

DECLARATION

I declare that this thesis is based on my original work on Establishment of Broodstock Conditioning Culture of Razor Clam *Solen regularis* Dunker, 1862 except for quotations and citations that had been acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

.....

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ABSTRACT

Natural stock of razor clam *Solen regularis* in western part of Sarawak had showed sign of overexploitation. However, effort to culture this species is facing a problem due to its natural annual reproductive cycle and the presence of endoparasite (trematode and nematode) within the broodstock gonad. Therefore, broodstock conditioning technique is essential in the provision of *S. regularis* for its larval culture. Thus, this study aimed to design a best broodstock conditioning culture system that can give better survival. Furthermore, impacts of broodstock conditioning toward Gonadal Condition Index (GCI), oocyte diameter and parasite prevalence on *S. regularis* were also compared between spawning and resting periods. Clams with shell length of more than 4 cm (4.04 – 5.79 cm) were obtained from intertidal area of Asajaya Laut, Asajaya Division for laboratory experiment. Two species of microalgae (*Isochrysis* spp. and *Chaetoceros* spp.) with mix ratio 1:1 were given daily to the broodstocks at approximately 1% of *S. regularis* mean dry meat. Experiments were performed in two phases. During Phase 1, the survival rate and growth rate of *S. regularis* broodstocks were tested with different treatments in order to establish a control treatment in terms of stocking density, sediment compactness, duration of culture and sediment type. In Phase 2, the broodstock conditioning experiments tested on different sediment thickness, seawater volume and seawater temperature for 25 days in two gonadal development stages (resting period and spawning period). During this Phase 2, the survival rate, growth rate, qualitative observation, gonadal improvement of broodstock and parasite prevalence were evaluated. Overall, results showed that the mean survival rate of razor clams ranged from $19.71 \pm 6.60\%$ to $71.30 \pm 28.91\%$. Among treatments, five treatments were significantly (*t*-test, $p < 0.05$) and treatment 10 L seawater volume and 30 °C seawater temperature

consistently have low survival rate. However, weight loss of razor clams was observed in all treatments due to insufficient amount of feed supplied during the conditioning culture. In term of shell growth, both gain and loss in shell length were observed throughout the study period with no specific trend which may be due to the damage of shell periostracum. Based on the results of mean survival rate, the broodstock conditioning culture system for ten clams can be set up in a tank with closed system containing 20 L seawater with daily 50% water exchange, 5 cm thickness of natural sediment and reared at 24 °C (ambient temperature). This system is considered as low cost and easy to handle. There was no significant difference pattern in mean survival rate between spawning and resting period, thus this indicates that the treatment design gave more influence on the survival than the broodstock physiology condition. After 25 days of conditioning culture, all broodstocks in both gonadal development stages showed increase in the mean GCI values compared to both control treatments (wild before conditioning treatment and wild after conditioning treatment). This broodstock conditioning technique also had successfully removed the endoparasite within the razor clam gonad content. In conclusion, this study had shed some important information on broodstock conditioning technique of *S. regularis* in small laboratory scale and this may lead to the development of a successful razor clam spat production in future.

Keywords: *Solen regularis*, broodstock conditioning, Gonadal Condition Index, survival rate, oocyte diameter, parasite prevalence

ABSTRAK

Stok semulajadi ambal Solen regularis di bahagian barat Sarawak telah menunjukkan tanda-tanda eksploitasi yang berlebihan. Walaubagaimanapun, usaha untuk mengkultur spesies ini menghadapi masalah akibat kitaran pembiakan tahunan semulajadi ambal dan kewujudan endoparasit (trematod dan nematod) pada gonad induk. Oleh itu, teknik pengkondisian induk sangat diperlukan oleh ambal demi pengkulturan larvanya. Maka, kajian ini menyasarkan untuk mencipta sistem kultur pengkondisian induk terbaik yang dapat memberikan kadar survival yang bagus. Seterusnya, kesan pengkondisian induk terhadap Indeks Kondisi Gonad (GCI), diameter oosit dan kelaziman parasit S. regularis juga dibandingkan antara fasa pelepasan gamet dan fasa rehat. Ambal-ambal dengan panjang cangkerang melebihi 4 cm (4.04 – 5.79 cm) diperolehi daripada kawasan pasang surut Asajaya Laut, Bahagian Asajaya untuk kajian makmal. Dua spesies mikroalga (Isochrysis spp. dan Chaetoceros spp.) dengan nisbah campuran 1:1 diberikan setiap hari kepada induk S. regularis pada anggaran 1% daripada min isi keringnya. Kajian ini telah dijalankan dalam dua fasa. Semasa Fasa 1, kadar survival dan kadar tumbesaran induk diuji dengan beberapa rawatan berlainan bagi mewujudkan rawatan kawalan dari segi kepadatan stok induk, kekompakan sedimen, tempoh pengkulturan dan jenis sedimen. Pada Fasa 2, ujikaji pengkondisian induk menguji ketebalan sedimen, isipadu air laut dan suhu air laut yang berbeza selama 25 hari pada dua peringkat perkembangan gonad (fasa pelepasan gamet dan fasa rehat). Semasa Fasa 2, induk telah dinilai dari segi kadar survival, kadar tumbesaran, pemerhatian kualitatif, penambahbaikan gonad dan kelaziman

parasit. Keseluruhannya, keputusan menunjukkan min kadar survival ambal berjulat dari $19.71 \pm 6.60\%$ to $71.30 \pm 28.91\%$. Antara kesemua rawatan, dua rawatan iaitu isipadu air laut 10 L dan suhu air laut $30\text{ }^{\circ}\text{C}$ kekal memberikan kadar survival yang rendah. Akan tetapi, pengurangan berat ambal telah dicerap pada semua rawatan kerana jumlah makanan yang diberikan tidak mencukupi semasa kultur pengkondisian. Dari segi tumbesaran cangkerang, penambahan dan pengurangan panjang cangkerang telah dicerap sepanjang tempoh kajian tanpa trend yang spesifik di mana mungkin berpunca daripada kerosakan periostrakum cangkerang. Berdasarkan keputusan min kadar survival, sistem kultur pengkondisian induk bagi sepuluh ekor ambal boleh dilakukan dalam satu tangki sistem tertutup yang mengandungi 20 L air laut dengan pertukaran harian 50% air, sedimen semulajadi setebal 5 cm dan dibela pada $24\text{ }^{\circ}\text{C}$ (suhu sekitar). Sistem ini dianggap sebagai berkos rendah dan mudah diuruskan. Tiada perbezaan yang signifikan pada min kadar survival antara fasa pelepasan gamet dan fasa rehat, maka ini menunjukkan rekabentuk rawatan memberikan lebih pengaruh ke atas survival berbanding dengan kondisi fisiologi induk. Selepas 25 hari kultur pengkondisian, semua induk pada kedua-dua fasa perkembangan gonad menunjukkan peningkatan min GCI berbanding dengan rawatan kawalan (liar sebelum pengkondisian dan liar selepas pengkondisian). Teknik pengkondisian induk ini juga telah berjaya menghilangkan endoparasit di dalam kandungan gonad ambal. Kesimpulannya, kajian ini telah menemui beberapa maklumat penting tentang teknik pengkondisian induk *S. regularis* pada skala kecil di makmal dan ini boleh membantu ke arah kejayaan membangunkan penghasilan benih ambal pada masa hadapan.

Kata kunci: *Solen regularis*, pengkondisian induk, Indek Kondisi Gonad, kadar survival diameter oosit, kelaziman parasit

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT.....	iii
ABSTRAK.....	v
TABLE OF CONTENTS	vii
LIST OF TABLES.....	xii
LIST OF FIGURES.....	xiii
LIST OF ABBREVIATIONS.....	xvii
CHAPTER 1: GENERAL INTRODUCTION	1
1.1 Research background.....	1
1.2 Broodstock conditioning and its importance	3
1.3 Problem statement	5
1.4 Objectives	6
CHAPTER 2: LITERATURE REVIEW	7
2.1 Razor clams and <i>Solen regularis</i> in Sarawak	7
2.1.1 Taxonomy	7
2.1.2 Distribution	7
2.1.3 Habitat.....	8
2.1.4 Reproduction cycle	8
2.2 Bivalve culture technique and broodstock conditioning process	9
2.2.1 Bivalve culture system and seawater	10

2.2.2	Substrate and sediment	11
2.2.3	Diet	12
2.2.4	Duration of culture for broodstock conditioning	13
2.2.5	Stocking density.....	14
2.3	Water quality on bivalve and broodstock conditioning culture	15
2.3.1	Salinity.....	15
2.3.2	Temperature	15
2.3.4	Water quality	16
2.4	Impact of broodstock conditioning to bivalve	17
2.4.1	Shell length and wet weight.....	17
2.4.2	Parasite prevalence	18
2.4.3	Gonadal condition index (GCI) and oocyte diameter	19
CHAPTER 3: METHODOLOGY		21
3.1	Broodstock preparation.....	21
3.1.1	Sediment	21
3.1.2	Seawater.....	21
3.1.3	Microalgae preparation	22
3.1.4	Razor clam sample collection and initial data recording.....	23
3.2.	General experimental setup design	25
3.3	Routine maintenance works of the broodstock culture.....	27
3.3.1	Monitoring of pH, temperature and pH of the water	27
3.3.2	Culture tanks maintenance during the conditioning experiment	27
3.3.3	Feeding of razor clam broodstock	27
3.4	Final data recording and cleaning process.....	28

CHAPTER 4: SURVIVAL, GROWTH AND QUALITATIVE RESPONSE OF	
<i>Solen regularis</i> DURING ESTABLISHMENT OF BROODSTOCK	
CONDITIONING CULTURE	30
4.1 Introduction.....	30
4.2 Materials and Methods	31
4.2.1 Phase 1 (Trial experiments).....	33
4.2.1.1 Experiment 1: Stocking density.....	33
4.2.1.2 Experiment 2: Sediment compactness.....	34
4.2.1.3 Experiment 3: Duration for broodstock conditioning.....	35
4.2.1.4 Experiment 4: Sediment conditioning	35
4.2.2 Phase 2	35
4.2.2.1 Control treatment.....	36
4.2.2.2 Experiment 5 and 6: Sediment thickness.....	36
4.2.2.3 Experiment 7 and 8: Seawater volume	37
4.2.2.4 Experiment 9 and 10: Seawater temperature	38
4.2.3 Shell length and wet weight.....	38
4.2.4 Routine maintenance works.....	39
4.2.5 Qualitative response.....	39
4.2.5.1 Burrowing time.....	39
4.2.5.2 Footprint on sediment.....	39
4.2.5.3 Mortality in sediment	40
4.2.5.4 Presence on sediment surface	40
4.2.5.5 Fecal excretion.....	40
4.2.5.6 Protrude of siphon	40

4.2.6	Data and statistical analysis	42
4.2.6.1	Survival rate.....	42
4.2.6.3	Shell length and wet weight	42
4.2.6.2	Statistical analysis and hypothesis.....	42
4.3	Results.....	43
4.3.1	Mean survival rate	43
4.3.1.1	Phase 1 (Trial Experiments)	43
4.3.1.2	Phase 2	45
4.3.1.3	Trend of razor clams broodstock survival	47
4.3.1.3.1	Sediment thickness	47
4.3.1.3.2	Seawater volume	48
4.3.1.3.3	Seawater temperature	49
4.3.2	Growth performance	50
4.3.2.1	Wet weight.....	50
4.3.2.2	Shell length	51
4.3.3	Qualitative observation	52
4.3.3.1	Burrowing time.....	52
4.3.3.2	Footprint on sediment.....	54
4.3.3.3	Mortality in sediment	55
4.3.3.4	Presence of razor clam on the sediment surface.....	57
4.3.3.5	Fecal excretion and pseudo fecal on sediment	59
4.3.3.6	Protrude of siphon	60
4.4	Discussion.....	62
4.5	Conclusion	66

CHAPTER 5: IMPACT OF BROODSTOCK CONDITIONING TO THE	
GONADAL CONDITION INDEX, OOCYTE DIAMETER AND	
PARASITE PREVALENCE	68
5.1 Introduction.....	68
5.2 Materials and Methods	69
5.2.1 Experimental set up and experiment design	69
5.2.2 Gonadal condition index (GCI)	70
5.2.3 Oocyte diameter.....	71
5.2.4 Parasite prevalence	71
5.2.5 Qualitative observation	72
5.2.6 Statistical analysis.....	72
5.3 Results.....	72
5.3.1 Gonadal condition index (GCI)	72
5.3.2 Oocyte diameter.....	74
5.3.3 Parasite Prevalence	75
5.3.4 Qualitative observation.....	76
5.4 Discussion.....	81
5.5 Conclusion	83
CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATIONS.....	85
REFERENCES ..	88
APPENDICES	105

LIST OF TABLES

		Page
Table 4.1	Experiment summary.	33
Table 4.2	Qualitative observations.	41
Table 4.3	Setting of hypotheses among all treatments.	43
Table 4.4	The mean survival rate (%) during Phase 1 (trial experiments).	45
Table 4.5	Survival rate (%) experiments in Phase 2.	46
Table 4.6	Mean wet weight (g) of <i>S. regularis</i> after the broodstock conditioning culture at two different gonadal development periods.	51
Table 4.7	Mean shell length of <i>S. regularis</i> after the broodstock conditioning culture at two different gonadal development periods.	52
Table 4.8	Burrowing time of the razor clams.	54
Table 4.9	Presence of razor clams footprint on sediment.	55
Table 4.10	Presence of dead razor clam in the sediment.	57
Table 4.11	Presence of razor clam on the sediment surface.	58
Table 4.12	Amount of fecal excretion released onto the sediment.	60
Table 4.13	Observation on protrude of siphon razor clams on Day 14 of culture period less of more than 50% of razor clam individuals.	61
Table 5.1	Summary of experimental set up in each trial and period.	70

LIST OF FIGURES

	Page
Figure 2.1	9
Variation of gonadal condition index (GCI) (mean $\pm$ S.D.) from March 2007 to February 2009 in males and females of <i>S. regularis</i> at Asajaya Laut with five stages of gonadal condition, (i) gonadal development, (ii) maturation, (iii) spawning period, (iv) spent and (v) resting period (Rinyod & Rahim, 2011).	
Figure 3.1	22
Summary of microalgae culture used throughout this experiment.	
Figure 3.2	24
<i>S. regularis</i> broodstock collection site at the intertidal area of Asajaya Laut, Sarawak.	
Figure 3.3	25
Carved razor clam with individuals number on the shell with consistent orientation.	
Figure 3.4	26
Arrangement of tanks from top view for two treatments with three replicates.	
Figure 3.5	26
The size of transparent glass aquarium that was used for conditioning throughout the experiment.	
Figure 3.6	28
Process of feeding the <i>S. regularis</i> broodstock culture.	
Figure 3.7	29
Illustration of work flow for processes in this experiment.	
Figure 4.1	32
Illustration of experiments in Phase 1.	
Figure 4.2	32
Illustration of experiments in Phase 2.	
Figure 4.3	34
Treatment 2A with pored PET bottles whereas Treatment 2B with standard design.	
Figure 4.4	36
The illustration of standard design or control treatment. This treatment contained 5 cm thickness of oven dried sediment and 20 L of seawater	

at 24°C. Each standard design tank contained 10 razor clams and conditioned for 25 days.

- Figure 4.5 Set up of tanks for the sediment thickness experiment. Right tank 37 contained 5 cm sediment thickness (standard design) as control treatment and left tank contained 3 cm sediment thickness.
- Figure 4.6 Set up of tanks for the seawater volume experiment. Right tank 37 contained 20 L of seawater (standard design) as control treatment and left tank contained 10 L of seawater.
- Figure 4.7 Set up of tanks for the seawater temperature experiment. Right tank 38 with 24°C temperature using standard design as control treatment and left tank with 30°C temperature.
- Figure 4.8 Trend of survival rate (%) of *S. regularis* throughout 25 days of culture 47 in sediment thickness experiment.
- Figure 4.9 Trend of survival rate (%) of *S. regularis* throughout 25 days of culture 48 in seawater volume experiment.
- Figure 4.10 Trend of survival rate (%) of *S. regularis* throughout 25 days of culture 49 in seawater temperature experiment.
- Figure 4.11 High mortality of razor clams was observed in a tank during the early 50 stage of conditioning culture experiment.
- Figure 4.12 Observation of razor clams burrowing into sediment. (a) Razor clams 53 presence on the sediment before burrowing into the sediment. (b) Razor clams had completely burrowed into the sediment.
- Figure 4.13 Example of razor clams footprint on sediment surface observed during 55 broodstock conditioning.

