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Table of contents

Volume 236

2019

◆ Previous issue
 Next issue ▶

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Open all abstracts

Preface			
OPEN ACCESS			011001
The 1st Internation	onal Conference on	Fisheries and Marine Science	
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS			011002
Organizing Com	mittee		
	View article	PDF	
OPEN ACCESS			011003
Conference Phot	os		
	View article	PDF	
OPEN ACCESS			011004
Peer review state	ement		
+ Open abstract	View article	PDF	
Papers			
OPEN ACCESS			012001
The Increase in f Light Intensities	3-carotene Content i	in Dunaliella salina from the Application of Different	
N Sugiati, E D Mas	sithah, W Tjahjaningsi	h and A A Abdillah	
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS			012002

Ammonia-eliminating potential of *Gracilaria* sp. And zeolite: a preliminary study of the efficient ammonia eliminator in aquatic environment

M R Royan, M H Solim and M B Santanumurti

+ Open abstract 🛛 🔤 View article 🛛 🏊 PDI	 Open abstract 	View article	🔁 PDF
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-			
OPEN ACCESS	avtrocallular anzum	a producing hasteria (protoclytic collulalytic and	012003
		e-producing bacteria (proteolytic, cellulolytic, and sive ponds in Tanggulrejo, Gresik	
OA Artha, Sudarno,	, H Pramono and LA S	Sari	
	View article	🔁 PDF	
OPEN ACCESS			012004
		t pond of pangasius fillet waste (<i>Pangasius</i> sp.) with ation medium due to the population growth of <i>Daphnia</i>	sp.
H P Alvian, E D Ma	asithah and M H Azha	r	
	View article	PDF	
e	survival rate in lettu densities of eel (<i>Me</i>	ce aquaponic systems (<i>Latuca sativa</i>) of eels in <i>pnopterus albus</i>)	012005
N K Portalia, L Suli	martiwi and B S Raha	rdja	
+ Open abstract	View article	🔁 PDF	
-	oondo Regency, Eas	umpback grouper (<i>Cromileptes altivelis</i>) in floating st Java, Indonesia	012006
+ Open abstract	View article	🔁 PDF	
•	f fungi on groupers Surabaya, East Jav	(<i>Epinephelus</i> sp.) in cage mariculture systems of the a	012007
E Yuliastuti, R Kuso	dawarti and Sudarno		
+ Open abstract	Tiew article	PDF	
OPEN ACCESS			012008
The spectrum of Spirulina platens	•	required to increase the production of phycocyanin	
H A Wicaksono, W	H Satyantini and E D	Masithah	
+ Open abstract	Tiew article	🔁 PDF	
OPEN ACCESS			012009

temperatures			
L Lutfiyah, D S Bud	di and M F Ulkhaq		
	View article	PDF	
OPEN ACCESS Preservation of co	ommon carp (<i>Cypri</i>	<i>inus carpio</i>) sperm using 0.9% NaCl and ringer's	01201
lactate solution			
D S Budi, L A Adav	viyah and L Lutfiyah		
+ Open abstract	View article	PDF	
OPEN ACCESS			01201
Comparison of th from <i>Sargassum</i>	• 、 ,	point, freezing point, and viscosity test) of biodiesels	
M B Santanumurti,	M R Royan, S H Sam	ara, S Sigit and M A Alamsjah	
+ Open abstract	View article	PDF	
quadricarinatus a	*	of ecdysis with the age of freshwater crayfish Cherax	
A H Fasya	View article	🔁 PDF	
+ Open abstract OPEN ACCESS		PDF	01201
+ Open abstract OPEN ACCESS Maximum density female offspring		<i>crocopa</i> culture able to produce parthenogenesis in	01201
+ Open abstract OPEN ACCESS Maximum density female offspring	y in the <i>Moina mac</i>	<i>crocopa</i> culture able to produce parthenogenesis in	01201
 + Open abstract OPEN ACCESS Maximum density female offspring A S Mubarak, D Jus + Open abstract OPEN ACCESS The oxygen contended 	y in the <i>Moina mac</i> sadi, M Z Junior and N T View article ent and dissolved or	 <i>crocopa</i> culture able to produce parthenogenesis in M A Suprayudi PDF xygen consumption level of white shrimp 	01201
 + Open abstract OPEN ACCESS Maximum density female offspring A S Mubarak, D Jus + Open abstract OPEN ACCESS The oxygen conta Litopenaeus vann 	y in the <i>Moina mac</i> sadi, M Z Junior and N Twiew article ent and dissolved or <i>namei</i> in the nanobu	 <i>crocopa</i> culture able to produce parthenogenesis in M A Suprayudi PDF xygen consumption level of white shrimp ubble cultivation system 	
 + Open abstract OPEN ACCESS Maximum density female offspring A S Mubarak, D Jus + Open abstract OPEN ACCESS The oxygen contended in the oxygen contended in the	y in the <i>Moina mac</i> sadi, M Z Junior and N To View article ent and dissolved or <i>namei</i> in the nanobushari, L Sulmatiwi, G I	 <i>Procopa</i> culture able to produce parthenogenesis in M A Suprayudi PDF xygen consumption level of white shrimp lable cultivation system Mahasri, Prayogo and LA Sari 	
 + Open abstract OPEN ACCESS Maximum density female offspring A S Mubarak, D Jus + Open abstract OPEN ACCESS The oxygen conta Litopenaeus vann 	y in the <i>Moina mac</i> sadi, M Z Junior and N Twiew article ent and dissolved or <i>namei</i> in the nanobu	 <i>crocopa</i> culture able to produce parthenogenesis in M A Suprayudi PDF xygen consumption level of white shrimp ubble cultivation system 	
 + Open abstract OPEN ACCESS Maximum density female offspring A S Mubarak, D Jus + Open abstract OPEN ACCESS The oxygen contended of the open abstract OPEN ACCESS The open abstract OPEN ACCESS The effect of the open abstract 	y in the <i>Moina mac</i> sadi, M Z Junior and M Twiew article ent and dissolved or <i>namei</i> in the nanobushari, L Sulmatiwi, G D Twiew article epiphytes of <i>Chaete</i>	 <i>Procopa</i> culture able to produce parthenogenesis in M A Suprayudi PDF xygen consumption level of white shrimp lable cultivation system Mahasri, Prayogo and LA Sari 	01201
 + Open abstract OPEN ACCESS Maximum density female offspring A S Mubarak, D Jus + Open abstract OPEN ACCESS The oxygen conta <i>Litopenaeus vann</i> D P Galang, A K As + Open abstract OPEN ACCESS The effect of the growth of <i>Gracilla</i> 	y in the <i>Moina mac</i> sadi, M Z Junior and M Twiew article ent and dissolved or <i>namei</i> in the nanobushari, L Sulmatiwi, G D Twiew article epiphytes of <i>Chaete</i>	erocopa culture able to produce parthenogenesis in M A Suprayudi	

