The Effect of Spirulina (*Arthrospira platensis*) Towards the Growth Performance of Asian Clam (*Corbicula fluminea*)

By

Mohamad Amin Bin Ibrahim

A report is submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

Faculty of Agro Based Industry
UNIVERSITI MALAYSIA KELANTAN
2018

DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

____

Student

Name: MOHAMAD AMIN BIN IBRAHIM

Date:

I certify that the report of this final year project entitled "The Effect of Spirulina (*Arthrospira platensis*) Towards the Growth Performance of Asian Clam (*Corbicula fluminea*)" by Mohamad Amin Bin Ibrahim, matric number F14B0137 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Sciene) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:

____

Supervisor

Name: Associate Prof. Dr. Lee Seong Wei

Date:

ACKNOWLEDGEMENT

In the name of Allah as The Almighty that giving me guidance and strong in completing this thesis, I also would like to express the deepest appreciation to my mother Pesah bt Man and other family members for their constantly support me without doubt.

I am grateful to my supervisor, Associate Prof. Dr. Lee Seong Wei, whose expertise, understanding, giving generous guidance and support make me possible to complete this interest topic of thesis. Her suggestion, comment and opinion always being my guidance for making this thesis complete. Her timely and efficient contribution helped me shape this thesis into the final form.

Next, infinity of thanks goes to my co-supervisor, Mr. Mohd Zhariff b Ramli, my project mates, Azree Faiz, Vinothiran, Siti Zanaria and Nur Shahiza whose always guide me in conducting the experiment; give an idea, and always support me all the time. Their idea and guidance really help me in completing this thesis.

In addition, I also wish to thanks to all my friends that always lend a help, sharing their opinion, knowledge and suggestion. Lastly, special thanks and appreciation also to those that not stated here who have giving a contribution directly or indirectly in making my thesis complete with success.

Thank you.

The Effect of Spirulina (*Arthrospira platensis*) Towards the Growth Performance of Asian Clam (*Corbicula fluminea*)

ABSTRACT

Asian clam or *Corbicula fluminea* is native clam species also known as 'etak' in Malaysia. It has been used as a food source by the local people there. It is such an exotic snack consumed as "smoked clam" or locally known as 'etak salai'. Nowadays, the population of this species in Kelantan has decline due to over-harvest activities. However, there is lack study of Asian clam and scientific information about their habitat and ecosystem in this country that are useful in order to preserve and conserve this native species from declining and extinction. This study is conducted to observe the feasibility of spirulina (*Arthrospira platensis*) as Asian clam feed for closed-culture system. Three replicates of culture tray in closed-culture systems were set up. Each replicate containing 40 samples marked and fed with dried spirulina. The weight (g), shell length (SL) and mortality of each sample are taken weekly for five weeks. Growth Performance Rate (GPR) and Survival Rate (SR) analyzed by using SPSS. Result from this study showed the average percentages (%) mean growth in shell length (SL) increment (SL) (mm) (±s.d.) for all replicates is 0.35 ± 2.03. The average percentage (%) mean of weight increment for all replicates is 0.3 ± 2.09. While the mortality rate is 15% and the survival rate is 85%. The findings of this research evaluated that the feasibility of spirulina as a feed to Asian clam is not effective.

Keywords: *Corbicula fluminea*, *Arthrospira platensis*, growth performance, feasibility and survival rate.

.

Kesan Spirulina (*Arthrospira platensis*) Ke Atas Pertumbuhan Asian Clam (*Corbicula fluminea*)

ABSTRAK

Etak atau *Corbicula fluminea* adalah spesies kerang tempatan yang juga dikenali sebagai 'etak' di Malaysia. Ia telah dijadikan sebagai sumber makanan oleh penduduk tempatan. Ia adalah sejenis snek eksotik yang digunakan sebagai "kerang salai" atau dikenali sebagai 'etak salai'. Pada masa kini, populasi spesies ini di Kelantan telah mengalami penurunan disebabkan oleh aktiviti penangkapan. Walau bagaimanapun, terdapat kekurangan kajian tentang etak dan maklumat saintifik mengenai habitat dan ekosistem mereka di negara ini yang berguna untuk memulihara dan memelihara spesies asli ini daripada berkurangan dan kepupusan. Kajian ini dijalankan untuk melihat kelayakan spirulina (*Arthrospira platensis*) sebagai makanan etak untuk sistem tertutup. Tiga replika dulang peliharaan dalam sistem tertutup telah ditetapkan. Setiap replika mengandungi 40 sampel yang ditanda dan diberi makan dengan spirulina kering. Berat (g), panjang cengkerang (SL) dan kematian setiap sampel diambil setiap minggu selama lima minggu. Kadar Prestasi Pertumbuhan (GPR) dan Kadar Kelangsungan Hidup (SR) dianalisis menggunakan SPSS. Hasil dari kajian ini menunjukkan, peratus (%) purata pertumbuhan dalam panjang cengkerang (SL) (mm) (± sd) bagi ketiga-tiga replika adalah 0.35 ± 2.03. Purata peratusan (%) kenaikan berat badan bagi ketiga-tiga replika pula adalah 0.3 ± 2.09. Manakala kadar kematian adalah 15% dan kadar survival adalah 85%. Penemuan kajian ini mendapati kelayakan spirulina sebagai makanan kepada etak adalah kurang berkesan.

Kata kunci: *Corbicula fluminea, Arthrospira platensis,* prestasi pertumbuhan, kemungkinan dan kadar hidup.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

mm	Millimeter
cm	Centimeter
m^2	Meter square
mL	Millimeter
g	Gram
Mg/L	Milligram per liter
ppt	Part per thousand
p.p.m.	Part per million
s.d.	Standard deviation
DO	Dissolve oxygen
pH	Potential of hydrogen
SR	Survival rate
RGR	Relative growth rate
SGR	Specific growth rate
L_1	Initial length
L^2	Final length
PIM	Particulate inorganic matter
POM	Particulate organic matter
WMW	Wet meat weight
LW	Live weight
DMW	Dry meat weight
AFDMW	Ash-free dry meat weight

LIST OF SYMBOLS

$^{\circ}c$	Celsius
>	More than
<	Less than
%	Percentage
H^1	Hypothesis accepted
H^0	Hypothesis not accepted

CHAPTER 1

INTRODUCTION

1.1 Asian Clam (*Corbicula fluminea*) in General

Asian clam or scientifically known as *Corbicula fluminea* is freshwater clam species locally known as 'etak' in Peninsular Malaysia. Asian clam has a pair of lateral teeth and three cardinal teeth observable inside their shell (Robinson, Asian Clam: An Exotic Aquatic Species, 2004). Asian clam is classified under phylum of mollusca, class of bivalvia and family of Corbulidae. It lives in freshwater but able to adapt up to 13 ppt of salinities for short periods (Shamsul et al., 2014). Asian clam can be found on intermediate substrate sizes unlike densities of other freshwater mussels that are not correlated with bottom type (Shamsul et al., 2014). Asian clam is a small clam with an inflated shell, slightly round to triangular in shape. The shell consists of numerous heavy concentric ridges (Shamsul et al., 2014). They are small bivalve with average size less than 25 mm and not exceed 50 mm (Mass, 2010). Asian clam is a hermaphrodite organism which is able to self-reproduce by itself (Moretzsohn et al., 2006). Fertilization may involve two individuals Asian clam which male will release their sperms where inseminating the eggs inside female body. The embryo will be brooded in the gills and the offspring are not released until the juveniles approximately 1 mm in size (Sheina, 2011). Sousa et al. (2008) mentioned that the life span of this species is ranging from one to five years. Asian clam burrows them into the sediments for feeding and to prevent from water currents movement using a strong muscle called a "foot" (Chandra, 2009).

