PDF

OPEN ACCESS 012058

The effect of bromelain enzyme on pineapple core on the relationship between platelet count and hematocrit value in carp (*Cyprinus carpio*) infested with *Argulus japonicus*

A B Fahturohman, Kismiyati and Y Rahmawati

+ Open abstract View article PDF

OPEN ACCESS 012059

Diversity of intertidal fishes in Porok Beach, Gunungkidul, Yogyakarta

E M Azizah, N Puspaningrum, D R Palupi, A A Fadlillah, A R Ismail, E T Nugroho, B M Effendi, F R Palupi and Z N Putri

+ Open abstract View article PDF

OPEN ACCESS 012060

Analysis of causes of changes in conditions of vannamei shrimp hepatopancreas indicators at PT. Lombang Sumber Rejeki Sumenep

T A Setyastuti, D Sukamto and I E Fawwaz

+ Open abstract View article PDF

OPEN ACCESS 012061

The effect addional omega-3 in feed on the gonadal maturity level and fecundity of female silver rasbora, *Rasbora argyrotaenia*

H Nabila, A S Mubarak and D S Budi

+ Open abstract View article PDF

OPEN ACCESS 012062

Climate change impact on shrimp (*Litopenaeus vannamei*) farming in Banyuwangi, East Java

M Yuniartik, E W Setyaningrum, S H Yuniari, S R Faturakhmat and H Prasetio

+ Open abstract View article PDF

OPEN ACCESS 012063

The Growth and Bacteriocin Productions of *Enterococcus Faecium* Cultured in Aerobic and Anaerobic Conditions

Muhamad Ali, Melinda Sanggu Artha, Muhamad Aidil Fadjar Suryadi, Djoko Kisworo and Muhamad Amin

+ Open abstract View article PDF

OPEN ACCESS 012064

Evaluation on feeding with sente leaves and enrichment on the conditioning of giant gourami (*Osphronemus gouramy*) broodstock

S Nashrullah, L A Sari and S Arshad

+ Open abstract View article PDF

OPEN ACCESS 012065

Molecular and phylogenetic analysis of *Sardinella lemuru* Bleeker 1835 at fishing ground Canggu-Bali inferred D-loop mutations of mtDNA

V A Winata, R D Kasitowati, F Iranawati, W S Pranowo and A Sartimbul

+ Open abstract View article PDF

OPEN ACCESS 012066

Description of Silver Barb *Barbonymus gonionotus* (Bleeker, 1849) (Cypriniformes: Cyprinidae) From Madura Island, Indonesia

F S Valen, V Hasan, F P Ottoni, A L Nafisyah, M Erwinda and A N Annisa

+ Open abstract View article PDF

OPEN ACCESS 012067

The Ecological Habitat of Spiny Lobster (*Panulirus* spp.): Case Study on Lobster Fishing Ground in Trenggalek, East Java, Indonesia

Muhamad Amin, Anis Fitria, Nur Aini Muslichah and Laila Musdalifah

+ Open abstract View article PDF

OPEN ACCESS 012068

Mass tilapia (*Oreochromis mossambicus*) mortality in floating net cages at Batur Lake, Bangli Regency, Bali Province: a case report

P A Wiradana, I K W Yudha and A T Mukti

+ Open abstract View article PDF

OPEN ACCESS 012069

The effects of different feeding rates on the growth of silver rasbora (*Rasbora argyrotaenia*)

L Nisak, Agustono and D S Budi

+ Open abstract View article PDF

OPEN ACCESS 012070

Extraction of Stingray Liver Oil (Dasyatis Sp) with Alkaline Digestion Method

M I Joesidawati

+ Open abstract View article PDF

OPEN ACCESS 012071

The effect of fortification *Moringa oleifera* leaves powder on calcium content in otak-otak products of *Clarias* sp.

T D Sulistiyati, E Suprayitno, H Djamaludin, J E Tambunan and U Muchayaroh

+ Open abstract View article PDF

OPEN ACCESS 012072

Application of Modified Starch in the Carragenan-Based Biodegradable Packaging from *Eucheuma cottonii* on Biodegradablility and Mechanical Properties

H S Islamiyah, M A Alamsjah and D Y Pujiastuti

+ Open abstract View article PDF

OPEN ACCESS 012073

The use of kailan (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.) and pakcoy (*Brassica rapa* L.) in the cultivation of striped catfish (*Pangasianodon hypophthalmus*) aquaponic system on blood glucose levels and oxygen consumption levels

D D Hairani, N N Dewi and B S Rahardja

+ Open abstract View article PDF

OPEN ACCESS 012074

First country record of the bearded gudgeon *Pogoneleotris heterolepis* (Günther, 1869) (Teleostei: Eleotridae) from Indonesia

Fitri Sil Valen, Veryl Hasan, Felipe P. Ottoni, Ayu Lana Nafisyah, Mugi Erwinda, Ana Nur Annisa and M A Adis

+ Open abstract View article PDF

OPEN ACCESS 012075

The use of local raw materials and fermented feeds for the growth of Giant Prawns (*Macrobrachium rosenbergii*)

Mulyasari, L Setijaningsih, R Samsudin, I Taufik and I I Kusmini

+ Open abstract View article PDF

OPEN ACCESS 012076

Zeylanicobdella arugamensis intensity and histopathology of hybrid grouper skin from soil and concrete ponds

M Nisa, G Mahasri and S Subekti

+ Open abstract View article PDF

OPEN ACCESS 012077

Destruction level on coral reef in the Ambon bay

D D Pelasula, C Y Manullang, M P Patria, S Wouthuyzen, J D Lekallete and S A Malik

