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Original Paper

APLICATION OF CHITOSAN FOR WATER QUALITY AND MACROBENTHIC FAUNA REHABILITATION IN VANNAMEI SHRIMPS (*LITOPENAEUS VANNAMEI*) PONDS, NORTH COAST OF SEMARANG, CENTRAL JAVA - INDONESIA

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ABSTRACT

Chitosan was widely known as natural food preservative such as noodle, meat ball, soy-tofu, wide variety of fresh vegetables, fruits, meat and fish products, etc. Chitosan is a non-toxic substances, has no side effect as human food and was made from the shell of shrimps (Penaeidae), or swimming crabs (Portunus pelagicus). Chitosan is primarily a polysacharids, with β-1,4-2 amino-2-deoxy- β- D-glucopyranose. The biopolymer has various noble functional characters such as anti-moth, anti-bacterial, coagulating agent for suspension, or heavy metals. This substance had been examined in the Lab of Natural Product, Diponegoro University and give a significant effect as bacteriostatic and bactericides for pathogenic bacteria such as Salmonela.sp; Pseudomonas, E.coli, B.subtilis, S.aureus, P.aeruginosa. In the study, liquid chitosan (200 ppm) was used as anti- harmful/ pathogenic bacteria in the brackish water pond so that can eliminate and control the bacterial or virus outbreak which so far is the main problems in the fish and shrimp culture activities. The use of chitosan in the brackish water ponds was carried out at a semi-traditional ponds at Mangkang Kulon north Semarang coastal area. The experiment had significantly decrease the suspended solid or increase water transparency, and the organic content (by 5 %) of the bottom susbtrate. Increase the abundance of important macro-benthic organism such as Polychaeta (7-fold) as important natural feed for the cultured fish and shrimp as well as increase the diversity index of total macro-benthic organism. Decrease the growth of disease vector benthic organism such as Cherithidae (Gastropods) by 24.5% . With the application of chitosan had significantly prevent the outbreak of fish and shrimp disease and increase up to 80 % the survival rate of Vanamae, as well as tiger shrimp Penaeus monodon, and polkadot grouper (Cromileptes,sp) in another field application.

Key words : chitosan; water-quality; rehabilitation; macro-benthic; vanamae

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INTRODUCTION

Chitosan is a biopolymer that has some unique characters that can be used for several applications and purposes, in small scale as well as in industrial scale comercially (Rha Chokyun, 1980). Rouget who was known the modification of chitin into chitosan in 1859 (Wibowo, 2003). Furthermore Wibowo (2003), define the chitosan as a long-chained polymer of 2-deocxy-2-amino-glucose or known as deacetilated chitin. Chitosan has a ($C_6H_{11}NO_4$) with molecular weight of 10^4-10^6 known also as β-1,4-2 amino-2-dioxy-D-glucose, a polymer with one amino (NH_2) cationic and 2 free-hydroxyl (OH) in each glucose ring. According to Hirano (1986) chitosan was firstly described in 1811 and named after Odier in 1823, but no further information on the use of chitosan as a cellulose biopolymer commercially. Suhardi (1993), the name of chitosan was used to refer to D–glucosamine, where some monosacharides in the polymer has an acetyl molecule. Muzzarelli in Hartoko, *at.al.,* (2007a) stated that chitin has 10% deacetylation degree and chitosan has a range of 90 – 100%. Chitosan is

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a poly electrolit cationic substances (positive charge) and ready to capture any negative ions. This is why chitosan was regarded as a very important bio-polymer (Roberts, 1992), such as to promote the growth of natural microbes (Sandford, 1991). The largest scale of chitosan use was in the water treatment for heavy metal and radio active pollutant. Chitosan is a coagulant that able to capture substances such as suspended solid, colloids and suspended solid in the water, then to sink or to float separation from the water (Widodo, *et.al.,* 2005).