Ridges on their shells help them burrow into sediments and stay anchored in the bottom when water currents move across them (Chandra, 2009).

Asian clam is a suspension-feeder and also applies pedal-feeder. They practices suspension feeder or also known as filter feeder by filtering phytoplankton and detritus from water column. They also use to feed on organic matter in the sediment by using their pedal or foot (Rosa, 2013). From the previous study by Shamsul et al. (2014) noted that juveniles collect fine organic materials from their substrate by pedal sweep and also by using their cilia to create an anterior suspension feeding current.

In short period of time, Asian clams are able to become highly invasive and can alter the food web and compete with native mussel species at high densities (Caffrey, 2017). It creates blockages in drinking and industrial water abstraction system that gives negative economic effects (Howells, 2015). Asian clam can filter large volume of water, depositing huge number of organic matter on the bottom and improve clarity of water (Lucy et al., 2012). According to Caffrey (2017), Asian clam can filter phytoplankton and bacteria effectively out of the water column and food from sediments when there were lack of food from the water column. That does be the reason why this species can be successful in so many different environments. They also have high filtration rates, metabolism, reproduction and tolerance to many ranges of habitat that also being a factor of their survival life (Caffrey, 2017)

1.2 Microalgae

Microalgae are most plentiful primary unicellular producers that can be found in all the aquatic systems. Subdivided into eukaryotic and prokaryotic algae, eukaryotic possessing defined cell organelles such as nuclei, chloroplasts, mitochondria, prokaryotes (cyanobacteria or blue-green) are primitive, possessing the simpler cellular structure of bacteria (Raja et al., 2014). Mainly aquatic invertebrates depend on microalgae for their whole life cycle nutrients. All growth stages of bivalve, crustaceans and some species of fish utilizes microalgae as their live feed (Malcata et al., 2012).

Raja et al. (2014) also stated that microalgae are feed stocks that rich with energy and too easy to adapt and grow in the photobioreactors or open ponds for high yields and multiple applications. *Nannochloropsis sp*., *Dunaliella sp*., *Scenedesmus sp*., exhibited exemplary biochemical profiles rich in high-quality protein, nutritious polyunsaturated fatty acids and antioxidant pigments (Kent et al., 2015). Different species of microalgae are significantly differing in their nutritional value. Its size, shape, digestibility that related to structure and composition of cell wall and biochemical composition are the factors that contribute to the nutritional value of microalgae (Malcata et al., 2012).

More than a few hundred of microalgal species have been tested as aquaculture feed a last decades ago, but just about 40 species are used to be grown in intensive cultivation systems including diatoms, flagellated and chlorococcalean green algae and also filamentous blue - green algae, especially spirulina (Becker, 2013). Spirulina applied in aquaculture to feeds on penaeid shrimp larvae, bivalve mollusc post larvae, brine shrimp and marine rotifers (Becker, 2013).

1.3 Spirulina

Spirulina *(Arthrospira platensis*) is a blue-green cyanobacterium from the Oscillateriaceae family that survived in alkaline and warm media. It is likely to be a highly hygienic food, because not many other microorganisms are able to survive such conditions (Gabriela et al., 2015). It is recognized by its peculiar shape of cylindrical trichomes that are arranged in a lefthanded helix throughout the filament. spirulina *(A. platensis*) is among the richest sources of proteins. Its protein content is about 60–70 % of its dry weight. This is an outstanding percentage since the vast majority of plant-based feeds contain only about 35 % (Gabriela et al., 2015).

According to Algorigin (2016), spirulina composed of all components that found in the ideal whole food which are protein, vitamins and minerals. It rich in protein content 55%-70% of its weight, half of amounts in others plant protein sources. Spirulina also provide full range of essential amino acids which is 47% of its total protein weight. ß-carotene, B1, B2, B12, and E are the vitamins that can be found naturally in spirulina. Spirulina are also rich in minerals which are an iron, magnesium, calcium and phosphorus (Falquet, 2017). The iron content in spirulina is 20 times more than wheat germ (Algorigin, 2016). Feeding spirulina in bivalve hatchery is recommended because of their low cost and the ability to fulfill the nutritional needs by some bivalve species under culture conditions (Arney et al., 2015). Spirulina spray-dried microalgae mix with *Schizochytrium* contained the biochemical constituents' essential to fulfill the nutritional needs of mussels (Sarkis, 2007).

1.4 Problem Statement

In Kelantan, Asian Clam or 'etak' is such a famous exotic snack consumed as smoked or known as 'Etak Salai'. Nowadays, the population of this species in Kelantan has decline. There is lack of study and scientific information about their habitat, feeding regimes and their growth performance that are useful in order to preserve and conserve this native species from declining and extinction. This research is carried out to investigate the feasibility of Spirulina (*A. platensis*) towards the growth performance of Asian clam (*C. fluminea*).

1.5 Hypothesis

H_0 : There is no effect of Spirulina (*Arthrospira platensis*) towards the growth performance of Asian clam (*Corbicula fluminea*).

H_a : There is an effect of Spirulina (*Arthrospira platensis*) towards the growth performance of Asian clam (*Corbicula fluminea*).

1.6 Objective

The objective of present study is to evaluate the growth performance of Asian clam (*C. fluminea*) fed by spirulina (*A. platensis*).

1.7 Scope of Study

This study focused on the observation of the growth performance of Asian clam (*C. fluminea*) on spirulina fed. The main goal is to observe the feasibility of spirulina (*A. platensis*) as Asian clam (*C. fluminea*) feed for closed-cultured system.

1.8 Significance of Study

The result from the study shows whether spirulina is suitable or not as an Asian clam feed. Thus, the data gained can be used for the future research and also can be used to establish a production of Asian Clam in a closed system as to commercialize this bivalve species.

1.9 Limitation of Study

There was a lack of study based of Asian clam in closed system. Hence, it is difficult to determine their path of feeding. In this experiment the Asian clams were fed by dropping a spirulina solution into a substrate randomly. Besides, the exact amount of feed filtrated by clam also cannot be quantified. That is the limitation in this experiment as the exact amount of feed that should be fed is not known. This may effect to over feeding or lack feeding.

CHAPTER 2

Literature Review

2.1 Feeding of Asian Clam

The study conducted by Arapov et al. (2010), described the feeding of bivalve species. According to the study, they stated that bivalves can be suspension-feeders or deposit-feeders, or even utilize both feeding method. Filter feeding process play important role in marine ecosystem. This mechanism control abundance of primary producers, zooplankton and larval stages of other aquatic species including bivalves. Phytoplankton was stated as a main food source for bivalves. Results verified that phytoplankton abundance in shallow areas could be strongly controlled by bivalve grazing. The finding of the study shows that the growth of bivalve is depended on quality of their diet; therefore from the point of aquaculture it is fundamental to know the best condition of bivalves having an optimum energy available to maximize their growth.

