P-ISSN : 2528 - 0678
E-ISSN : 2301 - 6159

JOURNAL OF MARINE AND COASTAL SCIENCE

Volume 9 No. 1/February 2020

FACULTY OF FISHERIES AND MARINE
UNIVERSITAS AIRLANGGA

Editorial Team

Articles

Effect of Red Ginger (Zingiber officinale Rosc.) Juice on Changes in the Anatomical Pathology of Gills and Amount of Blood Erythrocytes of Koi Fish (Cyprinus carpio koi) Infected by Myxobolus koi

DOI : 10.20473/jmcs.v9i1.20754
Nurul Kumalasari , Gunanti Mahasri , Sri Subekti
1-11
Abstract : 649
PDF : 1345

PDF

Correlation of Water Quality Against Prevalence of Ectoparasites in Tilapia (Oreochromis niloticus) in the Floating Net Cages Urban Farming Program in Surabaya, East Java

DOI : 10.20473/jmcs.v9i1.20756
Cintia Larasati , Gunanti Mahasri , Kusnoto kusnoto
12-20
Abstract : 1039
PDF : 3061

PDF

The Effect of Different Commercial Probiotics on the Diversity and Density of Sea Water Plankton in the Experiment Tub

DOI : 10.20473/jmcs.v9i1.20757
Mizar Desrialdi , Boedi Setya Rahardja , Endang Dewi Masithah
21-29
Abstract : 570
PDF : 1432

PDF

Addition of Crude Fish Oil (CFO) on Feed Toward Growth and Feed Conversion Ratio of Mud Crab (Scylla serrata)

DOI : 10.20473/jmcs.v9i1.20758
Kemala Hudita , Agustono Agustono , Widya Paramita Lokapirnasari
30-40
Abstract : 467
PDF : 1111

PDF

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

DOI : 10.20473/jmcs.v9i1.20759

Silvi Hardiyana , Boedi Rahardja Setya Rahardja , EndangMasithah Dewi Masithah

41-47

Studi Pemberian *Lactobacillus* spp. dan Barley Straw Terhadap Dinamika DO, pH dan Kelimpahan Plankton

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

Silvi Hardiyana1, Boedi Setya Rahardja[2] * dan Endang Dewi Masithah[3]

[1]Mahasiswa Program Studi Budidaya Perairan, Fakultas Perikanan dan Kelautan, Universitas Airlangga, , Surabaya, Indonesia
[2]Departemen Manajemen Kesehatan Ikan dan Budidaya Perairan, Universitas Airlangga, , Surabaya, Indonesia
[3]Departemen Kelautan, Fakultas Perikanan dan Kelautan, Universitas Airlangga, Surabaya, Indonesia

Koresponding: Boedi Setya Rahardja, Departemen Manajemen Kesehatan Ikan dan Budidaya Perairan, Universitas Airlangga, Surabaya, Indonesia

E-mail: bs_rahardja@fpk.unair.ac.id

Abstrak

Kegiatan usaha budidaya ikan dalam beberapa tahun terakhir ini banyak memperlihatkan kondisi kualitas air tidak mendukung kehidupan ikan yang dipelihara secara optimal dan semakin menurun daya dukungnya, salah satunya parameter DO dan pH. Sebagai tindakan penanganan yang dilakukan salah satunya dengan pemberian probiotik Salah satu bakteri yang biasa digunakan dalam pengaplikasian probiotik yaitu *Lactobacillus* spp. Bakteri gram positip *Lactobacillus* spp. banyak digunakan sebagai probiotik untuk memperbaiki kualitas airPenggunaan bakteri *Lactobacillus* spp. diharapkan dapat meningkatkan kualitas air yaitu mempercepat perombakan bahan organik dalam perairan dan menekan mikroorganisme merugikan sehingga dapat mempengaruhi dinamika nilai DO, pH dan meningkatkan nutrient dalam perairan. Barley straw adalah ekstrak bubuk jerami penghasil H_2O_2 yang berfungsi menghambat pertumbuhan mikroalga. Penggunaan ekstrak bubuk jerami telah menjadi lebih umum sebagai metode alternatif untuk mengendalikan pertumbuhan alga yang berlebihan dengan cara menghambat, bukan membasminya sehingga stabilisasi dari kelimpahan plankton dalam perairan dapat terkendali dengan baik.Untuk mempelajari peran bakteri *Lactobacillus* spp. dan *barley straw* dalam pengelolaan bahan organik dan kelimpahan plankton maka dilakukan penelitian Studi Pemberian *Lactobacillus spp.* dan *barley straw* terhadap dinamika DO,pH dan Kelimpahan Plankton.