012016

IOP Conference Series: Earth and Environmental Science, Volume 236, 2019 - IOPscience Growth monitoring of koi fish (Cypri nus carpio) in natural hatchery techniques in Umbulan, Pasuruan, East Java F P Putri and N N Dewi View article 🔁 PDF + Open abstract **OPEN ACCESS** 012017 Dynamic Ratio Correlation of N:P in relation to the Diatom Abundance in the Intensive System of the Vannamei (Litopenaeus vannamei) Shrimp Pond E D Masithah, D D Nindarwi, T Rahma and dan R R Satrya P I + Open abstract View article ື PDF **OPEN ACCESS** 012018 Dynamic ratio correlation of N:P on the abundance of Bluegreen algae in an intensive system in a white shrimp (Litopenaeous vannamei) pond E D Masithah, D D Nindarwi, A L A Suyoso and D Husin + Open abstract View article 🔁 PDF **OPEN ACCESS** 012019 Dynamic ratio correlation of N:P toward phytoplankton explosions in intensive systems of white shrimp pond E D Masithah, D D Nindarwi, D Husin and T Rahma 🔁 PDF + Open abstract View article **OPEN ACCESS** 012020 Development of water and nutrient management models to improve multitrophic

seafarming productivity

J A Surbakti, I A L Dewi, M A Alamsjah and M Lamid

+	Open abstract	View article	🔁 PDF
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Pond soil characteristic in reclaimed tidal lowlands and its correlation with the water quality for aquaculture

M Fitrani, I Wudtisin, M Kaewnern and R H Susanto

+ Open abstract View article 🔁 PDF

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012022

012021

The dynamics of total organic matter (tom) on sangkuriang catfish (*clarias gariepinus*) farming at upt ptpbp2kp and the effectiveness of freshwater bivalve (anodonta woodiana) in reducing the total organic matter with varying density

D Arfiati, C D G Putra, A H Tullah, S W A Permanasari and A W Puspitasari

View article 🔁 PDF + Open abstract

OPEN ACCESS	012023
Growth and morphological changes in relation to the maturation of male Japanese eel, <i>Anguilla japonica</i> injected with human chorionic gonadotrophin (HCG) in the different int the tropical region	
Y T Hee, F F Ching and S Senoo	
← Open abstract	
OPEN ACCESS	012024
Genetic diversity of the endangered species <i>Sphyrna lewini</i> (Griffith and Smith 1834) in Lombok based on mitochondrial DNA	
S Hadi, N P Anggraini, E Muttaqin, B M Simeon, B Subhan and H Madduppa	
← Open abstract	
OPEN ACCESS	012025
Sex ratio and size at first maturity of razor clam <i>Solen</i> sp. in Pamekasan and Surabaya coastal area, East Java, Indonesia	
N Trisyani, N I Wijaya and I Yuniar	
+ Open abstract 🔄 View article 🄁 PDF	
OPEN ACCESS Improving productivity and water quality of catfish, <i>Clarias</i> sp. cultured in an aquaponic ebb-tide system using different filtration E Setiadi, I Taufik, Y R Widyastuti, I Ardi and D Puspaningsih	012026
+ Open abstract 🔄 View article 🄁 PDF	
OPEN ACCESS Different substrate of trickling filter on growth, survival rate, and water quality of common carp (<i>Cyprinus carpio</i>) cultivation by using an intensive recirculation system E Setiadi, I Taufik, Y R Widyastuti, I Ardi and A Saputra + Open abstract The Widyastuti is the strate of the strate	012027
OPEN ACCESS Water quality dynamic, production and profitability of catfish, <i>Clarias</i> sp. cultured at different design construction of aquaponic Y R Widyastuti, E Setiadi, I Taufik and L Setijaningsih + Open abstract Image: View article	012028
OPEN ACCESS Effect of C:N ratio on the spore production of <i>Bacillus</i> sp. indigenous shrimp pond A Yuniarti, N B Arifin, M Fakhri and A M Hariati	012029
+ Open abstract 📄 View article 📂 PDF	

OPEN ACCESS			012020
Comparative Test		Digestive Tract of Vannamei Shrimp (<i>Litopenaeus</i> e Ponds in Ujungpangkah, Gresik	012030
D Ningrum, M Arief	and dan K T Pursety	0	
	View article	🔁 PDF	
OPEN ACCESS			012031
Growth Performan	nce of Laboratory-S	Scale Chaetoceros calcitrans in Different Containers	
M Jannah, M F Ulkh	aq, M H Azhar, Suciy	vono and dan W Soemarjati	
	View article	🔁 PDF	
OPEN ACCESS Study of the Dyna Managements in C	•	viversity of Plankton at Different Brackishwater Pond	012032
O Tilahwatih, E D M	fasithah and dan B S l	Rahardja	
+ Open abstract	View article	PDF	
OPEN ACCESS The Effect of Den Activated Carbon	nineralization Stage	e of Agar's Solid Waste on the Characterization of	012033
R Febrianto, Sudarno	o and R Kusdarwati		
+ Open abstract	View article	🔁 PDF	
•	•	ty of Cyanophyta in Different Pond Bases in eries and Marine Universitas Airlangga	012034
S Z Cahyani, E D M	asithah and Prayogo		
	View article	🔁 PDF	
(FCR) of Catfish (•	hin Water Against Growth Rate, Survival Rate	012035
	View article	🔁 PDF	
OPEN ACCESS Molecular identifi <i>forcipata</i> and <i>Uca</i>		enetic reconstruction of two fiddler crabs (Uca	012036
S Andriyono, H Prar	nono and H W Kim		
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS			012037