In the last 10 years since 1998, almost all shrimp-ponds area along the north coast of Java were ruined by pathogenic microbial desease (desease out break). This was caused by over carrying capacity as the impact of over feeding, thus uncontrolled feed waste, decrease of the water quality and finally desease out break and failure of shrimp culture, loss of capital and traumatic experience for fish farmers to this date (Hartoko, *et.al.,* 2007a,b,c,d). During the golden shrimp period 1975 – 1980, the main marine product of Semarang was marine shrimp espescially bottom/ demersal Penaeid and Metapenaeid fishery, that was caught using Trawl fishing gears. But in other point of views, demersal fishery is known as a highly *vulnerable* since these species has a very limited movement and passive, and tend to *over-fishing*. The length of Semarang coastal line is 21.6 km consist of fish and shrimp ponds about 1040 ha. Therefore, one of its potential resouces use is to develop shrimp pond production or *marine resource and fishery based industries and economics*.

The white shrimp American Vannamei (*Litopenaeus vannamei* Boone) was firstly entering Indonesia in May 2002, with import of 2,000 vannamei brood stock, and 5 million of post larvae from Hawai and Taiwan, and 300,000 from Latin America. About 110 species out of it belongs to genus Penaeid, and one of them is genera Litopenaeus, species *L.vannamei* (Haliman and Adijaya, 2005). According to Food and Agriculture Organization (FAO, 1989) there were about 343 shrimp species that can be cultured commercially.Vannamei shrimp have several names, internationally known as *Pacific White Shrimp*, *whiteleg shrimp* (English), *crevette pattes blances* (France), and *camaron patiblanco* (Spain). Before developed in Indonesia, Vannamei shrimps had already developed in south America, such as Ecuador, Mexico, Panama, Colombia, and Honduras. Their average production reach 10% of total world form culture and marine catch (Haliman and Adijaya, 2005). Vannamei shrimp was grouped into phyllum Arthropoda consist of thousand of species. The dominant is sub-phyllum Crustacea with have 3 pairs of walking legs, espescially ordo of Decapoda, such as *L.chinensi*, *L.indicus*, *L.japonicus*, *L.monodon*, *L.stylirostis*, and *L.vannamei* (Haliman and Adijaya, 2005). Spesific morphology is white transparent with a blueish chromatophores concentrated near to the telson and uropod. Having two rostrum teeth in the ventral and eight or nine rostrum teeth in the dorsal (Subaidah and Harjono, 2003). Vannamei shrimp belongs to subgenus Litopenaeus and the female species has a open thelycum without a cover or *seminal receptacle* (Wyban and Sweeney, 1991). The taxonomy of Vannamei shrimp (*L.vannamei*) was as follows : Phylum :Arthropoda, Subphylum : Crustacea, Class : Malacostraca, Subclass : Eumalacostraca, Superordo : Eucarida, Ordo: Decapoda, Subordo : Dendrobranchiata, Family : Penaeidae, Genus : *Litopenaeus,* Spesies: *Litopenaeus vannamei.* Vannamei shrimp is a nocturnal animal, with more activity during the night. Matting proces started with a sudden jump of the female and ejecting the egg cells. At the same time the male shrimp did ejecting the sperms. The mating process take about one minute. A couple of Vannamei weighted of 30 – 45 grams will produce about 100,000 – 250,000 eggs with diameter of 0.22 mm (Haliman and Adijaya, 2005).

Vannamei shrimp (*L.vannamei*) was considered as one spesies that can be developed to increase the national shrimp production, since it has high productivity, survival rate and fast growing period (3 months). More disease resistance compared to other species and wider salinity tolerance (Wyban, and Sweeney, 1991). With the rehabilitation and optimalization of the *L.vannamei* will make opportunity to support national shrimp industry. The study of chitosan application using two unit of 0.5 ha of shrimp pond, semi-intensive level with 30,000 30-days post larvae at Mangkang Kulon district, Semarang city. Objectives of the current research are to study the effect of chitosan to

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the macrobenthic fauna in the Vanname shrimp pond.

