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Blood cockles *Tegillarca granosa* growth performance

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Abstract
Blood cockles *Tegillarca granosa* culture in natural habitat is one of the way to increase national production in addition to harvesting natural resources today, but culture in pond is rare to Malaysia. Many factors need to be considered before the study of blood cockles in the pond is to be carried out. The presence of plankton as a food source is a key factor in obtaining optimal blood cockles growth. The study was conducted in two ponds with different treatments. Pond one used commercial probiotic for water treatment and pond two was as a control. Fertilization was carried out weekly while water exchange was carried out monthly. Measurement of water quality and chlorophyll a content were performed once a month. As a result of 12 month of culture, blood cockles from pond one average length was 22.85±1.80mm and for pond two average length was 25.36±3.00mm. Blood cockles length growth rate for pond one was 0.66mm/month while for pond two was 0.99mm/month.

Keywords: *Tegillarca granosa*, growth performance, water quality, blood cockles

Introduction
The aquaculture industry has made a significant contribution to the development of the country's fisheries industry through the production of high quality fish, shrimp and molluscs for the local and export markets. Shellfish commodities accounted for 3% of Malaysian aquaculture production in 2016 consisting of bloodcockles (*Tegillarca granosa*), green mussels (*Perna viridis*) and Oysters (Crassostrea spp.). The Department of Fisheries Malaysia (DOF) has targeted aquaculture production of 1.443 million metric tonnes by 2020 where shellfish have the potential to contribute 45,000 metric tonnes (3%) to achieve this target.

Shellfish production once peaked in 2010 with a production of 78,025.70 metric tonnes and accounted for 18.33% of marine aquaculture production under the National Aquaculture production. National shellfish production began to decline in 2011 to 57,544.40 tonnes and continued to decline to the level of 9,596.76 tonnes (3.1% of marine aquaculture production) in 2016 alone. The decline in shellfish landings has been detected in all major shellfish producing states namely Selangor, Perak, Penang and Kedah. The state of Selangor showed a very clear decrease from 41,410 mt in 2010 to only 2,023.65 mt in 2016. The state of Johor has started to become the largest shellfish producing state in the country, similar to the states of Selangor and Perak lately. Shellfish production in Johor in 2010 was 45.5mt and has increased every year to reach 1,263.21tm in 2016. This is also supported by stable shellfish seeding in the state of Johor so that the state of Johor can be a supplier of shellfish seeds to Perak and Selangor in 2020.

Currently, the state of Johor is a major shellfish producer in Malaysia and is seen to have great potential to become a major shellfish producing state apart from Selangor and Perak which have experienced issues of increasing natural mortality due to habitat quality changes, environmental changes and certain chemical pollution.

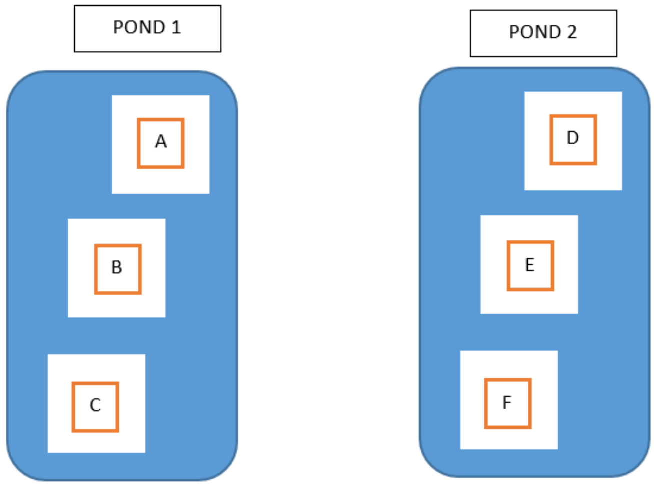
In 2016, a total of 1,260 metric tonnes of shellfish were produced in Johor through aquaculture activities. The highest quality and quality shellfish producers are from Pontian district (907 metric tons).

Objective
To determine the growth rate and survival of *Tegillarca granosa* by using commercial probiotic.