Kata kunci :Kualitas air, Bakteri ,Algistatik, Nutrient, dan Probiotik

Abstract

Fish farming activities in recent years, many shows water quality conditions do not support fish life are maintained optimally and decreases the carrying capacity, one DO and pH parameters. As a remedial action is carried out either by probiotic bacteria One commonly used in the application of probiotics is *Lactobacillus* spp. Gram positive bacterium *Lactobacillus* spp. widely used as probiotics to improve the quality of air Penggunaan *Lactobacillus* spp. is expected to improve the quality of water that is accelerating the overhaul of organic material in the water and suppress harmful microorganisms that can affect the dynamics of DO, pH and increasing nutrient waters. Barley straw is a powdered extract of hay-producing H_2O_2 which serves to inhibit the growth of microalgae. The use of straw extract powder has become more common as an alternative method for controlling excessive algae growth by blocking and not get rid of it so that stabilization of the abundance of plankton in the water can be controlled well.To study the role of Lactobacillus spp. and barley straw in the management of organic matter and plankton abundance Giving a research study *Lactobacillus* spp. and barley straw to the dynamics of DO, pH and Abundance of Plankton.

Keyword : Water quality, Bacteria, Algistatic, Nutrient, and Probiotics

1. Pendahuluan

Kegiatan usaha budidaya ikan dalam beberapa tahun terakhir ini banyak memperlihatkan kondisi kualitas air tidak mendukung kehidupan ikan yang dipelihara secara optimal dan semakin menurun daya dukungnya dan mempengaruhi parameter kualitas perairan salah satunya yaitu DO dan pH. Salah satu tindakan penanganan yang dilakukan adalah dengan pemberian probiotik dan bahan alam lainnya. Penggunaan probiotik ditujukan untuk memelihara kualitas air sebagai lingkungan hidup ikan. Sementara beberapa bahan alam yang biasa digunakan dalam pengendalian kualitas perairan antara lain *barley straw*, pelepah daun pisang, batu kapur (Ridwan, 2013).

Probiotik diaplikasikan untuk memperbaiki kondisi kualitas air dengan bertindak sebagai agen pengurai yang ditebarkan secara langsung ke air. Probiotik akan bekerja secara eksternal yaitu menguraikan senyawa toksik yang terdapat dalam air kolam seperti NH_3, NO_3, NO_2 juga menguraikan bahan organik, dan menekan populasi alga biru hijau. Salah satu bakteri yang biasa digunakan dalam aplikasi probiotik yaitu *Lactobacillus* spp.

Bakteri Gram positip *Lactobacillus* spp. banyak digunakan sebagai probiotik untuk memperbaiki kualitas air dengan mengupayakan populasi bakteri *Lactobacillus* spp. tetap dalam jumlah besar di dalam perairan kolam akan memacu perkembangan phytoplankton dan meningkatnya produksi DO. Penggunaan bakteri *Lactobacillus* spp. diharapkan dapat meningkatkan kualitas air yaitu mempercepat perombakan bahan organik dalam perairan dan menekan mikroorganisme merugikan sehingga dapat mempengaruhi dinamika nilai DO, pH dan meningkatkan nutrient dalam perairan.