According to Kramer-Wilt (2008), Asian clam is filter feeders. They primarily feed on phytoplankton as well as particulate organic matter and remove particles from water column. He also mentioned that Asian clam is an extremely efficient filter feeder, with higher filtration and assimilation rates compare to other fresh water clams. Asian clam feeds on various species of alga and particulate organic matter. Besides, Asian clam also capable to deposit feeding which it transports sediments across its labial palps by ciliary tracts on its foot. This it can be concluded that Asian clam is primarily a filter feeder and uses deposit feeding to supplement its diet when food resources are insufficient or particle size limits filter feeding.

Vaughn and Hakenkamp (2001) stated that burrowing bivalves such as Corbicula species filtering phytoplankton, bacteria and particulate organic. Corbicula also undergo deposit feeding by feeds on organic matter from the sediment. Their filtration rate are varies with species, size, temperature, particle size, flow regime, gill morphology and concentration. Filtration by this species can lead to a huge decrease of phytoplankton and other particles in the water column. They also affect nutrient dynamics in freshwater system via excretion. The rate of excretion is influenced by temperature, food availability and reproductive stage. The filter feeding rate of bivalve species can directly impact to ecosystem process. Thus, the loss of this native species and invasion of non-native bivalves, have a potential to alter rates of ecological processes.

2.2 Growth performance of Asian Clam

Research conducted by Gow et al. (2013) on age determination and growth rate of the freshwater clam *Galatea paradoxa* from the Volta River Estuary, Ghana, using tagging recapture experiment to get the growth increment data. They categorized the clam into three different size classes. Nine wooden boxes as replicate for each class (50 × 40 × 15 cm) filled with sandy sediment and fifty clams are put in each box. Each individual marked by etching a number on one side of the shell. The boxes then lowered to the bottom of the river and the data taken at the beginning and every two months. The data of the experiment shows that the growth in length was about 1-2 mm monthly between January and June. However, length growths drop to only one mm per month on

September to December which is the spawning season. The finding of this study shows that the species has an annual growth rate of ≈ 10 mm.

Research done by Serdar et al. (2007) about the efficiency of three different methods which are net, box and fenced group to maximize the carpet shell clam (*Tapes decussates)* production. The surface size of each method is 0.5 m^2 and carried out in triplicate. The shell length, width, height and total net weight were measured individually every month. While mortality rate was estimated by counting opened bivalve shells and broken-up shells caused by predators. This study conducted over one year. The outcome of the research shows that clam's shell length reached 34.13 ± 0.38 mm and total net weight 9.09 ± 0.27 g respectively. The significant differences in total wet weight and in shell length among culture methods is ($P < 0.05$). The findings of the study prove that hard plastic net method is the best method as the maximum growth and total wet weight and survival rate (64%) were obtained in the method.

Washitani et al. (2017) conducted a research to compare the survival rate of mangrove clam through field experiments in Mangrove Forests of Iriomote Island. Four plots were set across a mangrove forest and the survival rates among these plots. The different environment including salinity is compared for four months. Ten *Polymesoda* spp. were put into a metallic cage of 15 cm x 15 cm x 5cm sizes and 1 cm mesh. Each cage for each plot then placed at a location where there would be a continuous immersion in water. Their mortality, shell length, height and width were measured. The result shows that the survival rate is affected by salinity level. While the growth performance is the best at the changes of salinity is the lowest.

Adjei-Boateng et al. (2011) had a research to assess the optimum clam size and substratum type for the culture of *Galatea paradoxa* at the Volta estuary, Ghana. Samples of clams were divided into three classes of size which are small, medium and large. Each of classes containing 40 samples was marked individually by etching a numbered tag on the shell. Twelve rearing with size 30 x 30 x 10 cm were prepared for muddy substratum and sandy substratum. Each size classes were duplicated for muddy and sandy substratum treatment. The data for shell length was taken monthly while the weight taken only at the initial and at the end of the experiment. The water quality parameter were measured monthly along the experiment. The finding of the experiment shows that the culture of small sized clams is suitable on sandy substratum due to their high survival rates, while the muddy substratum appears most suitable for the culture of larger clams (>40 mm) because of their relatively higher survivorship and better growth performance.

Hart et al. (1998) carried out a research on growth and survival of the giant clams, *Tridacna derasa*, *T. maxima* and *T. crocea*, at village farms in the Solomon Islands. As much as 800 juveniles of each species with shell length sizes 20-30 mm shell length (SL) were divided equally into four replicate cages at each point. The growth and survival rate of the clams were monitored for 24 months. Environmental and rearing conditions were measured throughout this research. The finding of the research shows that *Tridacna derasa* had the best growth and survival, attaining a mean shell length (SL) of 150 mm ± 19.8 s.d., and mean weight of 710 g ± 26 s.d., after 24 months. Mean survival of *T. derasa* over this period was 92.2% ± 9.1 s.d. *T. maxima* grew to a mean size of 78.4 mm ± 14.9 s.d. in 19 months, and *T.crocea* reached 50.2 mm ± 8.1 s.d. in 22 months. After 19 months grow-out, survival of *T. maxima* was 38.9% ± 16.6 s.d., and survival of *T. crocea* after 17 months was 39% ± 22.6 s.d. From the findings shows that the factors affecting growth of all species are water temperature, exposure to wave action, water clarity and water flow.

Celik et al. (2009) carried out a research of the effects of environmental factors on growth and mortality of raft cultivated mussel (*Mytilus galloprovincialis* L.) cultivated in lantern nets in Black Sea. One year old mussels with uniform size were selected for the experiment and keep into three lantern nets and hanged from raft system with 1000 mussels per lantern. Monthly, 30 samples were taken from each lantern along 12 months period. For mortality, it measured by counting the empty shell or each lantern net. In order to determine monthly changes in shell length (SL), tissue weight (wet meat weight - WMW), live weight (LW), dry meat weight (DMW) and ash-free dry meat weight (AFDMW), sub-samples were taken. Temperature, salinity, chlorophyll-*a* (ch-*a*), seston (total particulate matter), particulate inorganic matter (PIM) and particulate organic matter (POM), were also determined monthly. The finding from the experiments shows that the specific growth rate (SGR) was the lowest on January which is 1.50%. Specific growth rate (SGR) is high (5.72%) in June when temperature is above 10°C and high availability of food. Their study also showed that number of environmental factors including water temperature, quantity and quality of food mainly affects growth in marine bivalves, but phytoplankton availability is the primary factor.

Ren et al. (2008) had a study on growth, mortality and reproduction of the transplanted Manila clam (*Ruditapes philippinarum* Adams & Reeve 1850) in Jiaozhou Bay. Seven sites were selected in sandy area which are 3 site each for age 1 and 2 while 1 site for age 3. The environmental factors including water temperature, salinity and dissolved oxygen (DO) were also monitored. The growth, mortality and reproduction of the transplanted Manila clam and the environmental effects were examined based on the data collected. The natural mortality was 35% from ages 1 to 2 and 47% from ages 2 to 3. The mean increments of individual gross weight were 3.88 for ages 2 and 4.02 g for ages 3, respectively, and the increments in shell length were 17.3mm and 6.5mm, which show that the enhanced clams grew well in Jiaozhou Bay. While the development of gonad for clams of ages 1 and 2 are almost synchronized in the similar reproduction cycle. The findings showed that the enhanced clams grew better and showed a similar growth trend to the local wild clams with the water temperature being the main factor affecting the growth.

CHAPTER 3

Materials and Methods

3.1 Sampling of Asian Clam

Sample of Asian clam has been taken from Sungai Pergau, Jeli, Kelantan at coordinate (5.693507, 101.727257) (Figure 3.1).