Materials And Methods

Shrimp pond preparations, construction and rehabilitation were carried out before the experiment. These include as rehabilitation of pond dyke holes and damages caused by crabs, and to rise the height of dyke with 0.5m more than the traditional ones, to anticipate possibility of tide flood in the area. Tide water inflow gate, deeping the bottom pond floor to make sure the water depth was about 60 cm and 100 cm at the surrounding canal inside the pond at mean sea level. Natural fertilizer had been applied in order to boost the growth of bluegreen algae, plankton, Copepods, and benthic organisms. The concentration of 100 ppm liquid chitosan in a 10 cm pond seawater was applied during the fertilization period (3 – 5 days) for water quality improvement, suppresion of parasitic growth and to increase Survival Rate (SR) of shrimp larvae. A 30-days of Vannamae post larvae were used to be reared to reach the juvenile size (about 5 cm length). Daily seawater exchange by means of high tide seawater inflow and pellet feeding in the morning and afternoon about 1 kg/day had been conducted throughout the study. A weekly chitosan solution (200 ppm) application during shrimp growing periode (after juvenile) from month-1 until month-3. A weekly measurement and samples of total suspended solid (TSS), chlorophyl-a, organic content and benthic organism in the substrate were collected during the three months of Vanamae shimp culuture in the pond.

Result And Discussion

Total Suspended Solid (TSS) in the chitosan treated Vannamae shrimp pond water in three month period has decreasing from 0.68 mg/l to 0.35 mg/l, while in a non-chitosan pond was 0.74 mg/l. After a grain size analysis on the samples of the pond substrate in Mangkang Kulon-Semarang revealed that the pond substrate was dominated by silt with percentage range of 68.62 – 73.22 %. With the application of chitosan in the shrimp pond had been able to increase the organic content in the substrate by 40% after three months period as shown in **Fig. 1.** As widely known that organic content in the substrate is a renewable and recycled materials or degrade organically by microorganisms without polluting the environments (Hartoko, 2010 in press). In this specific case, high organic content in the pond substrate was considered will in turn increase the growth of bottom blue-green algae, one of its role is as source of nitrogen. The higher the organic content, the higher is nitrogen content. The organic content can affect the shrimp pond fertility, but excessive organic content will endangered to the cultivated organisms in the ponds (Afrianto and Liviawaty, 1991). Classification on the organic content in the sediment according to Reynold (1971) was as follows : a very high organic content is more than 35%; high organic content : 17-35%; moderate organic content : 7-17%; low organic content : 3.5-7% and low organic content is less than 3.5%.

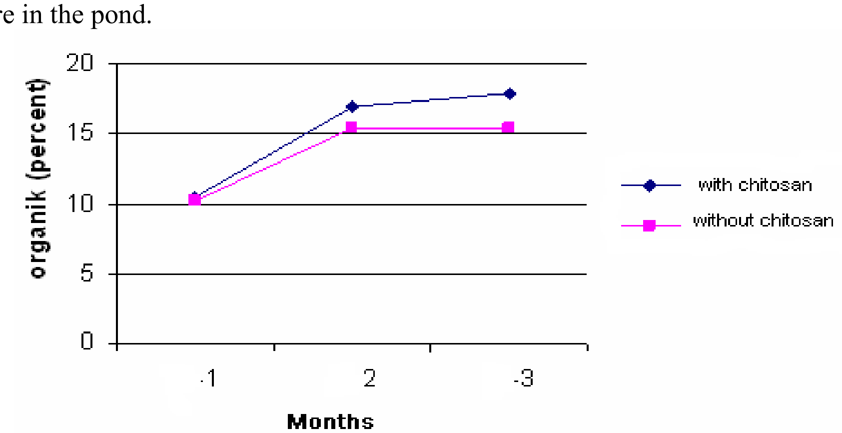

Fig 1 : Increase of organic content (percent) in the shrimp pond after three months chitosan treatment

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Identification on the macrobenthic samples had revealed the presence of two classes of macrobenthic Polychaeta (5 genera) and Gastropods (5 genera). Macrobenthic abundance in the chitosan treated shrimp pond was gradually crease by 1211 ind/m^2 (month-1), 1306 ind/m^2 (month-2) and 1352 ind/m^2 (month-3). While macrobenthic abundance in the control shrimp pond (no chitosan treatment) was found much lower than those chitosan treated varies from 708 ind/m^2, 691 ind/m^2, and 708 ind/m^2 in three months as shown in **Table 1** and **Table 2**. Diversity index in the chitosan treated shrimp pond were (2.22; 2.15 and 2.21) slowly decrease while in non-chitosan shrimp pond were (1.84; 1.87 and 1.92) slowly increase during three months. The similarity index of chitosan treated shrimp pond were 0.97; 0.98 and 0.96, and non-chitosan shrimp pond were 0.95; 0.90 and 0.92 as presented in **Table 3**.