Figure 4.14	The sediment turned into black if razor clams died inside the sediment.	56
Figure 4.15	Black coloured sediment can be observed from the side view of culture tank indicating the presence of dead razor clams inside the sediment.	57
Figure 4.16	Razor clam lying on the sediment in 3 cm sediment thickness treatment.	58
Figure 4.17	Dense amount of fecal excretion were released on top surface of sediment.	59
Figure 4.18	Treatment of high stocking density with 70 individuals of <i>S. regularis</i> in a tank. After Day 20 of culture, most razor clams buried their body with siphon protruded.	61
Figure 4.19	Damage of razor clams shell known as periostracum damage.	62
Figure 5.1	Comparison of mean GCI values between before conditioning and after conditioning (laboratory and from wild) during spawning period.	73
Figure 5.2	Comparison of mean GCI values between before conditioning and after conditioning (laboratory and from wild) during resting period.	73
Figure 5.3	Comparison of mean oocyte diameter values between before conditioning and after conditioning (laboratory and from wild) during spawning period.	74
Figure 5.4	Comparison of mean oocyte diameter values between before conditioning and after conditioning (laboratory and from wild) during resting period.	75
Figure 5.5	Comparison of parasite prevalence (%) values between before conditioning and after conditioning (laboratory and from wild) during spawning period.	76

- Figure 5.6 Comparison of parasite prevalence (%) values between before 76
conditioning and after conditioning (laboratory and from wild) during
resting period.
- Figure 5.7 Razor clams with full gonad content until end of the foot that were 77
obtained during spawning period in May 2016.
- Figure 5.8 Razor clam without any gonad content during resting period in 77
December 2016.
- Figure 5.9 Thin layer of foot flattened to examine the cell presence and also to 78
confirm the sex of razor clams during spent-resting period.
- Figure 5.10 Large oocyte diameter and higher number of cells in conditioned 79
broodstock during spawning period (40× magnification).
- Figure 5.11 Small number of oocyte cell and presence of empty gonad sack with 79
small oocyte diameter during resting period after the broodstock
conditioning (40× magnification).
- Figure 5.12 Sperms of wild razor clam that are clear without any parasite 80
prevalence. (40 × magnification)
- Figure 5.13 Parasites in the form of cyst (indicated by arrow) attached within the 80
gonad content.

LIST OF ABBREVIATIONS

GCI	Gonadal Condition Index
PSU	Practical Salinity Unit

CHAPTER 1

GENERAL INTRODUCTION

1.1 Research background

Bivalve is one of the protein sources being consumed by human. Until now, there are over 150 edible clam species in the world (National Marine Fisheries Service, 2011). Clams are nutritious seafood because they are low in fat but high in protein and have moderate amount of healthful minerals such as selenium, zinc, iron and magnesium (Idayachandiran, 2014). In some countries where their citizens have shellfish eating habit such as Japan, Spain and France, the demand for bivalves is very high making bivalves industries to be very important (Díaz & Sánchez, 2011). Bivalves have become as traditional foods in these countries and they become prime consumers and also principal producers (Struthers, 1993; Laing, 2001; da Costa & Martínez-Patiño, 2009).

Each year, landing of bivalves include captured and aquaculture operations is increasing. Food and Agriculture Organisation (FAO, 2011) reported that amount of bivalves landing in year 2014 is 16.073 million tonnes and this value keep on increasing each year (Helm et al., 2004). Thus, the bivalve industries (oysters, mussels, clams and scallops) form a significant part of world's fisheries production for human consumption.

In some areas near to the natural resources of bivalves, activities on bivalves harvesting provide subsistence income to the local people (Díaz & Sánchez, 2011; Rahim, 2011). Therefore, activities on catching and harvesting of bivalve especially clams species have become part of the traditional activities for that area (Carvalho et al., 2011; Rahim, 2011). In Portugal, harvesting of razor clams *Ensis siliqua* and *Solen marginatus* species at their natural beds of Ria de Aviro and Ria Formosa is a traditional activity since eighteenth

centuries (Miguel et al., 2011). In Malaysia, local coastal communities at western part of Sarawak namely Asajaya Laut and Buntal harvest Solenidae razor clams at commercial scale from November to December each year (Rahim, 2011). As a result, this drastically increase the fishing pressure and affect the natural population of razor clams.

Threats from anthropogenic activities can also reduce the natural stock of razor clams. Disturbance by human activities can change the landscape and ecosystem of coastal water and gives impact to benthic habitats (Brumbaugh et al., 2006; Veeravaitaya & Choowaew, 2011). For examples, deforestation and sand dredging will result in two major impacts: (1) alteration of the mudflat landscape due to increase or decrease of sediment at intertidal zone and, (2) changing the stability and compactness of sediment at intertidal areas (Hunt, 2005). The first impact will change the settlement sediment of spat in which razor clams need suitable sediment character for settlement process to succeed. This will result in low spat survival and failure of new recruitment of razor clams into the existing population (Pawlik, 1992). Furthermore, the second impact will result in sediment to become too soft or too compact for clams' burrowing activity (Ahn et al., 1993; Coen, 1995).

Fishing pressure on bivalves' population will continue to increase when market demand exceeds the natural supply. Effect of fishing pressure will become more serious when there is lack of sustainable management and bivalve spat production to recruit new population of razor clams to natural habitat each year (Carvalho et al., 2011). Moreover, the fishing pressure will be more severe on bivalve species that have specific spawning period in a year such as razor clam (Laing & Spencer, 2006; da Costa, 2012). In order to establish consistent production of razor clams, the knowledge on reproductive cycle, spawning periods and broodstock conditioning culture are crucial to study (Muranaka & Lannan, 1984; Utting, 1992; Robinson, 1994; Utting & Millican, 1997; Lannan et al., 1980a; Chavez-

Villalba et al., 2002; Liu et al., 2015). Therefore, requirement to improve in technique and technology of aquaculture for bivalves' production is important in order to maintain the bivalves' resources.

1.2 Broodstock conditioning and its importance

Advance technology on bivalves' culture is important in order to keep the bivalves industries from collapse (Brumbaugh et al., 2006; Laureta, 2014). Bivalves' production can be divided into three parts; broodstock conditioning, larvae rearing and spat production (Utting, 1992; Utting & Millican, 1997; Chávez-Villalba et al., 2002). Broodstock conditioning is carried out by manipulating the environmental condition in laboratory or hatchery setting on broodstock (Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; Argüello-Guevara et al., 2013). By mimicking the natural environment in the laboratory setting to reduce adult stress, the broodstock can be induced to reach at the optimum stage of gonadal development and also reduce the parasite prevalence (Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; Argüello-Guevara et al., 2013).

The outcomes of successful broodstock conditioning can give many benefits. For instance, the production of spat can be maintained throughout the year by adjusting early or later along the year (Matias et al., 2009). It also can increased the gonadal content, oocytes quality and larval survival by bringing the broodstock to an optimum state of gametogenesis production (Lannan et al., 1980; Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; da Costa et al., 2010; Argüello-Guevara et al., 2013). The quality of oocytes produced by broodstock conditioning showed positive correlation with survival of the embryo and larval growth (Bayne & Newell, 1983; Ahn et al., 1993; Thompson et al., 1996; Kenchington et al., 1998; Holbach et al., 2015). Bivalves broodstocks that are subjected to stress

conditioning or no conditioning at all resulted in reduction in fecundity and oocyte quality (Leal, 1994; da Costa, 2012; Marshall, 2012). They also produce fewer larvae and less competent larvae (Lannan et al., 1980; Gallagher & Mann, 1986). In contrast, suitable conditioning culture regimes help to reduce adult stress and can maximise the viability and survival of larvae (Bayne et al., 1975; Lannan, 1980).

By referring to the gonadal condition index (GCI) of each clams species, the information on reproductive cycles can be categorised into gonadal development, maturation, spawning period, spend and resting period (Darriba et al., 2005). In Sarawak, base line information on GCI and spawning period of *S. regularis* had been documented (Rinyod & Rahim, 2011). This all information is important for broodstock conditioning studies.

Problems in health and survival of bivalves also can be influenced by parasite presence in natural bivalve beds. The spreading of bivalves parasite and disease to new locations also can happened via import and export of live bivalve species (Bower et al., 1994; Berthe, 2005). In general, low number of parasites in bivalves gives no overt disease to the bivalve but when presence in significant number, this can cause debilitation behavioural changes, tissue destruction and broodstock death (Bower et al., 1994; Coen et al., 2004). The effects of parasites on bivalve broodstock can cause spawning failure by delaying spawning and destroying ripe oocytes (Ngo et al., 2003). Prevention of the parasite prevalence on broodstock is important for bivalve production culture but usage of antibiotic to reduce the parasite prevalence in aquaculture is not encouraged due to their negative impact to environment (Holbach et al., 2015). Thus, broodstock conditioning process is a way to reduce effect of parasite contamination.

Research on bivalves broodstock condition culture is getting more attention due to two main purposes. The first one is in the form of economic purpose because bivalve production could be performed in larger scale and consistent throughout the year. The second is the quality of larval production that are produce by healthy broodstocks are more safer to consume (Lucas & Beninger, 1985). However, a lot comprehensive research need to be conducted in order to obtain successful technique in razor clam from broodstocks conditioning, spawning and larval rearing (Berntsson et al., 1997).

1.3 Problem statement

The natural stock of *S. regularis* in western region of Sarawak showed sign of overexploitation due to unregulated fishing efforts which reduced the number of spawning adults and give negative impact on population structure of razor clams (Rahim, 2011). Moreover, reproductive organ of *S. regularis* shows preseneed of endoparasite (trematode and nematode) within the clam body cavity (Gelien & Rahim, 2009). The presence of parasite may reduce the health of *S. regularis* broodstock and thus affect the recruitment of *S. regularis*. According to previous study, clams which were infected with endoparasite could not spawn during the induce spawning experiment (Rahim, 2011). According to Rinyod (2012), the reproductive cycles of *S. regularis* in Sarawak is an annual cycle which includes specific month of spawning period and resting period. Recruitment of natural spat may be seriously affected if high prevalence of parasite occurred in razor clam broodstock during the spawning period. There is no established method to culture the broodstock or razor clam yet. Therefore, this study aimed to establish technique broodstock conditioning culture for *S. regularis* by evaluating broodstock survival, growth and broodstock conditioning impacts.

1.4 Objectives

The objectives of this research are: (1) to establish suitable conditions of broodstock of *S. regularis* at laboratory scale based on the survival and growth; (2) to evaluate the impact of broodstock conditioning of *S. regularis* on the gonadal condition index (GCI), oocyte diameter and parasite prevalence during spawning period and resting period.

CHAPTER 2

LITERATURE REVIEW

2.1 Razor clams and *Solen regularis* in Sarawak

2.1.1 Taxonomy

The taxonomy classification of *Solen regularis* according Cosel (2015) is as follows:

Phylum: Mollusca

Class: Bivalvia Linnaeus, 1758

Subclass: Heterodonta

Infraclass: Euheterodonta

Order: Adapedonta

Family: Solenidae

Genus: *Solen*

Species: *Solen regularis* Dunker (1862)

2.1.2 Distribution

Razor clams are distributed in tropical and subtropical areas and mostly can be found in the Indo-Pacific region. In Sarawak, *S. regularis* has its native distribution around intertidal mudflat, sandy bars and beaches specifically in the western part of Sarawak, particularly near Kuching and Samarahan Division (Ruhana et al., 2006; Rahim, 2011). The popular razor clamming areas in the western part of Sarawak are Asajaya Laut, Buntal, Bako, Muara Tebas, Sambir, Sebandi, Moyan and Sampun (Rahim & Tan, 2008).

2.1.3 Habitat

In general, razor clams are burrowing organisms at sandy muddy intertidal area from low intertidal until infralittoral zone (Darriba & Tajés, 2011). Razor clams can dig in and out of the sediment immediately and form a new vertical hole, in which it can ascend and descend (Gosling, 2015). According to da Costa (2012), *Solen* species prefers substrates with very fine grain, areas protected from swell and with medium-low salinities. Previous study in Sarawak showed that high density of razor clams was found in sediment that contain high sand and low silt and clay fractions (Loh, 2005). However, there are other factors that can influence the location of this species with various level of interaction between the physical and biological parameters (Bayne & Newell, 1983).

2.1.4 Reproduction cycle

In Sarawak, study of reproductive cycle of *S. regularis* at Asajaya Laut and Buntal is an annual cycle (Rinyod & Rahim, 2011). Based on the observation of mean gonadal condition index (GCI) pattern, it is suggested that razor clams have five stages of reproductive cycle: i) gonadal development (during the increment of GCI value); ii) maturation (during the maximum GCI values); iii) spawning (indicated by the decreasing in GCI value after the maximum); iv) spent (occurred after last spawning which indicated the end of active phase), and v) resting period (during the minimum GCI values). According to Figure 2.1 refer *S. regularis* from Asajaya, *S. regularis* spawned at least three times during the spawning period from end of March-April to September followed by a resting period from end of November to January in the next year (Rinyod & Rahim, 2011).

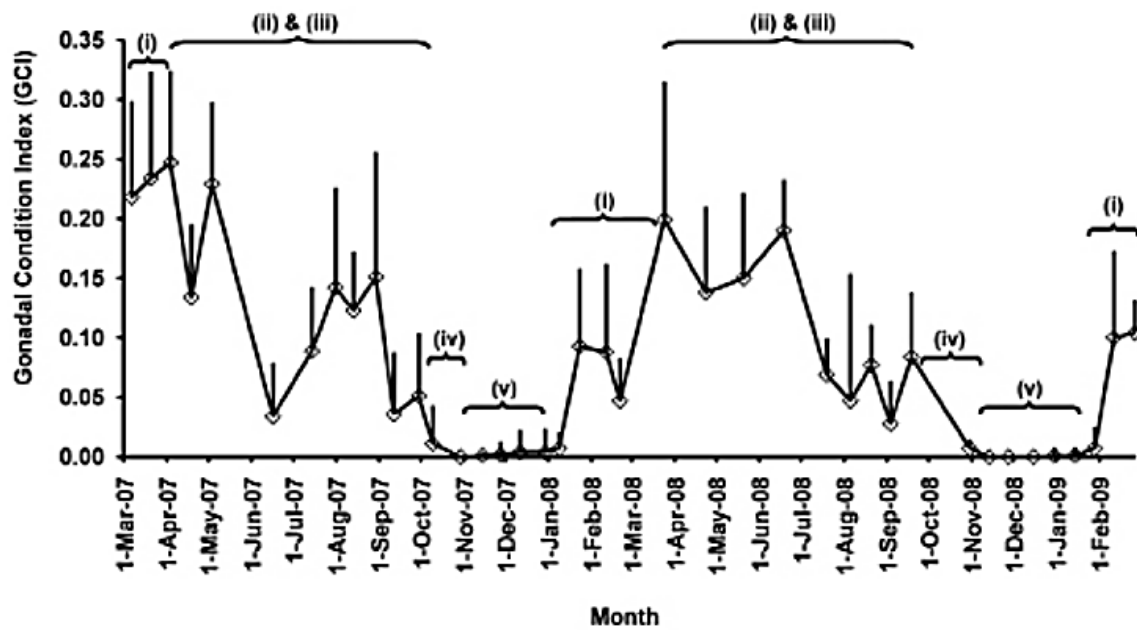


Figure 2.1: Variation of gonadal condition index (GCI) (mean $\pm$ S.D.) from March 2007 to February 2009 in males and females of *S. regularis* at Asajaya Laut with five stages of gonadal condition, (i) gonadal development, (ii) maturation, (iii) spawning period, (iv) spent and (v) resting period (Rinyod & Rahim, 2011).

2.2 Bivalve culture technique and broodstock conditioning process

In certain species of bivalves, the culture techniques also include the broodstock conditioning process (Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; Argüello-Guevara et al., 2013). By performing the broodstock conditioning on bivalve culture, it can improve the gonad quality of broodstock and also reduce parasite prevalence from the broodstock (Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; Argüello-Guevara et al., 2013).

Setting of bivalve culture in the laboratory or hatchery needs to mimic the environment of their natural habitat. It is important to reduce stress onto the broodstock in the new environment (Bayne et al., 1975; Utting & Millican, 1997). There are several fundamental aspects in establishment of bivalves broodstock conditioning culture. In broodstock culture management area, larger space is required in order to imitate the density

of razor clam in their natural habitat. Therefore, the stocking density of broodstocks is very important to be determined during the conditioning operation (Helm et al., 2004).