Barley straw adalah ekstrak bubuk jerami penghasil H_2O_2 yang berfungsi menghambat pertumbuhan mikroalga. Penggunaan ekstrak bubuk jerami telah menjadi lebih umum sebagai metode alternatif untuk mengendalikan pertumbuhan alga yang berlebihan dengan cara menghambat, bukan membasminya sehingga stabilisasi dari kelimpahan plankton dalam perairan dapat terkendali dengan baik (Lembi, 2012). Berdasarkan peranan bakteri *Lactobacillus* spp. dan *barley straw* dalam pengelolaan bahan organik dan kelimpahan plankton maka dilakukan penelitian mengkombinasikan keduanya terhadap dinamika DO, pH dan kelimpahan plankton.

2. Material dan Metode

Lokasi penelitian di Kolam Ikan Pendidikan Fakultas Perikanan dan Kelautan Universitas Airlangga Surabaya dengan tiga titik pengambilan sampel air (dua titik di ujung dan satu titik di tengah).

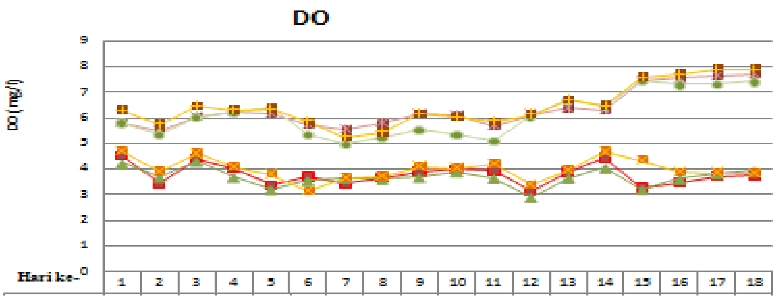
Pengukuran DO menggunakan DO meter (YSI 550A) yang juga sekaligus digunakan untuk mengukur suhu. Pengukuran pH menggunakan pH pen (TI senz pH) dan pH paper. Pengukuran ammonium dan ammonia serta kelimpahan plankton di Laboratorium Pendidikan Fakultas Perikanan dan Kelautan Universitas Airlangga Surabaya, sedangkan pengukurann nitrit, nitrat, fosfat, BOD dan COD dilakukan di Laboratorium Balai Riset dan Standarisasi Industri dan Perdagangan (Baristand Indag Surabaya), Kota Surabaya, Jawa Timur.

Pengambilan data awal dilakukan sebelum diberi produk komersial yang berisi bakteri *Lactobacillus* spp. dan *barley straw*. Pengambilan data awal yang terdiri dari DO, pH dan kelimpahan plankton yang dilakukan dua kali sehari pada pukul 05.00 dan 13.00 WIB sedangkan BOD, COD, NH_3, NO_3, NO_2 dan PO_4 dilakukan hanya satu kali sehari yaitu pada pukul 05.00 WIB.

Pemberian produk komersial yang berisi bakteri *Lactobacillus* spp. dan *barley straw* ke air Kolam Ikan Pendidikan FPK UNAIR pada pukul 03.00 WIB dengan dosis 1 tablet seberat 131 gram yaitu 0,13 ppm. Pengamatan parameter kualitas air dilakukan selama 16 hari. Pengambilan sampel COD dan BOD dilakukan 5 hari sekali pada pukul 05.00 WIB. Waktu pengambilan sampel COD dan BOD berdasarkan pada saat nilai DO terendah pada pengamatan pendahuluan. Pengambilan sampel NH_4, NH_3, NO_3, NO_2,dan PO_4 dilakukan 5 hari sekali yaitu pada pukul 05.00 WIB. Pengamatan kelimpahan plankton meliputi identifikasi plankton, kepadatan plankton, keanekaragaman plankton, keragaman plankton dan dominasi plankton. Identifikasi plankton dilakukan untuk mengetahui jenis-jenis plankton yang ada di perairan tersebut.