The molecules idea	titiontion and who	logenetic reconstruction of Palaemonid and Penaeid sl	arima
from the southern p		•	urimp
M J Alam, S Andriyon	C C		
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS			01203
Morphometric chara Estuary, Surabaya	acteristics of Fur	Cockles (Anadara spp.) in Wonokromo and Juanda	
P B Pamungkas, K T F	Pursetyo, J Triastuti	and N N Dewi	
	View article	PDF	
OPEN ACCESS			012039
Stock status of ark o Surabaya, Indonesia	,	app.) based on dredge fishing of the east coast of	
N N Dewi, K T Purset	yo, O P Darmono, I	F R Fachri, F S Puspitasari and A Damora	
+ Open abstract	View article	PDF	
OPEN ACCESS			01204
OPEN ACCESS The distribution pat Sidoarjo, East Java	terns and biomas	s of bivalves in Segoro Tambak estuary, Sedati,	012040
The distribution pat Sidoarjo, East Java		s of bivalves in Segoro Tambak estuary, Sedati, sithah, A M Sahidu and K T Pursetyo	012040
The distribution pat Sidoarjo, East Java			012040
The distribution pat Sidoarjo, East Java S H Liyana, L A Sari, T + Open abstract OPEN ACCESS	N N Dewi, E D Ma	sithah, A M Sahidu and K T Pursetyo	
 The distribution path Sidoarjo, East Java S H Liyana, L A Sari, E Open abstract 	N N Dewi, E D Ma	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia	012040
The distribution pat Sidoarjo, East Java S H Liyana, L A Sari, T + Open abstract OPEN ACCESS	N N Dewi, E D Ma	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia Kenconojati	
The distribution pat Sidoarjo, East Java S H Liyana, L A Sari, T + Open abstract OPEN ACCESS Inventorization of re	N N Dewi, E D Ma	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia	
 The distribution path Sidoarjo, East Java S H Liyana, L A Sari, I Open abstract OPEN ACCESS Inventorization of results Suciyono, M A Azhar, Open abstract 	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia Kenconojati PDF	01204
 The distribution path Sidoarjo, East Java S H Liyana, L A Sari, I Open abstract OPEN ACCESS Inventorization of results Suciyono, M A Azhar, Open abstract 	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article the effect of calci	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia Kenconojati PDF um hydroxide and sodium bicarbonate treatment on	
 The distribution path Sidoarjo, East Java S H Liyana, L A Sari, I Open abstract OPEN ACCESS Inventorization of results Suciyono, M A Azhar, Open abstract OPEN ACCESS Dynamic study on the N/P ratio and plate 	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article the effect of calci ankton abundanc	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia Kenconojati PDF um hydroxide and sodium bicarbonate treatment on e	01204
 The distribution path Sidoarjo, East Java S H Liyana, L A Sari, I + Open abstract OPEN ACCESS Inventorization of results Suciyono, M A Azhar, + Open abstract OPEN ACCESS Dynamic study on t 	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article the effect of calci ankton abundanc	sithah, A M Sahidu and K T Pursetyo PDF aan Island, Banyuwangi, East Java, Indonesia Kenconojati PDF um hydroxide and sodium bicarbonate treatment on e	01204
The distribution path Sidoarjo, East Java S H Liyana, L A Sari, T + Open abstract OPEN ACCESS Inventorization of re- Suciyono, M A Azhar, + Open abstract OPEN ACCESS Dynamic study on t the N/P ratio and pla M R N Tsany, E D Ma + Open abstract OPEN ACCESS	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article the effect of calci ankton abundanc sithah, B S Rahardj View article	sithah, A M Sahidu and K T Pursetyo PDF an Island, Banyuwangi, East Java, Indonesia Kenconojati PDF um hydroxide and sodium bicarbonate treatment on e to and D D Nindarwi PDF	01204
The distribution path Sidoarjo, East Java S H Liyana, L A Sari, T + Open abstract OPEN ACCESS Inventorization of re- Suciyono, M A Azhar, + Open abstract OPEN ACCESS Dynamic study on the N/P ratio and pla M R N Tsany, E D Ma + Open abstract OPEN ACCESS	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article the effect of calci ankton abundanc sithah, B S Rahardj View article	sithah, A M Sahidu and K T Pursetyo PDF an Island, Banyuwangi, East Java, Indonesia Kenconojati PDF um hydroxide and sodium bicarbonate treatment on e io and D D Nindarwi	01204
The distribution pat Sidoarjo, East Java S H Liyana, L A Sari, I + Open abstract OPEN ACCESS Inventorization of re Suciyono, M A Azhar, + Open abstract OPEN ACCESS Dynamic study on t the N/P ratio and pla M R N Tsany, E D Ma + Open abstract OPEN ACCESS Distribution pattern Sidoarjo, East Java	N N Dewi, E D Ma View article eef fish on Tabuh M F Ulkhaq and H View article the effect of calci ankton abundanc sithah, B S Rahardj View article s and the biomas	sithah, A M Sahidu and K T Pursetyo PDF an Island, Banyuwangi, East Java, Indonesia Kenconojati PDF um hydroxide and sodium bicarbonate treatment on e to and D D Nindarwi PDF	01204

OPEN ACCESS

Optimization of diatom Haslea ostrearia cultivation in different mediums and nutrients

012044

S Arsad, C Stavrakakis, V Turpin, P Rossa, Y Risjani, L A Sari, F S Prasetiya and J-L Mouget

	Tiew article	🔁 PDF	
OPEN ACCESS			012045
Coastal ecosystem	m model based on e uwangi Region, Ea	environmental suitability and carrying capacity of the st Java, Indonesia	012013
E W Setyaningrum,	, Maghdalena, A T K I	Dewi, M. Yuniartik and E D Masithah	
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS			012046
*	l succession of sess oastal Waters of Sal	ile macrofouling organisms on the artificial structure bah, Malaysia	
M A M Affandy, J M	Madin, K P Jakobsen a	and M Auluck	
+ Open abstract	View article	🔁 PDF	
-		ght of metal halide lamps and LED lamps with mini	012047
purse seine opera			
-	rfiati, T D Lelono and	_	
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS Status of coral di North Bali island	-	mised health syndromes on Pemuteran shallow reefs,	012048
W Karim			
	View article	PDF	
OPEN ACCESS			012049
Management opt institutional appr		rtificial coral reefs in Indonesia: strengthening in	
Rudianto and Ahma	ad Zainul		
	View article	🔁 PDF	
OPEN ACCESS			012050
	ecretor and non-salt s to habitat zonation	secretor mangrove seedlings with varying salinity n	
M Basyuni, Ramay	ani, A Hayullah, Prayı	unita, M Hamka, L A Putri and S Baba	
	View article	🔁 PDF	
OPEN ACCESS			012051
Distribution of <i>C</i> Sumenep regency		nd Fungia Consinna coral on Mamburit island,	