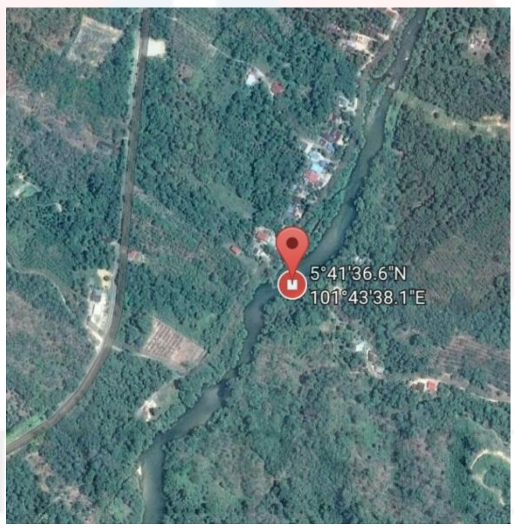

Figure 3.1: Location of sampling at Sg. Pergau.

Sources: (Google Maps, 12th November 2017)

Sg. Pergau is located in Jeli, Kelantan that has a large population of Asian Clam usually harvested by villagers and 'etak' traders. The point of sampling is upstream freshwater river with sandy gravel substrate. The water level depth is shallow and has low current flow. The samples were taken using sieve tray. The clam that trapped in the sieve with different size then put in a tank supplied with continuous aeration. A total of 300 clams with different size were collected and brought to Aquaculture Laboratory at Universiti Malaysia Kelantan Jeli Campus and kept in a holding tank at for a week. In holding tank the samples were let to acclimatize to adapt with new environment condition. After a week the samples were transferred to experimental tank.

3.2 Tank Preparation

Three glass tanks with size (122 cm x 46 cm x 46 cm) were used as the experimental tank. All the tanks filled with dechlorinated water volume 168,360 cm^3 as the height of water is 30 cm. Water pump Aqua San AS-1000 with flow rate 14.97 per/hour is used for all the replicate tanks. A plastic tray with size (50 cm x 36 cm) is used as culture tray. The culture trays were placed in each experimental tank. In order to imitate the natural habitat of Asian clam, sand is used as a substrate. From the analysis using sedimentation method from British Standard the sand percentage composition is 99.5% while silt and clay are 0.4 % and 0.1 % respectively. Sand thickness for each tray is set to 2 cm.

Spirulina (*Arthrospira platensis)* was used a food source. The amount of feed given is based on preliminary study. In the study the highest filtering rate is 1.015 mg/L for ten hours. However, in Cheng (2015) stated that the filtration rates for Asian Clam from the Red Clay Reeks is 2.62 mg/L. Dried Spirulina is grinded using mortar and mix with water by ratio 1g Spirulina to 10ml of water. Each tank fed by dropping using Pasteur Pipette randomly at their opening siphon and rest deposited into substrate. Water pump stopped every time before feeding and switch-on back two hours later to ensure the Spirulina were deposited in the substrate.

A week after acclimatization in a holding tank, 120 clams were selected to be transferred into experimental tank. An individual were marked with numbered by tagging on the shell numbering from 1-40 for each replicate tanks. Then, their initial individual weight and shell length (SL) were measured and recorded. The clams were placed randomly in a culture tray containing sand substrate. The tray then placed in the experimental tank filled with dechorinated water and water pump is switched on.

Environmental parameters in experimental tank were monitored weekly along the experiment by using YSI Pro Plus Multi-Parameter (YSI, USA). All the variables including pH, dissolved oxygen (DO), salinity (ppt) and temperature (ºC) were taken from all the replicate tanks. Along 5 weeks of the experiment the water pH range from 7.45 – 7.62, dissolved oxygen (DO) range from 6.16 mg/L – 6.95 mg/L while salinity and temperature recorded in range 0.01 ppt - 0.02 ppt and 25 ºC – 27 ºC respectively.

3.3 Estimation of Growth Indices

Based on all the data taken, at the end of this experiment the indices of growth were calculated. The relative growth rate (RGR), shell length increment and survival rate were identified. For data analysis, the data analyzed in one–way ANOVA method and Spearman's correlation by using IBM SPSS Statistics V2.2 software.

CHAPTER 4

RESULTS

4.1 Growth of Weight and Shell Length

The average percentages (%) mean growth in shell length (SL) increment (SL) (mm) (±s.d.) for all replicates is 0.35 ± 2.03. The average percentage (%) mean of weight increment for all replicates is 0.3 ± 2.09. However, there is no significance difference for all the replications.

Table 4.1.1: Table shows the average percentage (%) mean growth in shell length (SL) increment (±s.d.) and Percentages (%) mean growth in shell length (SL) increment (mm) (±s.d.) for all replicates of Asian Clam (*C. fluminea*). The data has no significance difference.

	Percentage (%) mean of weight increment (±s.d.)	Percentages (%) mean growth in shell length (SL) increment (mm) (±s.d.)
Average	0.3 ± 2.09	0.35 ± 2.03

*the data was rescale times by -1

Based on the Table 4.1.2 the correlations between percentages (%) mean growths in weight (g) increment and percentages (%) mean growth in shell length (SL) (mm) increment for Asian clam (*C. fluminea*) is 0.253. The results show that is weak correlation between mean growth in weight (g) and (SL). Shell length (SL) and weight (g) significantly correlated at $p<0.01$. Any increment of shell length (SL) either growth in weight (g) are not influenced by one another.

Table 4.1.2: Table shows correlation between percentages (%) mean growths in weight (g) increment and percentages (%) mean growth in shell length (SL) increment for Asian Clam (*C. fluminea*).

Correlations

		gram	mm
Spearman's rho	gram	1.000	.253
	mm	.253	1.000

The mean of weight increase from 2.213 g on week 1 to 2.237 g on week two but decrease suddenly from 2.27 g on week three to 2.21 g on week five (Figure 4.1.1). The data used a cumulative mean from all replicates for each week.

