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2022

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**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

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## Preface

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The 4<sup>th</sup> International Conference on Fisheries and Marine Sciences (INCOFIMS) Surabaya Indonesia, 29 September 2021

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

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Potential of feed supplements on morphometric and gonad weight of fish exposed to microplastics

Q A'yun, F S Musthoza, S Supartini, D Utari, I Listiani, H Triwahyudi, N Fikriyah, N Suprpti and A Hayati

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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, Agustono, Arimbi, W Rahmawati and E D Masithah

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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

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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

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

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

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

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Quality Enhancement in Seaweed Dodol Using Edible Film Carrageenan Packaging

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

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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

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

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

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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

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

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

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H C Pratiwi, B S Rahardja and W Tjahjaningsih

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

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Quality improvement formulation of salak chili 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

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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

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## 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

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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

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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

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

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Identification of *Escherichia coli* and *Salmonella* on Fishery Product from Juanda Airport, East Java, Indonesia

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Risk analysis of catfish cultivation (*Pangasius hypophthalmus*) business in Gondosuli Village, Gondang, Tulungagung

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Evaluation of the culture of *Spirulina sp.* with Walne nutrient plus vitamin B12, KCl, NPK, ZA CaO and urea

A L A Suyoso, L A Sari, P D W Sari, D D Nindarwi and dan S Arsad

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Potential feed substitute of *Cirripectida sp.* flour on body weight gain, feed conversion ratio, feed consumption of buck

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Analysis of polycyclic aromatic hydrocarbons (PAHs) bioremediation by hydrocarbonoclastic degrading bacteria (*Gordonia terrae*)

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Trophic ecology and length-weight relationship of *Hypoatherina temminckii* (Bleeker, 1854) in the seagrass ecosystem of Semak Daun Island, Kepulauan Seribu

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Effectiveness of striped catfish (*Pangasianodon hypophthalmus*) cultivation in aquaponic system with three different plant against ammonia (NH<sub>3</sub>), nitrite (NO<sub>2</sub>), and nitrate (NO<sub>3</sub>)

S Andriani, N N Dewi and B S Ranaidja

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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

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

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

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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

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

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

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

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

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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*

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Diversity of intertidal fishes in Porok Beach, Gunungkidul, Yogyakarta

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

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

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

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

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

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

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

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

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

M I Joesidawati

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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

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

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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

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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

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

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

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Destruction level on coral reef in the Ambon bay

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

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

Nabila Aulia, Sapto Andriyono and Eka Saputra

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

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Use of microbubble generator on the growth vannamei shrimp culture

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

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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*

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

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

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

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

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**Abstract.** This study aimed to evaluate Pacific white shrimp larval quality from several shrimp breeding centers in East Java. parameters measured include 1) bolitas, 2) abundance of *Vibrio* spp. (water and shrimp body), and 3) muscle gut ratio (MGR). Larval samples were collected from 8 hatcheries located in East Java. The results showed that the percentage of bolitas in the hepatopancreas organ of shrimp larval in ponds 6, 7, and 8, respectively was <35%. Larval guts were found in all hatcheries, except for the 2<sup>nd</sup> pond as many as 60%. The highest abundance of *Vibrio* spp. (yellow colonies) in water samples and fry bodies was found in the 6<sup>th</sup> pond ( $26.5 \times 10^3$  CFU mL<sup>-1</sup>) and the 4<sup>th</sup> pond ( $76.9 \times 10^3$  CFU gr<sup>-1</sup>). The abundance of *Vibrio* spp. (green colony) and the highest water sample was found in the 3<sup>rd</sup> pond ( $3.8 \times 10^3$  CFU mL<sup>-1</sup>), while the larval was not detected. The larval found the highest percentage of MGR in the 3<sup>rd</sup> pond (87.5%), and the lowest was in the 7<sup>th</sup> pond (57.50%). Overall, our results demonstrate the infection of *Vibrio* spp. has involvement in the incidence of zoea syndrome in pacific white shrimp fry in hatcheries.

## 1. Introduction

The aquaculture sector in Indonesia occupies a strategic position in the development of national economy. Shrimp cultivation is one of the most prominent aquaculture industries today [1,2]. White shrimp, *Litopenaeus vannamei* is one of the most important commodities. The production value of aquaculture including *L. vannamei* in Indonesia in 2020 was 7.45 million tons and is targeted to 10.32 million tons by 2024 [3]. Management of *L. vannamei* is also related with its nutritional value such as protein, various types of fatty acids, vitamins, and main minerals for human health [4].