	09 AM	IOP Conference	ce Series: Earth and Environmental Science, Volume 236, 2019 - IOPscienc	e
Sa	awiya, D Arfiati, C	Guntur and U Zakiyah		
+	Open abstract	Tiew article	PDF	
01	PEN ACCESS			012
Di	iversity species	and condition of se	agrass ecosystem in Teluk Awur and Prawean Jepara	
ΙF	Riniatsih, A Amba	riyanto, E Yudiati, R H	Hartati, W Widianingsih and R T Mahendrajaya	
+	Open abstract	View article	🔁 PDF	
	PEN ACCESS			012
	opper (Cu) and phytoplankton	• • •	icity on growth, chlorophyll-a and carotenoid content	
D	Hindarti and A.W	. Larasati		
+	Open abstract	Tiew article	🔁 PDF	
01	PEN ACCESS			012
	0	cteristic and Micro East Sinjai, South S	Environment of Mangrove Rehabilitation Forest at Sulawesi	
Н	Setiawan			
+	Open abstract	View article	PDF	
Tł De	eposits of Heav		<i>atiotesas</i>) as Phytoremediator for Concentration and Tilapia (<i>Oreochromis niloticus</i>) Gills	012
	Open abstract	View article	PDF	
01	PEN ACCESS			012
		•	Pb, Cd and Zn Reduction in NPK Fertilizer Waste d (<i>Gracillaria</i> sp.), Blood Clam (<i>Anadara</i> sp.), and Zeo	lite
A	R K Sari, R K Ha	rryes, F A Anggraini, I	M A Alamsyah and dan A Ahadi	
+	Open abstract	View article	PDF	
01	PEN ACCESS			012
		-	<i>acilaria</i> Sp. Seaweed Biofilter and <i>Anadara granosa</i> the Level of Mercury (Hg) Heavy Metal	
J A	A Spespatri, B S R	ahardja and A A Abdi	llah	
+	Open abstract	Tiew article	PDF	
01	PEN ACCESS			012
			f Seaweed (<i>Gracillaria</i> sp.), Blood Clamp (<i>Anadara</i> the Reduction of Heavy Metal Copper (Cu)	
gr	anosa), and Ze			

+ Open abstract	View article	PDF	
	· · ·	Heavy Metal on Seaweed (<i>Gracilaria</i> sp.) in strict, Sidoarjo District	012059
O Ardiyansyah, Sud	larno and Rosmanida		
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS Identification of I Mangrove Forest	•	l Isolates in Sediment Ecosystem of Gunung Anyar	012060
P B Utomo, Sudarno	o and B S Rahardja		
+ Open abstract	Tiew article	PDF	
Banjar Kemuning	g River, Sedati, Sido	•	012061
R F Saputra, E D M	asithah and P D Wula	nsari	
+ Open abstract	View article	🔁 PDF	
Dadapan, Sedati S	oral Variation of Bi Sub-District, Sidoar T Pursetyo, Prayogo a		012062
+ Open abstract	View article	🔁 PDF	
OPEN ACCESS Distribution Patte Sedati, Sidoarjo,		f Bivalve in Juanda and Segoro Tambak Estuary in	012063
S H Liyana, E D Ma	asithah and A M Sahid	lu	
+ Open abstract	Tiew article	🔁 PDF	
<i>marina</i> at Mangro W C Dermawan, Pr	ove Ecotourism Wo ayogo and B S Rahard	lja	012064
+ Open abstract	Tiew article	🔁 PDF	
Saronggi Sumene	(Pb) Value Compar ep Marine, Madura, F Pursetyo and S A Su		012065
• *	-		

	View article	🔁 PDF	
-	ition of lemuru oil ((<i>Monopterus albus</i>)	to commercial feed to increase the content of EPA	012066
A Imanisa, M B San	ntanumurti, M Lamid a	and Agustono	
+ Open abstract	View article	PDF	
OPEN ACCESS Effect of lysine in	addition to comme	ercial feed on crude protein and the energy	012067
	ourami (<i>Osphronem</i>		
D Setiyawan, S H S	amara, Agustono and	MAAArif	
+ Open abstract	View article	PDF	
1	apain enzyme to co (Anguilla bicolor)	mmercial feed against protein retention and feed	012068
DA Liono, M Arief,	Prayogo and W Isron	i	
+ Open abstract	Tiew article	PDF	
and energy retent	ion of tilapia Oreoc	and W P Lokapirnasari	012069
(Cyprinus carpio)	C	ed on the survival rate, and growth of Common carp ation of Freshwater Culture (IBAT) in Punten, Batu. M Sahidu	012070
+ Open abstract	View article	🔁 PDF	
	f Phytase Enzymes ayer and Fish Feed	and SEM Analysis in order to increase the Quality of	012071
M Lamid, Anam Al-	Arif and S H Warsito		
	Tiew article	PDF	
•		ankton abundance and diversity in relation to white consumption in intensive ponds	012072

D D Nindarwi, E D Masithah, D Zulian and A LA Suyoso

	View article	PDF	
-	-	neal with <i>Spirulina platensis</i> meal in practical diets val, and color enhancement of percula clownfish <i>Amphi</i>	012073 prion
S Hudaidah, B Putri,	S H Samara and Y T	Adiputra	
	View article	🔁 PDF	
OPEN ACCESS			012074
		otic Addition on Commercial Feed against ilapia Feed (<i>Oreochromis Niloticus</i>)	
D Taufik, M Arief an	d H Kenconojati		
+ Open abstract	Tiew article	🔁 PDF	
	ing Synbiotics Into Dumbo Catfish (<i>Cla</i>	Commercial Feed Towards Protein Retention and <i>rias</i> sp.)	012075
H Syevidiana, M Ari	ef and I S Hamid		
	Tiew article	PDF	
Feed Convertion H	Ratio to Tambaqui (tono and S H Kencond	nercial Feed on Growth Rate, Feed Efficiency, and <i>Colossoma Macropomum</i>) ojati	012076
+ Open abstract	View article	PDF	
	-	moke in Commercial Feed on Total Bacteria of bia's Kidney (<i>Orechromis niloticus</i>)	012077
M Rahmawati, Suda	rno and S Subekti		
	Tiew article	🔁 PDF	
	ginosa Bacteria on	moke in Commercial Feed Towards Total Gastrointestinal Tract Tilapia (<i>Oreochromis Niloticus</i>) PDF	012078
OPEN ACCESS Antibacterial activ room temperature	rity of honey in pres	serving high-pressure cooked milkfish stored at	012079