2.2.1 Bivalve culture system and seawater

The culture system for bivalve culture depends on many factors such as bivalve species, location of hatchery, production scale, infrastructures and logistic capability (Lannan et al., 1980; Helm et al., 2004; Pronker, 2008). For example, the running flow water exchange system equipped with filtration components to filter the water source intake was used on some bivalves for rearing (Loosanoff et al., 1953). In most cases, conditioning takes place in flow-through systems, which may or may not include an element of water recycling and filtration system in order to conserve cultured microalgae that are added as feed (Helm et al., 2004). However, for the flow-through systems, the location of hatchery should be as close as possible to the source of seawater intake (Helm et al., 2004).

On the other hand, tanks with seawater recirculation system have been used in the broodstock conditioning of some bivalve species (Díaz & Sánchez, 2011; Menasveta et al., 2001). Normally, the total volume of water in the system needs to be drained and refilled at least twice weekly in order to maintain the quality of the culture for this system and to prevent build-ups of bacteria and metabolites (Helm et al., 2004). Maintaining bivalve broodstock culture in a closed recirculating raceways system with filtration component help in removing ammonia excretion by the organism (Hadley & Whetstone, 2007). With filtered seawater, it can help in reducing mortality of bivalve and preventing fouling of worm and tunicate compared to unfiltered seawater (Loosanoff et al., 1953). This technique is enough to offer higher survival and quicken the growth rate of broodstock clam (Pfeiffer et al., 1999).

However, Manila clam (*Ruditapes philippinarum*) cultured with unfiltered seawater showed better survival and growth rate compared to those in filtered seawater (Yan et al., 2006).

Culture of bivalves needs sufficient high quality seawater supply for maintaining the quality of seawater and culture itself (Menasveta et al., 2001). This is because bivalves are filter feeders that are very strict in physiological requirements such as water temperature, ammonia level, salinity and oxygen which must be maintained at optimum range (Helm et al., 2004). Low quality of seawater supply from the coastal water of central and northern Adriatic had caused high mortality to the culture of *Chamelea gallina* during the experiment (Angioni, 2010). Thus, water parameters must be monitored continuously during bivalve culture to prevent huge loss.

2.2.2 Substrate and sediment

Razor clams are burrowing organisms and showed some preferences for particular sediment types. According to Alexander et al. (1993), clams species are classified as substrate generalist which are slow to moderate burrowers and can live in the broadest range of sediment textures from very coarse to mud sediment. Razor clams can feed more efficiently if there are kept in the suitable substrate and burrowing in the suitable substrate help to prevent shell gaping due to their weak abductor muscle (da Costa et al., 2010). Furthermore, substrate is also needed in larval settlement and spat production of razor clam because spats being reared without substrate showed deformities in their shells (Tezuka et al., 2013; da Costa et al., 2010). Substrate has to be provided progressively to acclimatise spats for on growing conditions in natural beds (da Costa et al., 2011).

The sediment also can influence the growth rate and survival rate of razor clam. This is the main challenge in developing culture techniques for razor clams since this species

require substrate to allow burrowing and preventing shell gaping (Cromie & McDonough, 2006). Two broodstock holding systems on razor clam *E. siliqua* were investigated in order to prevent the shell gaping: (1) with sand (which mimics natural conditions) and (2) using plastic rods (to which the razor clams were attached with elastic bands and suspended around the inside of the holding tank (Cromie & McDonough, 2006). Higher survival rate was observed in *E. siliqua* that used sand system (85%) compared with the plastic rod system (only 30%) after 33 days (Cromie & McDonough, 2006). This showed that the use of sediment for holding shells of razor clams is more preferable in broodstock conditioning technique. In other technique, artificial substrate like clay stone can be used as replacement for the natural substrate (da Costa et al., 2011). The culture of razor clams is being constrained by their need to be buried and that they are sensitive to manipulation, making spat handling and cleaning difficult. Moreover, for large-scale production, it is important to reduce the volume of the sand because it is quite difficult to handle.

2.2.3 Diet

The success of bivalve aquaculture also depends on the production of microalgae as their diet. Moreover, the preparation of microalgae needed for bivalve broodstock conditioning must be in large quantities and high quality. The general diet used for bivalve culture including razor clams comprise mixed unicellular algae from diatoms (*Skeletonema costatum*, *Chaetoceros* spp., *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*) and flagellates (*Chlamydomonas coccoides*, *Nannochloropsis oculata*, *Dunaliella tertiolecta*, *Pyramimonas suecica*, *Isochrysis* spp., *Monochrysis lutheri* and *Pseudoisochrysis paradoxa*) (Laing, 1991). Most bivalve cultures used *Chaetoceros* spp. and *Isochrysis* spp. as the common life feed (Elisabeth et al., 2008; Golez et al., 2011;

Marshall, 2012; Argüello-Guevara et al., 2013). Mixed diet of *Chaetoceros* spp. and *Isochrysis* spp. gave the highest survival in the angelwing clams *Pholas orientalis* broodstock culture (Golez et al., 2011).

In broodstock conditioning of bivalve species, the volume of microalgae required is based on the mean dry meat weight of the adult which is usually between 1 to 4% (Utting, 1992; Leal, 1994; Utting & Millican, 1998; Connor et al., 2000; Helm et al., 2004). However, rations exceeding 6% did not show any significant effect toward the gonadal development of broodstock (Helm et al., 2004). Moreover, the combination of suitable temperature and diet showed improvement in bivalve growth and gonad content (Helm et al., 2004). Furthermore, the effect of light intensity can also influence the feeding activity of angelwing clams which feed better in total darkness (Golez et al. 2011 as cited in Gosling, 2015).

2.2.4 Duration of culture for broodstock conditioning

In broodstock conditioning of bivalves, there are many factors that influence the duration of culture (Lannan, 1980; Gallager & Mann, 1986; Liu et al., 2008). Duration of broodstock conditioning culture refers to the number of days of broodstock being held before they are ready to spawn after some improvement in their gonad content. However, the number of conditioning days itself is not a good indicator of the time required to reach a condition ready for spawning, since it is dependent on the initial physiological condition of the animals (Lannan et al., 1980). The time required to develop mature oocytes depends on animal origin, the gonad maturation stage at the time of collection and the period of the year (Robinson, 1992). In general, the duration of culture for maintaining and conditioning broodstock clams in a closed system raceway ranged from 2 to 8 weeks and can reach up to 6 months (Hadley & Whetstone, 2007).

For example, the clams *Ruditapes philippinarum* in Italy needs 6 to 8 weeks in order to become ready for spawning in winter and early spring, but this period becomes progressively shorter as nearing the natural spawning period (Ojea et al., 2008). This showed that the length of the conditioning period is seasonally dependent (Helm & Pellizzato, 1990). In addition, the duration of broodstock conditioning also dependent on the availability and quantity of food during conditioning (Sastry, 1979).

2.2.5 Stocking density

Stocking density of bivalve culture is an important factor to be considered because it influences both the growth and survival of the bivalve reared (Marshall, 2012). Slow growth rate and weak condition were observed in both clams and oysters as the stocking density increased (Marshall & Dunham, 2013). Moreover, in a closed culture system, high stocking density will result in increased amount of metabolic wastes in the water and giving rise to detrimental effects (Yan et al., 2006).

On the other hand, high stocking density of razor clam *E. siliqua* can give advantage to them because the neighbouring clams will prevent the shell gaping and reduce the energy necessary to keep shells closed (da Costa et al., 2011). This is due to the weight of neighbours pushing one another. However, too high stocking density of razor clam should be prevented in order to ensure the clams can get the optimal feed supply for their growth (Fréchette, 2010; Shakir et al., 2014).

Until now, suggested values for stocking densities of broodstock conditioning vary considerably for all bivalve groups. In general, Utting (1992) suggested approximately 50 individuals of bivalve (20 to 25 g live weight) per 200 L tank for a flow through system, while Chew et al. (1987) suggested a much lower stocking density of one to two individuals

bivalve per 50 L of water for a static water system. But Hadley and Whetstone (2007) stated that stocking density for razor clam broodstock culture can be one clam per 4 L of water for static water system.

2.3 Water quality on bivalve and broodstock conditioning culture

2.3.1 Salinity

Marine bivalves are euryhaline that can tolerate wide range of salinity since they inhabit the estuaries and bays which are subjected to pronounce salinity fluctuations due to evaporation, rainfall and inflow from rivers (Gosling, 2015). In general, the adult bivalve's salinity tolerance range are 4 to 33 PSU (Gosling, 2015). *Perna viridis* broodstocks showed 50.00% survival in an experiment after two weeks of exposure to salinities of between 24 and 80 PSU although the salinity of *P. viridis* natural habitat range from 27 to 34 PSU (Gosling, 2015). Another example is *Mya arenaria* that lives in estuary and being constantly exposed to changes of salinity from 10 to 25 PSU (Verween et al., 2007). Davis (1955) stated that range of salinity can influence the gonadal development of bivalves. In too low salinity (5 PSU) or too high salinity (39 PSU), the development of oocyte will be limited and interrupted the fertilisation process. However, the bivalves can produce larvae constantly at 20 PSU (Davis, 1955).

2.3.2 Temperature

Several studies had suggested that temperature plays an important role in determining the success of broodstock conditioning for various bivalve species (Utting & Millican, 1997; Chávez-Villalba et al., 2002; Halper, 2009; Matias et al., 2009). Temperature has positive impacts towards clams growth and gonad improvement (Laing et al., 1987; Argüello-

Guevara et al., 2013). By knowing the optimal temperature tolerance of the broodstock, adjustment of temperature can improve the gonadal development and gamete production including reduced time to gamete maturation (Gallager & Mann, 1986; Fabioux et al., 2005). Moreover, temperature also can increase number of oocytes produced (Utting & Doyou, 1992), more rapid accumulation of lipid and protein in the gonad (Martinez et al., 2000) and better quality larvae in terms of survival, growth and metamorphosis (Robinson & Breese, 1984; Utting & Doyou, 1992).

However, range of temperature for clams culture needs to be evaluated because they have specific range and optimum temperature to produce good gonad condition (Mann & Glomb, 1978; Heasman et al., 1996). Otherwise, bivalve broodstocks that were held at high temperatures may fail to ripen (Heasman et al., 1996), lose their good condition (Thompson et al., 1996), show declining carbohydrate levels (Mann, 1979), or produce smaller size of oocytes (Honkoop & Beukema, 1997).

2.3.4 Water quality

Quality of water during bivalve culture can affect their survival and health. During the bivalve culture operation, ammonia will be produced as a result of excretory metabolism and monitoring on the ammonia level is important because it may be toxic to the cultured organism. In a closed and re-circulating water system, effect of water quality can be significant whereby the nitrogenous wastes and dissolved carbon dioxide will accumulate and depletion of dissolved oxygen can occur (Borsuk et al., 2002). Under high temperatures of the culture system, metabolic rate of organisms will increase (Hamburger et al., 1983) and oxygen solubility is lower (Mann, 1979). Some benthic organisms like bivalves can tolerate low dissolved oxygen level (Honkoop & van der Meer, 1998) but at hypoxia condition will

result in lower metabolic rate and adjustment of ventilation rate (Willson & Burnett, 2000) which will reduce the growth and survival rate of bivalve.

Unsuitable water quality level inside a culture system will restrict the improvement of gonadal development. In a culture system, the effects of low water quality on adult bivalves can be reduced by aeration, ‘air stripping’ using recirculation technology to remove carbon dioxide and bio filtration to remove ammonia (Willson & Burnett, 2000). Another way of preventing low water quality is to avoid over stocking density during the culture (Yan et al., 2006). In a closed water system, the water exchange can help to maintain favourable dissolved oxygen levels (Southgate et al., 2008). In a closed and recirculating system, the rate of water exchange is difference between bivalve species. For instance, the water exchange for hard clam broodstock was carried out three times per week and the water input was also treated with ultraviolet (UV) light and undergoes 25 µm filtration (Hadley & Whetstone, 2007). For the broodstock conditioning of bivalves, the growth and survival rate were significantly faster in unfiltered water than the filtered water (Yan et al., 2006).

2.4 Impact of broodstock conditioning to bivalve

2.4.1 Shell length and wet weight

Documentation on increment of shell length and wet weight of bivalves is important during the establishment of bivalves’ culture. The growth of shell length and gain in wet weight of bivalves are used to evaluate the condition of bivalve broodstock depending on the culture system (Serdar et al., 2007; Albuquerque et al., 2009; Ojea et al., 2009; Breen et al., 2017). For razor clam *E. macha*, the elongation of shell as individuals growing give them an adaptive advantage to become effective burrowers (Barón et al., 2004). The values of minimum and maximum growing of shell length and wet weight of bivalves differ among

species (Gosling, 2015). Commonly, the shell growth and total wet weight of bivalves increased steadily over the years especially for larvae stage (López-Alvarado, 2015).

The growth of shell length and wet weight of bivalves are also influence by many factors such as temperature, salinity and food availability (Chew et al., 1987; Laing et al., 1987; Bacher & Goulletquer, 1989; Goulletquer et al., 1999; López-Alvarado, 2015). For example, it was found that the stocking density of hard clam affect on the increase of the total weight rather than on the shell length (López-Alvarado, 2015). In most bivalves broodstock conditioning studies, the growth rate and wet weight of clams are not emphasize but in this experiment, the shell length and wet weight were evaluated as part of the establishment of the *S. regularis* broodstock culture technique.

2.4.2 Parasite prevalence

Parasite prevalence is one of the significant cause for mortalities on bivalves' culture. There are 169 summaries of worldwide marine parasite species such as platyhelminthes, protozoa, fungi, snails and trematode (Bower et al., 1994). For example, culture of hard clam *Mercenaria mercenaria* in three state of Canada (Massachusetts, New Jersey and Virginia) had recorded a catastrophic mortality with 80 to 95% of mortality instances after being infected by Quahog Parasite Unknown (QPX) (Calvo & Burreson, 2002). Another common parasite for bivalve broodstock is parasitic snail which feeds on bivalve broodstock via trunk to suck body fluids from the mantle of the clams (Struthers, 1993). Symptoms of the prevalence can be observed by bleaching or loss of mantle colour, an incomplete extended mantle, shell gaping and sporadic mortality (Ellis, 1997).

In Sarawak razor clam *S. regularis*, the presence of endoparasites namely trematode and nematode within the clam's body cavity had been reported (Gelian & Rahim, 2009). The

trematode was identified as a digenean trematode of the Family Gymnophallidae, but the nematode could not be further identified. The site of prevalence is mainly within the gonad and digestive tract. Infected clams could not be successfully induced in the spawning experiments (Gelian & Rahim, 2009).

In addition, hard clams that are subjected to stress associated with high stocking densities and poor husbandry will have higher risk of parasite prevalence problems (Calvo & Burreson, 2002). For endoparasites, the parasite prevalence can be reduced with the water exchange during the culture of bivalve. This is because the parasite have a planktonic phase of lifecycle that hatch from the oocytes and escape from their host to the water column (Sanil & Vijayan, 2011). By doing the water exchange process during broodstock conditioning, it can break the production cycle of the parasites (Bower et al., 1994; Poulin et al., 2005; Petersen & Garcia-ortega, 2008; Bamio, 2011; Golez et al., 2014; Gosling, 2015; O'Connell-Milne, 2015;). Some hatcheries will remove and clean all ectoparasites that are visible on the shell of bivalve (mostly hard shell bivalve) to prevent the spreading of the parasite prevalence (Ellis, 1997; Coen et al., 2004).

2.4.3 Gonadal condition index (GCI) and oocyte diameter

Temperature and nutritional plankton supply are the two main factors that affect the gonadal development and reproductive cycle of bivalve during broodstock conditioning, either in accelerating gametogenesis or in retarding gonadal maturation (Gallager & Mann, 1986; Berntsson et al., 1997; Utting & Millican, 1997; Martínez et al., 2000; Navarro et al., 2000; McNeill & Columbia, 2010; Argüello-Guevara et al., 2013). The speed of oocyte growth and oocyte size of scallop *Argopecten purpuratus* increased with changes of temperature (16 and 22 °C) (Navarro et al., 2000). Another study by Ojea et al. (2008) on clam *R. decussatus*

showed higher gonadal condition index and oocyte size after being conditioned under the gradual increase of temperature regime. This further enhance the larval production of *R. decussatus* and reduced the overall hatchery production costs.