Method and materials

Study Site and experiment design

The study was performed in Muar, Johore the earthen ponds are designated as Pond one and Pond two which both ponds were 0.25ha each. The water depth of ponds ranged between 1.2 and 1.5 m. Water depth is not usually a limiting factor in mollusc culture, however it will determine what culture method can be used (FAO., 1988). Probably the most important aspect with regard to water depth is to avoid long exposure period during the extreme low water spring tides when benthic mollusc such as cockle are cultured. With cockle culture, where planting and harvesting is carried out from a vessel, the culture area should have a water depth of about 1 to 2 m mean tide level (FAO., 1988). Pond one was treated with commercial probiotic whereas pond two was kept as a control pond that is without commercial probiotic. Ponds were initially prepared by drying, tilting and liming.



Stocking

Blood cockle seeds were obtained from Bagan Panchor, Perak. The seeds were stocking 300 seeds/m^2in the experiment. The seeds sizes were in the range from 14.00mm to 15.00mm. These were measured before being transferred into culture ponds. 30 seeds samples were recorded every time from March 2019 to February 2020.

Water Quality Management

Water quality analysis divided into two types of parameter, in-situ and ex-situ. For in-situ, daily measurement was done for salinity, pH, temperature, dissolved oxygen, and turbidity. For ex-situ, monthly measurement was done for nutrient parameters such as nitrate, nitrite, ammonia, ferum and phosphate, as well as total suspended solids (TSS), alkalinity and 'Biological Oxygen Demand' (BOD).

Physical parameters of water such as salinity, temperature, pH, dissolved oxygen and chlorophyll-a were measured monthly using multivariate devices (YSI Model Pro DSS). For turbidity, it was measures by secchi disk. Water samples were collected and nutrients in water were analysed for nitrite, nitrate, ammonia, phosphate, ferum and by using a Shimadzu Uvmini-1240 UV-vis spectrophotometer, meanwhile alkalinity (Titrimetric, method APHA, 1995). Water samples were transported to the laboratory after collection and analysed. Total suspended solids (TSS): known volumes of water samples (100-1000 ml) were filtered through pre-washed, dried and -weighed Whatman GF/F Ø47 mm. After drying, the filters were weighed again and the dry weights were recorded. Water exchanges were carried out in pond at 20% level once a month during high tide.

Blood cockles sampling

The growth rates of cockle in the Pond 1 were plot A, B and C while pond 2 were plot D, E and F. The comparison of growth rates were between the two pond and among the plots. Thirty sample of individual shell from each pond and the experimental plot/replicate were sampled randomly monthly for measurements of shell length and weight. Shell length (mm) of the sampled clams was measured along the greatest anterior-posterior direction using Vernier calliper. The wet weight of clam (g) was measured using a 3-digit digital balance. Growth was expressed as absolute growth rates of the shell length (AGRL) was a follows: AGRL (mm/month) = (average final shell length (AGL_1) – average initial shell length)/ culture period (Δt), where AGL_1 is the length (mm) at a specified time, AGL_o is the length (mm) at time 0, and Δt is the time difference (months) of t_o and t_1.

Average environmental factors were calculated using the recorded values from March 2019 to February 2020. In this study, production potential of blood cockles was determined by the growth rate of the clam in each pond.

Statistical analysis

Statistical analysis was performed by using Microsoft office excel-2010 data sheet analysis tool pack. One- way ANOVA and regression was applied to physical and chemical, AGL and AGRL variables in order to compare 2 Ponds and to evaluate the interaction between them. Regression analysis was used to examine the relationship between average growth rate of blood cockles and environmental factors. Relationship analysis between average chlorophyll-a and TSS of two ponds was also performed. Significance level was $p<0.05$.

Results and Discussion

Water Quality

Water quality is determined by various physio-chemical and biological factors, as they may directly or indirectly affect its quality and consequently its suitability for the distribution and production of fish and other aquatic animals (Moses, 1983). All living organisms have tolerable limits of water quality parameter in which they perform optimal. A sharp drop or an increase within the limits has adverse effects on their body functions (Davenport, 1993: Kiran, 2010). So, good water quality is very essential for survival and growth of blood cockle. Good water quality is characterised by adequate oxygen, proper temperature, transparency and limited levels of metabolites affecting blood cockle culture.