D A Hakim, W Tjah	njaningsih and Sudarno	0	
	View article	🔁 PDF	
OPEN ACCESS Bacterial compos marina leaf litter	-	ntestinal tract of Uca spp crabs fed on Avicennia	012080
M A B Kareho, E D	Masithah and W Tjah	ıjaningsih	
	View article	🔁 PDF	
	etween ectoparasite imp (<i>Litopenaeus ve</i>	e infestation and the total plate count of <i>Vibrio</i> sp. in <i>annamei</i>) in ponds	012081
G Mahasri, Rozi, A	T Mukti, W H Satyan	tini and N M Usuman	
	View article	🔁 PDF	
OPEN ACCESS In vitro study of a bacteria	an ethanolic extract	of coffea leaves to inhibit freshwater pathogenic	012082
H Kenconojati, MF	Ulkhaq, DS Budi and	MH Azhar	
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Gills and swimming leg histopathologies in pacific white shrimp (*Lithopenaeus vannamei*) from ponds exposed to the immunogenic membrane proteins of *Zoothamnium penaei*

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	View article	🔁 PDF	

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	View article	🔁 PDF	

OPEN ACCESS			012100
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	View article	PDF	
Indonesia		s (Class Demospongiae) collected from Biak, o, D C Pratiwi and M A P Panjaitan	012102
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 OPEN ACCESS Antibacterial activity in <i>Litopenaeus</i> shut E Saraswati and AS + Open abstract 	rimp <i>vannamei</i>	ngulata herb extract on white feces diseases (WFD)	012103
	rps (Cyprinus carp	gcl2) on the Changes in Hematology and Blood <i>io</i>)	012104
with Heavy Metal	Cadmium (Cd) in	thological Liver (Mystus nigriceps) Accumulated Ketingan Estuary, Sidoarjo - East Java, Indonesia	012105
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,	OPEN ACCESS Texture profile of (BGF) and wheat	-	from composite flour Bruguiera gymnorrhiza flour	01210
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	OPEN ACCESS The potential of s	seaweed waste (grad	cilaria sp. and eucheuma cottonii) as a medium	01211
(density fiberboard	d (mdf)-based pot m	naterial for better water use efficiency in tomato plants	
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	+ Open abstract	View article	🔁 PDF	
,		oeptides derived from Angiotensin I Conve	m the chymotrypsin hydrolysate of soft shelled turtle erting Enzyme	0121
	D Y Pujiastuti and J	L Hsu		

OPEN ACCESS			012114
	-	ntion of <i>Kappaphycus alvarezii</i> and <i>Eucheuma</i> e cream raw material product	
I Suryani, D I Perma	ta Sari, D M Astutik a	and A Abdillah	
	Tiew article	🔁 PDF	
OPEN ACCESS			012115
Detection of antib market	iotic-resistant Saln	nonella sp. in the seafood products of Surabaya local	
H Pramono, A Kurni	awan, N Andika, T F	Putra, M A R Hazwin, S Utari, A P Kurniawan, E D Masithah an	nd
A M Sahidu			
	View article	PDF	
OPEN ACCESS			012116
The effect of kapp properties of milk	e	ification on the physicochemical and organoleptic	
D Darmawan, L Sulr	nartiwi and A A Abdi	llah	
	View article	🔁 PDF	
	murium bacteria in	ctric field (HVEF) to reduce <i>Escherichia coli</i> and a red snapper (<i>Lutjanus</i> sp.) fillets	012117
OPEN ACCESS			012118
	n the quality contro	pport consumption bacteria in a fish quarantine ol and safety of fishery products at Tanjung Priok, Jakart	a
Open abstract	View article	🔁 PDF	
OPEN ACCESS			012119
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OPEN ACCESS	012121
DPPH scavenging property of bioactives from soft corals origin palu bay, Central Sulawesi, Indonesia	
W A Tanod, U Yanuhar, Maftuch, D Wahyudi and Y Risjani	
+ Open abstract Tiew article PDF	
OPEN ACCESS	012122
Extraction of bioactive compounds fruit from <i>Rhizophora mucronata</i> using sonication method	
Ernawati, E Suprayitno, Hardoko and U Yanuhar	
← Open abstract	
OPEN ACCESS	012123
Harvesting Chaetoceros gracilis by flocculation using Chitosan	
WAA Yamin, S R M Shaleh, F F Ching, R Othman, M Manjaji-Matsumoto, S Mustafa, S Shigeharu an	t
G Kandasamy	
+ Open abstract 📄 View article PDF	
OPEN ACCESS	012124
The Use of Rajungan (Portunus Pelagicus) Shells as Flour in Wet Noodles Ingredient	
A Rahma, A A Abdillah and E Saputra	
+ Open abstract	
OPEN ACCESS	012125
The Potential of Lindur Fruit Flour (<i>Bruguiera Gymnorrhiza</i>) in Reducing Oil Absorption of Milkfish Nugget during the Deep Frying Process	
A Widyastuti, AA Abdillah and Laksmi Sulmartiwi	
+ Open abstract	
OPEN ACCESS	012126
Chemical and Sensory Characteristics of Flakes Made from Seaweed (<i>Eucheuma cottonii</i>) and Soybean (<i>Glycine max</i> (L.) Merill)	012120
I R Firdarini, Kismiyati and A Manan	
+ Open abstract 🔄 View article 🄁 PDF	
OPEN ACCESS	012127
The Effect of Maltodextrin Concentration on the Characteristics of Snappers' (<i>Lutjanus</i> sp.) Peptone	
R Ningsih, Sudarno and Agustono	
+ Open abstract	
OPEN ACCESS	012128