3. Hasil dan Pembahasan

Hasil Dinamika DO

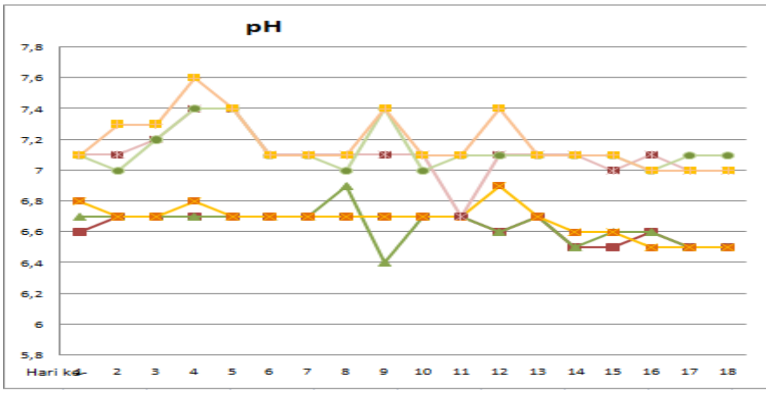


Gambar 1. Grafik dinamika DO

Selama penelitian nilai dinamika DO masih dalam tingkat wajar dan masih baik untuk budidaya. Nilai DO pada pagi hari berkisar antara 3,0 mg/l -4,0 mg/l dan pada siang hari pada siang hari berkisar antara 5mg/l - 7 mg/l. Nilai tersebut merupakan nilai yang masih bisa diterima untuk ikan budidaya agar dapat bertahan (Harada, 1978). Menurut Saliman (2005), perairan yang mengandung 5 mg/liter oksigen pada suhu 20 sampai 30ºC masih dipandang sebagai air yang cukup baik untuk kehidupan ikan.

Pada pukul 05.00 WIB hari pertama penelitian yaitu hari sebelum penebaran dinamika DO memiliki nilai pada titik 1 sebesar 4,5 ppm, titik 3 4,2 ppm dan titik 5 sebesar 4,7 ppm. Pada hari ke-2 nilai DO pada tiap titik mengalami penurunan. Hal ini diduga aktifitas bakteri *Lactobacillus* spp. yang terkandung pada produk komersial yang menyebabkan percepatan perombakan bahan organik dalam perairan sehingga penggunaan oksigen terlarut dalam perairan menjadi meningkat.

Hasil Dinamika pH



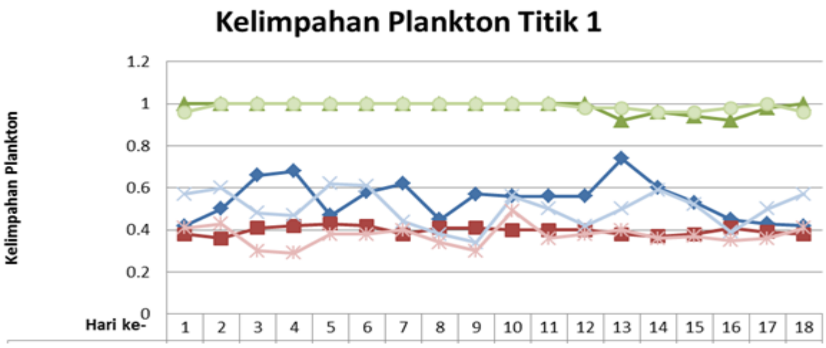
Gambar 2. Grafik dinamika pH

Selama penelitian nilai dinamika pH masih dalam tingkat wajar dan berada pada kisaran 6-6,4 pada pukul 05.00 WIB dan 7-7,4 pada pukul 13.00 WIB. Menurut data dinamika pH tersebut dapat disimpulkan kadar keasaman dalam perairan masih dalam taraf ideal dan efektif dalam pembudidayaan. Perairan dengan pH < 4 merupakan perairan yang sangat asam dan dapat menyebabkan kematian makhluk hidup, sedangkan pH > 9,5 merupakan perairan yang sangat basa yang dapat menyebabkan kematian dan mengurangi produktivitas perairan.

Perairan kolam dengan nilai pH berkisar pH 6,7 – 8,6 bisa dikatakan normal hal ini dikarenakan karena kedalaman kolam dangkal, kenaikan dan penurunan pH sejalan dengan kedalaman, diikuti kenaikan konduktivitas (Hardjojo dan Djokosetiyanto, 2005).