Table 1. Abundance, Diversity Index and Similarity Index of Chitosan and Non-chitosan pond

No		With Chitosan; Mean	Without Chitosan
1	Abundance	1250 ind/m^2	700 ind/m^2
2	Diversity Index	2.2	1.8
3	Similarity Index	0.97	0.93

Table 2. Individual and relative abundace of macrobenthic organisms in month-1

No.	Genera	With chitosan		Without chitosan	
		IA (Ind/m^2)	RA (%)	IA (Ind/m^2)	RA (%)
	Polychaeta				
1.	*Nephthys* sp	142	11.69	-	-
2.	*Capitella* sp	173	14.29	31	4.44
3.	*Nereis* sp	**204**	**16.88***	79	11.11
4.	*Marphysa* sp	126	10.39	-	-
5.	*Prionospio* sp	110	9.09	-	-
	*) highest number				
	Gastropoda				
6.	*Telescopium* sp	110	9.09	142	20.00
7.	*Terebralia* sp	126	10.39	157	22.22
8.	*Cerithidea* sp	126	10.39	142	20.00
9.	*Turitella* sp	47	3.90	63	8.89
10.	*Melanoides* sp	47	3.90	94	13.33

IA : Individual Abundace (ind/m^2)
RA : Relatif Abundance (%)

Table 3. Diversity and Similarity Index of Macrobenthic Organisms After Three Months Chitosan Treatment in the Vannamae Shimp Pond

Period :	Diversity Index		Similarity Index	
	Pond with chitosan	Pond without chitosan	Pond with chitosan	Pond without chitosan
Month-1	2.224	1.844	0.966	0.948
Month -2	2.154	1.873	0.980	0.901
Month -3	2.213	1.918	0. 961	0.922

Relative abundance was known as percentage number to the total of individu in a given area (Odum, 1971), and considered that relative abundance as important indicator based on total individu as response of the treatment. While diversity index is statement on the overview on the community structure sistematically so that can be more easy to analyse information on the number of the genera or species in a given area. Furthermore it was stated that diversity index is define as comparations on the number of genera or species in a given area . Where the more high number of the diversity index will have higher number of species. Lee *et al.,* (1978) and Knobs (1978) in Anggoro (1992), shown the level of shrimp pond suitability based upon the Diversity index (H') into four classes as shown in **Table 4**.

Table 4. Suitability classes of shrimp ponds water quality based upon the macrobenthic diversity index

Diversity index of macrobenthic	Water quality status for shrimp pond :
< 1,0	Heavy polluted, water quality is difficult and not suitable to be used for shrimp or marine culture activities
1 – 1,5	Moderate to heavy polluted, not suitable for shrimp or marine culture
>1,5 -2,0	Light to moderate pollution, suitable for Bivalve, oyster, Milkfish or seaweed culture
> 2,0	Light pollution, suitable for Bivalve, oyster, Milkfish, shrimp or seaweed culture

Source : Anggoro 1992

Individual macrobenthic abundance in the first month samples was shown in **Fig. 2 and Fig. 3** revealed an important finding that the dominant genera in the chitosan treated pond was *Nereis* sp (Polychaeta – which is an edible natural feed for cultivated shrimp), with total of 10 genera of macrobenthic, such as *Nephthys* sp, *Capitella* sp, *Nereis* sp, *Marphysa* sp, *Prionospio* sp, *Telescompium* sp, *Terebralia* sp, *Turitella* sp, *Cerithidea* sp and *Melanoides* sp. While non-chitosan ponds were only 7 genera was found, that are Capittela.sp; *Nereis* sp, *Telescompium* sp, *Terebralia* sp, *Turitella* sp, *Cerithidea* sp and *Melanoides* sp.