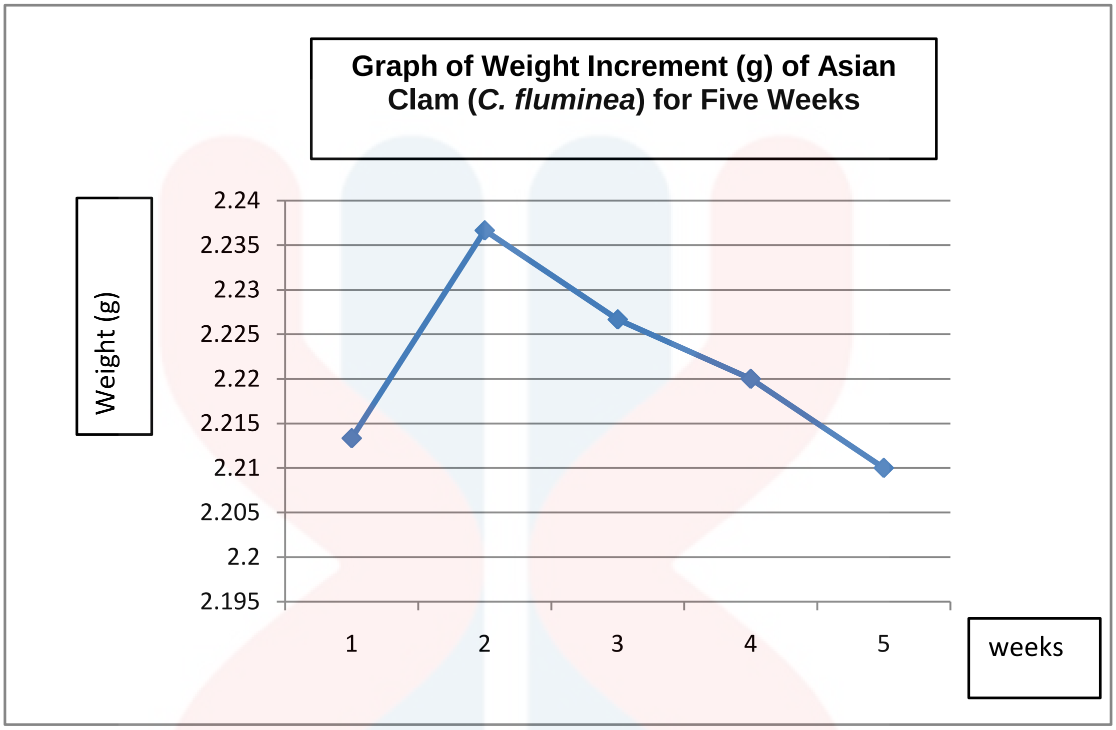

Figure 4.1.1: the graph shows the weight increment (g) of Asian Clam (*C. fluminea*) for 5 weeks.

The mean of shell length (SL) of Asian clam (*C. fluminea*) raise slightly from 19.083 mm on week one to 19.187 mm, 19.23 mm, 19.287 mm on week two, week three, week four and lastly raise to 19.33 mm on week five (Figure 4.1.2). There is no declined of mean of shell length (SL) along the five weeks of the experiment.

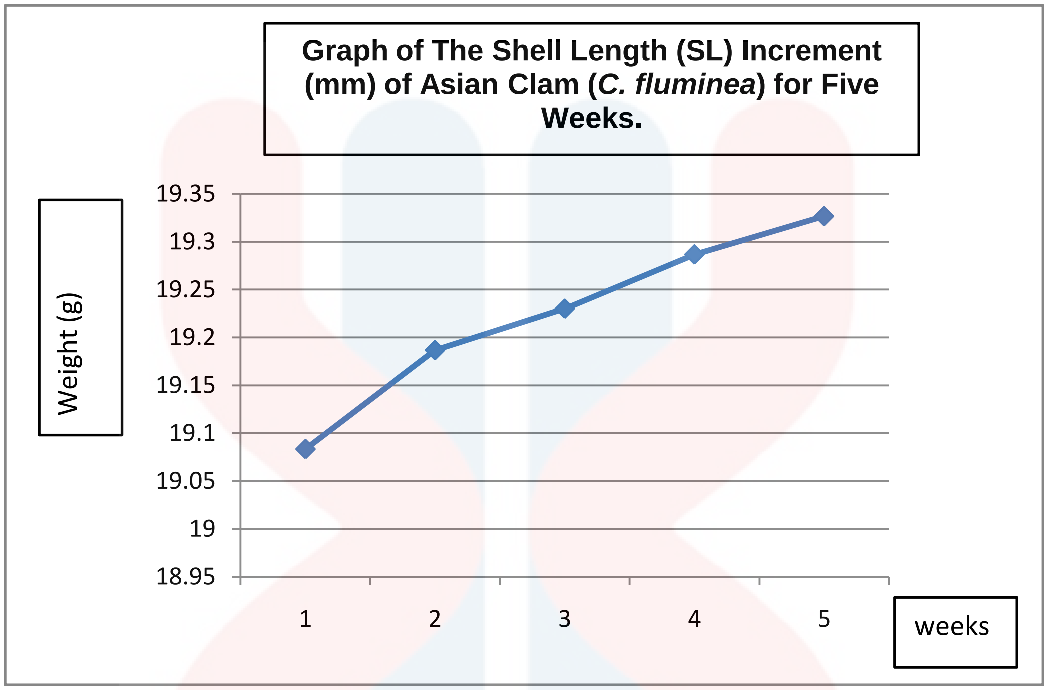

Figure 4.1.2: the graph shows the shell length (SL) increment (mm) of Asian clam (*C. fluminea*) for five weeks.

4.2 Mortality

The total numbers of samples are 120 for all three replicates. The total numbers of mortality along the experiments are six out of 120 samples. The total number of samples that survived is 114 samples. So, the mortality rate is 5 % while the survival rate is 95 %. The mortality rate % mean (± s.d.) is 5.0 ± 2.5 along the experiment. While the survival rate % mean (± s.d.) is 95 ± 2.5. The average percentage of mortality for week one recorded is zero. In week two and three the percentage of mortality is 0.83 % while for week 3 and 5 is 1.67 % (Figure 4.2.1).

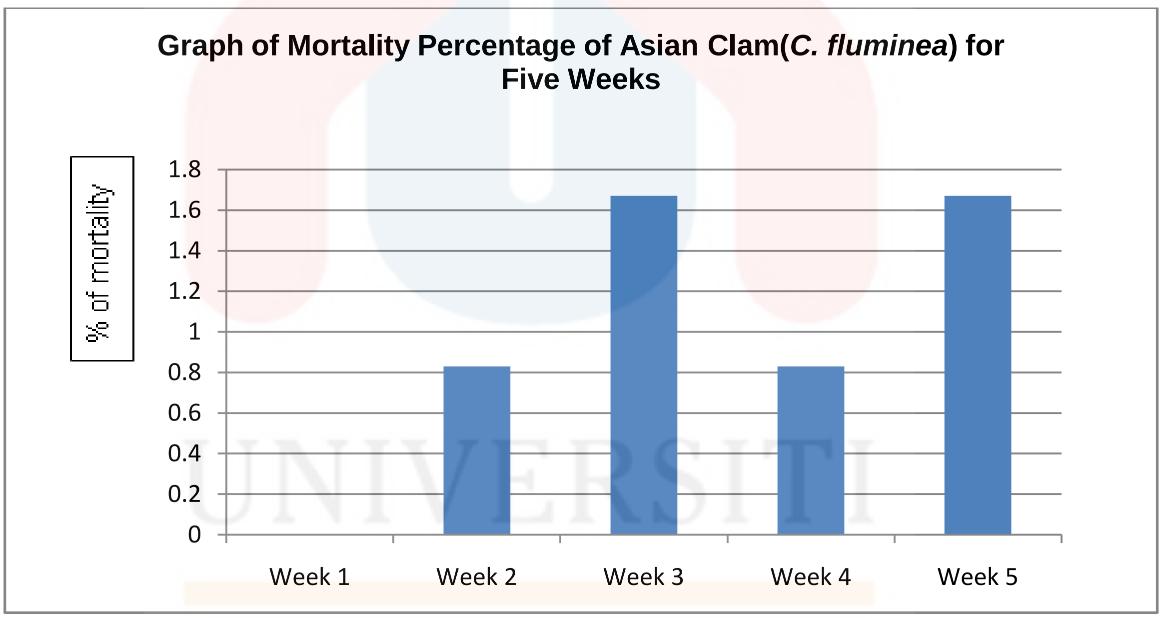

Figure 4.2.1: The graph shows the average percentage of mortality of Asian clam (*C. fluminea*) for five weeks of the experiment.

4.3 Filtering Rate

From the present study, the filtration rate for sediment with live clam is the highest. It shows that sediment is important for clam compared to other treatments without sediment that has the lowest rates of filtration. Based on this result of filtration rates, the amounts of feed given to the clams were identified.