+ Open abstract View article PDF

OPEN ACCESS 012078

The effect of differences extraction solutions on the gelatin characteristic fishbone of bader bang (*Barbonymus balleroides*)

A Y Prasetya, J Triastuti and E Saputra

+ Open abstract View article PDF

OPEN ACCESS 012079

The Effect of Turmeric (*Curcuma domestica*) Addition ont The Consumer Acceptance Level of Milkfish Crackers

Nabila Aulia, Sapto Andriyono and Eka Saputra

+ Open abstract View article PDF

OPEN ACCESS 012080

Characterization of Dry Noddles with Additional of Pedada (*Sonneratia caseolaris*) Mangrove Flour as Alternative Food Resource

F Muhammad, S Andriyono and D Y Pujiastuti

+ Open abstract View article PDF

OPEN ACCESS 012081

Use of microbubble generator on the growth vannamei shrimp culture

P N Rizky, L B R Ritonga, K Primasari and Nasuki

+ Open abstract View article PDF

OPEN ACCESS 012082

The effect of bromelain enzyme on pineapple core on the relationship between platelet count and hematocrit value in carp (*Cyprinus carpio*) infested with *Argulus japonicus*

A B Fahturohman, Kismiyati and Y Rahmawati

+ Open abstract View article PDF

OPEN ACCESS 012083

Examination of White Spot Syndrome Virus (WSSV) in White Shrimp (*Litopenaeus vannamei*) and Tiger Prawn (*Penaeus monodon*) with *Polymerase Chain Reaction* (PCR) Method

A N Fadilah and A H Fasya

+ Open abstract View article PDF

OPEN ACCESS 012084

Correlation Between Water Quality to Blood Glucose of Cantang Grouper (*E. fuscoguttatus* x *E. lanceolatus*) as an Indicator of Stress in Floating Net Cage

C N P Wibowo, L Sulmartiwi and S Andriyono

+ Open abstract View article PDF

OPEN ACCESS 012085

Dietary combination of maggot and commercial feed enhance the growth rate and feed conversion ratio of snakehead fish (*Channa striata*)

A A Wallady, B S Rahardja and H Kenconojati

+ Open abstract View article PDF

OPEN ACCESS 012086

The effect of *Ocimum sanctum* L. crude extract on haematology of *Cyprinus carpio* infected by *Aeromonas hydrophila*

A M Halim, A B Cahyanurani and A A Aonullah

+ Open abstract View article PDF

OPEN ACCESS 012087

Characteristics of turtle laying habitat on vemara beach, Banyuwangi Regency, East Java

W Isroni, N Maulida, M Irfan Muhajir, P Surya Lesmana and D Aji Ismail

+ Open abstract View article PDF

OPEN ACCESS 012088

Providing a combination of chicken manure and broth in a different concentration to the production count of *Daphnia magna*

Rizky Firdha Yuniar, Yudi Cahyoko and Luthfiana Aprilianita Sari

+ Open abstract View article PDF

OPEN ACCESS 012089

Effect of probiotics addition on total organic matter and survival rate of catfish (*Clarias* sp.) maintenance using recirculating aquaculture system (RAS)

A Paranita, B S Rahardja and Prayogo

+ Open abstract View article PDF

OPEN ACCESS 012090

Differences In Livelihood Activities Householdes of Sasak Farmers and Sasak Fishermen In The Province of West Nusa Tenggara

Adriana Monica Sahidu, Arya Hadi Dharmawan, Arif Satria, Soeryo Adiwibowo and Ali Khomsan

+ Open abstract View article PDF

OPEN ACCESS 012091

Accumulation of heavy metals Pb and Hg in feather shells *(Anadara antiquata)* in Lekok Coastal Waters, Pasuruan Regency

W Isroni and N Maulida

+ Open abstract View article PDF

OPEN ACCESS 012092

The effect of salinity on the expression of heat shock protein 70 (hsp70) in the gills and kidneys of srikandi tilapia (*Oreochromis niloticus*)

M Winarti, L Sulmartiwi and L Lutfiyah

+ Open abstract View article PDF

OPEN ACCESS 012093

Management Analysis of Paddle Wheel For Efficiency Operational Cost Shrimp Culture Based on Oxygen Budget Capacity

Alauddin MHR

+ Open abstract View article PDF

OPEN ACCESS 012094

Analysis of organic waste loading (total suspended solid) from shrimp culture activity in mangara bombang coastal area – South Sulawesi Province

Alauddin MHR

+ Open abstract View article PDF

OPEN ACCESS 012095

Banana blossom addition to increase food fiber in tuna (*Thunnus* sp.) floss product as functional food for degenerative disease's patient

Hardoko, E Suprayitno, T D Sulistiyati, B B Sasmito, A Chamidah, M A P Panjaitan, J E Tambunan and H Djamaludin

+ Open abstract View article PDF

OPEN ACCESS 012096

The abundance of *Vibrio* sp. bacteria on *liptopenaeus vannamei* grow out - pond in CV. Lautan Sumber Rejeki Banyuwangi

A Asmarany, S Jayanti and N U Mahbubah

+ Open abstract View article PDF

OPEN ACCESS 012097

Bioacumulation of Heavy Metals Pb and Hg in Green Shells (*Perna viridis*) in Pasuruan Waters Based on Different Seasons

W Isroni and N Maulida

+ Open abstract View article PDF

OPEN ACCESS 012098

Distribution of microalgae and omega-3 as food for *Sardinella lemuru* in Bali Strait