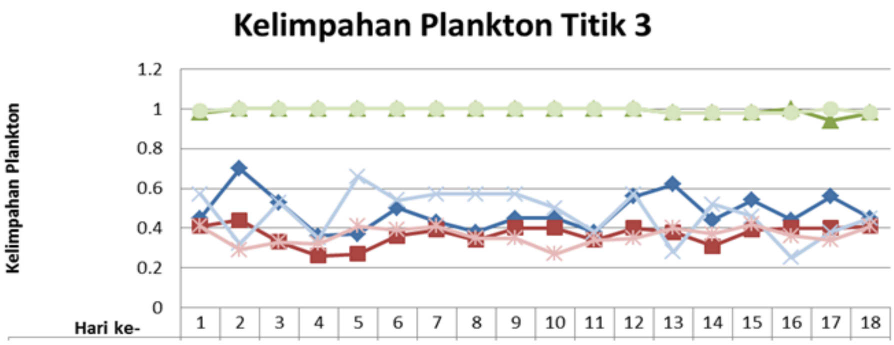
Pada pukul 05.00 WIB hari pertama penelitian yaitu hari sebelum penebaran dinamika pH memiliki nilai pada titik 1 sebesar 6,7 , titik 3 6,7 dan titik 5 sebesar 6,8. Pada hari ke-2 (setelah penebaran) nilai pH dalam keadaan stabil, titik 1 dan titik 3 memiliki nilai 6,7 dan titik 5 bernilai 6,8. Pada hari ke-3 sampai hari ke-8 titik 1 dan titik 3 masih menunjukan nilai yang stabil, namun pada titik 5 terdapat penurunan pada hari ke-3 yang diduga karena banyaknya bahan organik pada titik 5 hal tersebut sesuai dengan hasil uji BOD dan COD yang mengalami kenaikan nilai. Pada hari ke-4, titik 5 mulai stabil kembali dan mengalami peningkatan kembali pada hari ke-5 hal tersebut dikarenakan banyaknya aktifitas fitoplankton yang berfotosintesis dalam perairan terutama pada titik 5.

Hasil Dinamika Kelimpahan Plankton



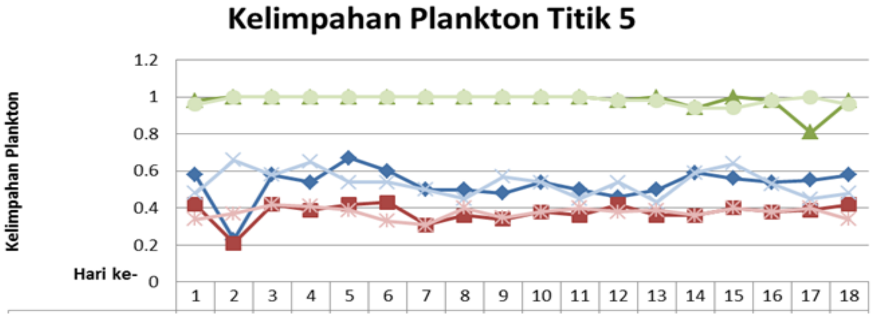
Gambar 3. Dinamika plankton titik 1

Sebelum pengaplikasian produk komersial yang berisi *Lactobacillus* spp. dan barley straw, kolam memiliki kecerahan 30 cm dan berwarna hijau pekat yang telah didominasi oleh fitoplankton yang berasal dari golongan Cyanophyta (*Calothrix* sp.) dan Chlorophyta (*Chlorella* sp.), beberapa dari golongan Diatom air tawar seperti *Nitszchia curvula*, dan beberapa zooplankton. Setelah ditebar produk komersial yang berisi *Lactobacillus* spp. dan *barley straw*, kolam berwarna hijau muda dengan kecerahan tetap 30 cm. Namun pada identifikasi, banyak ditemukan zooplankton dan diatom yang terdiri dari golongan Rotatoria, Entomostraca dan Cyclops.