However, there are other factors that contribute to gonad maturation and oocyte size of bivalve that underwent the broodstock conditioning. This is because the maturation of oocytes for bivalve species also depends on animal origin, the gonad maturation stage at the time of collection, quality of microalgae supply, type of culture system, presence of substrate, salinity of seawater and duration of conditioning period (Utting & Millican, 1997; Laing & Spencer, 2006; da Costa et al., 2012).

CHAPTER 3

METHODOLOGY

3.1 Broodstock Preparation

3.1.1 Sediment

Natural sediment for substrate in this experiment was obtained from Asajaya Laut intertidal area where the razor clam broodstock were collected. The sediment was wet sieved through 1 mm mesh with tap water to remove large macrofauna, large sediment particles, wood and debris. Then, the sediment was oven dried overnight at 60 °C. After experiments, the sediment will be retreated again and reused for the next experiments. In the standard design (control condition), 36 L glass conditioning tanks were filled with 5 cm sediment thickness and 20 L of unfiltered seawater. These tanks were left overnight to ensure all sediments settled down. The texture of sediment used comprised of 25.58% clay, 23.34% silt and 28.21% very fine sand. The other minor fractions are fine sand (6.67%), medium sand (7.71%) and coarse sand (8.53%). Analysis grain-size of sediment was followed the standard wet sediment analysis protocol (Singer et al., 1988).

3.1.2 Seawater

The seawater used in this study was obtained during high tide at the hatchery located at Santubong Fisheries Research Institute, Sarawak. The salinity of seawater ranged from 23 to 28 PSU. Seawater used in broodstock conditioning culture was filtered through 179 µm mesh net filter to remove any suspended particles. The seawater was filtered through glass microfiber filter paper (Sartorius, grade MGC, pore size 1.2 µm) using electric vacuum pump (GAST, DOA-P504-BN) for microalgae culture.

3.1.3 Microalgae preparation

Two species of marine microalgae (*Isochrysis* spp. and *Chaetoceros* spp.) were used to feed the razor clam broodstocks. Those microalgae were obtained from the Microalgae Unit, Santubong Fisheries Research Institute (FRI), Sarawak. New batch of microalgae were prepared and obtained every six months.

The 50 ml flat bottom glass containers were used as a starter culture for microalgae culture, 2 L conical flask for batch microalgae culture and 10 L round semi-transparent plastic tank for large batch microalgae culture. For starter and batch culture, the microalgae were proliferated in SW II media. However, for larger batch culture the *Chaetoceros* spp. (5 L volume) and *Isochrysis* spp. (10 L volume) were proliferated in commercial KW21 medium (1 ml/L) added with sodium metasilicate 45 g/ L (PES C). The microalgae cultures were maintained at 22 to 24 °C temperature and illuminated for 24 hours under full-spectrum florescent bulb (Philip Lifemax TLD 36W/840) at between 2.65 to 3.22 Klux (Hanna Luxmeter, HI 97500). The media used in starter and small batch culture of microalgae were sterilised with autoclave machine (TOMY, SX-700) but for 5 L and 10 L large batch microalgae culture used filtered seawater without sterilised (Figure 3.1).

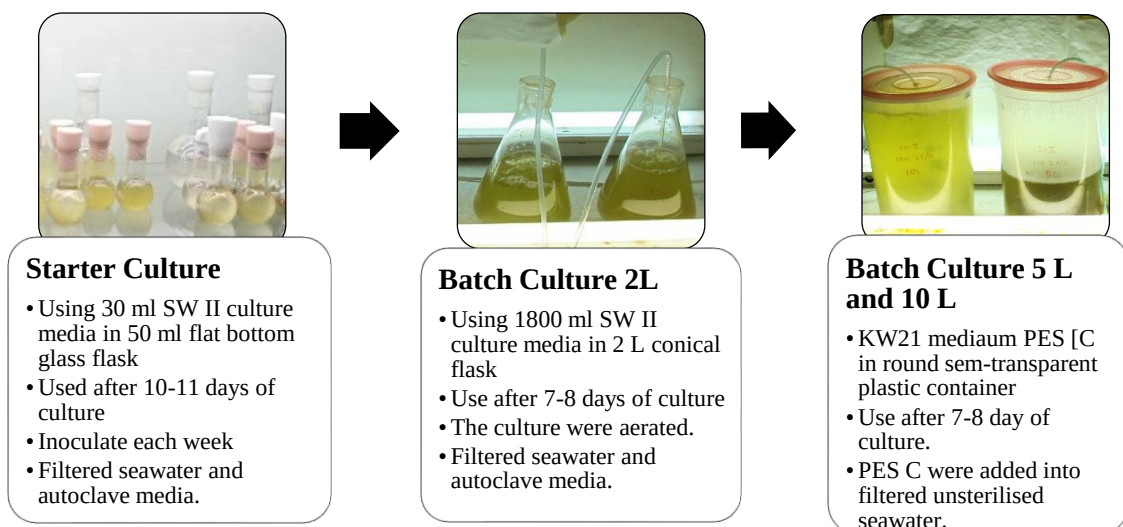


Figure 3.1: Summary of microalgae culture used throughout this experiment.

In general, the 5 L *Chaetoceros* spp. culture and 10 L *Isochrysis* spp. culture used within three to five days for feeding before used from new batch in order to maintain the quality of microalgae. The overall process for microalgae culture for preparation until ready to feed the broodstock take about 24 to 27 days.

3.1.4 Razor clam sample collection and initial data recording

In this study, a total of 649 razor clams (*S. regularis*) with shell length range 4.04 – 5.79 cm were used. The clams were collected manually by local people at low tide of spring tide where the intertidal areas of Asajaya Laut (N 01° 36', E 110° 36') were exposed (Figure 3.2). Collections were performed during the spring tide (except some collection of samples during Chapter 5 had during low tide of neap tide) from November 2015 until December 2016. During sampling (approximately 3 hours), the clams were kept in plastic bag containing seawater without aeration and later inside cooler box containing ice during transportation to Aquatic Invertebrate Laboratory UNIMAS. These clams remained in the chilled seawater for about 4 hours in total until all the initial data recording work finished.

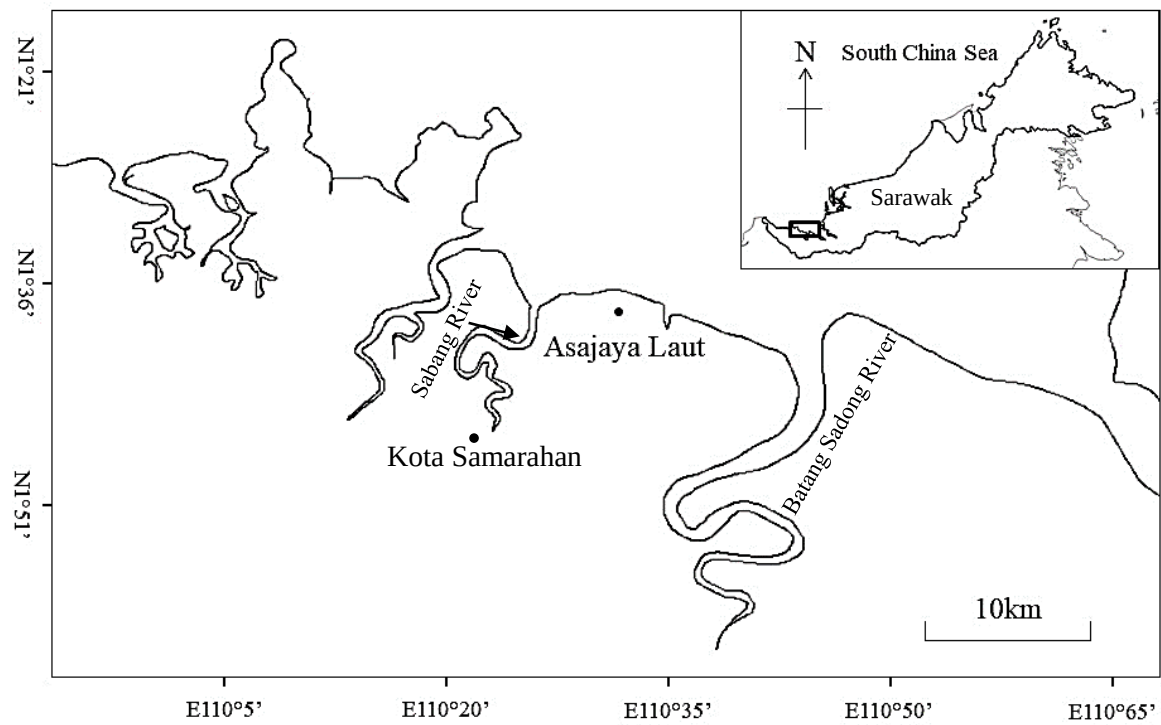


Figure 3.2: *S. regularis* broodstock collection site at the intertidal area of Asajaya Laut, Sarawak.

In the laboratory, each clam was marked individually by carving only the left valve shell with a sharp knife (Figure 3.3). This is to aid the monitoring of any increment in total shell length and wet weight at the end of the culture period (Mamat, 2010). After that, the initial total shell length and total wet weight of each of razor clams were recorded using dial calliper (Mitutoyo, 5005-671; ± 0.02 cm) and digital balance (SHIMADZU, BL 220H; ± 0.000 g), respectively. Total shell length was measured from the two end of shell at hinge side and wet weight was measured in a petri dish containing seawater for each sample. For each experiment, initial value of Gonadal Condition Index (GCI) were calculated for 10 to 15 randomly-chosen razor clams. Detail calculation on GCI will be explained in Methodology of Chapter 5.

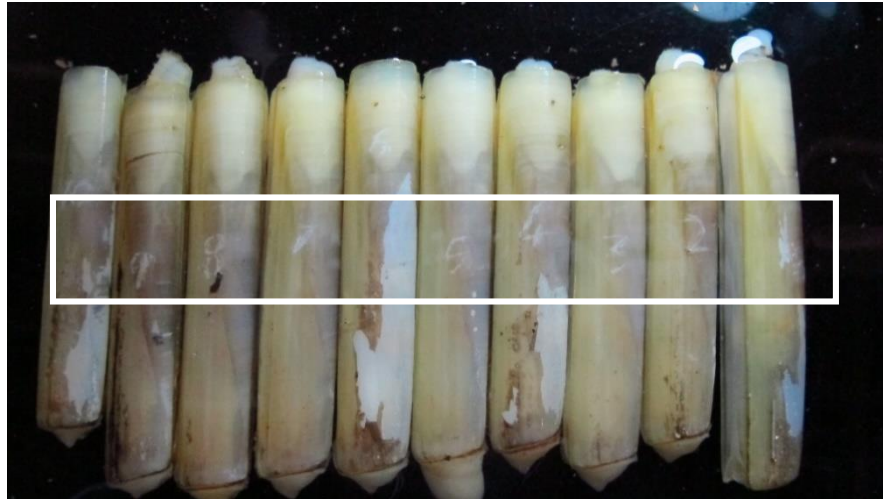


Figure 3.3: Carved razor clams with individual number on the shell with consistent orientation.

3.2. General experimental setup design

This experiment was conducted in November 2015 until December 2016. Each experiment evaluated one parameter with two different conditioning treatments. For preliminary study, Experiment 1 was conducted using only two tanks mean did not have any replicate. Experiment 2 until Experiment 10 were conducted with three replicates simultaneously, giving six tanks for each experiment per setup (Figure 3.4). The size of transparent glass conditioning tanks used is 40×30×30 cm (Figure 3.5). Preparation of these conditioning tanks containing sediment as substrate and unfiltered seawater was conducted one day before sampling to ensure the compactness of sediment in the seawater is uniform. All the broodstock conditioning cultures were aerated 24 hours and were kept in dark condition except during routine maintenance works involving water exchange, culture maintenance and feeding the razor clams with microalgae.

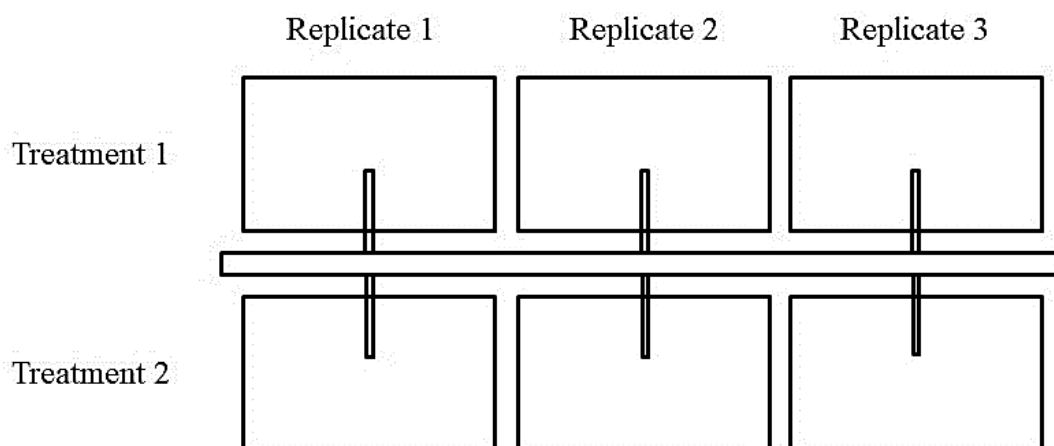


Figure 3.4: Arrangement of tanks from top view for two treatments with three replicates.

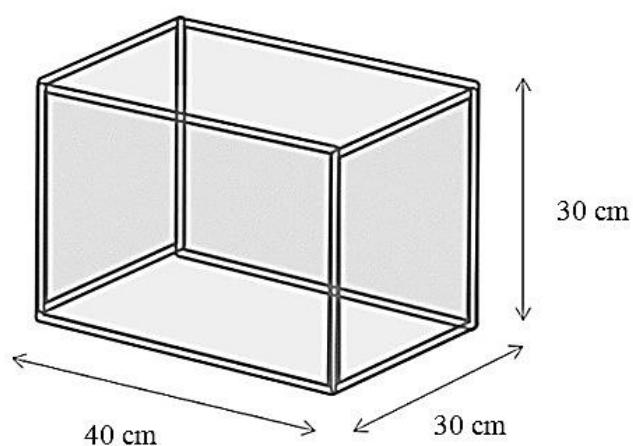


Figure 3.5: The size of transparent glass aquarium that was used for conditioning throughout the experiment.

The razor clams were acclimatised to room temperature in several plastic cups filled with chilled seawater from the sampling area and remain for several minutes before they were transferred into the conditioning tank. This is to prevent drastic changes of temperature between the cold seawater to conditioning seawater temperature.

3.3 Routine maintenance works of the broodstock culture

3.3.1 Monitoring of pH, temperature and pH of the water

Broodstocks were cultured for 25 days and seawater salinity was maintained at 26 to 28 PSU throughout the experiments. The salinity was checked using salinometer (Milwaukee, MA887) and NaCl powder was added if the salinity was lower than 26 PSU. Temperature of seawater was kept at 24 ± 2 °C and being monitored using thermometers. The salinity and temperature were recorded daily but pH value were monitored once a week using the bench pH meter (EUTECH instruments, PH700).

3.3.2 Culture tanks maintenance during the conditioning experiment

During the broodstock conditioning experiments, about 50% of seawater volume was exchanged daily with new unfiltered seawater. Fecal on the surface of sediment and any suspended materials in the water column were siphoned out gently from the tank using a two cm diameter tube. Any dead razor clams found inside the sediment were removed carefully using a large forceps. If the meat of clam disintegrated from the shell, the rotten meat was siphoned out including the infected sediment and new sediment was added into the tank in order to maintain the sediment thickness.