Mean water temperature, salinity, pH, ammonia, nitrite and nitrate did not vary significantly ($p>0.05$) between the two ponds during rearing period. Overall measurement of the physical and chemical parameters of water resulted categorized in reasonable values ranges for blood cockle culture activities in pond 1. In the study the range of salinity between 17 to 28ppt these shown the blood cockles were survival. Japanese recorded between 26 to 30ppt for survival of the cockles (Nakamura, 2005). Generally, the dissolved oxygen (DO) recorded in those ponds from 4.0 mg/L to 7.4 mg/L. In the previous study, Pahri *et al.* (2006) [13] reported the DO around cockles farming area in Jeram, Selangor ranged between 4.028mg/L to 5.895 mg/L. According to Malaysia Marine Water Quality Criteria and Standard (MWQCS), categorized in Class 2 which is DO must be at 5 mg/L to serve marine life, fisheries, coral reefs, recreational and marine culture. While the Class E category needs the DO to be 4 mg/L to fit for mangroves, estuarine and river-mouth water. Particularly, there is a lack of research regarding on how the concentration level of dissolved oxygen influence the blood cockles. However, most bivalves possess the LC50 >2mg/L (Vaquer-Sunyer & Duarter, 2008).

In the study, water temperature recorded range from 28.34 °C to 31 °C. Water temperature is considered to have an impact on bivalve recruitment and growth (Mahapatro *et al*., 2009). Water temperature recorded in area study is similar within to cockle farming site in Pontian, Batu Pahat and Muar. Water temperature was one of the physical parameters that play a role in the life and growth of aquatic biota. Temperature directly affect aquatic organism, especially in the photosynthesis of aquatic plants, metabolic processes, and reproductive cycles.

Other physical parameter, pH recorded range from 7.37 to 8.7. The previous study, pH ranges from 7.67 to 8.03 was recorded in Chilika Lagoon, India which is home to several bivalves (Mahapatro *et al*., 2009). Meanwhile, the *T. granosa* farming in Jeram, Selangor recorded pH range from 6.57 to 7.82 (Pahri *et al*., 2016) [13]. pH acted as the environmental factors that influenced the growth of benthic organisms. It controlled the breakdown of the elements such as phosphorus and nitrogen in water will be affected the phytoplankton to use those elements for growth. The appropriate pH for aquatic animal was in range of 6.5 to 9.0. The high or lower pH would be harmful to the living, mostly be dangerous when pH<4 or pH>11. pH of sediment affected the distribution and activities of marine organisms. Low acidity condition restricted to shell synthesis and decomposition. Changes in pH may be caused by reduced salinity, but fairly drastic differences over a period of time are necessary to cause difficulties with bivalves.

Blood cockles live depend tightly on salinity, they close their shell when salinity falls from 32 to 16ppt, and shell remain closed at salinity below 16ppt (Davenport *et al*., 1986). This prevent clam from feeding during conditions of low salinity. In salinity of 28 to11ppt, *T.granosa* feeds only sporadically and no feeding activities were detected at salinity of 6ppt. Broom also mentioned that salinity above 23ppt would allow *T. granosa* to feed relatively efficiently while salinity less than 20ppt would result in decreased feeding efficiency and activity ((Broom, 1985) [2].

In nature, growth of mollusc is known to be supported by food availability i.e., phytoplankton abundance (Gosling, 2003), which in this case is represented by chlorophyll-a concentration. Apart from phytoplankton, other organic particles were also found to be sources of most clams (Meshram, A.M., 2015). In the muddy habitat where sediment is easily suspended, benthic microalgae and a mixture of detritus or microorganisms attached to detritus could serve as food for clam (Broom, 1985; Gosling, 2003) [2]. In decomposition process, nutrient such as nitrogen, phosphorus and phosphate had been released to enhance a suitable condition for algae growth. During the study period, the level ammonia, nitrite, nitrate, phosphate and ferum were relatively low in Pond 1 than Pond 2. Ammonia concentration fluctuated largely during the culture period for pond 2. In context with ammonia, pond 2 has higher average value of end the culture period. This might be because of the use of nitrifying bacteria in the form probiotics. As these bacteria are known to convert ammonia to nitrite and then to nitrate, low levels of ammonia and nitrite observed in Pond 1 compared to Pond 2 can be supported. Phosphorus occurs mainly in the form phosphate and this element is recognized to be the most important critical factor in the maintenance of pond fertility (Boyd, 1982) [3]. Rao (2001) [17] reported that the probiotic bacteria utilize phosphate for their body metabolic activities and thus diminish this nutrient in pond waters. Probiotic bacteria are known to improve water quality in many ways. Heterotrophic bacteria necessitating some organic sources of carbon in addition to inorganic forms for growth have a significant role in the decomposition of organic matter and production of particulate food materials from dissolved organics (Jana and De, 1990) [10]. There are many studies on the relationship between heterotrophic bacteria and water quality (Guo *et al*., 1988; Fang *et al*., 1989; Liu *et al*., 1992) [7, 5, 10]. Heterotrophic bacteria are known to utilize nitrogen rich substances and release ammonia or ammonium salts (Jana and Barat, 1983) [9]. However, as Pond 1 is treated with probiotics, which convert ammonia to nitrates, relatively low levels of ammonia compared to Pond 2 were observed.