On the other hand, the rapid growth of national shrimp production is followed by major inhibiting factors such as the emergence of various diseases that actually reduce the development of the shrimp industry [5,6]. It was reported that since 1990s there have been various viral and bacterial disease outbreaks in shrimp farming systems such as white spot syndrome virus (WSS), taura syndrome virus



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(TSV), viral covert mortality disease (VCMD), Enterocytozoon hepatopenaei (EHP), Infection of shrimp hemocyte iridescent virus (SHIV), [7–11] and acute hepatopancreatic necrosis disease (AHPND) which have caused losses of up to 1 billion dollars annually [12]. Until one of the serious diseases that often occurs in *L. vannamei* hatcheries is the case of *Zoea Syndrome*. There are still lack concrete reports that explain the emergence of this disease, but several cases show that this outbreak is capable of causing mass death in the zoea larval stage of *L. vannamei* that is characterized by the empty larval digestive tract [13].

This outbreak is also called “*Syndrome Zoea-2*” and has been found in *L. vannamei* hatcheries in countries such as India, Ecuador, Mexico, and the United States. Sathish Kumar *et al.* [14] reported that shrimp larvae infected with this disease experienced a decrease in molting activity which eventually led to premature death. The death of the zoea *L. vannamei* stage that occurs in hatcheries is also suspected by the involvement of biotic and abiotic factors [14].

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Environmental and animal health management efforts are very important to prevent and control the spread of disease [15,16]. In this paper, we report the identification of (i) hepatopancreas and intestinal organ bolus, (ii) abundance of *Vibrio* spp., and (iii) muscle gut ratio (MGR) of Pacific white shrimp larvae reared in hatcheries in East Java, Indonesia. Our research is expected to provide initial information regarding the importance of monitoring the health of Pacific white shrimp larvae, especially for relevant authorities in preventing the spread of *Zoea Syndrome* in hatcheries.

## 2. Material and methods

Pacific white shrimp nauplius was obtained from eight shrimp hatcheries in East Java, Indonesia. There were 400 Nauplius taken from hatchery and put into an oxygenated clear plastic bag. Clinical symptoms of *Zoea Syndrome* (ZS) were observed manually and microscopically using a light microscope. The parameters observed included Bolitas (hepatopancreas and intestines) and the color of larvae that have entered the zoea-1 and 2 stages. The ideal bolity percentage had a value of <30% [17].

The total number of *Vibrio* spp. (yellow and green colonies) on water samples and *Vibrio* spp. (yellow colonies) of shrimp larvae were determined by culturing the Thiosulfate-citrate-bile salts-sucrose (TCBS) Agar (Merck) media, then incubated at room temperature for 24 hours. The calculation of the number of grown bacterial colonies is expressed in CFU<sup>-1</sup> (water) and CFU<sup>-1</sup> (shrimp larvae body) [17,18].

The assessment of digestive tract of shrimp was carried out by observing intestinal peristaltic movements and muscle:intestinal ratio in the last abdominal segment of shrimp larvae (muscle:intestinal ratio was 4:1). The indication of good digestive tract health had a value of >75% [19].

Data were analyzed using SPSS 23.0 software (IBM, USA). To find out the differences in each hatchery, the data were interpreted using the Brown-Forsythe test (P value < 0.0001). Data was displayed in images and graphs using GraphPad Prism 8.0.1 software.