Gambar 4. Dinamika kelimpahan plankton titik 3

Perubahan warna kolam diduga disebabkan karena berkurangnya kepadatan dari fitoplankton akibat reaksi *barley straw* yang menghasilkan H_2O_2 yang memiliki sifat algistatik dimana dinding sel dari fitoplankton yang dihancurkan (Ivanka, 2016). Kelimpahan plankton di titik 1 memiliki nilai yang paling rendah dibanding titik 3 dan titik 5, hal ini disebabkan karena di titik 1 memiliki nilai DO yang paling rendah, diduga akibat bahan organik yang menumpuk pada titik tersebut. Pada titik 5 memiliki nilai kelimpahan plankton yang paling tinggi. Hal tersebut dikarenakan pada titik 5 banyak menerima sinar matahari, sehingga fitoplankton dapat berfotosintesis dengan baik pada titik tersebut.



Gambar 5. Dinamika kelimpahan plankton titik 5

Hal ini baik karena kandungan protein zooplankton lebih tinggi dibandingkan pada fitoplankton. Namun rantai kehidupan di perairan sebenarnya saling berkaitan, apabila kepadatan fitoplankton menurun dikhawatirkan kepadatan zooplankton akanberkurang juga sehingga kepadatan fitoplankton dan zooplankton harus dalam keadaan seimbang. Rotatoria yang mendominasi adalah dari genus *Branchionus*, *Euchlanis* dan *Tetramanotrix*. Plankton genus tersebut mampu bertahan terhadap perubahan kondisi perairan, zooplankton mampu menyerap makanan atau nutrisi dari bahan organik yang ada di perairan dan juga diatom, dengan kata lain, zooplankton tidak hanya mendapat nutrisi dari fitoplankton saja (Setiawan, 2013).

4. Kesimpulan

Pemberian bakteri *Lactobacillus* spp. dan *barley straw* pada kolam dengan dosis 0,13 ppm mampu meningkatkan dan menstabilkan kondisi DO, nilai pH di perairan dan mampu mengurangi kepadatan fitoplankton dan membuat zooplankton dominan di perairan.

Daftar Pustaka

Anggraeni, G. (2014). Analisa perubahan kualitas air akibat pembuangan lumpur Sidoarjo pada muara Kali Porong. Surabaya. *Jurnal Teknik Pomits*, 2(1):1-5.

Boyd, C. E. (1982). Water quality management for pond fish culture. Amsterdam: . Elsevier Scientific Publishing Company.

Arinardi, O.H., Sutomo, Trimaningsih, Asnaryanti, & Riyono. (2009). Kisaran kelimpahan dan komposisi plankton predominan di perairan kawasan timur Indonesia. Jakarta: Pusat Penelitian dan Pengembangan Oseanologi Lembaga Ilmu Pengetahuan Indonesia.

Bellinger, E. G., & Sigee, D.C. (2010). Freshwater algae identification and use as bioindicators. United Kingdom: John Wiley & Sons Ltd.

Anggoro, S., Soedarsono, P., & Suprobo, H.D. (2013). Penilaian pencemaran perairan di Polder Tawang Semarang ditinjau dari aspek saprobitas. *Journal of Management of Aquatic Resources*, 2(3):109-118.

Amelia, C. D., Hasan, Z & Mulyani, Y. (2012). Distribusi spasial komunitas plankton sebagai bioindikator kualitas perairan di Situ Bagendit Kecamatan Banyuresmi, Kabupaten Garut, Provinsi Jawa Barat. *Jurnal Perikanan dan Kelautan*, 3(4):301-311.

Fidiastuti, H. R. (2014). Potensi bakteri indigen dalam biodegradasi air sungai. *Saintifika*, 6(1):29-39.

Isnansetyo, A & Kurniastuty. (1995).Teknik kultur phytoplankton dan zooplankton. Yogyakarta: Penerbit Kanisius.

Ivanka. (2016). Cara baru menghilangkan alga (*green water*) tanpa zat kimia. https://getbusygardening.com/keeping-pond-water-clear-natural-way/

Lembi, CA. (2002). Aquatic plant management barel straw for algae control. United States: Purdue University.

Indrayani, N., Anggoro, S., & Suryanto, A. (2014). Indeks trofik saprobik sebagai indikator kualitas air di Bendung Kembang Kempis Wedung, Kabupaten Demak. *Diponegoro Journal of Maquares* (*Management of Aquatic Resource*), 3(4):161-168.