The chlorophyll-a content of water was positively with growth rate of Pond 1 and Pond 2 (rs=0.52, rs=0.73). High growth rates of blood clam were observed in the Pond 2 of greatest concentration of chlorophyll-a. In the nature, growth of mollusc is known to be supported by food availability, i.e., phytoplankton abundance (Gosling E, 2003.), which in this case is represented by chlorophyll-a concentration. Apart from phytoplankton, other organic particles were also found to be food sources of most clam (Meshram AM, 2005). In the contrast to chlorophyll-a concentration, a negative trend was found between TSS and growth rate of blood cockles in 2 Ponds. This could be explained by negative correlation between TSS and chlorophyll-a. Pond 2 with high TSS were observed to exhibit low concentration of chlorophyll-a, except Pond 1. This indicates that TSS might be composed of inorganic particles which cannot be utilized by blood cockle as food and consequentially result low growth rate. For Pond 1, where high TSS and high chlorophyll a concentration was found together, a low growth rate was also observed. It is possible that the phytoplankton was not fully consumed by blood cockle due obstacles to filter feeding. Bivalves molluscs are not good at filtering water with high concentration of suspended solid (Broom MJ, 1985) [2]. Thus, making them unable to perform proper feeding. Appropriate concentrations of suspended solid could enhance growth of the bivalves.

Table 1: Results of water quality test carried out at *T. granosa* culture.

Parameter	Pond 1	Pond 2	P-value
Temperature (ºC)	30.25±0.80	30.24±0.90	p>0.05
pH	8.28±0.34	8.16±0.31	p>0.05
Salinity (ppt)	20.20±1.75	20.03±1.64	p>0.05
Dissolved oxygen (DO) mg/L	6.57±0.78	5.45±0.75	P<0.05
Biochemical oxygen demand (BOD) (mg/L)	2.76±1.94	2.75±1.44	p>0.05
Turbidity	84.0±17.73	42.85±9.9	P<0.05

Total suspended solids (TSS) (mg/L)	95.6±42.84	128±50	*p*>0.05
Alkalinity (mg/L)	149±19.87	140±23.01	*p*>0.05
Phosphate (PO^4) (mg/L)	0.01±0.01	0.02±0.01	*p*>0.05
Nitrite (NO^2)	0.02±0.03	0.03±0.02	*p*>0.05
Nitrate (NO^3) (mg/L)	0.03±0.02	0.03±0.01	*p*>0.05
Ammonia (NH^3) (mg/L)	0.03±0.02	0.14±0.30	*p*>0.05
Ferum (Fe) (mg/L)	0.03±0.02	0.04±0.03	*p*>0.05
Chlorophyll a	6.12±3.05	10.34±3.19	*p*>0.05