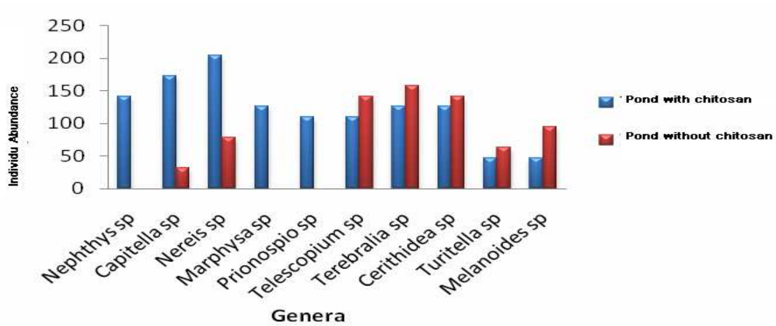

Fig. 2 Histogram of the first month macrobenthic individu abundance

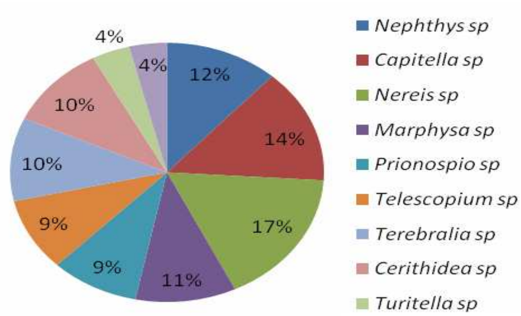

Fig. 3 Pie diagram of the first month macrobenthic abundance

Table 5. Individual and relative abundance of macrobenthic organisms in month-2

No.	Genera	Pond with chitosan		Without chitosan	
		IA(Ind/m^2)	RA(%)	IA (Ind/m^2)	RA(%)
	Polychaeta				
1.	*Nephthys* sp	173	13,25	63	9,09
2.	*Capitella* sp	157	12,05	47	6,82
3.	*Nereis* sp	220	16,87	31	4,55
4.	*Marphysa* sp	173	13,25	0	0
5.	*Prionospio* sp	126	9,64	0	0
	Total	849		141	
	Gastropoda				
6.	*Telescopium* sp	142	10,84	157	22,73
7.	*Terebralia* sp	126	9,64	142	20,45
8.	*Cerithidea* sp	126	9,64	173	25
9.	*Turitella* sp	63	4,82	47	6,82
10.	*Melanoides* sp	0	0	31	4,55
	Total	457		550	
	Grand Total	1306	100	691	100

IA : Individu Abundance (ind/m^2)
RA : Relatif Abundance (%)

Individual and relative abundance of macrobenthic organism of month-2 was shown in **Table 5,** with total of 1306 ind/m^2 found in pond with chitosan and was found 691 ind/m^2 in pond without chitosan. The highest genera was *Nereis* sp (Polychaeta), with total of 9 genera of macrobenthic that were *Nephtys* sp, *Capitella* sp, *Nereis* sp, *Marphysa* sp, *Prionospio* sp, *Telescompium* sp, *Terebralia* sp, *Cerithidea* Sp, and *Turitella* sp found in pond with chitosan treatment. While in pond without chitosan were only 7 genera : *Nephthys* sp, *Nereis* sp, *Marphysa* sp, *Telescompium* sp, *Cerithidea* sp, *Melanoides* sp, and *Terebralia* sp as shown in **Fig. 4** and **Fig. 5.**

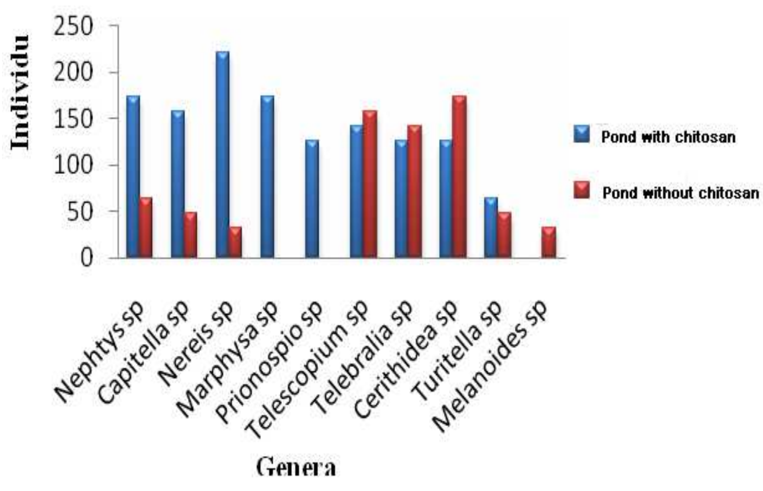

Fig. 4. Histogram of marcobenthic organisms in month-2 substrate samples in Vannamae shrimp pond