Pramitha, S., Aunurohim & Trisnawati, I. (2010). Analisis kualitas air Sungai Aloo, Sidoarjo berdasarkan keanekaragaman dan komposisi fitoplankton. Surabaya: Biologi FMIPA Institut Teknologi Sepuluh November.

Wildada. (2011). Kandungan dan fungsi khusus *Lactobacillus* spp. Yogyakarta: Fakultas Pertanian Universitas Gadjah Mada.

Sholichin, M. (2012). Teknologi pengelolaan air limbah. Malang: Jurusan Teknik Pengairan Universitas Brawijaya.

Wijaya, T. S & Hariyati, R. (2009). Struktur komunitas fitoplankton sebagai bio indikator kualitas perairan Danau Rawapening Kabupaten Semarang Jawa Tengah. Artikel Imiah. Semarang: Laboratorium Ekologi dan Biosistematika Jurusan Biologi F. MIPA UNDIP.

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: 1438/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 bulan Februari tahun 2020 dengan judul sebagai berikut:
Provision Study of Lactobacillus* spp. *and Barley Straw Against Dynamics of DO, pH and Plankton Abundance

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari:
1. *American Fisheries Society* (AFS, 2014) yang berjudul *Guideline for the Use of Fishes in Research* yang menyebutkan bahwa: untuk memenuhi kaidah biosecurity bahan penelitian yang bersifat patogen telah diatur penggunaannya, selain itu juga menyebutkan bahwa penelitian dalam kondisi laboratorium baru mengatur tentang hewan percobaan berupa ikan hidup (hal 11,12,43 ; terlampir), dan
2. *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guideline on the Care and Use of Fish in Research, Teaching and Testing* yang menyebutkan bahwa: pedoman tersebut hanya digunakan untuk hewan uji berupa ikan hidup (Kelas: Chondrichthyes, Agnatha, dan Osteichthyes) dan Avertebrata (Kelas: Cephalopoda) (hal 13,14 ; terlampir).

Sedangkan dalam penelitian tersebut tidak menggunakan bahan penelitian yang bersifat pathogen, juga tidak menggunakan ikan hidup tetapi menggunakan mikroorganisme (bakteri dan plankton) sebagai bahan penelitiannya. Sehingga penelitian tersebut tidak perlu dilakukan ***Uji Ethical Clearence.***

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

Surabaya, 27 April 2023

Mengetahui,
Dekan FPK Unair

UNIVERSITAS AIRLANGGA FAKULTAS PERIKANAN DAN KELAUTAN

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

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

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

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

3.2 Biosecurity

Biosecurity can be defined in many ways (Falk et al. 2011); it is the process of taking precautions to minimize the risk of introduction and spread of infectious organisms into or among populations. The FAO defines biosecurity as a strategic and integrated approach that encompasses the policy and regulatory frameworks that analyze and manage risks in the sectors of food safety, animal life and health, and plant life and health, including associated environmental risk. FAO declared that “biosecurity covers the introduction of plant pests, animal pests and diseases, and zoonoses, the introduction and release of genetically modified organisms (GMOs) and their products, and the introduction and management of invasive alien species and genotypes” (FAO 2013, http://www.fao.org/biosecurity/). Disease occurrence is dependent on the health of the animals, the condition of the environment, and the presence of a pathogen at levels sufficient to negatively affect health (see section 7.2 Confinement, Isolation, and Quarantine). The term "biosecurity" is also relevant to environmental biodiversity. Generally, regulations, appropriate permits (see section 3.4 Permits and Certificates), and other specific concerns regarding biosecurity within a country are addressed within the guideline documents mentioned herein. New Zealand, by virtue of its unique geographic isolation and economic agricultural base, has a plethora of legislation dealing with biosecurity issues (Biosecurity Act 1993).

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

3.3 Federal, State, and Local Regulations

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

7. Laboratory Activities

7.1 General Principles

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

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

7.2 Confinement, Isolation, and Quarantine

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

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

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

Canadian Council on Animal Care

guidelines on:

the care and use of fish in research, teaching and testing

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

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