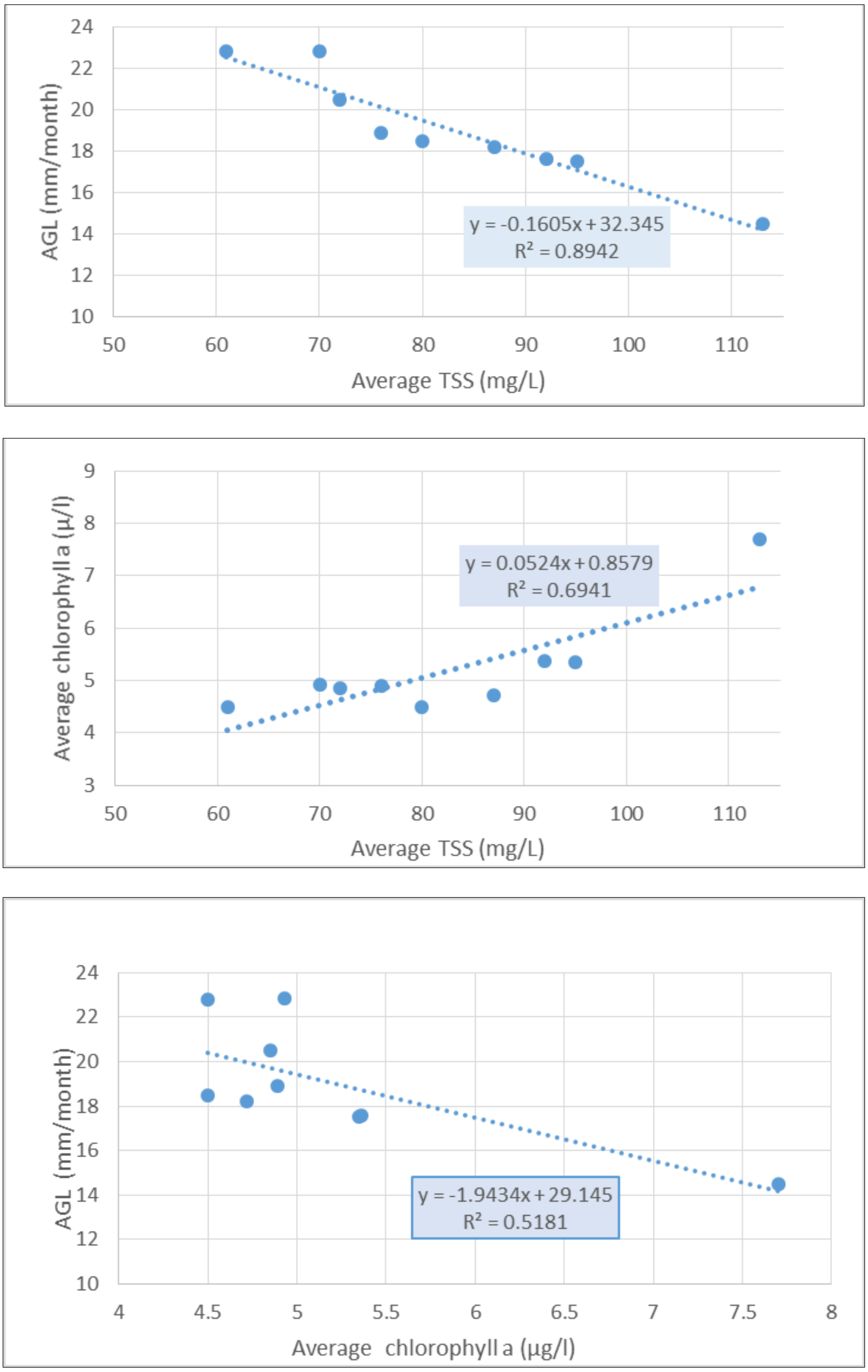


Fig 1: Relationship between the environmental factor (TSS and Chlorophyll a) and average growth length of blood cockles in pond 1