B Suprakto, T Harijono, I G P G R Yudana, A Kiswanto and A Fauziah

+ Open abstract View article PDF

OPEN ACCESS 012099

Application of Sanitation and Hygiene In Improving The Quality of Products From Shells In The Collective Business Group (Kub) of Shell Fishers In Banjar Kemuning Village, Sedati District, Sidoarjo Regency

Dwitha Nirmala, Wahju Tjahjaningsih and Endang Dewi Mashitah

+ Open abstract View article PDF

OPEN ACCESS 012100

Effect of acid, base and mixed treatment towards characteristics gelatin of bader bang (*Barbonymus balleroides*) skin

N I Oktavia, J Triastuti and D Nirmala

+ Open abstract View article PDF

OPEN ACCESS 012101

Evaluation of hatching rate and survival rate of giant freshwater prawn (*Macrobrachium rosenbergii*) in Installation of Brackish Water Culture Mayangan, Probolinggo, East Java, Indonesia

T A A Wiguno and N N Dewi

+ Open abstract View article PDF

OPEN ACCESS 012102

The design of turbo jet surface aerator to enhance dissolved oxygen level in shrimp pond

S D Nugroho, C Z Pratiwi and S Holil

+ Open abstract View article PDF

OPEN ACCESS 012103

The abundance of alien fish species flowerhorn (*Cichlasoma trimaculatum* (GÜNTHER, 1867) in its fishing ground area at Lake Mahalona, South Sulawesi

S H Nasution, A M Muchlis and H T Cinnawara

+ Open abstract View article PDF

OPEN ACCESS 012104

Morphological Analysis of Sili (*Macrognathus* sp.) in Some Regencies in East Java Province

H Meirikayanti, Mufasirin and A S Mubarak

+ Open abstract View article PDF

OPEN ACCESS 012105

Providing feed from a mixed of chicken broth and chicken manure with different dosages on nutritional content *Daphnia magna*

Suryaning Trinita Setyawan, Yudi Cahyoko and Luthfiana Aprilianita Sari

+ Open abstract View article PDF

OPEN ACCESS 012106

Water replacement to reduce tetracycline residue level in the liver of tilapia (*Oreochromis* sp.)

W Pawestri, N Hakimah and M J Pangestika

+ Open abstract View article PDF

OPEN ACCESS 012107

The effect of different stocking densities on specific growth rate and survival rate of striped snakehead (*Channa striata*) culture in bucket system

H Latifah, Prayogo and B S Rahardja

+ Open abstract View article PDF

OPEN ACCESS 012108

The effects of exogenous dietary enzyme on the growth of giant gourami (*Osphronemus goramy*)

A N Imaniy, M Lamid and D S Budi

+ Open abstract View article PDF

OPEN ACCESS 012109

The effect of different stocking densities on ammonia (NH_3) and nitrate (NO_3) concentration on striped snakehead (*Channa striata*) culture in the bucket

A Meidiana, Prayogo and B S Rahardja

+ Open abstract View article PDF

OPEN ACCESS 012110

Evaluation of three species coral (*Acropora branching*) transplantation, case study; pantai tirtawangi Banyuwangi East Java

D P Anggara, B S Rahardja and Suciyono

+ Open abstract View article PDF

OPEN ACCESS 012111

Estimation of planting season for food crops based on water balance model at center of food crops, Parimo Regency, Central Sulawesi Province

Fathurrahman, Abd. Syakur, Rio Marthadi, Inrianto Kadeko and Ramal Yusuf

+ Open abstract View article PDF

OPEN ACCESS 012112

Molecular identification and potency of scalloped spiny lobster (*Panulirus homarus*) study from Kodang Merak Beach, South Malang

R Ramdhani, L Sulmartiwi and S Andriyono

+ Open abstract View article PDF

OPEN ACCESS 012113

Seasonal variation of water quality of three urban small lakes in West Java, Indonesia

Sulastri and Ira Akhdiana

+ Open abstract View article PDF

OPEN ACCESS 012114

Microplastic pollution in lower Cimandiri River, Indonesia: early detection on the occurrence, abundance and distribution

C Henny, Triyanto, T Suryono, D Rohaningsih, G P Yoga and A Waluyo

+ Open abstract View article PDF

OPEN ACCESS 012115

The effect of *Caesalpinia sappan* extract on body weight and carcass weight of bucks (*Cuniculus forma domestica*) exposed to heat stress

+ Open abstract View article PDF

JOURNAL LINKS

Journal home

Journal scope

Information for organizers

Information for authors

Contact us

Reprint services from Curran Associates

The addition of *Spirulina platensis* extract in feed on gill histopathology and survival rate of *Osphronemus gouramy* after infected with *Aeromonas hydrophila*

W H Satyantini[1*], Agustono[1], Arimbi[2], W Rahmawati[3] and E D Masithah[4]
[1]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, C Campus, Surabaya 60115 Indonesia
[2]Faculty of Veterinary Medicine, Universitas Airlangga, C Campus, Surabaya 60115 Indonesia
[3]Study Program of aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, C Campus, Surabaya 60115 Indonesia
[4]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, C Campus, Surabaya 60115 Indonesia
*E-mail: woro_hs@fpk.unair.ac.id

Abstract. Prevention of disease in *Osphronemus gouramy* due to infection with *A. hydrophila* can be done by using immunostimulants, one of which is using *Spirulina platensis* extract. The purpose of this study was to determine the effect of *S. platensis* extract on gill histopathology and survival rate of *O. gourami* after being infected with *A. hydrophila*. The fish were divided into three groups: fish without addition of *S. platensis* extract and infected with PBS (K-); without giving the *S. platensis* extract and infected with *A. hydrophila* (K+); and given extracts of *S. platensis* at 75, 150 and 300 mg/kgof feed and infected with *A. hydrophila*. The results showed the lowest value of gill damage was obtained in fish with the addition of 75 mg/kg of feed of *S. platensis* extract (1.22) and the highest damage was in K+ (2.67). The highest survival rate was found in the addition of 75 mg/kg of feed of *S. platensis* extract (91.67%) and the lowest was in K+ (70.83%). It can be concluded that the addition of *S. platensis* extract75 mg/kg of feed proved to be the most effective in reducing gill damage and increasing the survival rate of fish.