3.3.3 Feeding of razor clam broodstock

Two species of microalgae (*Isochrysis* spp. and *Chaetoceros* spp.) with 1:1 mix ratio were given daily to the broodstocks at approximately 1% of mean dry meat of *S. regularis*. The mean dry meat of *S. regularis* (0.357 g) were calculated based on to 10 individuals obtained in December 2015. The amount of dry (organic) weight for *Isochrysis* spp. and *Chaetoceros* spp were 2.20×10^{-5} g and 7.00×10^{-5} g, respectively (Helm & Bourne, 2004). The process of

feeding the *S. regularis* broodstock culture is showed in Figure 3.6. Amount of microalgae to be fed to the razor clams of each tank were determined after checking the residue of microalgae in each tank. Several formulae were used to determine the amount of microalgae given to *S. regularis* in each tank:

$$(1) \text{ Density of microalgae per clam } (\times 10^6 \text{ cells}) = 1\% \times \frac{(\text{mean dry meat of clam})}{\text{weight of microalga (g)}}$$

$$(2) \text{ Volume of microalgae needed per clam (in ml / L)} = \frac{(1)}{\text{Density microalgae culture}}$$

$$(3) \text{ Total volume of microalgae for each conditioning tank} = (2) \times \text{no. clams left in each tank}$$

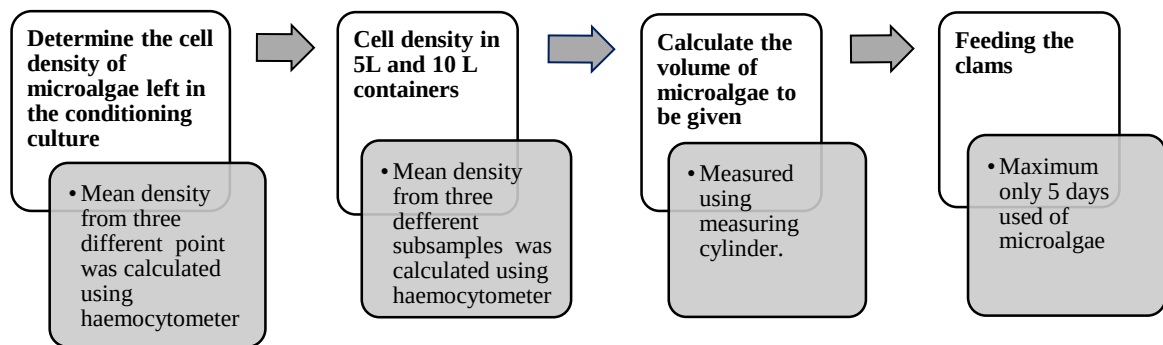


Figure 3.6: Process of feeding the *S. regularis* broodstock culture.

3.4 Data recording and cleaning process

At the end of each experiment, all clams that survived were collected. The final reading of total shell length and wet weight were recorded according to the number carved on the shell of each razor clam. The seawater was siphoned out and sediment was washed with tap water and oven dried at 60 °C overnight before it can be used again for next experiment. All tanks were washed and open air dried.

Illustration on work flow for all steps (Figure 3.7) covers from microalgae preparation until statistical analysis. All batches of broodstock culture followed the same steps except during broodstock culture design and data recording.

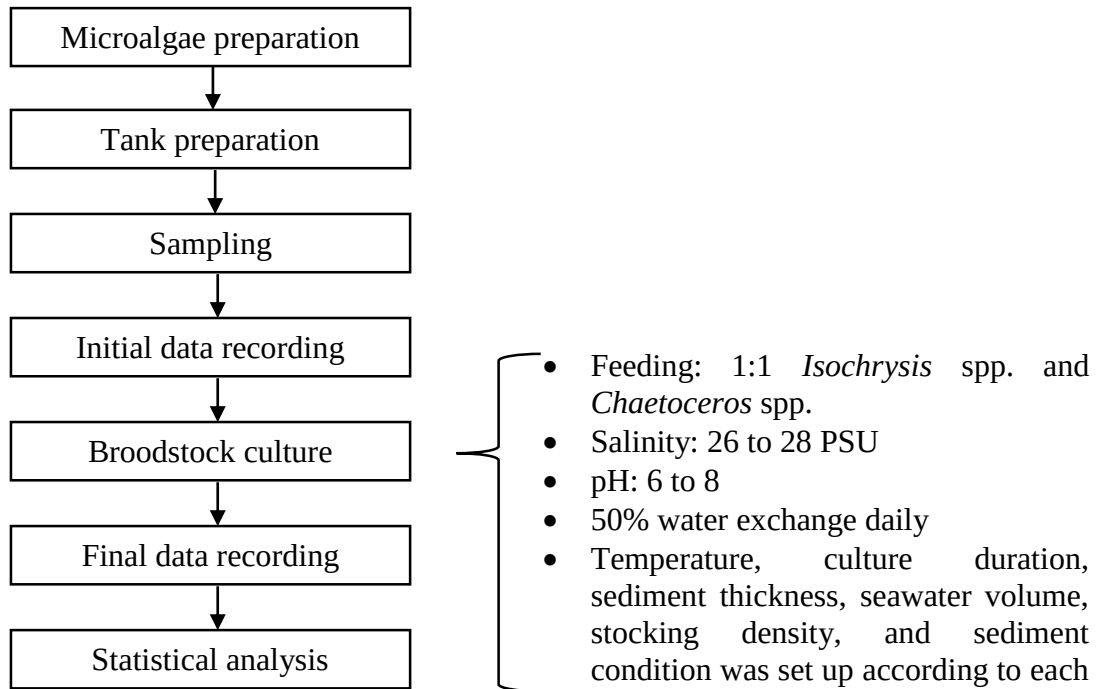


Figure 3.7: Illustration of work flow for processes in this experiment.

CHAPTER 4

SURVIVAL, GROWTH AND QUALITATIVE RESPONSE OF *Solen regularis* DURING ESTABLISHMENT OF BROODSTOCK CONDITIONING CULTURE

4.1 Introduction

Broodstock conditioning is one of the significant practices applied for the bivalve culture such as razor clam in the hatcheries. Manipulating the physical and nutritional parameters during broodstock conditioning practices may affect the clams gonadal development, either in accelerating gametogenesis or in retarding gonadal maturation (Gallager & Mann, 1986). Moreover, the issue of endoparasite prevalence in the gonad of the *Solen regularis* could be resolved using broodstock conditioning technique as being reported in certain bivalve species (Golez et al., 2014; Gosling, 2015). Trials of induce spawning in the laboratory showed that razor clam broodstocks become weak and die after spawning has been induced (Rahim, 2011). In order to carry out the broodstock conditioning of razor clams, it is important to design or set up the culture tank that will result in highest survival and growth of the razor clam broodstocks.

Documentation on survival rate and growth of razor clam broodstocks during preliminary experiment to set up broodstock conditioning tank is important to ensure the clam broodstocks are at optimal health condition along the culture period. Razor clam broodstocks under the stress environment during culture will have problem with tissue growth, tissue composition and survival rate reduction (Carmichael et al., 2004). Studies of survival rate, life tolerance and clam's growth on particular parameters could be executed by set up mimicking the natural environment at laboratory scale. Previous studies showed that physical parameters such as temperature, sediment, seawater volume and seawater

temperature have been tested in order to establish optimum bivalve broodstock culture (Muranaka & Lannan, 1984; Heasman et al., 1996; Thompson, Wilson et al., 1996; Chávez-Villalba et al., 2002; Mamat, 2010; Marshall, 2012; Holbach et al., 2015).

This chapter focused on survival rate, growth and qualitative response of razor clams during broodstock conditioning culture. Broodstock survival rate that ranged from 50.00% to 90.00% from stocking density indicates the setting of conditioning system was suitable for that particular bivalve species (Utting & Millican, 1997; da Costa et al., 2011). Therefore, the objectives of this chapter were to evaluate the effect of several parameters setting towards the survival rate and growth of broodstock *S. regularis*.

4.2 Materials and Methods

In this research, Experiment 1 to 4 are referred as Phase 1 which is the preliminary stage (Figure 4.1). The best outcome of survival rate from Experiment 1 to 4 were then applied as standard design in Phase 2 experiments. During Phase 2, experiments on survival rate and growth were evaluated with several parameter settings and also between two main periods of gonadal development (Figure 4.2). Overall setting of all experiments with their treatment setting were summarized in Table 4.1.

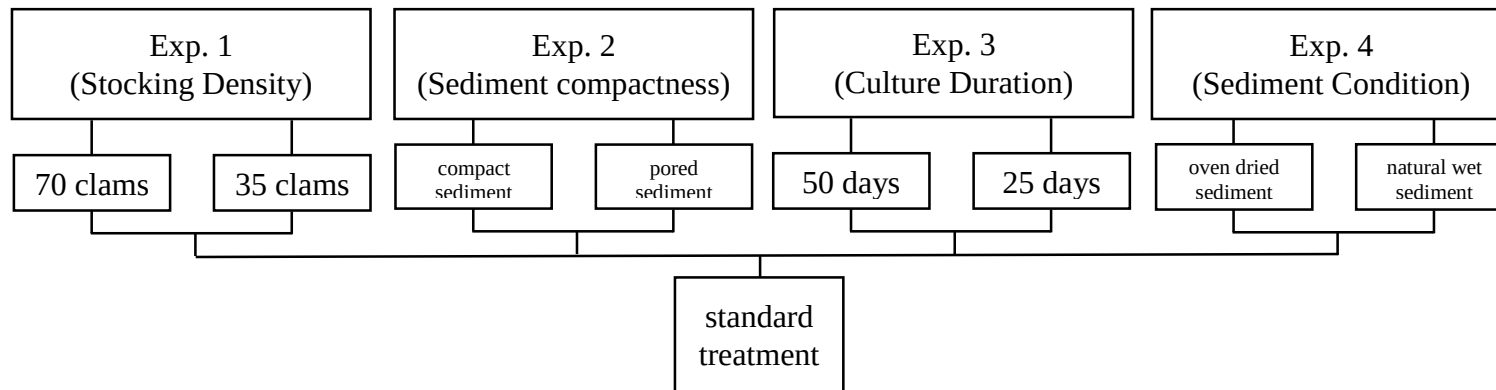


Figure 4.1: Illustration of experiments in Phase 1.

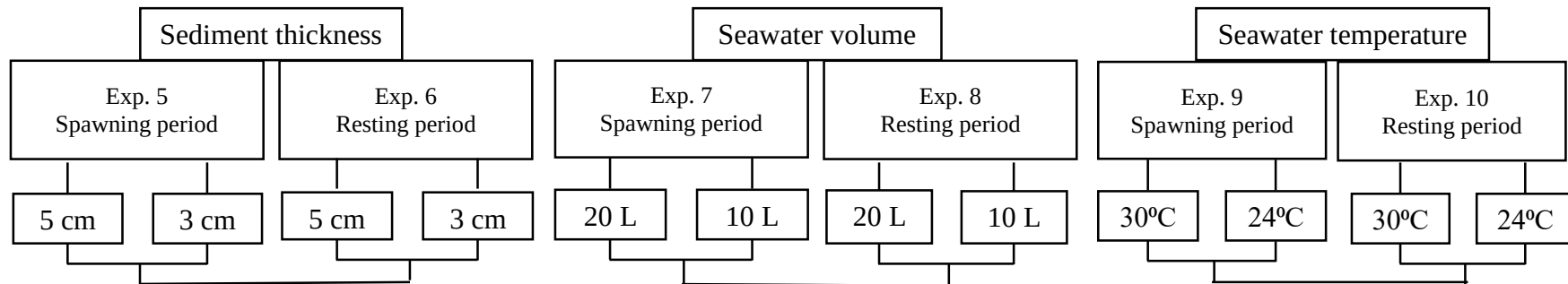


Figure 4.2: Illustration of experiments in Phase 2.

Table 4.1: Experiment summary

No	Experiments (period)	Treatment name: setting	Date
1	Stocking density (r)	1A: 70 clams 1B: 35 clams	30 Nov 2015
2	Sediment compactness (gd)	2A: less compact 2B: compact	27 Jan 2016
3	Culture duration (gd)	3A: 50 days 3B: 25 days	27 Jan 2016
4	Sediment conditioning (s)	4A: Oven dried 4B: Natural wet	18 Apr 2016
5	Sediment thickness (s)	5A: 5 cm (ctrl) 5B: 3 cm	4 May 2016
6	Sediment thickness (r)	6A: 5 cm (ctrl) 6B: 3 cm	7 Dec 2016
7	Seawater volume (s)	7A: 20 L (ctrl) 7B: 10 L	25 May 2016
8	Seawater volume (r)	8A: 20 L (ctrl) 8B: 10 L	7 Dec 2016
9	Seawater temperature (s)	9A: 30 °C 9B: 24 °C (ctrl)	25 May 2016
10	Seawater temperature (r)	10A: 30 °C 10B: 24 °C (ctrl)	7 Nov 2016

s = spawning period, r = resting period, gd = gonadal development period, ctrl = control treatment and using the standard design. All experiments have replicates except for Experiment 1

4.2.1 Phase 1 (Trial experiments)

In this phase, four trial experiments were carried out prior to establishment of the standard design of broodstock conditioning culture which later acts as control treatment. The trial experiments were conducted to determine the stocking density, duration of conditioning culture, sediment compactness and sediment conditioning. In this phase, the survival rate and qualitative response of razor clam between treatments were recorded.

4.2.1.1 Experiment 1: Stocking density

The purpose of this experiment was to evaluate the effect of stocking density on the survival of razor clams. Two different stocking densities (during resting period) of *S. regularis* were

tested: Treatment 1A used 70 clams per tank, while Treatment 1B used 35 clams per tank with 0.12 m² sediment surface or 0.006 m³ volume. This experiment had no replicate and was conducted for 25 days in two tanks containing 20 L of seawater, 5 cm thickness of oven dried sediment and reared at 24 °C.

4.2.1.2 Experiment 2: Sediment compactness

The purpose of this experiment was to evaluate the effect of sediment compactness on the survival of 10 razor clams per tank. Two different degree of sediment compactness (during gonadal development period) were tested in triplicate: Treatment 2A used sediment with pored PET bottles while Treatment 2B used sediment without pored PET bottles in the tanks (Figure 4.3). These pored PET bottles make the sediment layer thicker to maintain sediment volume and less compact. The sediment moved through the small pores when clams burrow into the sediment, so it may reduce the compactness between the sediments particles and facilitate the burrowing process. For other settings, this experiment was conducted for 25 days using 20 L seawater, oven dried sediment and at 24 °C. Clams from this treatment were directly used for Experiment 3. From Experiment 2 onwards, the amount of razor clams was limited to 10 individuals per tank due to limited numbers of broodstock and algae culture capacity.

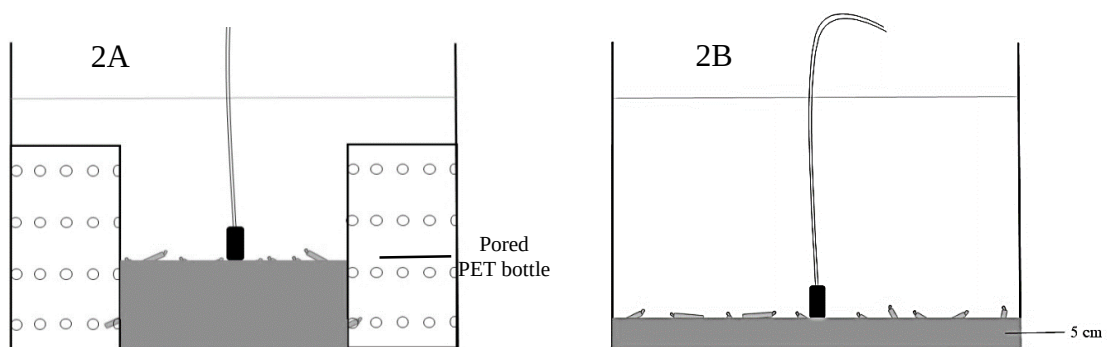


Figure 4.3: Treatment 2A with pored PET bottles whereas Treatment 2B with standard design.

4.2.1.3 Experiment 3: Duration for broodstock conditioning

The purpose of this experiment was to evaluate the effect of culture duration on the survival of 10 razor clams per tank. Two different culture durations (during gonadal development period) were tested in triplicates: Treatment 3A tanks were maintained until 50 days of culture, while Treatment 3B cultured only for 25 days. For other settings, this experiment was conducted using 20 L seawater with temperature of 24°C and 5 cm thickness of oven dried sediment standard design.

4.2.1.4 Experiment 4: Sediment conditioning

The purpose of this experiment was to evaluate the effect of different sediment conditioning on the survival of 10 razor clams per tank. Two different sediment conditioning were tested (during spawning period): Treatment 4A used oven dried sediment (at 60 °C overnight), while Treatment 4B used untreated natural sediment. For other settings, this experiment was conducted for 25 days in tanks containing 20 L seawater with temperature of 24 °C and 5 cm sediment thickness as standard design.

4.2.2 Phase 2

During Phase 2, another three parameters (sediment thickness, seawater volume and seawater temperature) were evaluated using best condition achieved in Experiment 2 until Experiment 4 as constant variables. In Phase 2, each parameter was evaluated at two gonadal development periods; i.e. resting period and spawning period of *S. regularis*. In this phase, results were evaluated base on survival rate and growth of razor clam between treatments and between two periods of gonadal development.