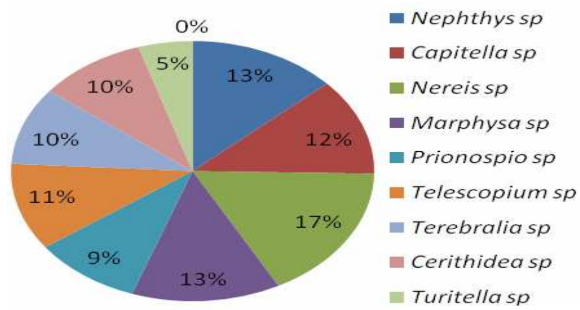

Fig. 5. Pie diagram of relative abundance macobenthic organisms in month-2 substrate samples in Vannamae shrimp pond

Table 6. Individual and relative abundance of macrobenthic organisms in month-3

No.	Genera	Pond with chitosan		Pond without chitosan	
		IA(Ind/m^2)	RA(%)	IA(Ind/m^2)	RA(%)
	Polychaeta				
1.	*Nephthys* sp	189	13,95	0	0
2.	*Capitella* sp	173	12,79	47	6,67
3.	*Nereis* sp	236	17,44	47	6,67
4.	*Marphysa* sp	157	11,63	31	4,44
5.	*Prionospio* sp	157	11,63	0	0
	Total	912		125	
	Gastropoda				
6.	*Telescopium* sp	110	8,14	142	20,00
7.	*Terebralia* sp	94	6,98	142	20,00
8.	*Cerithidea* sp	126	9,30	173	24,44
9.	*Turitella* sp	47	3,49	63	8,89
10.	Melanoides sp	63	4,65	63	8,89
	Total	440		583	
	Grand Total	**1352**	100	708	100

IA : Individu Abundance (ind/m^2)
RA : Relatif Abundance (%)

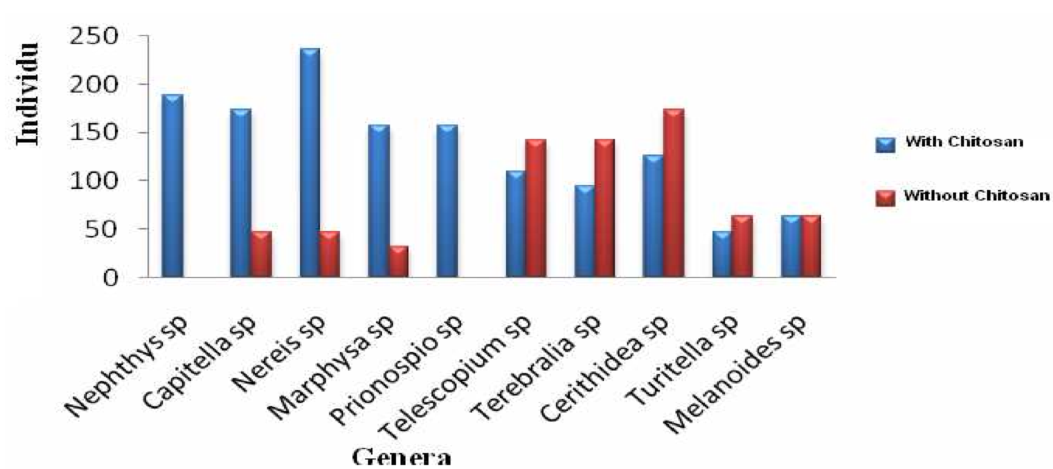

Fig. 6 Histogram of marcobenthic organisms in month-3 substrate samples in Vannamae shrimp pond