1. Introduction

Giant Gourami (*Osphronemus gouramy*) is a consumption fish that is much favoured by the people of Indonesia [1]. A serious problem in the fish farming of giant gourami is the emergence of diseases caused by *Aeromonas hydrophila*, namely Motile *Aeromonas Septicemia* (MAS). *A. hydrophila* can infect through injured body surfaces or gills [2]. The gills are the main organs that work in the diffusion mechanism between blood and water so that the gills have a great chance of being exposed to pollutants in the waters [3]. Damage to the gills can cause gill function to be not optimal and can interfere with respiration, causing death.

Efforts to control the disease, fish farmer still use antibiotics or other chemicals, but the use of these antibiotics is not environmentally friendly because it causes *A. hydrophila* to be resistant to some chemicals. Disease prevention can be done by using immunostimulants [4]. One of the algae that can be used as an immunostimulant is *Spirulina sp.*[5].

Suggested by [6] that hot water extraction *Spirulina platensis* can release chemical compounds in the form of polysaccharides that can stimulate the immune system in fish and shrimp. Polysaccharides are known to be able to make contact with intestinal epithelial cells or cellular components in the intestine that are associated with lymphoid tissue resulting in immune cell activity [7]. Said by [8] that

lipopolysaccharide (LPS) can stimulate the activity of cellular defence responses, in this case activating phagocytosis, melanisation, encapsulation, nodulation and coagulation activities.

Control of disease expansion must be carried out as early as possible so that disease outbreaks do not occur, causing economic losses [8]. Based on this, it is necessary to conduct research to determine the effect of hot water extract of *Spirulina platensis* on gill histopathology and survival rate of giant gourami after infected with *A. hydrophila*).

2. Methods

2.1. Procedure

The test animals used were giant gourami with a size range of 9-10 cm and a weight of 10-12 grams. Prior to the research, giant gourami was given acclimatization treatment for approximately one week, after which they were put into an aquarium equipped with an aerator. Making hot water extraction of *Spirulina platensis* based on the method of [9] with a ratio of 1:10, namely 20 g of *Spirulina platensis* flour was added to 200 ml of water, then boiled and stirred for 1 hour at 90°C. Then it was centrifuged at 3000 rpm for 30 minutes to separate the precipitate and supernatant. The supernatant was dried using the freeze-drying process and obtained a dry weight of 3.382 g. The resulting extract was mixed with commercial feed according to the treatment dose and was given binder in the form of 2 grams of egg white and 15 ml of PBS (1% of the total feed). The feed mixture was stirred homogeneously and dried in an oven at 50-60°C for 6 hours.

Before being challenged, *A. hydrophyla* was passed (malignant) first. Passage is done by infecting healthy giant gourami with *A. hydrophyla*. *A. hydrophyla* was then isolated on the part of the organ or skin that was injured due to infection with *A. hydrophyla*. The bacteria were ready to be cultured and then inoculated on Tryptic Soy Agar (TSA) media for 24 hours at a temperature of 30°C, after which they were identified through observation of colony shape and biochemical tests. The giant gourami that had been given *Spirulina platensis* extract for 14 days was then challenged with *A. hydrophyla*.

The research treatment given was A (positive control) fish injected with bacteria but not fed extract, B (negative control) where the fish were not injected with bacteria and not fed extract. Treatments were C (75 mg/kg feed), D (150 mg/kg feed), E (300 mg/kg feed) and were infected with 0.1 ml of *A. hydrophyla* with a total of 10^8 CFU/ml [10]. The main parameters observed in this study were gill histopathology and survival rates of giant gourami. Supporting parameters observed were water quality measurements consisting of temperature, pH, dissolved oxygen (DO) and ammonia.

2.2. Data Analysis

The data obtained from the scoring results of the gill histopathology were analysed by the Kruskall-Wallis test and if there was a significant difference between the treatment groups ($p < 0.05$), then continued with the Mann-Whitney test [11]. While the survival rate data were analysed using the Analysis of Variance (ANOVA) statistical test with a 95% confidence interval and continued with Duncan's multiple distance test to determine the difference between treatments [12].

3. Results and Discussion

3.1. Clinical Sign

Giant Gourami (*Osphronemus gouramy*) which has been infected with *A. hydrophila* was observed for clinical signs that appear through observation of wounds and behaviour of giant gourami. The results showed that in treatments A, C, D and E there were wounds and dilation at the injection site, scales fell off and bleeding around the injection site. On the 3rd day post-infection, fish treatment A showed that the injection site was getting wider and the fish flesh was exposed. Observation of behaviour in treatments A, C, D and E, fish experienced decreased swimming movements, tilted swimming and fish tended to cluster at the aeration source and swim on the surface. In treatment B (control -) the fish looked normal and there was no dilation at the injection site, the fish movement was active and there was no change.

A. hydrophila can be isolated from the internal organs of fish and from skin wounds caused by *A. hydrophila* attacks. The isolated bacteria were then cultured on TSA media for 24 hours at 30°C. Colonies of *A. hydrophila* that had been inoculated and grown on TSA media were then identified to

determine whether the species that attacked carp was caused by *A. hydrophila* or not. Bacterial identification was carried out at Balai Karantina Ikan Kelas II Perak Surabaya. The results of the biochemical test showed a positive result that the bacteria that attacked the giant gourami was a type of *A. hydrophila*.