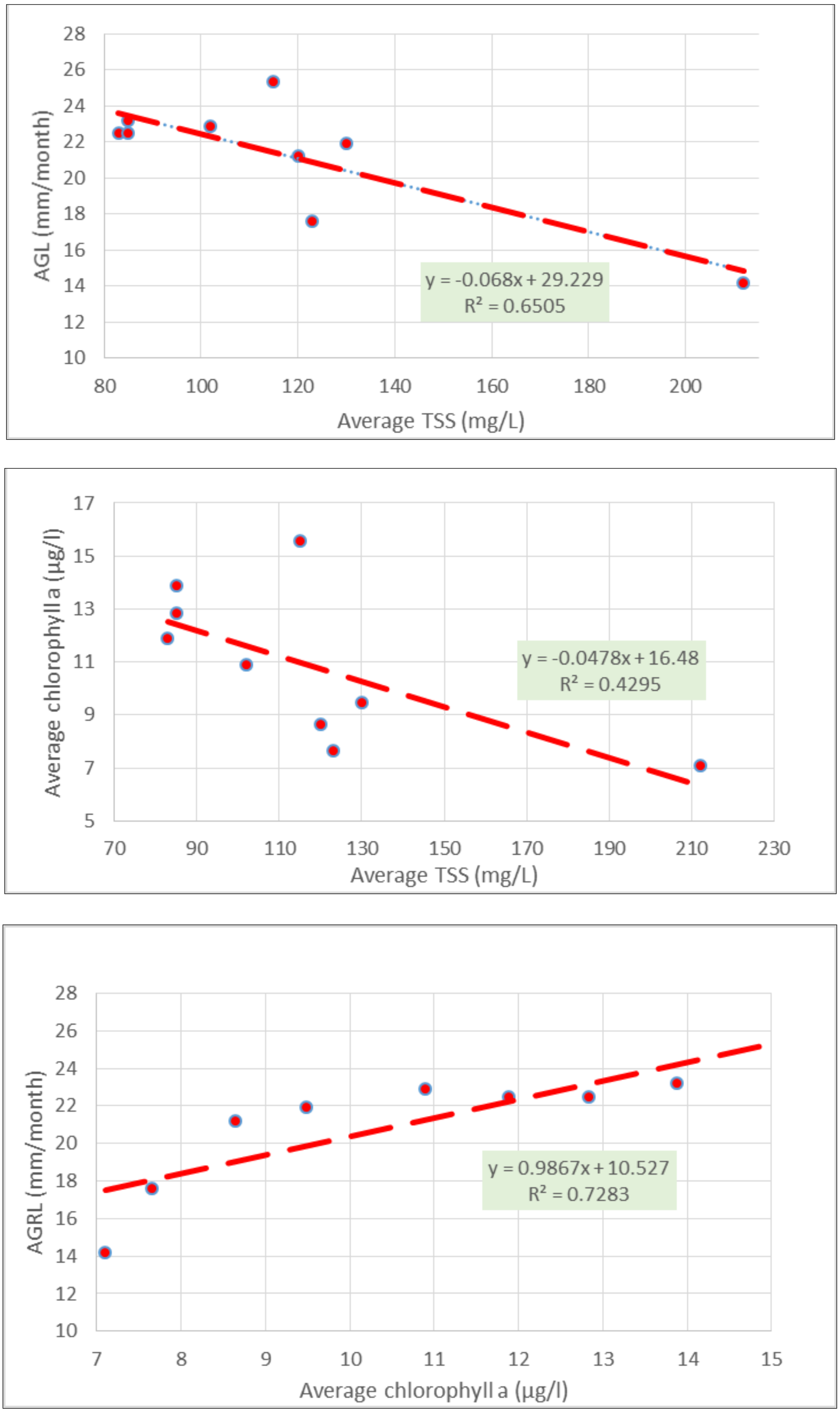


Fig 2: Relationship between the environmental factor (TSS and Chlorophyll a) and average growth length of blood cockles in pond 2

Blood cockles growth rate

Blood cockles length growth rate pond 1 was 0.66 mm/month while for pond 2 was 0.99mm/month. It is quite low if compare natural culture in brackish water where their length growth was 2.7+0.52mm/month (Amirul A.M.J. *et al*) with their water salinity 26.92+4.79.ppt. Weight growth rate of blood clams for pond 1 was 0.33 g/month while for pond 2 was 0.41 g/month. Both were very low compare to others studies (Monissa S., 2018) that growth was ranged 0.6-0.8 g/month. Blood cockles in Phang-nga Bay were reported to have growth rates 0.89g/month Senagulp A.P (1985) Pattani Bay was up to 1.55 g/month (Tookwinas *et al*., 1987) ranged 1.01-1.36 g/month in Ban Don Bay (Vichaiwattana, *et al*., 2008). High chlorophyll a concentration was found in pond 2, their turbidity was 42.85±9.9cm (secchi disc) compare to pond 1 (84.0±17.73 cm) almost doubled. This suggest that low turbidity promoted to produce abundant phytoplankton, also high growth rates of blood clam with high concentration of chlorophyll a.