From the graph in the figure 4.3.1 the filtration rates recorded by sediment with live clam is the highest along ten hour period which is 1.015 mg/L compared to other treatment. The second highest is no sediment with live clam with 0.67 mg/L and 0.66 mg/L for sediment with shell. Filtration rate for no sediment with shell is the lowest which is 0.547 mg/L.

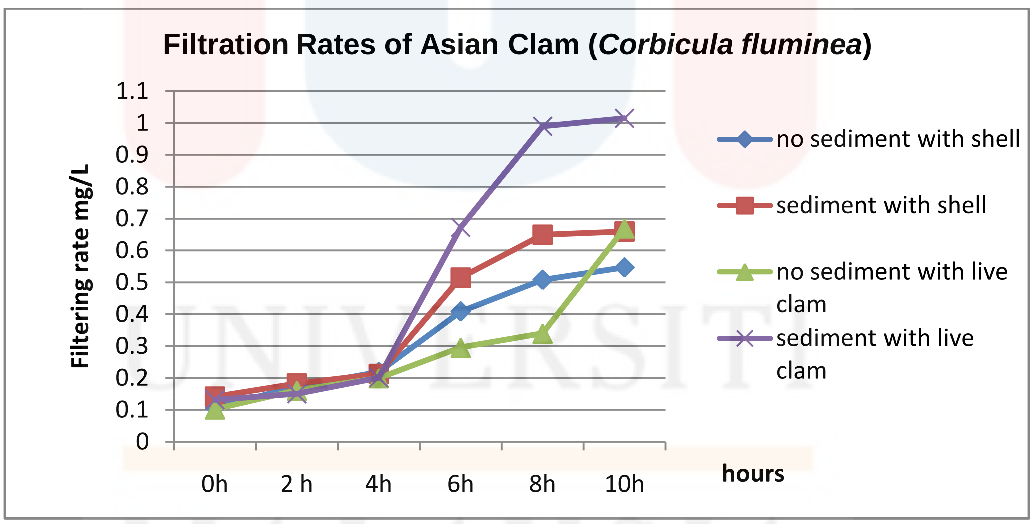

Figure 4.3.1: The graph shows the filtration rates of Asian clam (*C. fluminea*) for all the treatments for ten hours.

CHAPTER 5

DISCUSSION

5.1 Growth Performance

The total period of this experiment is five weeks. The time length of the experiment was set as 10% from their life span which ranging from one to five years (Sousa *et al*., 2012).

Growth rates for Asian Clam (*C. fluminea*) have no significance different among all the replicates (Table 4.1.1). The mean percentage (%) of growth increment in weight (±s.d.) for Asian clam (*C. fluminea*) was evaluated along the period of experiment. The highest percentage (%) mean of growth in weight increment (±s.d.) is 0.36 ± 1.57 while the lowest is 0.26 ±1.29. This results shows that the percentage of increment are too small for all the three replicates.

Figure 4.1.1 shows a weekly mean of weight increment in gram (g). This graph indicates that the uses of spirulina as a feed not sufficed for nutrient requirement of Asian Clam. Although, Nuhu (2013) mentioned that spirulina is among the richest sources of protein as high as 60-70%, however the results in the experiment show contradict. Arney et al., (2015) have used the spray-dried spirulina (*A. platensis*) to feed on the Pacific geoduck clam (*Panopea generosa*) and the result shows that growth rate was significantly higher in individuals compared to those given *Schizochytrium* sp. It may be effectively for a marine bivalve but not for Asian clam which is a freshwater clam. This might be a factor of the low growth increment in this experiment. However, mixed spray-dried of *Schizochytrium* and spirulina that containing biochemical constituents are necessary to satisfy the needs of nutritional value of mussel are able to enhance juvenile mussel growth (Sarkis, 2007). Thus, the uses of spirulina solely feed to Asian Clam might be not effective and should be mixed with other microalgae to ensure a higher growth rates.

The quality of spirulina fed might also be a factor to low growth increment. The quality and grade of spirulina that supplied and used in this experiment was not known. The purity and low-grade of the spirulina may not 100% and mixed with other microalgae. spirulina with low-grade contains compression agents or lubricants agents (Algorigin, 2017).

Temperature also is one of the factors that can affect the growth rate. Bayne et al. (1976) stated that when temperature is high and food is available, growth rate will be high. The study conducted by Celik et al. (2009) found that the highest shell growth rate of mussels recorded during spring and summer due to high temperature and food availability. Weber et al. (2015) stated that increasing water temperature increases metabolic rate, while decreasing temperatures will decrease metabolic rate, affecting both growth and reproduction of clams.

Figure 4.1.2 is a graph of a weekly mean increment of shell length (SL) in mm of Asian clam (*C. fluminea*). The trend of the graph shows the rose steadily from week one to week five. The mean increment of shell length (SL) from the initial to final week is 0.247 mm. It indicates that feeding with spirulina can influence the shell length (SL) increment of Asian clam. Spirulina contained high calcium which is 468 mg from 100 g composition (Gutiérrez-Salmeán et al., 2015). The calcium content might provide shell development although the effectiveness in the increment of Asian clam's shell is not favorable. The effectiveness may not favorable because the length period of the experiment which represents 10% of their life span was not strong enough to represent their whole life cycle. The weight (g) increment and shell length (SL) also correlated to each other. However, the correlation between these two parameters is too weak (0.253). Therefore, it can be considered that increment of shell length (SL) and growth in weight (g) was not influenced by each other.

5.2 Mortality

The total number mortality of Asian clam for experimental period was six individuals from 120 samples (Table 4.2.1). The mortality rate is recorded at 15% while the survival rate is 85%. High of survival rate shows that the feeding of spirulina not hazardous, harmful, toxicity to the Asian clams. The result of ammonia test shows that the content is 0.5 mg/L indicate that it is not harmful and hazardous. In order to maintain and prevent high content of ammonia the water in experimental were changed every two days. High content of ammonia will reduce the availability of oxygen in the water thus, will turn the water to toxicity and can lead to mortality. Furthermore, the water parameter of each tank was monitored weekly to ensure the preferable condition. The water pH range from 7.45 – 7.62, dissolved oxygen (DO) range from 6.16 mg/L – 6.95 mg/L while salinity and temperature recorded in range 0.01 ppt - 0.02 ppt and 25 ºC – 27 ºC respectively. This might be the factor of high survival rate along the period of experiments

CHAPTER 6

CONCLUSION AND RECOMMENDATION

As a conclusion the use of spirulina as a feed is not favorable to the growth performance of Asian Clam (*Corbicula fluminea*). In the present study, the finding shows that use of spirulina as a feed not suitable to the growth of Asian clam.

Other sources of feed that have high or even better nutritional value than spirulina could be used in future study, to evaluate the effectiveness towards the performance of Asian clam. For example, animal and plant waste from farming activities could be treated and use as feed, which it is also easy to obtain and low cost.

REFERENCES

Adjei-Boateng, D., Essel, M. K., & Agbo, N. W. (2011). Growth and survival of the freshwater clam, *Galatea paradoxa* (Born 1778) cultured on different substrata. *Aquaculture Research*, 1–7.

Arapov, J., Ezgeta–Bali, D., Peharda, M., & Nincevic, Z. (2010). Bivalve Feeding-How and What They EatT? *Ribarstvo*, 105—116.