3.2. Gill Histopathology

Gills are the main respiratory organs in fish that work in the diffusion mechanism of respiratory gases between blood and water. The gills are formed from hardened cartilage arches with several filaments consisting of primary lamellae that have many branches and are called secondary lamellae [13]. The results of microscopic examination showed gill damage in the form of secondary lamellae hyperplasia, lamellae congestion and fusion with varying degrees of damage, ranging from slight to severe. Gill damage of giant gourami (*Osphronemous gouramy*) in each treatment can be seen in Figure 1.

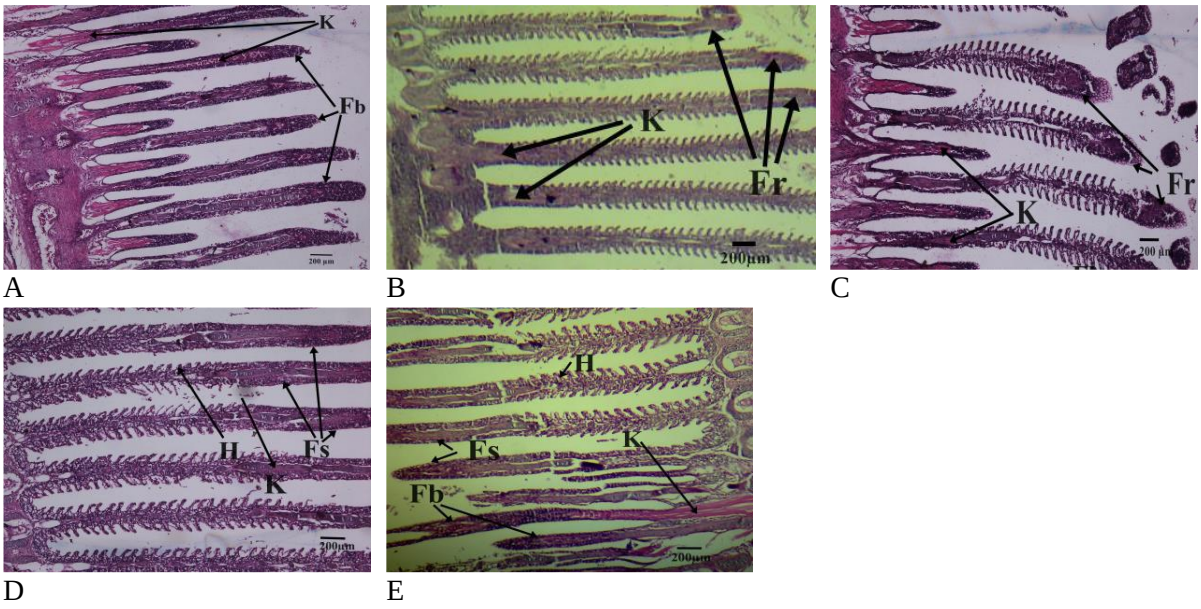

Figure 1. Histopathological Result of Giant Gourami Gill in Treatments A, B, C, D and E. Staining HE, Magnificationon200 times.
Description: A: control +, B: control -, C: dose 75 mg/kg feed, and E: dose 300 mg/kg feed.
⟶ Indicates damage to the gills, F indicates fusion, r: slight, s: moderate and b: severe, K indicates congestion and H indicates hyperplasia.

The results of the examination based on the score and average gill damage are presented in Table 1.

Table 1. Average Histopathological Data on Giant Gurame Gill Damage

Treatments	Average of Gill Scoring ±SD
A (control +)	2.667^{a} ± 0.471
B (control -)	1.000^{c} ± 0.000
C (75 mg/kg feed)	1.222^{b} ± 0.157
D (150 mg/kg feed)	2.222^{ab} ± 0.314
E (300 mg/kg feed)	2.333^{ab} ± 0.942

Note: Different superscript letter notations in the same column indicate that there is a significant difference between treatments. (F count >F table 0.05).

Determination of the level of gill damage at various degrees of infection was carried out using the scoring method. Based on the Kruskal Wallis test, there were significant differences between treatments in histopathological gill damage ($p<0.05$). Treatment B obtained the lowest average damage value of 1,000, this was because in treatment B it was not infected with bacteria so that the fish's functional system and immune system were normal. Gill damage in this treatment is thought to be due to the influence of cultivation media or less sterile equipment, so that contaminants, disease or bacteria can easily attack the gills.

The lowest average level of damage in the treatment was obtained in treatment C (75 mg/kg feed) which was 1.222. Treatment D (150 mg/kg feed) had an average score of 2.222 while treatment E (300 mg/kg feed) was 2.333. Treatment C got the lowest score (slight fusion level), it is suspected that this treatment is the right concentration of *Spirulina platensis* extract added in giant gourami feed. *Spirulina platensis* contains biologically active compounds including polysaccharides and lipopolysaccharides (LPS) [14]. LPS will be recognized by a receptor, TLR-4 which is found on the surface of leukocytes. TLR-4 functions as a dimer, and relies on the small protein MD-2 to recognize LPS [15]. The released LPS binds to lipopolysaccharide binding protein (LBP) with the help of the TLR-4 receptor and forms an LBP-LPS complex [16]. The LBP-LPS complex with the help of the TLR-4 signal will stimulate macrophages, neutrophils and monocytes to produce and release proinflammatory cytokines [17]. These cytokines function to activate phagocytic activity against bacterial cells so that bacteria cannot reproduce.