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Physics and Chemical Characteristics of Sargassum Sp. Seaweed with Addition of Sodium Algina Stabilizer to Different Concentrations				
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Dynamic ratio correlation of N:P toward phytoplankton explosions in intensive systems of white shrimp pond

E D Masithah¹*, D D Nindarwi², D Husin³, T Rahma³

¹Department of Marine, Faculty of Fisheries and Marine, Airlangga University, Surabaya 60115. Indonesia

²Department of Aquaculture, Faculty of Fisheries and Marine, Airlangga University, Surabaya 60115, Indonesia

³Study Program of Aquaculture, Faculty of Fisheries and Marine, Airlangga University, Surabaya 60115, Indonesia

*Coresponding author: endang_dm@fpk.unair.ac.id

Abstract. Phytoplanktons are a supporting microorganism within the activities of vannamei shrimp cultivation. Phytoplankton are useful to control the color and clarity of the water, increasing the dissolved oxygen (DO), controlling the growth of weeds in the bottom of the pond, and absorbing the abundance of organic compounds in the water such as ammonium, nitric and nitrate. This research aimed to find out the influence of the ratio dynamic of N:P toward the abundance of phytoplankton in an intensive system shrimp pond. The main parameters observed were ammonium, nitric, nitrate and phosphate, as well as the abundance of phytoplankton. The study was being held in the laboratory of PT Surya Windu Kartika Banyuwangi, and measurements were also taken of the supporting parameters of pH, clarity, and salinity. The results of the data analysis and study revealed that the dynamic composition of ammonium, nitric, nitrate and phosphate influences the dynamic composition of the phytoplankton in the water. The increasing nitric levels affect and increase the composition of Bacillariophyceae and decrease the abundant composition of Cyanophyceae. An increase in the level of ammonium in the water causes an increase in the abundant composition of Cyanophyceae and a decrease in the abundant composition of Bacillariophyceae, and dynamic PO4 influences the dynamic abundant composition of phytoplankton classified as Bacillariophyceae and Cyanophyceae.

1. Introduction

Phytoplanktons are a supporting microorganism in the context of the activities of Vannamei shrimp cultivation. Phytoplanktons are important as natural feed due to its characteristic as the foundation of the food chain in the aquatic system [1]. In the activities of intensive shrimp cultivation, phytoplankton are also important when it comes to controlling water color and turbidity, as well as increasing the level of dissolved oxygen (DO) in the water, restraining the growth of the moss in the bottom of the pond, and also absorbing harmful organic compounds like ammonia, nitrite and nitrate, which are necessary nutrients for growing phytoplankton [2].

The diversity and abundance of phytoplankton is useful as stability parameter regarding the nature of the water; a high diversity of phytoplankton species and the prevalence of a high number of individuals of each species means that the water quality is in a range that is suitable for the growth of cultivation organisms [2]. An excess density of plankton shows there to be a discrepancy in the aquatic

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condition; an increase of plankton density in daylight can cause a high saturation level of oxygen up to 250% and this generate eboligas in gills; this causes death in shrimp. At night, there will be a lack of oxygen because of the high respiration process of the phytoplankton [3].

The problem formulated in this research was: 'Does the influence of the ratio of dynamic N:P have an effect on the abundance of phytoplankton in an intensive system shrimp pond?' The aim of this research was to discover the influence of the ratio of dynamic N:P on the abundance of phytoplankton in an intensive system in a shrimp pond.

The benefit of this research was that it has been able to provide information related to the influence of the N:P conversion ratio in the context of affecting the diversity and abundance of phytoplankton in one of the intensive system shrimp ponds. Therefore the potential of various species of phytoplankton can be predicted.

2. Methodology

2.1 Tools and materials

The tools used in this research were as follows: a DO meter, spectrophotometer, secchi disk, refractometer, pH pen, plankton net, bucket, ropes, hemocytometer, drop pipette, microscope, glass object, glass cover, sample bottle, burette, stative, clamp, a 100 ml measurement glass, a 300 ml Erlenmeyer glass, a Erlenmeyer 500 ml glass, a volume pipette of 2 ml and a filler/bulb. The materials being used in this research were: a water sample from the intensive vannamei shrimp pond in PT. Surya Windu Kartika in Banyuwangi Regency, a PP indicator, 1% aquadest, ammonia test kit, the reagent of water hardness, nitrite, nitrate and phosphate.

2.2 Research methods

The research methodology used was a survey, which is a research method that aims to describe both existing and ongoing phenomena. The research was applied without manipulating or changing the free variables, in order to describe the real condition [4]. The research plan was done by the measurement of the water quality at 3 sample points to observe the water quality and 12 sample points to observe the abundance of phytoplankton. A sample is taken at the 12 sample points in the intensive shrimp pond of PT. Surya Windu Kartika. Samples to observe the water quality of DO, temperature, pH, salinity, water level, abundance and the diversity of the plankton were taken twice a day at 4 PM, depending on the availability of sunlight. The measurement of the water quality parameters of ammonium, ammonia, nitrite, nitrate, and phosphate were taken once a day at 7 AM. The sample plankton was taken using a plankton net, and were then observed under a microscope using the direct measurement method using a Sedgwick Rafter at 100 to 400 times enlargement, twice repeated. The water quality observation of ammonium, ammonia, nitrite, nitrate and phosphate was directly performed at 3 sample points using a sample bottle, and they were then observed using the spectrophotometer adjusted to the water. The research preparation stage was applied to prepare the necessary tools and materials, i.e. pH paper, secchi disk, plankton net, bailer, hand counter, hemocytometer, dropping pipette, microscope, object glass, cover glass, test kit, sample bottle, and lugol. The water samples, as the material to observe the diatom abundance, were taken at 3 stations, which were the pond plots with 4 points in each corner of the intensive system for data clarification. The samples were taken with filtered water using a plankton net, to calculate the density. The samples had to be directly brought to the laboratory to be observed and analyzed. The samples were observed at 100 and 400 times the enlargement under a binocular microscope with the direct calculation done using a hemocytometer. The samples needed to be directly observed to maintain the quality of the phytoplankton. The samples to observe the ammonium, nitrite, nitrate, and phosphate were taken in the morning at 5 AM, while the water samples to observe the plankton and water quality were taken in the afternoon at 4 PM.