4.2.2.1 Control treatment

As mention previously, the standard design was used in Experiment 5 to Experiment 10 (Figure 4.4). The standard design consisted of 10 clams per tank and the conditioning culture period was set for 25 days. The sediment for all experiments used recycled sediment from previous experiments by drying it in the oven at 60 °C whole night. Standard design tanks were setup with 5 cm sediment thickness, 20 L of seawater at 24 °C for all experiments.

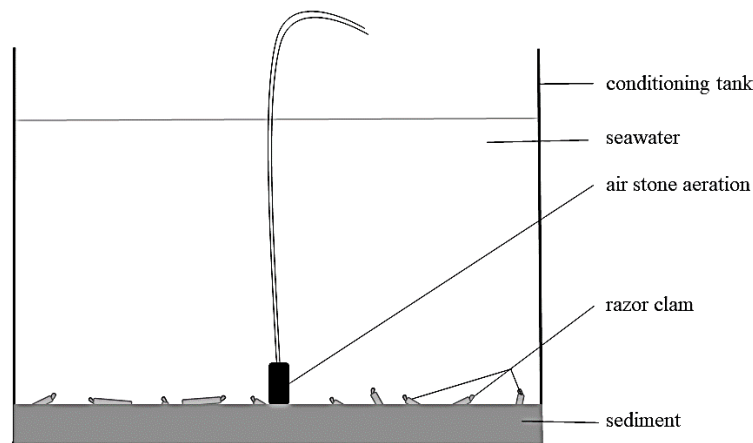


Figure 4.4: The illustration of standard design or control treatment. This treatment contained 5 cm thickness of oven dried sediment and 20 L of seawater at 24°C. Each standard design tank contained 10 razor clams and conditioned for 25 days.

4.2.2.2 Experiment 5 and 6: Sediment thickness

The purpose of this experiment was to evaluate the effect of different sediment thickness on the survival rate and growth performance of razor clams in two period of gonadal development (resting and spawning periods, Figure 4.5). Two different sediment thickness tested were as follows;

- a) Experiment 5 during spawning period: Treatment 5A (5 cm sediment thickness) and Treatment 5B (3 cm sediment thickness).
- b) Experiment 6 during resting period: Treatment 6A (5 cm sediment thickness) and Treatment 6B (3 cm sediment thickness).

Other settings were followed the standard design.

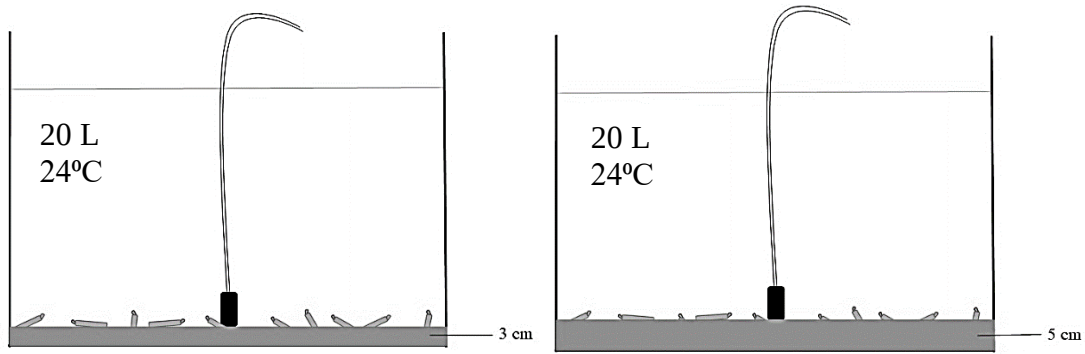


Figure 4.5: Set up of tanks for the sediment thickness experiment. Right tank contained 5 cm sediment thickness (standard design) as control treatment and left tank contained 3 cm sediment thickness.

4.2.2.3 Experiment 7 and 8: Seawater volume

The purpose of this experiment was to evaluate effect of different seawater volume on the survival rate and growth performance of razor clams in two period of gonadal development (resting and spawning periods, Figure 4.6). Two different seawater volume tested were as follows;

- a) Experiment 7 during spawning period: Treatment 7A (20 L seawater volume) and Treatment 7B (10 L seawater volume).
- b) Experiment 8 during resting period: Treatment 8A (20 L seawater volume) and Treatment 8B (10 L seawater volume). Other settings followed the standard design.

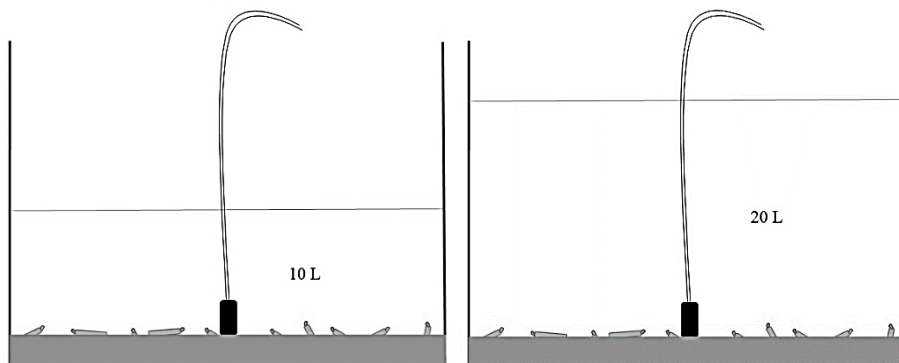


Figure 4.6: Set up of tanks for the seawater volume experiment. Right tank contained 20 L of seawater (standard design) as control treatment and left tank contained 10 L of seawater.

4.2.2.4 Experiment 9 and 10: Seawater temperature

The purpose of this experiment was to evaluate the effect of different seawater temperature on the survival rate and growth performance of razor clam in two period of gonadal development (resting and spawning periods, Figure 4.7). Two different seawater temperature tested were as follows;

- a) Experiment 9 during spawning period: Treatment 9A (30 °C seawater temperature) and Treatment 9B (24 °C seawater temperature).
- b) Experiment 10 during resting period: Treatment 10A (30 °C seawater temperature) and Treatment 10B (24 °C seawater temperature).

A submersible aquarium heater (TIANRUN, stainless steel electronic heater 300W W-300HG) was used to heat up seawater to 30 °C. Other settings followed the standard design.

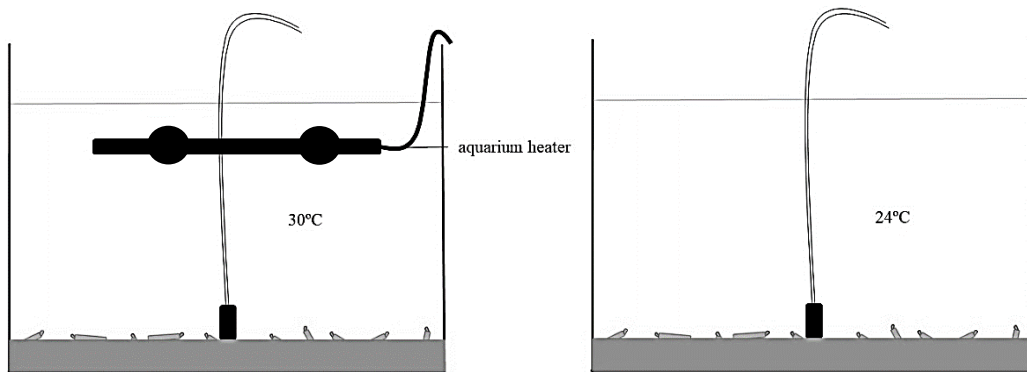


Figure 4.7: Set up of tanks for the seawater temperature experiment. Right tank with 24°C temperature using standard design as control treatment and left tank with 30°C temperature.

4.2.3 Shell length and wet weight

Data on growth of razor clam shell length were evaluated during Phase 2 experiments only.

In the laboratory, each clam was tagged individually by carving on the shell with number respectively for all individuals so that the increment of shell length and wet weight could be obtained at the end of the culture period for each razor clams (modified from Mamat, 2010).

At the beginning of experiment, shell length and total wet weight were measured using dial

calliper (0.02 cm; Mitutoyo, 5005-671) and digital balance (0.000 g; SHIMADZU, BL 220H), respectively. Total shell length was measured from the two end of shell at hinge side, while wet weight was measured by placing the clams in the petri dish containing seawater. Measurement of shell length and wet weight were performed only to broodstock that survived after 25 days of conditioning. The data were recorded accordingly with the same clams following their individual number on the shell.

4.2.4 Routine maintenance works

The routine maintenance works of broodstock conditioning were as explained in Chapter 3.

4.2.5 Qualitative response

4.2.5.1 Burrowing time

Observation was made on the time taken for all clams in a conditioning tank to burrow after they were transferred into the conditioning tank on Day 0 of culture period. The time taken for burrowing into sediment may indicate the favourable of sediment and level of weakness of the clams on Day 0 (Day 1 of conditioning was counted on the next day). Observation on time taken was recorded as; (i) below 5 minutes, (ii) between 5 to 10 minutes or (iii) more than 10 minutes (Table 4.2).

4.2.5.2 Footprint on sediment

Daily observation on any track presence by footprint of razor clam crawling on the sediment was made from Day 0 to Day 25 of culture period. These crawling imprints on sediment indicate unfavorable of treatment and difficulties to burrow into sediment (Table 4.2).

4.2.5.3 Mortality in sediment

Daily observation on sign of dead clam in sediment or formation of black spot at the sediment surface was conducted from Day 0 to Day 25 of culture period. This black spot was due to dead razor clam in the sediment. If this happened, the decaying clam and black sediment were siphoned out from the tank and replaced with new sediment (Table 4.2).

4.2.5.4 Presence on sediment surface

Observation were recorded as presence or absent of razor clam that came out from their burrows and lying on the sediment from Day 0 to Day 25 of culture period. Lying of clams on sand may indicate unfavourable of parameter setting tested, difficulties to stay burrowing in sediment or they are dying. Any unresponsive clam was removed from the tank (Table 4.2).

4.2.5.5 Fecal excretion

Daily observation was made on amount of fecal on the sediment during the culture period starting from Day 0 to Day 25. Amount of fecal indicates the health and condition of clams in the tank that was scored as (i) little, (ii) moderate or (iii) dense depend on surface of the sediment (Table 4.2). Scored amount of fecal are illustrated as in Appendix 3.

4.2.5.6 Protrude of siphon

Observation was made on the number of razor clams with protruding siphon during Day 14. Protrude of siphon indicates the health of clams in the treatment condition. The number of razor clams that protrude their siphon was recorded either more than 50% or less than 50% during Day 14 from the total population of razor clams in each tank (Table 4.2).

Table 4.2: Qualitative observations.

Observation	Burrowing time	Footprint	Mortality in sediment	Presence on sediment	Fecal excretion	Protrude of siphon
Data recorded	• < 5 minute • 5 -10 minute • > 10 minute	• Presence = + • Absent = -	• Presence = + • Absent = -	• Presence = + • Absent = -	• Little • Moderate • Dense	• < 50% • > 50%
Observation duration (days)	On Day 0	0 - 25	0 - 25	0 - 25	0 - 25	On Day 14

4.2.6 Data and statistical analysis

4.2.6.1 Survival rate

The survival rate for each tank per day was calculated as ' $(N_t/N_0) \times 100$ ', where ' N_t ' is the number of live clams remain after dead and weak clams were removed from the tank and ' N_0 ' is the number of clams at the beginning of the experiment.

While the trend of survival throughout the conditioning culture was shown by daily mean survival for each treatment and was calculated as total mean survival data from three tanks (same treatment). Evaluation of clam survival rate was calculated after 25 days of conditioning except for Treatment 2B (50 days).

4.2.6.3 Shell length and wet weight

Data on shell length of razor clams was calculated as differences of shell length in 25 days only to the survived clams in each treatment. The wet weight was evaluated by difference between initial wet weight and wet weight after conditioning of razor clams for each treatment.

4.2.6.2 Statistical analysis and hypothesis

For statistical analysis, two treatments in each experiment were tested with independent-sample *t*-test to determine any significant differences of mean survival after conditioning for 25 days using Minitab version 17 software with significant level set at 95% confident intervals. The null hypothesis (H_0) and alternative hypothesis (H_a) between the two treatments in each experiment for *t*-test were summarised in Table 4.3. The statistical analysis for *t*-test was not included for Experiment 1, because there is no replicate in the experiment.

Table 4.3: Setting of hypotheses among all treatments.

No	Experiment	Treatment	Experiment design and Survival rate
2	Sediment compactness (trial, gd)	2A: less compact 2B: compact	H₀: => $\mu_A = \mu_B$ H ₀ : => There is no significant difference of survival rate between treatment A and treatment B through this experiment.
3	Culture duration (trial, gd)	3A: 25 days 3B: 60 days	
4	Sediment condition (trial, s)	4A: Oven dried 4B: Natural wet	
5	Sediment thickness (s)	5A: 5 cm (ctrl) 5B: 3 cm	
6	Sediment thickness (r)	6A: 5 cm (ctrl) 6B: 3 cm	H_a: => $\mu_A \neq \mu_B$ H _a : => There is significant difference of survival rate between treatment A and treatment B through this experiment.
7	Seawater volume (s)	7A: 20 L (ctrl) 7B: 10 L	
8	Seawater volume (r)	8A: 20 L (ctrl) 8B: 10 L	
9	Seawater temperature (s)	9A: 30 °C 9B: 24 °C (ctrl)	
10	Seawater temperature (r)	10A: 30 °C 10B: 24 °C (ctrl)	

s = spawning period, r = resting period, gd = gonadal development period, ctrl = means control treatment and using the standard design. Trial means experiments were at trial stage and did not have control. All experiments have replicates except for Experiment 1

4.3 Results

4.3.1 Mean survival rate

In general, 14 out of 20 treatments showed 50% and more survival rate until the end of culture period for all treatments.

4.3.1.1 Phase 1 (Trial Experiments)

The mean survival rate of razor clams *S. regularis* for Phase 1 ranged from $49.42 \pm 24.57\%$ to 70.47% (Table 4.4) which used broodstock from three stages of gonadal development stage.

At the end of Experiment 1 (stocking density), 70 individuals of razor clam showed higher survival rate (70.47%) compared with 35 individuals of razor clams (65.47%). In this experiment, no statistical analysis can be performed due to no replicate was performed.

For Experiment 2 (sediment compactness), less compact sediment showed higher mean survival rate ($69.20 \pm 1.64\%$) compared to compact sediment ($68.04 \pm 5.69\%$) but there was no significant difference in mean survival rate in *t-test* analysis ($p = 0.843$). The compact sediment design was more convenient and easier to monitor compared to less compact sediment design and thus compact sediment was chosen as the standard design in this study.

For Experiment 3 (culture duration), 25 days of culture period showed higher mean survival rate ($69.77 \pm 2.61\%$) compared with 50 days of culture period ($61.01 \pm 1.61\%$) with significant difference between mean survival rate (*t-test*, $p = 0.000$). Culture duration of 25 days was chosen as standard design in this study because it is less time consuming and giving high survival rate of broodstocks.

For Experiment 4 (sediment conditioning), oven dried sediment showed higher mean survival rate ($54.80 \pm 14.37\%$) than natural wet sediment ($49.42 \pm 24.57\%$) but there was no significant difference between these two sediments conditioning (*t-test*, $p = 0.491$). The natural wet sediment was chosen as standard design since it is less time consuming to prepare compared with oven dried sediment. The natural wet sediment can be used directly from the field and can be reused for the next experiment.

Table 4.4: The mean survival rate (%) during Phase 1 (trial experiments)

No	Experiment	Treatments	Mean survival rate at end of culture period (%)
1	Stocking density (r)	1A: 70 clams	70.47
		1B: 35 clams	65.47
2	Sediment compactness (gd)	2A: less compact	68.04 ± 5.69
		2B: compact	58.26 ± 2.48
3	Culture duration* (gd)	3A: 50 days	61.01 ± 1.61
		3B: 25 days	69.77 ± 2.61
4	Sediment condition (s)	4A: Oven dried	54.80 ± 14.37
		4B: Natural wet	49.42 ± 24.57

s = spawning period, r = resting period, gd = gonadal development period. All experiments have three replicates except for Experiment 1 (stocking density)

4.3.1.2 Phase 2

The overall mean survival rate of *S. regularis* on last day of culture during Phase 2 experiments ranged from $19.71 \pm 6.60\%$ to $71.30 \pm 28.91\%$ (Table 4.5) which used broodstocks from two stages of gonadal development.