Cockle growth performance within plot study

The average increment of growth in of cockle growth in Plot

A, Plot B and Plot C of the Pond 1 is 19.80±3.19, 19.72±4.09 and 19.22±2.42 mm per month, respectively (60 and 360 day), as can be seen in Figure 4. Among all three plots, plot A shows the highest average growth increment followed by plot B and plot C as the lowest average growth increment. One-way ANOVA reveals that there was a no significant difference (p>0.05) in growth increment of cockle between Plot A with two other cockle Plots (Plot B and Plot C),

The average increment of growth in of cockle in in of cockle growth in Plot D, Plot E and Plot F of the Pond 2 is 20.43±3.04, 20.82±3.45 and 22.48±4.50 mm per month, respectively (60 and 360 day), as can be seen in Figure 4. Among all three plots, plot F shows the highest average growth increment followed by plot E and plot D as the lowest average growth increment. One-way ANOVA reveals that there was a no significant difference (p>0.05) in growth increment of cockle between Plot F with two other cockle Plots (Plot E and Plot D).

Table 2: Blood cockle growth performance in both ponds.

Days of culture	Pond 1	Pond 2
0	14.51±1.76	14.24±1.92
60	17.41±2.09	17.60±1.78
120	17.57±2.00	21.18±2.59
180	18.18±2.13	22.67±2.55
240	20.46±1.86	22.46±2.27
300	22.84±1.80	23.19±2.68
360	23.71±2.96	25.06±2.99

Table 3: Pond Performance Details

Details	Pond 1	Pond 2	P-value
Area (Ha)	0.25	0.25	-
Initial Stocking	300/m²	300/m²	-
Stocking Date	24/3/2019	24/3/2019	-
AGRL (mm/d)	0.022±0.01	0.033±0.01	p>0.05
AGL (mm/month)	19.04±2.67	21.26±3.35	p>0.05
Survival rate (%)	90.86±4.1	91.42+3.37	P<0.05

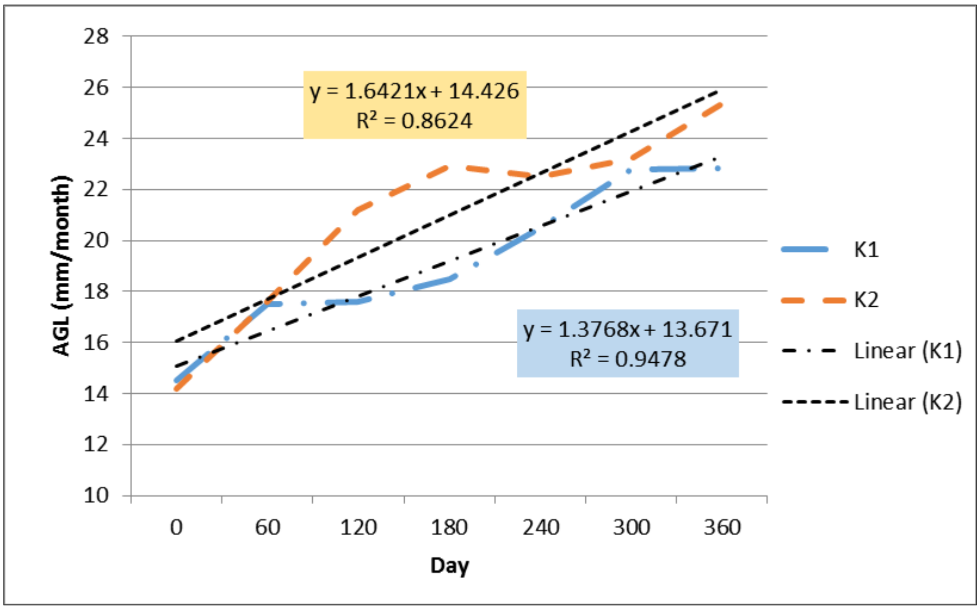


Fig 3: Growth curve of *Tegillarca granosa* within each pond 1 from 0 day to 360 days