Treatment D and E had a higher level of damage, namely moderate fusion and some severe fusion, this is presumably because the concentration of *Spirulina platensis* extract given was too much so that it was toxic. This is related to the role of LPS in activating macrophages to produce cytokines. Cytokines in large quantities or uncontrolled products can cause oxidative stress, harm the body and can cause tissue damage in the host [18]. This is thought to cause higher fusion in treatments D and E.

Treatment A (control +) got the highest average score of 2.667, which means the level of secondary lamellae damage is getting higher. This is presumably due to the decreased immunity of fish due to the attack of *A. hydrophila*, so that bacteria can easily attack the target organ, namely the gills. Gill damage due to infection with *A. hydrophila* will cause disturbances in the respiration process so that the transport of respiratory gases (O_2 and CO_2) also does not run normally, which ultimately causes fish death [19].

3.3. Survival Rate

Data on the survival rates of giant gourami (*Osphronemus gouramy*) after infection with *A. hydrophila* are presented in Table 2.

Table 2. Survival Rates of Giant Gourami (*Osphronemus gouramy*) after Infection with *A. hydrophila*

Treatments	Survival Rates (%) ±SD
A (control +)	70.833^{a} ± 0.098
B (control -)	91.667^{b} ± 0.291
C (75 mg/kg feed)	91.667^{b} ± 0.291
D (150 mg/kg feed)	83.333^{ab} ± 0.125
E (300 mg/kg feed)	87.500^{ab} ± 0.000

Note: Different superscript letter notations in the same column indicate that there is a significant difference between treatments. (F count >F table 0.05).

Based on the ANOVA test on the effect of treatment on survival showed a significant value ($p <0.05$). The highest survival rate of giant gourami was obtained in treatment B (negative control) which was 91.667% and C, which was 91.667%. Treatment B (negative control) got the highest survival value because the fish were not infected with *A. hydrophila* so that the fish's body functions continued to run normally. According to [20], in relatively low concentrations LPS can function as an immunomodulator, which can increase the nonspecific immune system to attack bacteria. In this study, the lowest extract dose, namely treatment C (75 mg *Spirulina platensis* extract/kg feed) obtained the highest survival rate compared to other treatments. It is suspected that the treatment is the right concentration which causes the fish's functional system is not disturbed.

Treatment D (150 mg *Spirulina platensis* extract/kg feed) and E (300 mg *Spirulina platensis* extract/kg feed) had lower survival values. This is presumably due to the addition of too much *Spirulina platensis* extract so that the lipopolysaccharide content is toxic to fish. Excessive addition of lipopolysaccharide can induce the production and release of inflammatory cells such as Reactive Oxygen Species (ROS) which can cause chain reactions, and also trigger several types of infection (inflammatory) in macrophage cells and other cells [17];[21].

The lowest survival was found in treatment A (positive control) which was 70.833% where in this treatment the giant gourami were infected with bacteria but were not given feed containing *Spirulina platensis* extract. The low survival rate was influenced by the high level of damage to the gill lamellae in treatment A due to the attack of *A. hydrophila*. This gill damage causes disturbances in the respiration process so that O_2 transport also does not run normally, which ultimately causes fish death.

3.4. Water Quality

Water quality according to [22] is the nature of water and the content of living things, energy substances, or other components in the water. The range of measurement results of water quality parameters during the maintenance period of giant gourami (*Osphronemus gouramy*) is presented in Table 3.

Table 3. Water Quality Data during Research

Water quality parameters		Treatments				
		A (K+)	B (K-)	C	D	E
Temperature (ºC)	Morning	27-29	27-29	27-29	27-29	27-29
	Afternoon	28-30	28-30	28-30	28-30	28-30
DO (mg/l)	Morning	5.4-6.2	5.75-6.15	5.6-6.27	4.96-6.17	5.18-6.05
	Afternoon	5.75-6.25	5.45-6.05	5.5-6.16	5.2-6.12	5.7-6.08
pH	Morning	7	7	7	7	7
	Afternoon	7	7	7	7	7
Ammonia (mg/l)	Morning	0.5-1	0.5	0.5-1	0.5-1	0.5-1
	Afternoon	0.5-1	0.5	0.5	0.5-1	0.5

The results of water quality measurements obtained during the study generally indicate that the water quality is still in the optimal range to support the maintenance of giant gourami. Based on observations of water temperature in the morning, the range is between 27-29°C and in the afternoon between 28-30°C. This condition is in accordance with the statement by Handajani [23] that the optimal temperature for giant gourami is 25°C–30°C. Temperatures below 21°C will reduce the growth rate of fish so that it will reduce the ability of hormones to change sex [24].

DO (dissolved oxygen) is the level of oxygen dissolved in water. Dissolved oxygen (DO) content ranged from 4.96-6.27 mg/L. According to [25] the optimal value of dissolved oxygen in the waters is 4.4-7.6 mg/L. The degree of acidity (pH) during the study was still in the normal range of 7, this is in accordance with the statement by [23] that the optimal pH for giant gourami is 7-8. Giant gourami will experience growth disturbances if the pH is <4 or >11, which is an unfavourable condition for fish/lethal. Ammonia content during the study ranged from 0.5-1 mg/L. This water condition is quite normal for fish growth because according to [25] the ideal ammonia level for life is less than 1 ppm. Toxicity will increase as the pH decreases and the dissolved oxygen decreases.