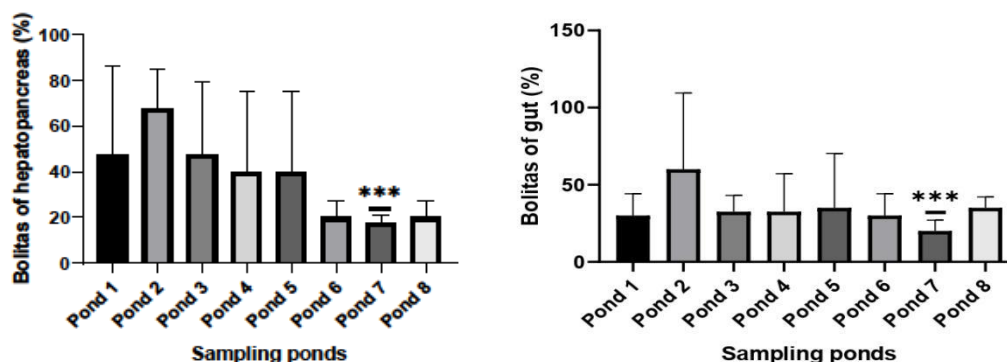
## 3. Result and discussion

### 3.1. Bolitas

Bolitas are organ disorder of shrimp larvae in the hepatopancreas and intestines. The percentage of bolitas in the hepatopancreas organ of shrimp larvae showed different values in each pond (Figure 1). The highest bolus percentage was found in hatchery 2, which was 67.50±17.68%, the lowest percentage was found in hatchery 7 that was 17.50±3.53% (P<0.0001). It is similar to what happened in the hepatopancreas organ, the highest percentage of bolus in the intestines of shrimp larvae was also found in the 2<sup>nd</sup> pond that was 60.00±49.50%, the lowest percentage was found in the 7<sup>th</sup> hatchery pond that was 20.00±7.07% (P<0.0001).

Our findings showed that larval conditions of Pacific white shrimp in several hatchery centers in East Java experienced bolus. Beside bolitas, the health condition of shrimp larvae can be observed through the necrosis, swelling and cytoplasm. Karunasagar *et al.* [20] and Austin & Zhang [21] explained that bolitas disease can cause hepatopancreas degeneration, thereby inhibiting the digestive glands due to swelling. The white shrimp culture management approach by observing the development and condition of the hepatopancreas and intestines that can have a profound and positive effect on the shrimp culture system [22]. Studies have also reported that diverse gut bacterial communities have a positive correlation with disease development and shrimp development [23]. The high percentage of bolitas in our findings could be caused by the influence of a less stable hatchery environment that affects the health condition of white shrimp larvae. In addition, natural feed that is not clean or possibly contaminated by toxic agents can also cause this disorder. It should be recalled, that under ideal conditions, the hepatopancreas of the shrimp plays an important role in regulating the innate immune system as a vital organ that is responsible for the digestion and absorption of feed nutrients [24].

Another interesting finding reported that beside the gut, microbiota that can be found in the hepatopancreas organ showed the condition that can respond the variations in environmental and health conditions and closely related to the type of host food [24,25]. Recent findings explained that the health condition of shrimp was indicated by the dominance of the general *Didymella* and *Filobasidium* in the intestine and *Pyrenochaetopsis* in the hepatopancreas organ. However, the sick shrimp group had a higher abundance of the genus *Candida* and can be used as a bioindicator to assess the health of shrimp larvae [26]. AHPND cases caused by *Vibrio parahaemolyticus* were also able to affect the bacterial community in the stomach and hepatopancreas of shrimp to be dysbiotic [27].

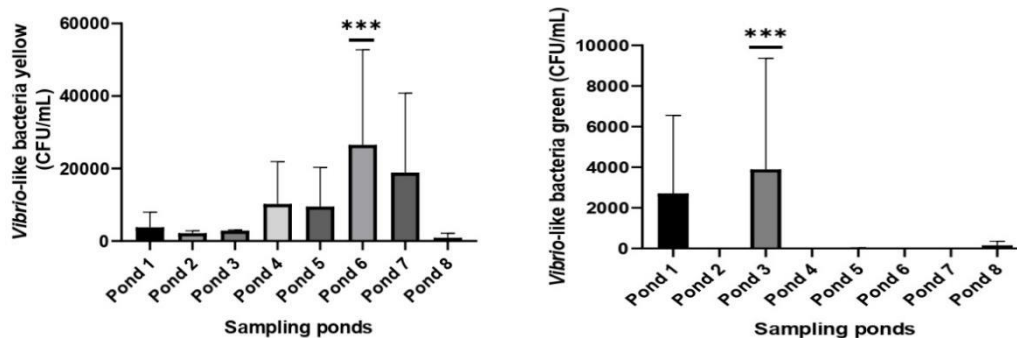


**Figure 1.** The incidence of bolus in the hepatopancreas and intestines of Pacific white shrimp larvae in hatcheries, East Java. Remarks: \*\*\* showed a significantly different value ( $P < 0.0001$ ) based on the Brown-Forsythe test

### 3.2. The abundance of *Vibrio spp.*

ch as rivers, estuaries, seas, and even deep sea waters. In aquaculture activities, water quality parameters and nutrients accumulated in the water column may affect the presence of *Vibrio* [28]. Our results showed that the abundance of *Vibrio spp.* that varies in each Pacific white shrimp larvae hatchery in East Java. In rearing water, the number of *Vibrio spp.* The highest yellow color was found in the 6<sup>th</sup> pool that was  $26.5 \times 10^3$  CFU mL<sup>-1</sup>, while the lowest number of yellow colonies in the water sample was found in the 8<sup>th</sup> pool that was  $0.9 \times 10^3$  CFU mL<sup>-1</sup>. Furthermore, the highest green-colonies were found in the 3<sup>rd</sup> pool water sample that was  $3.8 \times 10^3$  CFU mL<sup>-1</sup>, and green-colonies were not detected in the 2<sup>nd</sup>, 6<sup>th</sup>, 7<sup>th</sup> pool (Figure 2).