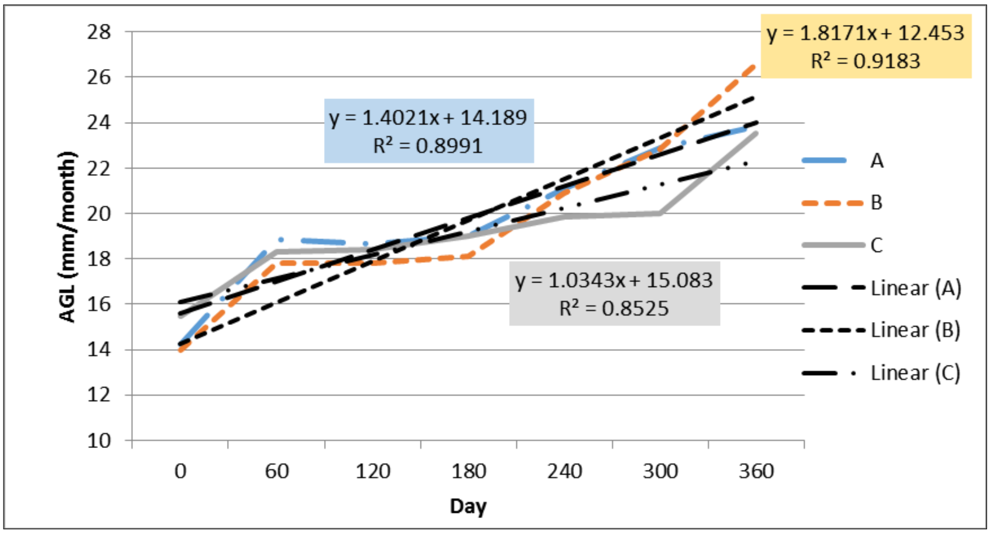


Fig 4: Growth curve of *Tegillarca granosa* within each study plot A, B and C of Pond 1 from 0 day to 360 days.

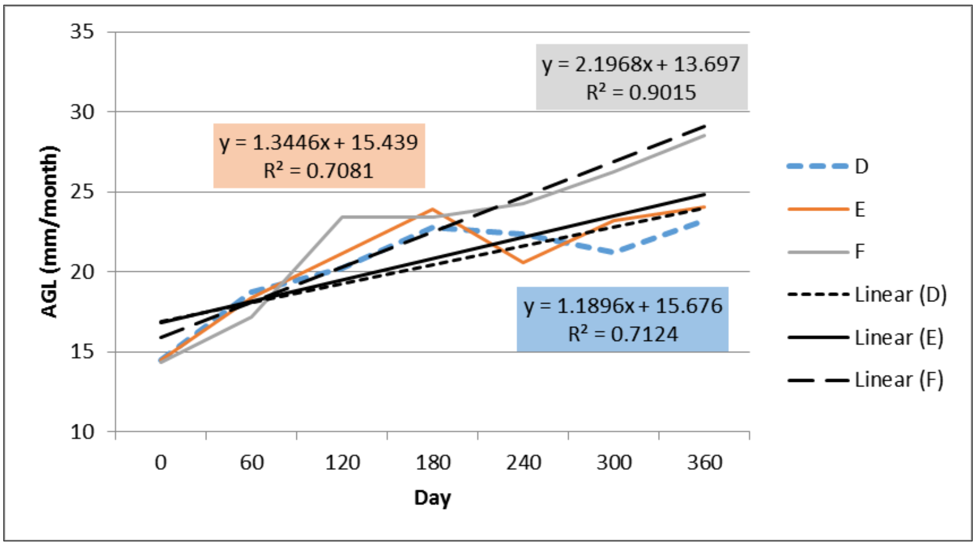


Fig 5: Growth curve of *Tegillarca granosa* within each each study plot D, E and F of pond 2 from 0 day to 360 days

Conclusions

Comparing the growth performance of blood cockles (*T.granosa*) cultured in the two ponds, the Pond 2 achieved better result than Pond 1. The general conclusion obtained from the present study is that the probiotic played major role in maintaining optimum water quality parameter in Pond 1 especially dissolved oxygen, ammonia, nitrate, nitrite and phosphates throughout the culture period. Probiotic are known to improve water quality in many ways. Growth rates for pond 1 was lower than pond 2. That was even lower than normal growth rates in culture farm. These finding indicate environment factor should be in optimum condition to let the blood cockles grow with best performance. Suitable environment have influential on the growth of clam.

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