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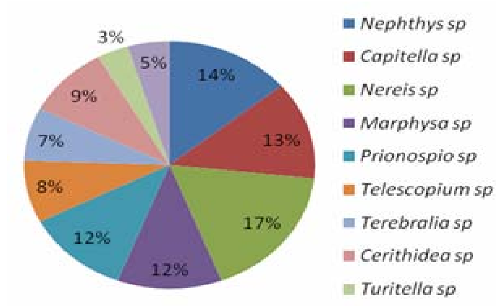

Fig. 7 Pie diagram of relative abundance macrobenthic organisms in month-3 substrate samples in Vannamae shrimp pond

Table 7. Development of Polychaeta and Gastropods in Vannamae shrimp pond

	Macrobenthic Abundance (Ind/m^2)	
	Pond with chitosan	**Pond without chitosan**
Bl-1. Polychaeta	755	110
Gastropods	456	598
Bl-2. Polychaeta	849	141
Gastropods	457	550
Bl-3. Polychaeta	912	125
Gastropods	440	583
	1352*	**708**

As shown in **Table. 6,** total macrobenthic abundance of pond with chitosan in month-3 was 1352 ind/m^2 and in pond without chitosan was 708 ind/m^2. Meanings that with application of chitosan had significantly increase the total macrobenthic in the shrimp pond substrate. **Fig. 7** shows that the dominant macrobenthic abundance in pond with chitosan was *Nereis* sp 17,44 % (236 Ind/m^2). With total number of 10 genera : *Nephthys* sp, *Capitella* sp, *Nereis* sp, *Marphysa* sp, *Prionospio* sp, *Telescompium* sp, *Terebralia* sp, *Turitella* sp, *Cerithidea* sp and *Melanoides* sp. More specifically total individu of Polychaeta was 912 ind/ m^2. While in pond without chitosan total number of genera was only 8 : *Nephthys* sp, *Nereis* sp, *Marphysa* sp, *Telescompium* sp, *Terebralia* sp, *Turitella* sp, *Cerithidea* sp and *Melanoides* sp with abundance of Polychaeta was only 125 ind/m^2 . The data as shown in **Table. 6** explain that with application of chitosan in the Vannamae shrimp pond had consistency in increasing the abundance of the important natural feed biomass Polychaeta from 755, 849 and 912 ind/m^2, and contrary decreasing the organic feeding competitor and disease vector Gastropods in the shrimp pond.

CONCLUSION

With the application of chitosan treatment into the shrimp-pond water had revealed that chitosan can : (a) lower the water turbidity; (b). increase the organic content in the shrimp-pond substrate; (c) had significantly increase the number of total macro benthic precisely Vannamae shrimp, but contrary decrease the disease vector Gastropods such as Cerethidae; (d). increase the diversity index of macro benthic organisms.

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Appendix-1.

Shrimp pond condition before rehabilitation (left-above) and after rehabilitation (right-below)

Inside canal, out-let canal and Vanamei size - 50 (weight 20 gram) after harvest

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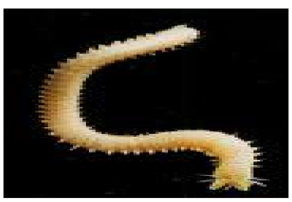
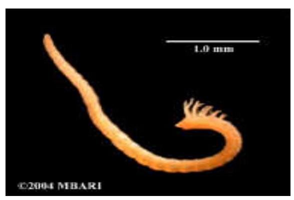
Chitosan biopolymer produced by the Organic Technology Lab,UNDIP used in the experiment

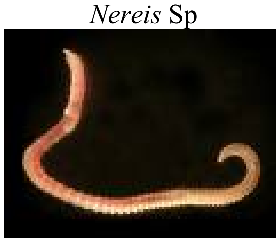
Appendix 2 . Photomicrograf of Macrobenthic duting the study

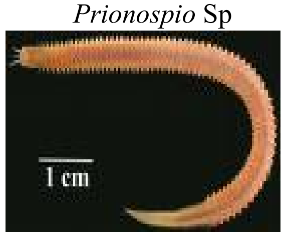

Nereis Sp



Prionospio Sp


Nephthys Sp


Capitella Sp



Marphysa Sp


Melanoides Sp


Cerithidea Sp


Terebralia Sp

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Turitella Sp

Telescopium Sp

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