Arney, B. L., Liu, Wenshan, Forster, Ian, McKinley, et al. (2015). Feasibility of dietary substitution of live microalgae with spray-dried *Schizochytrium* sp. or spirulina in the hatchery culture of juveniles of the Pacific geoduck clam (*Panopea generosa*). *Aquaculture*, 117-133.

Barrera, F. M. (2006). *Occurrence of the Asian Clam, Corbicula fluminea, in the Jim Naismith Reservoir, Ingleside, Texas, April 2006.* Texas: Texas A&M University-Corpus Christi (TAMUCC).

Becker, E. W. (2013). Microalgae for aquaculture: nutritional aspects. In A. R. Hu, *Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2nd Edition* (p. 736). Arizona: Wiley-Blackwell.

Caffrey, D. J. (2017). *Asian Clam*. Retrieved November 12th, 2017, from Inland Fisheries Ireland: https://www.fisheriesireland.ie/Invasive-species-list/asian-clam.html

Chandra, S. (2009). *Tahoe: State of The Lake Report 2009 Asian Clam.* Reno: UC Davis Tahoe Environmental Research Center.

Composition of Spirulina. (2016). Retrieved November 12th, 2017, from Algorigin: https://www.algorigin.ch/en/composition-contents-spirulina

Falquet, J. (2017). *The Nutritional Aspects of Spirulina.* Retrieved November 27th, 2017, from Antenna Foundation: https://www.antenna.ch/wp-content/uploads/2017/03/AspectNut_UK.pdf

Gow, Adjei-Boateng, D., & Wilson. (2013). Age Determination and Growth Rate of the Freshwater Clam *Galatea paradoxa* (Born 1778) from the Volta River Estuary, Ghana. *Journal of Aquatic Science*, 31-38.

Gutiérrez-Salmeán, G., Fabila-Castillo, L., & Chamorro-Cevallos, G. (2015). Nutritional and toxicological aspects of Spirulina (Arthrospira). *Nutricion Hospitalaria*, 34-40.

Hart, A. M., Bell, J. D., & Foy, T. P. (1998). Growth and survival of the giant clams, *Tridacna derasa*, *T. maxima* and *T. crocea*, at village farms in the Solomon Islands. *Aquaculture*, 203–220.

How to identify a "good" Spirulina ? (n.d.). Retrieved December 1st, 2017, from Algorigin: https://www.algorigin.ch/en/good-spirulina

Kent, M., Welladse, H. M., Mangott, A., & Li, Y. (2015). Nutritional Evaluation of Australian. *Plos One*, 14.

Lucy, F. E., Karatayev, A. Y., & Lyubov. (2012). Predictions for the spread, population density, and impacts. *Aquatic Invasions*, 465–474.

M. Yesim Celik, S. K. (2009). Effects of environmental factors on growth and mortality of raft cultivated mussel (*Mytilus galloprovincialis L.*) cultivated in lantern nets in Black Sea. *Aquaculture, Aquarium, Conservation & Legislation*, 97-108.

Malcata, Guedes, A. C., & Xavier, F. (2012). Nutritional Value and Uses of Microalgae in Aquaculture. In D. Z. Muchlisin, *Aquaculture* (pp. 59-78). Rijeka: InTech.

McMahon, M. A. (1999). Effects of temperature and temperature acclimation on survival of zebra mussels (*Dreissena polymorpha*) and asian clams (*Corbicula fluminea*) under extreme hypoxia. *The Malacological Society of London*, 317–325.

Nuhu, A. A. (2013). Spirulina (Arthrospira): An Important Source of Nutritional and Medicinal Compound. *Journal of Marine Biology*, 8.

Raja, R., Shanmugam, H., & Carvalho, I. S. (2014). Biomass from Microalgae: An Overview. *Oceanograph*, 7.

Richmond, A. (2008). In A. Richmond, *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (p. 584). Blackwell Publishing.

Robinson, M. (2004). *Asian Clam: An Exotic Aquatic Species.* Massachusets: DCR Massachusets.

Sarkis, S. (2007). *Installation and Operation of a Modular Bivalve Hatchery.* Rome: FAO.

Serdar, S., LoK, A., Kose, A., Yildiz, H., Acarli, S., & Goulletquer, P. (2007). Growth and survival rates of carpet shell clam (*Tapes decussatus Linnaeus*, 1758) using various culture methods in Sufa (Homa) Lagoon,. *Aquacultural Engineering*, 89-99.

Sheina. (2011). *Species profile: Asian clam*. Retrieved November 26th, 2017, from Invasivore.org: http://invasivore.org/2011/05/species-profile-corbicula-fluminea-the-asian-clam/

Sousa, Ilarri, M., & Ronaldo. (2012). *Corbicula fluminea* Müller (Asian Clam). In R. A. Francis, *A Handbook of Global Freshwater Invasive Species* (p. 484). New York: Routledge.

Sousa, R., Antunes, C., & Guilhermino, L. (2008). Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. *Annales de Limnologie - International Journal of Limnology*, 85-94.

Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, 1431-1446.

Washitani, Y., Hayakawa, R., Li, M., & Shibata, S. (2017). Comparing the Survival Rate of Mangrove Clam, *Polymesoda* (Geloina) spp.(Solander 1876) Through Field Experiments in Mangrove Forests of Iriomote. *Zoological Science*, 223-228.

Weber, K., Sturmer, L., Hoover, E., & Baker, S. (2015). *The Role of Water Temperature in Hard Clam Aquaculture.* Retrieved December 14th, 2017, from University of Florida IFAS Extension: http://edis.ifas.ufl.edu/fa151

Yiping Ren, B. X. (2008). Growth, mortality and reproduction of the transplanted Manila clam (*Ruditapes philippinarum* Adams & Reeve 1850) in Jiaozhou Bay. *Aquaculture Research*, 1759-1768.

Zaween Najjah Mohamad Shamsul, A. E. (2014). Assessment of Physico-Chemicals Water Quality, Substrate Compositions and Phytoplankton in Relation to The Density of *Corbicula fluminea* In Pattani River, Southern Thailand. *International Journal of Advanced and Applied Sciences*.

APPENDIX A

Table A.1 One-way ANOVA for weight increment (g) and shell length increment (mm).

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
gram	Between Groups	.784	2	.392	.075	.928
	Within Groups	2137.549	409	5.226		
	Total	2138.334	411			
mm	Between Groups	4.566	2	2.283	.341	.711
	Within Groups	2740.225	409	6.700		
	Total	2744.791	411			

Table A.2 Descriptives table for weight increment (g) and shell length increment (mm).

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
gram	1	136	.28797	3.415988	.292919	-.29133	.86728	-29.197	23.034
	2	120	.26250	1.228109	.112110	.04051	.48449	-8.028	8.273
	3	156	.36359	1.571431	.125815	.11505	.61212	-7.273	9.091
	Total	412	.30919	2.280956	.112375	.08828	.53009	-29.197	23.034
mm	1	136	.4965	4.31818	.37028	-.2358	1.2288	-7.85	46.19
	2	120	.2625	1.22811	.11211	.0405	.4845	-8.03	8.27
	3	156	.2815	.52938	.04238	.1978	.3652	-1.05	2.79
	Total	412	.3469	2.58425	.12732	.0967	.5972	-8.03	46.19

Table A.3 Spearman's correlation table for weight increment (g) and shell length increment (mm).