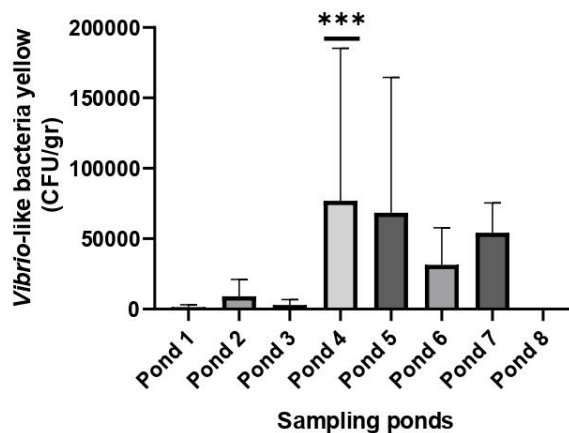
**Figure 2.** Abundance of Vibrio-like bacteria yellow and Vibrio-like bacteria green in water samples cultured the Pacific white shrimp larvae grown in TCBS agar media. Remarks: \*\*\* showed a significantly different value ( $P < 0.0001$ ) based on the Brown-Forsythe test. CFU: Colony Forming Units.

The *Vibrio* genus is often associated with various species of aquatic animals such as fish, crustaceans, and mollusks. The existence of this genus can affect the development of the larval stage to adult, in various diseases of aquatic organisms [29]. The genus *Vibrio* was also demonstrated by the formation of biofilms on surfaces submerged using water. In our findings, Vibrio-like bacteria yellow tended to be found more frequently in all water samples in the hatchery when compared to Vibrio-like bacteria green colonies that were only found in three water samples (Figure 2). Selective culture media (TCBS) caused sucrose-positive strains to form yellow colonies, whereas sucrose-negative strains formed green colonies [30].

Generally, the appearance of *Vibrio* in aquatic crustaceans can be associated with the emergence of infectious disease as a bioindicator with the pathogen. In fact, it is indicated by a large reduction in shrimp production as a consequence of the vibriosis outbreak and the diseases such as acute hepatopancreatic necrosis (AHPND) threatening shrimp farm operations [31]. The appearance of Vibrio-like bacteria yellow can be caused by the high concentration of organic matter in the rearing water column [32,33]. Our results are slightly different from those of Luis-Villasenor *et al.* [34] who obtained Vibrio-like bacteria in water samples of juvenile Pacific white shrimp cultured with biofloc at  $1.57 \times 10^4$  CFU  $\text{ml}^{-1}$ .

In contrast to the appearance of Vibrio-like bacteria yellow found in all water samples, Vibrio-like bacteria green was only found in water samples of n three hatcheries (Figure 2). Based on the reports from Aryal [35], *Vibrio* spp. with green appearance on TCBS Agar selective media can be associated with *Vibrio parahaemolyticus*. The existence of *V. parahaemolyticus* is currently being highlighted, especially for its potential in reducing aquaculture productivity to human health [36]. Our initial findings suggest that the incidence of *Zoea Syndrome* demonstrated by the occurrence of Bolitas may be associated with the emergence of this bacterium, the further research is needed to elucidate this.

The abundance of *Vibrio* spp. on the shrimp larvae body was only highlighted in Vibrio-like bacteria yellow, the Vibrio-like bacteria green was not found in all samples (Figure 3). The highest abundance of Vibrio-like bacteria yellow was found in shrimp bodies from the 4th pond, namely  $76.9 \times 10^3$  CFU  $\text{gr}^{-1}$ , while the lowest abundance was found in the 1st and 3rd ponds of  $1.3 \times 10^3$  CFU  $\text{gr}^{-1}$  and  $9.1 \times 10^3$  CFU  $\text{gr}^{-1}$ .