Experiment on sediment thickness showed that mean survival rate of razor clam culture during spawning period with 3 cm sediment thickness was higher ($71.30 \pm 28.91\%$) than 5 cm sediment thickness treatment ($64.27 \pm 20.12\%$) with significant difference between these two treatments (*t-test*, $p = 0.000$). During resting period, mean survival rate on of razor clam cultured with 5 cm sediment thickness was higher ($64.27 \pm 2.22\%$) than 3 cm sediment thickness ($30.72 \pm 0.20\%$) and there is significant difference between these two treatments (*t-test*, $p = 0.000$). In term of qualitative observation, razor clams cultured in 5 cm sediment thickness showed better response and this will be explained later in this chapter. Therefore, the 5 cm sediment thickness was chosen as standard design.

Experiment on seawater volume showed that razor clam cultured with 20 L seawater for both stage of gonadal development was higher in survival rate ($64.27 \pm 2.22\%$ for spawning period and $69.47 \pm 16.98\%$ for resting period) compared to 10 L seawater (19.71

$\pm 6.60\%$ for spawning period and $36.52 \pm 9.33\%$ for resting period). The *t*-test between two different seawater volume during spawning (*t*-test, $p = 0.000$) and resting (*t*-test, $p = 0.000$) period were significantly different. Thus, 20 L seawater volume design which gave higher survival rate was chosen as standard design in this study.

For seawater temperature experiment, *t*-test analysis between treatment set at 24 °C showed significant higher survival rate than 30 °C for both periods of gonadal development ($p = 0.000$ for spawning period and $p = 0.000$ for resting period). Therefore, 24 °C seawater which is also the ambient laboratory temperature was chosen as the standard design for the broodstock culture.

Between the two periods of gonadal development (spawning period and resting period), the results of mean survival rate of razor clam were not depending on the period of gonadal development but more on the design treatment of the experiment.

Table 4.5: Survival rate (%) of experiments in Phase 2.

No	Experiment	Treatment	Mean survival rate at end of culture period (%)
5	Sediment thickness (s)	5A: 5 cm (ctrl)	64.27 $\pm$ 20.12
		5B: 3 cm	71.30 $\pm$ 28.91
6	Sediment thickness* (r)	6A: 5 cm (ctrl)	64.27 $\pm$ 2.22
		6B: 3 cm	30.72 $\pm$ 0.20
7	Seawater volume* (s)	7A: 20 L (ctrl)	64.27 $\pm$ 2.22
		7B: 10 L	19.71 $\pm$ 6.60
8	Seawater volume (r)	8A: 20 L (ctrl)	69.47 $\pm$ 16.98
		8B: 10 L	36.52 $\pm$ 9.33
9	Seawater temperature* (s)	9A: 30 °C	40.27 $\pm$ 5.13
		9B: 24 °C (ctrl)	64.49 $\pm$ 14.21
10	Seawater temperature* (r)	10A: 30 °C	38.40 $\pm$ 2.55
		10B: 24 °C (ctrl)	63.78 $\pm$ 13.96

s = spawning, r = resting, ctrl = control treatment, * indicate the treatment that were significant different at 95% confident interval.

4.3.1.3 Trend of razor clams broodstock survival

The overall trend of *S. regularis* broodstock culture survival was analysed according to manipulation of sediment thickness, seawater volume and seawater temperature in two different gonadal development periods.

4.3.1.3.1 Sediment thickness

Generally, during spawning period survival rate of broodstock were not stable and decreased until Day 13 and Day 14 for both sediment thickness designs. The mean survival rate became stable at 53.33% (5 cm) and 63.33% (3 cm) until end of culture (Figure 4.8). During resting period, generally trend survival rate for 5 cm sediment thickness decreased gradually until Day 19 and remained stable till the end of culture period. In contrast, the survival rate trend for 3 cm sediment thickness decreased drastically until Day 7 and became stable form day 13 at 20.00% until the end of culture period.

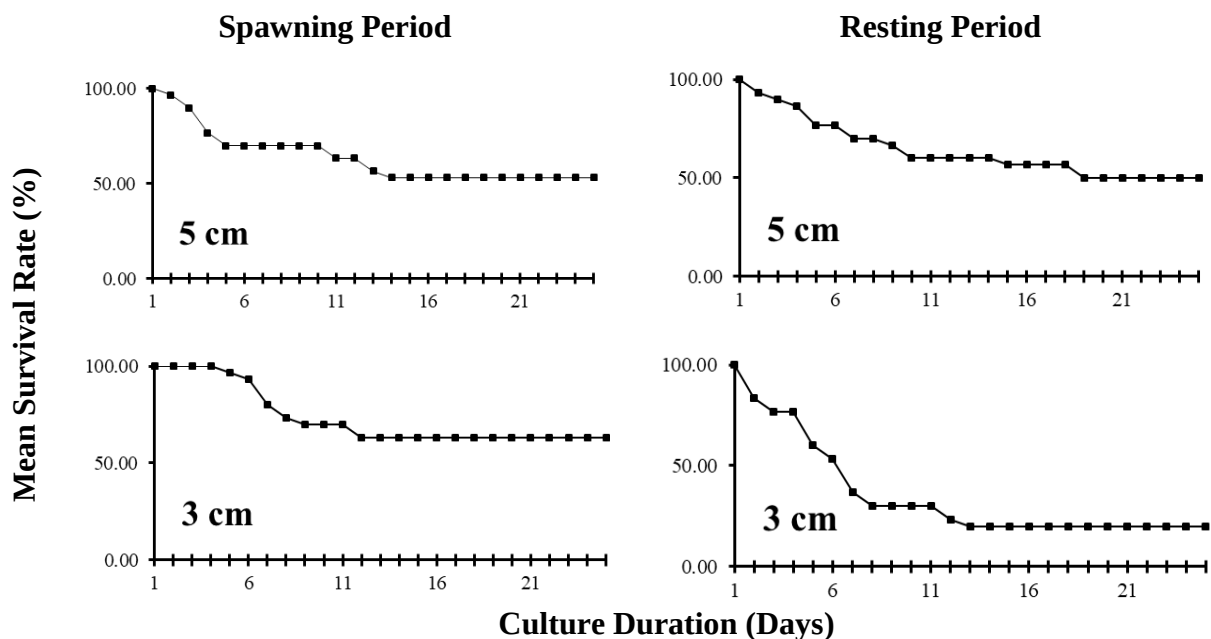


Figure 4.8: Trend of survival rate (%) of *S. regularis* throughout 25 days of culture in sediment thickness experiment.

4.3.1.3.2 Seawater volume

Seawater volume experiment showed that trend of survival rate with 10 L seawater decreased drastically along the culture period compared to 20 L seawater (Figure 4.9) for both period of gonadal development. During spawning period, 20 L seawater decreased gradually at Day 19 and remained stable at 50.0% survival rate until end. Culture in 10 L seawater, the survival rate trend decreased drastically until Day 14 (10.00%) and later decreased gradually until 6.67%. During resting period, cultures in 20 L seawater were more stable from Day 5 until Day 16. For 10 L seawater, the trend decreased gradually until below than 7% of survival rate.

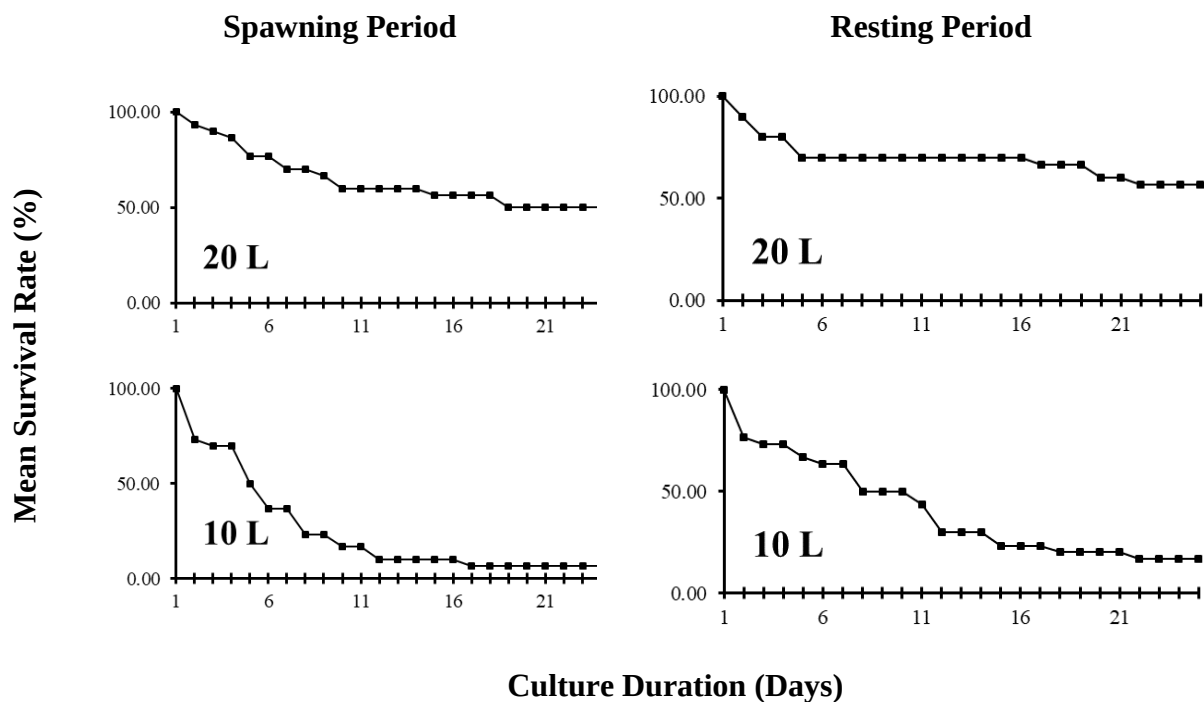


Figure 4.9: Trend of survival rate (%) of *S. regularis* throughout 25 days of culture in seawater volume experiment.

4.3.1.3.3 Seawater temperature

Cultures in 30°C seawater temperature showed drastic decrease survival rate compared to 24°C for both periods (Figure 4.10). During spawning period, trend of survival rate for 30°C cultures decreased drastically until Day 9 (33.33%) and then decreased gradually until the end. For 24°C cultures, the survival rate decreased gradually along the culture period. During resting period, culture in 30°C showed drastic decrease until Day 12 and recorded a very low survived of 6.67%. For 24°C cultures, the survival of razor clam was stable at 63.33% from Day 5 until Day 12 and decreased slowly until the end of culture period.

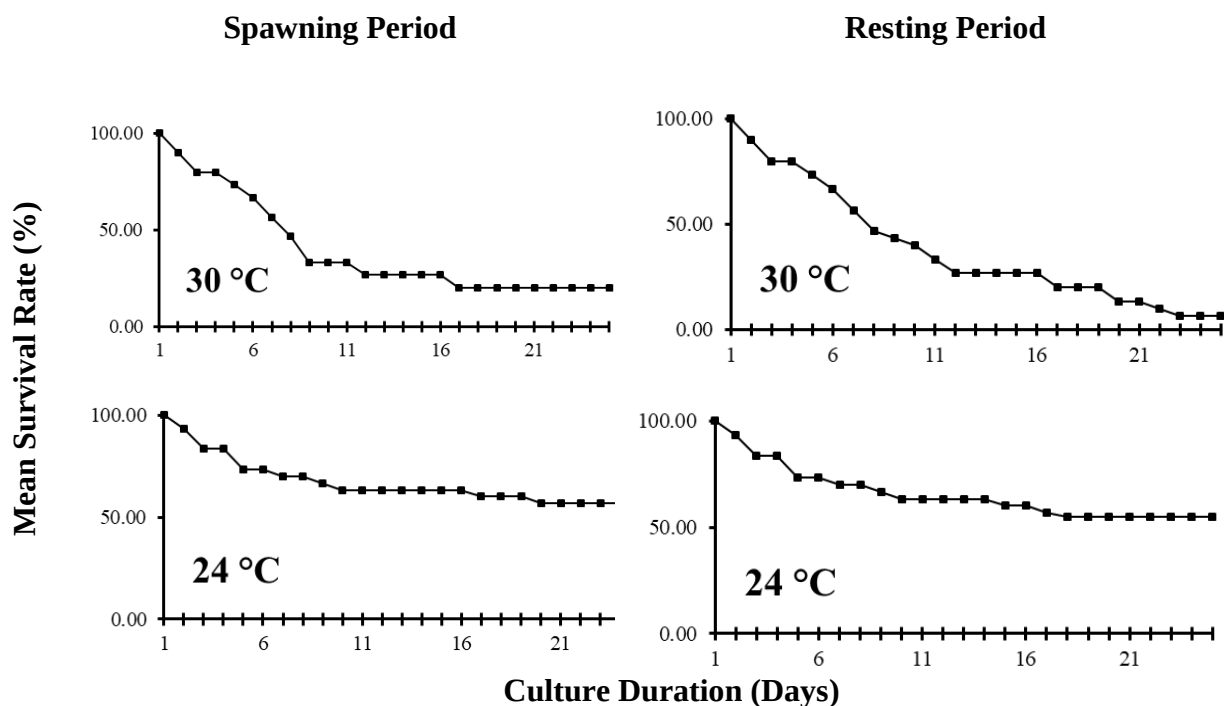


Figure 4.10: Trend of survival rate (%) of *S. regularis* throughout 25 days of culture in seawater temperature experiment.

In general, the survival of broodstock conditioning cultures became stable only after 7 days. But high mortality of razor clams was observed in some culture treatments especially in experiments using 10 L seawater volume and 30 °C of seawater temperature (Figure 4.11).

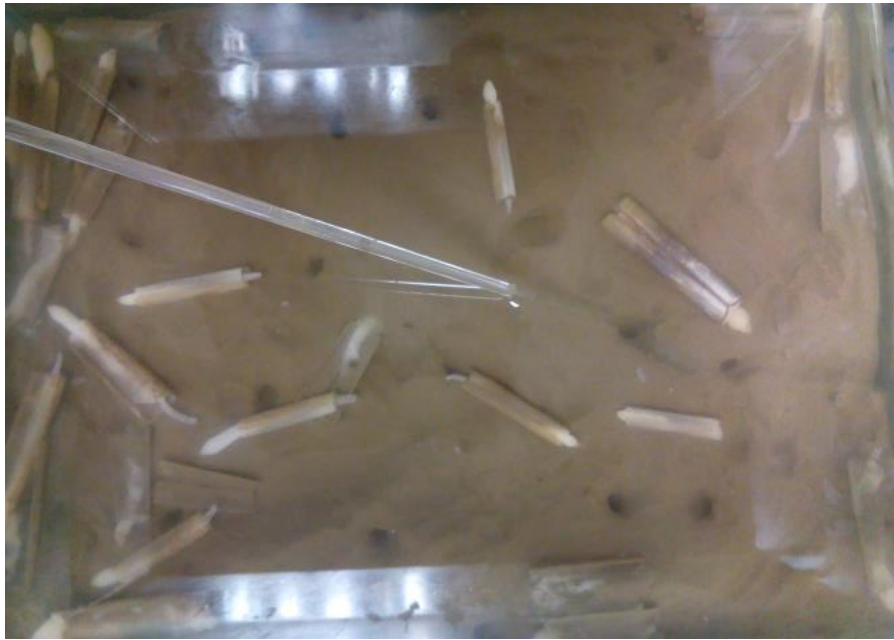


Figure 4.11: High mortality of razor clams was observed in a tank during the early stage of conditioning culture experiment.

4.3.2 Growth performance

4.3.2.1 Wet weight

All treatments showed that razor clams suffered weight loss after the conditioning culture period. The overall of *S. regularis* mean wet weight after broodstock conditioning ranged from -0.395 ± 0.424 g to -2.009 ± 0.879 g (Table 4.6).

For sediment thickness experiment, the mean wet weight loss during spawning period using 3 cm sediment thickness treatment was lower (-0.476 ± 0.795 g) than 5 cm sediment thickness treatment (-0.650 ± 0.838 g). However, during resting period, the mean wet weight loss using 3 cm sediment thickness treatment was higher (-0.434 ± 0.892 g) than 5 cm sediment thickness treatment (-0.395 ± 0.424 g).

Seawater volume experiment during spawning period showed that the mean wet weight loss of culture in 20 L seawater was lower (-0.543 ± 0.698 g) than those in 10 L

seawater ($- 1.227 \pm 0.620$ g). However, during resting period, the mean wet weight loss of cultures in 20 L seawater was higher ($- 1.703 \pm 0.760$ g) compared to these in 10 L seawater ($- 0.673 \pm 0.137$ g).