3. Results and discussion

3.1 Results

The results of this research consist of the main data and supporting data. The main data is the numbers involved in the ratio of N:P and the abundance of phytoplankton. The supporting data was water quality, including the acidity level (pH) and clarity.

3. 1.1 Grade of N: P ratio

The data for the ratio of N:P was obtained from the data of the total nitrogen in the ammonia, ammonium, nitrate, and nitrite compared to phosphate; this then became the grade of the ratio of the dynamic N:P. The data used was the result calibration done every 3 days. The results of the ratio of the dynamic N:P can be seen in the following graphic.

The measurement result of the N:P ratio movement in plot 1 showed a stable level of nitrate at DOC 33-48, and a high fluctuation at DOC 15 by 15 mg/L. It then decreased again the next day. The dynamic condition of ammonium occurred in plot 1 with an adequate fluctuation and there was a frequent level of contact between ammonium and nitrate at DOC 27-30, 30-34, 45-48 and 55-58. In plot 1, there was a high level of ammonium and nitrate at DOC 31, 49, 52, and 55. The phosphate level in plot 1 was generally stable and increased at DOC 46-61.

The measurement result of the nitrite and phosphate in plot 4 showed a highly dynamic situation at DOC 51 until the end of the observation session, with a nitrite increase occurring in DOC 51, 57 and 60 with the highest level of nitrate being up by 25 mg/L at DOC 60. The level of nitrite as an intermediate nutrient significantly increased at DOC 60 of 20 mg/L. The other nutrients in plot 4 didn't occur significantly or in a dynamic condition. The nitrite level in plot 4 significantly increased to 20 mg/L, which is above the threshold of 1.0 mg/L. This can be dangerous to live organisms in the aquatic system [5].

The observation result of the dynamic situation of nitrate and phosphate in plot 7 showed that there was no significant dynamic situation from the initial observation until DOC 48. The increased and decreased levels of ammonia, nitrite, nitrate, and phosphate in DOC 24-48 were about 0.1 - 3.5 mg/L. The dynamic level of nitrate significantly increased at DOC 57 and 60 to be the highest at 60 ml/L, while the other nutrients such as ammonia, nitrite, nitrate, and phosphate did not significantly increase; they only went up about 5-15 mg/L in DOC 51-60.

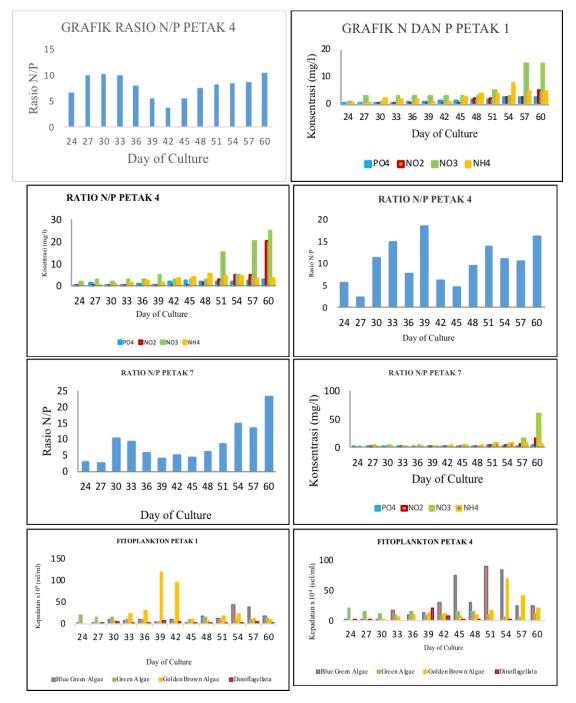
3.1.2 Plankton abundance

We discovered phytoplankton in all of the observation stations, consisting of 19 genus from 4 classes. The 4 classes were Cyanophyceae (6 genus), Bacillariophyceae (9 genus), Chlorophyceae (3 genus) and Dinophyceae (1 genus). All of the discovered genus could be seen in Table 4. The abundance of phytoplankton in this research consists of the density level in relation to the total number of cells in the water and the percentage composition of phytoplankton in the water. The data was taken by observation every 3 days.

The observation results of the phytoplankton abundance in plot 1 were generally dominated by phytoplankton of the Bacillariophyceae class. The increased dynamic situation of Bacillariophyceae started at DOC 30 and gradually increased until it achieved the highest density at DOC 39. It then decreased with no significant dynamic situation present until the end of the observation session. Significantly increased phytoplankton also occurring in the class of Cyanophyceae, which increased significantly at DOC 54. The phytoplankton of Clorophyceae and Dinoflagellate didn't have a significant condition, but their existence in plot 1 was always consistent. This shows the stability condition of the water in plot 1. There was a significant dynamic situation at the end of the observation session at DOC 54.

The observation results of the phytoplankton abundance in plot 4 showed there to be highly dynamic conditions between the phytoplankton of class Cyanophyceae. The phytoplankton of class Cyanophyceae started to have a dynamic condition at DOC 42 and achieved the highest density at DOC 51; this shows the dynamic condition of Cyanophyceae in decreased aquatic condition. In plot 4,

the dynamic condition of the phytoplankton of class Bacillariophyceae was generally stable, with the highest density at day 54. In general, the highest abundance of phytoplankton in plot 4 occurred at DOC 54, with the domination of class Cyanophyceae and Bacillariophyceae. The observation results of the phytoplankton abundance in plot 7 showed that the highest density was of the phytoplankton of class Bacillariophyceae at DOC 39. Besides that, the phytoplankton of class Bacillariophyceae significantly fluctuated in condition from DOC 33 to 39, with the fluctuations increasing and decreasing until the end of the observation session. The phytoplankton class of Blue-green algae did not occur in a dynamic condition and were more stable compared to Bacillariophyceae; the highest growth of Cyanophyceae occurred at DOC. In plot 7, the phytoplankton of class dinoflagellate highly increased at DOC 54.