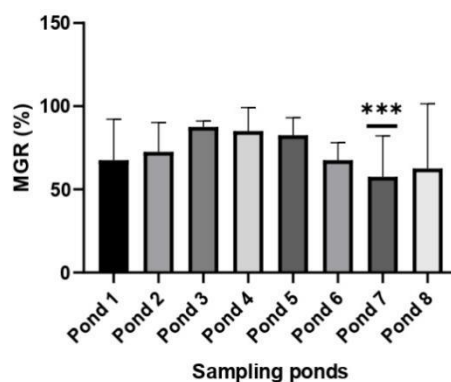


**Figure 3.** The abundance of *Vibrio*-like bacteria yellow in Pacific white shrimp larvae grown in TCBS agar media. *Vibrio*-like bacteria green data were not detected in all samples. Remarks: \*\*\* showed a significantly different value ( $P < 0.0001$ ) based on the Brown-Forsythe test. CFU: Colony Forming Units.

The emergence of *Vibrio* spp. on shrimp body is normal considering this bacterium is a natural microflora in the body of wild and cultured shrimp. However, when the natural defense mechanisms of shrimp are suppressed due to disease and environmental stressors, these bacteria will become “opportunistic pathogens” for shrimp [37]. The intensive culture systems, both larval and juvenile shrimp species are often exposed to stress conditions due to high stocking densities that lead to the emergence of secondary vibriosis [38]. Thus, our findings can be used as an initial reference in efforts to manage the health of Pacific white shrimp fry which will be cultivated in an intensive system by the relevant authorities.

### 3.3. Muscle gut ratio (MGR)

The highest MGR measurement of Pacific white shrimp larvae was shown in the 3<sup>rd</sup> hatchery that was  $87.50 \pm 3.5\%$ , while the lowest MGR value was statistically shown in shrimp larvae from the 7<sup>th</sup> hatchery that was  $57.50 \pm 38.89\%$ . On average, overall results showed that the MGR values found in shrimp larvae from ponds 1, 6, 7, and 8 were still below the values from ideal larval health conditions ( $>75\%$ ) (Figure 4).



**Figure 4.** *Vibrio*-like bacteria green data were not detected in all samples. Remarks: \*\*\* showed a significantly different value ( $P < 0.0001$ ) based on the Brown-Forsythe test.

The good MGR of shrimp larvae had a tail muscle condition that covered more than 50% of the tail width in the 6<sup>th</sup> segment. For poor tail muscle condition, it was shown by the muscle stretched more than 50% of the tail width in the 6th abdominal segment. Next, overall poor muscle condition was indicated by the occurrence of discoloration and thinness of the tail muscles. Some cases also find the presence of lumpy nematocysts that come out of the digestive tract and can be an indication of the emergence of an infection. In our case, the clot was represented by gregarine appearing in the larval gut.

The condition of the shrimp hatchery in not optimal environment may trigger the decrease in the MGR value. The behavior of shrimp that always exposes the water column may cause the exchange of microflora between the environment and the digestive system [39]. It is possible to cause unfavorable conditions or destabilization of the digestive microflora. Furthermore, the shrimp digestive system that is directly connected to the environment can be an entry point for various chemical or biological pollutants such as protozoa, bacteria, fungi, and viruses that are directly responsible for decreasing the profitability of shrimp production. On the other hand, intestinal disease is also associated with several names such as White Feces Syndrome (WFS) [40], White Gut Disease (WGD) [41], and White Muscle Disease (WMD) [41].

Good water quality management and aquaculture activities need to be carried out to reduce the incidence of Zoa Syndrome case. Some careless mismanagement lead to the emergence of *Vibrio* as an opportunistic pathogen that is able to delay early moulting for three to four days and zoea death at stage zoea-II in hatcheries with a stocking capacity of 100 million Nauplius [14]. By ensuring good management practices, following by disinfection periods, proper termination between larval production cycles, and reducing the number of stocking days of naupli, and improving the quality of natural feed (microalgae) through series dilutions, ensuring good circulation, and separating units (maturation, spawning), larval rearing, algal culture, water supply, and labor units) can help reduce the incidence of zoea syndrome in Pacific white shrimp fry [14].

#### 4. Conclusion

Overall, *Zoea Syndrome* is also characterized by the empty of larvae intestines due to impaired appetite since the early stage (zoea-1). We expect the involvement of ZS with the abundance of *Vibrio* spp., although it needs further research to confirm this by using a molecular approach. However, our initial research may provide the important information for the relevant authorities to take policy in monitoring aquatic animals health especially *L. vannamei* at an early stage.

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## 6. Acknowledgments

This study was supported by Universitas Dhyana Pura, Badung, Bali and Universitas Airlangga, Surabaya, East Java, Indonesia. We also thank all Pacific white shrimp farmers who have granted research permits and related authorities such as the Department of Marine Affairs and Fisheries, East Java who supported our initial research.