4. Conclusion

The conclusion of this study is that the addition of hot water extract of *S. platensis* has an effect on the histopathology of giant gourami gills, namely reducing the average damage to gill lamellae and can increase the survival of giant gourami after infected with *A. hydrophila*. The best dose of hot water extract *Spirulina platensis* which affects the histopathology of gills and the survival of giant gourami after infected with *A. hydrophila* is a dose of 75 mg/kg of feed (Treatment C). Based on this research, it is recommended that the application of adding *Spirulina platensis* hot water extract to giant gourami feed should use an effective dose of 75 mg extract/kg feed. Furthermore, it is hoped that there will be

further research on the benefits of *Spirulina platensis* extract in treating diseases and normalizing damage to important fish organs so that fish survival will also increase.

5. References

[1] Puspowardoyo, H and Abbas S D 1992 *Membudidayakan Gurami Secara Intensif* (Yogyakarta: Kanisius) p 78.
[2] Kabata, Z 1985 *Parasites and Disease of Fish Cultured in The Tropic* (London and Philadelphian: Taylor and Francis) p 318.
[3] Saputra, H M, Netti, M and Putra, S 2013 *Jurn Bio.* 2 138-144.
[4] Johny, F Roza, D K Mahardika, Zafrandan A P 2005 *Jurn Pen Per Ind*. 11 75-83.
[5] Simanjuntak, S B I 2002 *Histologi Organ Limphoid Ikan Patin Jambal (Pangasius djambal Bleeker) yang Diberi Immunostimulan Spirulina*. Thesis (Bogor: Institut Pertanian Bogor) 62 hal.
[6] Tayag, C M Lin Y C, Li C C, Liou C H, and J C Chen 2010. *Jurn Fish and Shellfish Imm*. 28 1-8.
[7] Gershwin, M E and A Belay 2008 *Spirulina in Human Nutrition and Health* (London: Taylor and Francis) p 2-9.
[8] Alifuddin, M 2002 *Jurn Akua Ind*. 87-92.
[9] Hayashi, O, Ono, S, Ishi, K., Shi Y H, Hirahashi, T, and T Katoh 2006 *Jour of Aplied Tech*. 1-18.
[10] Harikrishnan R, Nisha Rani M, Balasundaram C 2003 *Aqua*. 221 41–50.
[11] Kusriningrum, R S 2012 *Perancangan percobaan* (Surabaya: Universitas Airlangga) p 92.
[12] Fujaya, Y 2004 *Fisiologi Ikan. Dasar Pengembangan Teknik Perikanan* (Bandung: Penerbit Rineka Cipta) p179.
[13] Bhowmik, D, Dubey J, and M Sandeep 2009 *World Jour of Dai & Fo* Sci. 2 1-3.
[14] Triantafilou, M, Triantafilou, K 2002 *Trends Immunol*. 23 301–304.
[15] Yustina, A R, Suardita, K dan Agustin, D 2012 *Jour of Bio Sci*. 14 140-144.
[16] Beumer, C, Marty W, Willem R, Danielle R, Ruud B, and Willem S 2003 *The Jour of Pharm and Exp Ther.* 307 737-744.
[17] Munasir, Z 2001 *Sar Ped*. 2 193 – 197.
[18] Wilson, J M and P Laurent 2002 *Jour of Exp Zoo*. 293 192-213.
[19] Wang, X, and P J Quinn 2010 Subs Bio. 1-25.
[20] Darwadi, R P, Aulanni'am dan Chanif, M 2013 *Kim Stu Jour*. 1 133-139.
[21] Effendi, H 2003 *Telaah Kualitas Air: Bagi Pengelolaan Sumber Daya dan Lingkungan Perairan* (Yogyakarta: Kanisius) p 258.
[22] Handajani, H, 2006 *Jur Ilm Huk*.13 1-7.
[23] Cholik, F., Artati and Arifuddin, 1979 *Pengelolaan Kualitas Air* (Jakarta: Pusat Penelitian dan Pengembangan Perikanan. Direktorat Jenderal Perikanan) p 50.
[24] Boyd, C E 1982 *Water Quality Management for Pond Fish Culture* (Auburn, USA: International Centre for Aquaculture Experiment Station, Auburn University) p 163.

Acknowledgments
Authors would like to thank the granting of research funds from DRPM of the Ministry of Research Technology and Higher Education.

UNIVERSITAS AIRLANGGA
FAKULTAS PERIKANAN DAN KELAUTAN
Kampus C Mulyorejo Surabaya 60115 Telp. (031) 5911451 Fax (031) 5965741
Laman : https://fpk.unair.ac.id, e-mail : info@fpk.unair.ac.id

SURAT KETERANGAN

Nomor: 1439/UN3.FPK/KP/2023

Yang bertanda tangan di bawah ini:

Nama	: **Dr. Eng. Sapto Andriyono, S.Pi., MT.**
NIP	: 197909252008121002
Pangkat/Golongan	: Penata/III - C
Jabatan	: Wakil Dekan III

Dengan ini menerangkan bahwa:

Nama	: **Dr. Endang Dewi Masithah, Ir., MP.**
NIP	: 196909121997022001
Pangkat/Golongan	: Pembina/IV - A
Jabatan	: Lektor Kepala

Telah melakukan penelitian yang dipublikasi pada tahun 2022 dengan judul sebagai berikut:
The addition of Spirulina platensis extract in feed on gill histopathology and survival rate of Osphronemus gouramy after infected with Aeromonas hydrophila

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: Penggunaan mortalitas sebagai titik akhir dapat diterapkan jika sedikit atau tidak ada informasi yang berkaitan dengan tujuan penelitian yang tersedia pada spesies yang diminati atau variabel eksperimental yang diterapkan, (hal 6 ; 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: ikan yang telah diketahui cara pembiakannya secara berkelanjutan dan juga jenis ikan komersil dinyatakan tidak memerlukan protokol (hal 16 ; terlampir).