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Figure 1. (1) N: P ratio of plot 1 during research, (2) Ammonium, Nitrite, Nitrate and Phosphate Plots 1 during the study, (3) N: P ratio of plot 4 during research, (4) Graphs of Ammonium, Nitrite, Nitrate and Phosphate Plot 4 During Research, (5) N: P Ratio 7 during Research, (6) Ammonium, Nitrite, Nitrate and Phosphate Plot 7 During Research, (7) Phytoplankton Plot 1 During Research, (8) Phytoplankton Plot 4 During Research, (9) Phytoplankton Plot 7 During Research

3.2 Discussion

The diatom nutrients in the aquatic system are important for the existing life of microorganisms, in particular in aquatic cultivation where the existence of microorganisms like phytoplankton and bacteria is important in relation to the success of cultivation activities. The most influencing nutrients in the lives of phytoplankton are divided into macro elements and microelements, which is determined based on the needs of the phytoplankton towards the elements. Among the various nutrients, there are 2 important nutrients that play the role of being a divider factor in the lives of phytoplankton i.e. N and P [6].

N and P are important nutrients in the viability of phytoplankton, both in water in general and in cultivation water. The two nutrients play a role as the media of energy transfer in the cells and also as an essential element in the formulation of the phytoplankton cells [7]. The influence of N and P is not only limited to the ratio dynamic N:P in the water, but it is also a dynamic form of each element, like ammonium and nitrate in water and the dynamic condition of phosphate and nitrite.

Nitrate and ammonia are the most dominant form of the N elements that influence the dynamic condition of the phytoplankton in the water. This is because nitrate is formed of N, which is directly absorbed by the phytoplankton. Ammonia is the initial material in the nitrification process in cultivation water, and it stimulates the growth of certain phytoplankton. According to previous studies [6], the increase in the content of nitrate in the aquatic system is able to increase the domination of Bacillariophyceae and decrease the domination of Cyanophyceae. Meanwhile, an increase of the ammonia in water is potentially decreasing the population of Bacillariophyceae and increasing the density of Cyanophyceae.

Based on the research, the observation data from 3 observation stations (plot 1 DOC 33 and 51; plot 4 DOC 27, 33, 51 and 57; and plot 7 DOC 27, 36 and 51) showed there to have been an enhancement of the nitrate level in the cultivation water affected by the increase in the domination of Bacillariophyceae and a decrease in the phytoplankton class of Cyanophyceae. Besides that, the decrease in the nitrate level caused a decrease in the Bacillariophyceae domination in the water. It aligns with the statement in a previous study by [6] on the enhancement level of NO₃, which is able to increase the abundance of Bacillariophyceae in an aquatic system.

Based on the results of the data analysis of the correlation of the decreased dynamic condition and dynamic abundance of phytoplankton, there are 4 conditions that lead to phytoplankton dynamic change in the water. This includes crashing between NO₃ and NH₄ causing a dynamic change in the Bacillariophyceae asin plot 1 at DOC 30 and 54; plot 4 at DOC 30; and plot 7 at DOC 33 and 39. The enhancement of PO₄ influences the dynamic condition between the nitrate level and the phytoplankton like in plot 1 at DOC 54 and in plot 7 at DOC 39. This is because the enhancement of the PO₄ levels in the water is affected by the decrease in the ratio of N:P as supported by the Bacillariophyceae growth. The significant enhancement of the NO₂ levels influences the dynamic of the nitrate in relation to the dynamic condition of the phytoplankton in the water, as in plot 4 on DOC 54 and 16 and plot 7 on DOC 57 and 60. This happened because the NO₃ at a level of 1.0 ppm was toxic toward aquatic organisms, including phytoplankton [5].

Ammonia is an organic compound that influences the dynamic condition of the phytoplankton in water, in particular, cultivation water. This is because the existence of ammonia in the water, in general, is increasing along with the increase in the age of the cultivation. According to the previous studies [6], the enhancement of the ammonia level in the water is able to decrease Bacillariophyceae

and increase the domination of Cyanophyceae. This is suitable for the observation results in plot 1 at DOC 30, 42, 48 and 54, plot 4 at DOC 30, 33, 42 and 45 and plot 1 DOC 39, 42, 48 and 51. This is where the ammonia level increased and was followed by the domination and enhancement of Bluegreen algae. The decreased level of ammonia in the water also showed there to be a decrease in the domination of phytoplankton class of Cyanophyceae. It is suitable as seen in the observation data in plot 1 at DOC 33, 39 and 57, plot 4 at DOC 57 and plot 7 at DOC 36 and 60.

There are several conditions that lead to an exception in the correlation of the ammonium and phytoplankton dynamic condition, such as an intensive fluctuating condition of the nitrate level in the water. This influences the domination of Cyanophyceae in the water. It is possible that this happens because high levels of nitrate may prompt Cyanophycean growth, like in plot 4 on DOC 27 and 60. Meanwhile, in conditions where there are lower nitrate levels or there is a crash of nutrients between ammonium and nitrate, this can push Cyanophyceae growth. The PO₄ dynamic condition in the water is also important in relation to phytoplankton domination in the water as in plot 4 DOC 39 and 48. Cyanophyceae domination is more affected by the availability of PO₄ than ammonia in that aquatic system. As in the previous study [8], water condition with PO₄ levels higher than 1.10 ppm can cause the phytoplankton domination of Cyanophyceae.

A condition where the water nutrients are not consumed by phytoplankton can be seen in plot 1 DOC 39, 57 and 60, plot 4 DOC 39, 57 and 60 and plot 7 DOC 60. In this condition, nitrate as dividing factor is abundant but the phytoplankton, in general, is decreased or increased. The phytoplankton class of dinoflagellate is a bioindicator of discrepancies in aquatic systems. Therefore, the nutrient concentration in water is not fully absorbed by the phytoplankton. Generally, nutrients cannot be absorbed because of several factors, such as a low level of DO, a high pH in the water, a high level of nitrite, a high level of ammonia and also significant changes in the water temperature [8].

4. Conclusion

Based on the results of the data analysis and the discussion related to the correlation of the ratio of N:P towards the abundance of phytoplankton, it can be concluded that the value of the ratio of N:P influences the composition of the phytoplankton class in the water of Vannamei shrimp cultivation. The dynamic composition of ammonia, nitrite, nitrate, and phosphate influences the dynamic composition of phytoplankton in aquatic systems. The enhancement of the nitrate level increases Bacillariophyceae and decreases the abundant composition of Cyanophyceae and decrease the abundant composition of Cyanophyceae and decrease the abundant composition of PO₄ influences the dynamic composition of the phytoplankton classes of Bacillariophyceae and Cyanophyceae.

5. References

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