Correlations

			gram	mm
Spearman's rho	gram	Correlation Coefficient	1.000	.253**
		Sig. (2-tailed)	.	.000
		N	412	412
	mm	Correlation Coefficient	.253**	1.000
		Sig. (2-tailed)	.000	.
		N	412	412

**. Correlation is significant at the 0.01 level (2-tailed).

Table A.4 Percentage (%) mean of growth in weight increment (±s.d.) for Asian Clam (*C. fluminea*).

Replication	Percentage (%) mean of weight increment (±s.d.)
1	0.29±3.42
2	0.26±1.29
3	0.36±1.57

*the data was rescale times by -1

Table A.5 The mean percentage (%) of mortality rate and survival rate of Asian clam (*C. fluminea*) (± s.d.) for all replicates.

Mortality rate % mean (± s.d.)	Survival rate % mean (± s.d.)
5.0±2.5	95±2.5

Table A.6 Percentage (%) mean growth in shell length (SL) increment (±s.d.) for Asian Clam (*C. fluminea*). The data has no significance difference.

Replication	Percentages (%) mean growth in shell length (SL) increment (mm) (±s.d.)
1	0.50±4.32
2	0.26±1.23
3	0.28±0.53

*the data was rescale times by -1

Table A.7 Filtration rates of Asian Clam (*C. fluminea*) in different treatments.

Treatment	Mean ± s.d.
No sediment with shell	0.327±0.176
Sediment with shell	0.394±0.246
No sediment with live clam	0.295±0.194
Sediment with live clam	0.527±0.398

APPENDIX B

Figure B.1 Sampling of Asian Clams at Sg. Pergau, Jeli.

Figure B.2 Samples of Asian Clam collected during sampling.

Figure B.3 Holding tank used to place Asian Clamping for acclimatization.

Figure B.4 Plastic tray used to place Asian Clam and sand substrate in experimental tank system.

Figure B.5 Sand used as substrate for Asian Clam culturing.

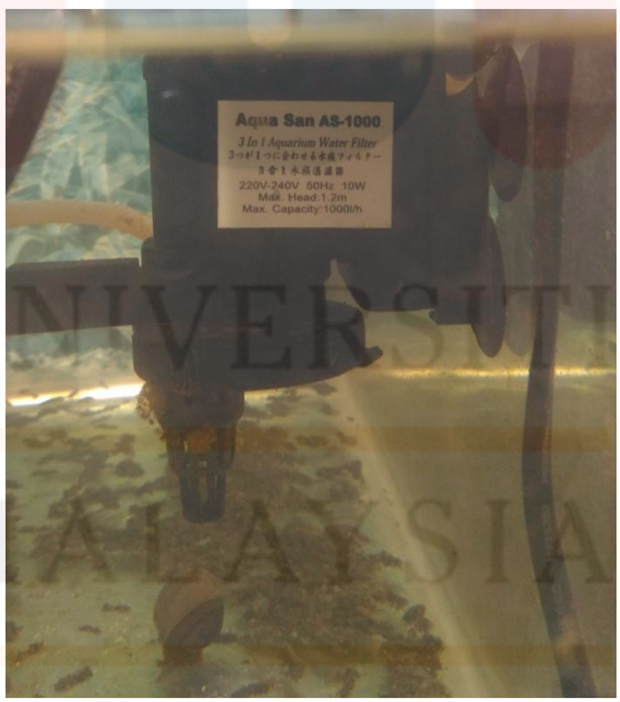

Figure B.6 Aerator pump used in experimental tank.

Figure B.7 Closed-culture experimental tank system for Asian Clam culturing.

Figure B.8 Each sample tagged with individual number ID mark.

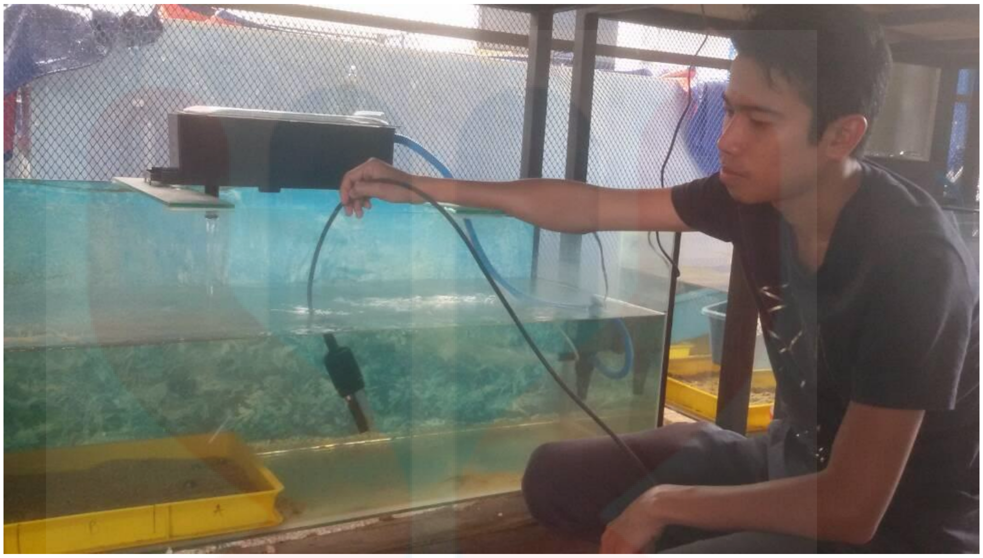

Figure B.9 Monitoring water parameter using YSI Pro Plus Multi-Parameter

Figure B.10 Ammonia Test Kit used for monitoring ammonia level in experimental tank.

Figure B.11 Asian Clam burrowing and anchoring them in a sand substrate.

Figure B.12 Weighing Asian Clam weight using analytical balance.

Figure B.13 Measuring the shell length of Asian Clam using Vernier caliper.

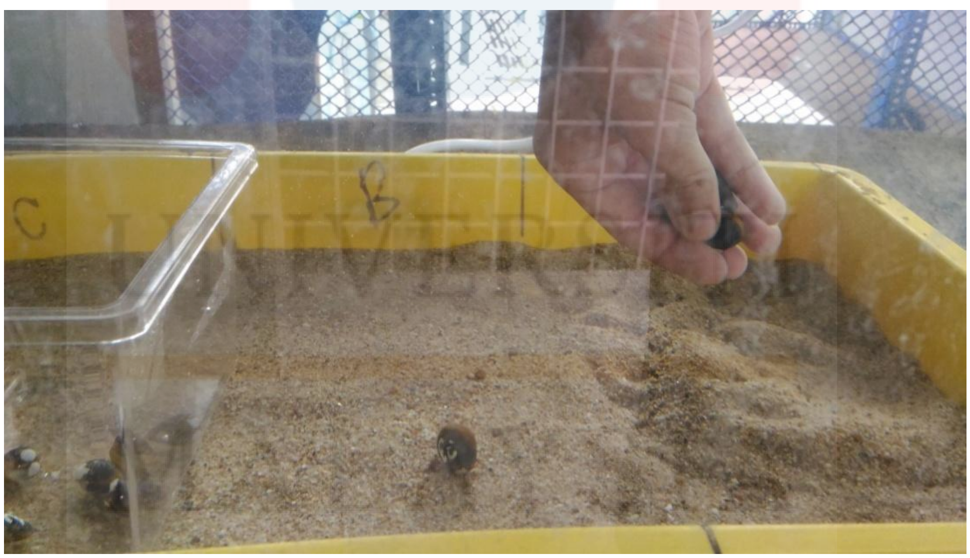

Figure B.14 Collecting sample of Asian Clam from culturing tray for data collection.