Penelitian tersebut menggunakan hewan percobaan larva ikan gurami. 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

Mengetahui,
Dekan FPK Unair

Prof. Moch. Amin Alamsjah, Ir., M.Si., Ph.D.
NIP. 197001161995031002

Wakil Dekan III FPK Unair

Dr. Eng. Sapto Andriyono, S.Pi., MT.
NIP. 197909252008121002

Guidelines for the Use of Fishes in Research

Use of Fishes in Research Committee members:
J. A. Jenkins, Chair, H. L. Bart, Jr., J. D. Bowker, P. R. Bowser, J. R. MacMillan, J. G. Nickum, J. D. Rose, P. W. Sorensen, and G. W. Whitledge on behalf of the American Fisheries Society; J. W. Rachlin and B. E. Warkentine on behalf of the American Institute of Fishery Research Biologists; and H. L. Bart on behalf of the American Society of Ichthyologists and Herpetologists

American Fisheries Society
Bethesda, Maryland
2014

A suggested citation format for this book follows.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland.

Cover art: Close-up photograph of Brown Trout, *Salmo trutta*, from the South Fork of the Cache la Poudre River, Colorado, taken by James Rose in 2010.

Printed in the United States of America on acid-free paper.

Library of Congress Control Number 2014943876
ISBN 978-1-934874-39-4

American Fisheries Society Web site address: *www.fisheries.org*

American Fisheries Society
5410 Grosvenor Lane, Suite 100
Bethesda, Maryland 20814
USA

Table of Contents

Studies of early life stages may require very large numbers of individuals. In all cases, studies should be designed to use the fewest animals necessary to reliably answer the questions posed. The use of adequate numbers to establish variance and to ensure reliability is essential so as to prevent needless repetition of the study (ASIH et al. 1987, 1988). A true "replicate" is the smallest experimental unit to which a treatment can be applied independently. Pseudoreplication can result from wrongly treating multiple samples from one experimental unit as multiple experimental units or from using experimental units that are not statistically independent (Heffner et al. 1996). Statistical power analysis can improve designs of experiments (Peterman 1990). Conducting statistical power analyses ensures the development of study designs that have the appropriate statistical power to accomplish research objectives.

2.4 Mortality as an Experimental Endpoint

In laboratory studies, experimental endpoints, other than death of the experimental subjects, should be developed unless mortality is required by the study protocol. The use of mortality as an endpoint is appropriate when one or both of the following criteria are met: (1) Little or no information pertaining to research objectives is available on the species of interest or the experimental variable being imposed (e.g., short-term, limited mortality studies may be used to develop experimental limits for subsequent sublethal studies), and (2) mortality data are required, or at least preferred, by a sponsoring agency to provide a basis for criteria development as part of a regulatory process. Studies that require mortality endpoints include, but are not limited to, those concerning the effects of pathogens and parasites, toxicological research, and physiological tolerance.

2.5 Fish Health Management: Control of Pathogens and Parasites

In laboratory studies involving fishes, healthy subjects are prerequisites for reliable data (Jenkins 2011a), unless an infectious disease is part of the experimental protocol. Fish used in research must be free of any notable microbial presence that could indicate a diseased condition. Fish free from infectious fish pathogens generally will be satisfactory; however, an unrecognized disease condition, even at chronic or nonlethal levels, can seriously confound research results (Lawrence et al. 2012). The source of fish used in research will, in general, influence their health status. Fish raised in captivity have a level of health oversight that will not occur in wild-caught fish. When inquiring about the health status of fish at a culture facility, the researcher can request specific information including any available fish health inspection reports. When fish are brought into a laboratory setting from the wild, the researcher should expect that microorganisms are present. If no disease symptoms are apparent, this is no guarantee that these wild-caught fish are free from problematic disease organisms. Once those fish are in a laboratory setting, the culture conditions and associated stressors will be very different from those in the natural environment, whereby an active disease event can develop. Many laboratories will administer formalin baths to newly arrived fish during an acclimation period (see section 7.3 Acclimation to Laboratory Conditions). The goal is to eliminate external protozoa and monogeneans from the

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.

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.

Mr John Batt, Dalhousie University
Dr Kristina Bennett-Steward, Bioniche
Mr Cyr Couturier, Memorial University
Dr Larry Hammell, University of Prince Edward Island
Dr Chris Harvey-Clark, University of British Columbia (Chair)
Mr Henrik Kreiberg, Fisheries and Oceans Canada
Dr George Iwama, Acadia University
Dr Santosh Lall, National Research Council
Dr Matt Litvak, University of New Brunswick at St John
Dr Don Rainnie, University of Prince Edward Island
Dr Don Stevens, University of Guelph
Dr Jim Wright, University of Calgary
Dr Gilly Griffin, Canadian Council on Animal Care

In addition, the CCAC is grateful to former members of CCAC Council: Ms Susan Waddy, Fisheries and Oceans Canada; Dr Jack Miller, University of Western Ontario; and Dr Choong Foong, Dalhousie University; and to Dr David Noakes, University of Guelph who provided considerable assistance in preliminary phases of this project. CCAC thanks the many individuals, organizations and associations that provided comments on earlier drafts of this guidelines document. In particular, thanks are extended to representatives of Fisheries and Oceans Canada, Environment Canada, the Canadian Aquaculture Institute, the Canadian Food Inspection Agency and the Canadian Society of Zoologists.

ISBN: 0–919087–43–4

Canadian Council on Animal Care
1510–130 Albert Street
Ottawa ON CANADA
K1P 5G4

http://www.ccac.ca

TABLE OF CONTENTS

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 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

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 information 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):

- 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) 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