For seawater temperature experiment, both gonadal development periods showed higher mean wet weight loss in 30 °C seawater temperature during spawning period ($- 0.945 \pm 0.532$ g) and resting period ($- 2.009 \pm 0.879$ g) compared to 24 °C seawater temperature.

Table 4.6: Mean wet weight (g) of *S. regularis* after the broodstock conditioning culture at two different gonadal development periods.

Treatments	Wet weight (g)	
	Spawning Period	Resting Period
5 cm sediment thickness	$- 0.650 \pm 0.838$	$- 0.395 \pm 0.424$
3 cm sediment thickness	$- 0.476 \pm 0.795$	$- 0.434 \pm 0.892$
20 L seawater volume	$- 0.543 \pm 0.698$	$- 1.703 \pm 0.760$
10 L seawater volume	$- 1.227 \pm 0.620$	$- 0.673 \pm 0.137$
30 °C seawater temperature	$- 0.945 \pm 0.532$	$- 2.009 \pm 0.879$
24 °C seawater temperature	$- 0.653 \pm 0.414$	$- 1.703 \pm 0.760$

(- = wet weight loss)

4.3.2.2 Shell length

Different kinds of broodstock culture treatment in different gonadal development period resulted in either shell length gain or shell length loss. The overall of *S. regularis* mean shell length after broodstock conditioning ranged from $- 1.77 \pm 2.34$ mm to 2.79 ± 1.83 mm (Table 4.7).

In sediment thickness experiment during spawning period showed that both 5 cm and 3 cm sediment thickness recorded almost similar mean shell length gain (0.71 ± 0.16 mm and 0.70 ± 0.15 mm), respectively compared to during resting period which recorded mean shell length loss ($- 0.27 \pm 1.06$ mm and $- 0.34 \pm 0.66$ mm), respectively.

For seawater volume experiment, mean shell length gain was observed during resting period for both seawater volume used (20 L = 2.79 ± 1.82 mm and 10 L = 0.34 ± 0.97 mm) while mean shell length loss (20 L = -0.57 ± 1.87 mm and 10 L = -1.77 ± 2.34 mm) happened in both treatments during spawning period.

In seawater temperature experiment, mean shell length gain was observed in 24 °C cultures for both period of gonadal development (0.75 ± 1.74 mm during spawning period and 2.79 ± 1.83 mm during resting period) and 30 °C during spawning period (0.23 ± 1.52 mm). However, mean shell length loss (-0.57 ± 1.83 mm) happened in 30 °C cultures during resting period.

Table 4.7: Mean shell length of *S. regularis* after broodstock conditioning culture at two different gonadal development periods.

Treatments	Shell length (mm)	
	Spawning Period	Resting Period
5 cm sediment thickness	0.71 ± 0.16	-0.27 ± 1.06
3 cm sediment thickness	0.70 ± 0.15	-0.34 ± 0.66
20 L seawater volume	-0.57 ± 1.87	2.79 ± 1.82
10 L seawater volume	-1.77 ± 2.34	0.34 ± 0.97
30 °C seawater temperature	0.23 ± 1.52	-0.57 ± 1.83
24 °C seawater temperature	0.75 ± 1.74	2.79 ± 1.83

(- = shell length loss, + = shell length gain)

4.3.3 Qualitative response

4.3.3.1 Burrowing time

The first observation was on the time taken for all razor clams to burrow into the sediment during Day 0 of culture period (Figure 4.12). Time taken for all razor clams to burrow into sediment may reflect the condition of razor clams on Day 0 of conditioning period. Among all treatments, only two treatments showed less than 5 minutes, eight treatments 5 to 10 minutes and eight treatments more than 10 minutes for razor clams to burrow into the

sediment (Table 4.8). In Phase 1, two different stocking density treatments showed similar results (more than 10 minutes) for razor clam to burrow into the sediment. In contrast, the sediment compactness showed different response time in which razor clams burrowed faster in compact sediment than the less compact sediment. Sediment condition experiment however did not show any differences in time for razor clam to burrow into sediment.

In Phase 2, the 5 cm sediment thickness treatment during spawning period showed less time (5 – 10 min) was taken for razor clams to burrow compared to 3 cm sediment thickness. But in resting period, razor clams took more than 10 minutes for burrowing into sediment. Experiment of seawater volume showed that the clams burrowed faster during spawning period in both volumes. But seawater temperature did not show any differences in time taken for razor clam burrowing into sediment between two different period of gonadal development and both temperatures.

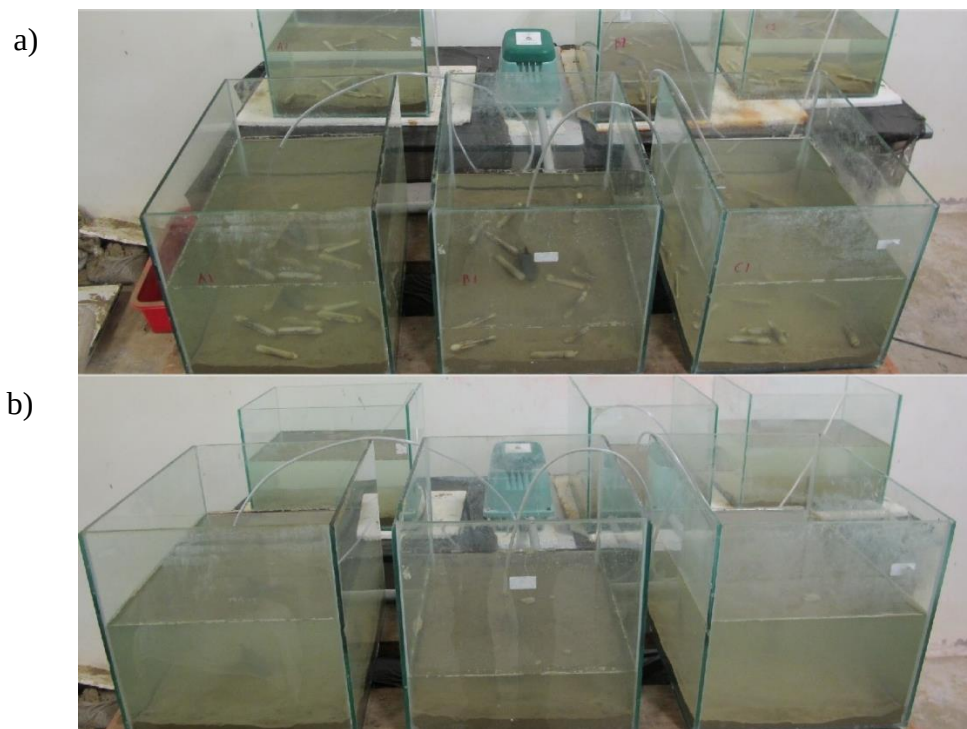


Figure 4.12: Observation of razor clams burrowing into sediment. (a) Razor clams presence on the sediment before burrowing into the sediment. (b) Razor clams had completely

burrowed into the sediment.

Table 4.8: Burrowing time of the razor clams.

Observation	Duration clams burrowing in sediment	
70 clams (r)	> 10 min	
35 clams (r)	> 10 min	
Less compact sediment (gd)	> 10 min	
Compact sediment (gd)	5 - 10 min	
Oven dried sediment (s)	< 5 min	
Natural sediment (s)	< 5 min	
	Spawning	Resting
5 cm sediment thickness	5 - 10 min	> 10 min
3 cm sediment thickness	> 10 min	> 10 min
20 L seawater volume	5 - 10 min	> 10 min
10 L seawater volume	5 - 10 min	> 10 min
30 °C seawater temperature	5 - 10 min	5 - 10 min
24 °C seawater temperature	5 - 10 min	5 - 10 min

(s = spawning period, r = resting period, gd = gonadal development period)

4.3.3.2 Footprint on sediment

Presence of *S. regularis* footprint on sediment surface during broodstock conditioning culture is shown in Figure 4.13. Results showed that 14 out of 18 treatments had presence of *S. regularis* footprint on sediment surface (Table 4.9). All trial treatments showed presence of the footprint. During resting period, majority of treatments had presence of *S. regularis* crawling footprint on sediment surface except in treatment using 5 cm sediment thickness. However, during spawning period, three treatments (3 cm sediment thickness, 10 L seawater volume and 24 °C seawater temperature) presence of *S. regularis* footprint on sediment while another three treatments were absent. Cultures in 3 cm sediment thickness, 10 L seawater volume and 24 °C seawater presence of footprint during both general development periods.



Figure 4.13: Example of razor clams footprint on sediment surface during broodstock conditioning.

Table 4.9: Presence of razor clams footprint on sediment.

Observation	Footprint on sediment	
70 clams (r)	+	
35 clams (r)	+	
Pored PET bottle (gd)	+	
Compact sediment (gd)	+	
Oven dried sediment (s)	+	
Natural wet sediment (s)	+	
	Spawning	Resting
5 cm sediment thickness	–	–
3 cm sediment thickness	+	+
20 L seawater volume	–	+
10 L seawater volume	+	+
30 °C	–	+
24 °C	+	+

(s = spawning period, r = resting period, gd = gonadal development period, + = presence, – = absent)

4.3.3.3 Mortality in sediment

Dead razor clams inside sediment may indicate the condition of treatment was very unfavourable to the razor clams. Presence of dead *S. regularis* in the sediment during conditioning culture were indicated by black coloured sediment (Figure 4.14 and Figure

4.15). Normally, razor clams will come out of sediment and died on the sediment surface. The dead razor clams need to be removed from the treatment tank immediately before the sediment turned into black colour and reduced the survival rate of others razor clams. Results showed that seven treatments had occurrence of dead *S. regularis* in sediment while 11 treatments were absent (Table 4.10). Treatment with high stocking density (70 clams) during trial also had presence of dead *S. regularis* in sediment compared to 35 razor clams. Dead clams were also recorded in less compact sediment. Two different type of sediment showed absent of dead clam inside sediment. The 5 cm sediment thickness did not show any dead clam inside sediment for both period of gonadal development but mortality was recorded in 3 cm sediment thickness during resting period. Treatment with 10 L seawater volume and 30 °C seawater temperature of both periods showed presence dead of *S. regularis* in sediment.

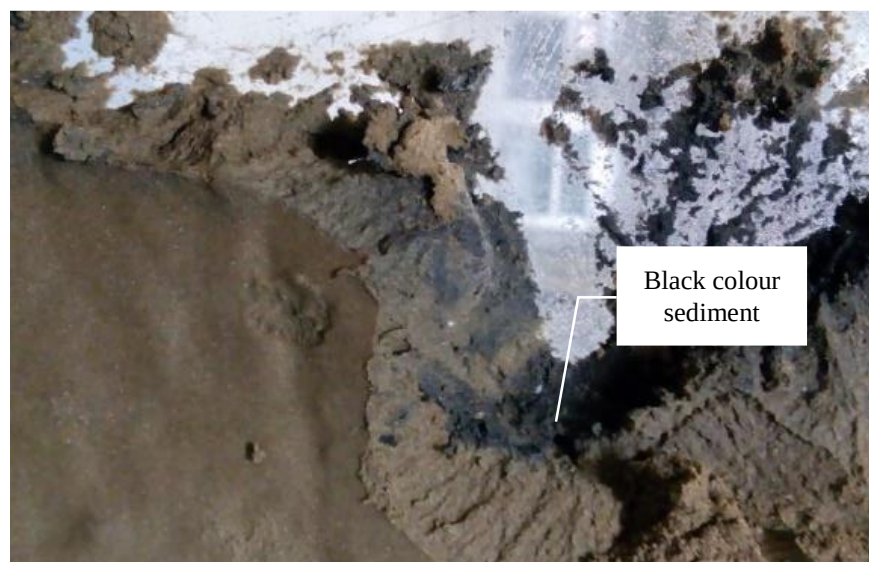


Figure 4.14: The sediment turned into black if razor clams died inside the sediment.

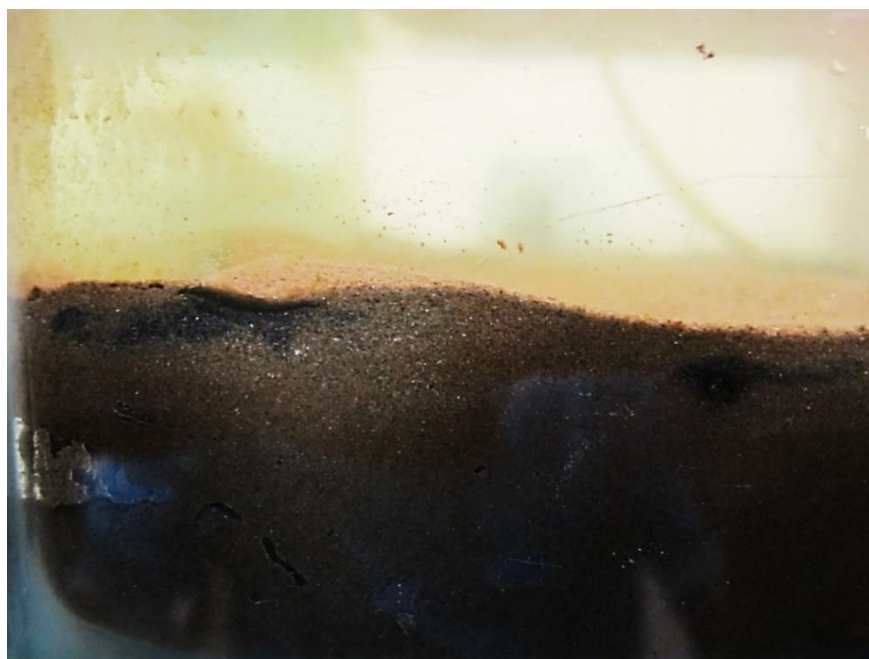


Figure 4.15: Black coloured sediment can be observed from the side view of culture tank indicating the presence of dead razor clams inside the sediment.

Table 4.10: Presence of dead razor clam in the sediment.

Observation	Dead clams inside sediment	
70 clams (r)	+	
35 clams (r)	–	
Pored PET bottle (gd)	+	
Compact sediment (gd)	–	
Oven dried sediment (s)	–	
Natural wet sediment (s)	–	
	Spawning	Resting
5 cm sediment thickness	–	–
3 cm sediment thickness	–	+
20 L seawater volume	–	–
10 L seawater volume	+	+
30 °C seawater temperature	+	+
24 °C seawater temperature	–	–

(s = spawning period, r = resting period, gd = gonadal development period, + = presence, – = absent)

4.3.3.4 Presence of razor clam on the sediment surface

The fourth observation was on the presence of *S. regularis* lying on or near the surface of sediment (Figure 4.16) and normally this happened starting from Day 7 onwards of

conditioning. Results showed that 10 treatments had presence of such behaviour and eight treatments were absent (Table 4.11). High stocking density, compact sediment and both type of sediment treatment showed presence of razor clam near the sediment surface. Treatments 3 cm sediment thickness and 10 L seawater volume for both periods had presence of *S. regularis* lying on the sediment surface. Both different temperature setting between two gonadal developments inconsistently showed presence of razor clam near the sediment surface.



Figure 4.16: Razor clam lying on the sediment surface in 3 cm sediment treatment.

Table 4.11: Presence of razor clam on the sediment surface.

Observation	Lying of razor clam on sediment	
70 clams (r)	+	
35 clams (r)	—	
Pored PET bottle (gd)	—	
Compact sediment (gd)	+	
Oven dried sediment (s)	+	
Natural wet sediment (s)	+	
	Spawning	Resting
5 cm sediment thickness	—	—
3 cm sediment thickness	+	+
20 L seawater volume	—	—
10 L seawater volume	+	+
30 °C seawater temperature	+	—
24 °C seawater temperature	—	+

(s = spawning period, r = resting period, gd = gonadal development period, + = presence, — = absent)

4.3.3.5 Fecal excretion and pseudo fecal on sediment

The amount of fecal being released on top surface of sediment can be clearly observed starting from Day 7 onwards of conditioning (Figure 4.17). Higher amount of fecal produced may indicate that the razor clams are feeding in good condition. Results showed that five treatments had dense amount of fecal on top surface of sediment, seven treatments had moderate amount of fecal and six treatments had little amount of fecal (Table 4.12). Cultures using oven dried sediment and both period at 3 cm sediment thickness treatment showed little amount of fecal released during this experiment. Higher stocking density of razor clams (70 individuals per tank) showed more excretion was produced. In this study, culture in 30 °C seawater temperature during spawning and resting period showed dense amount of fecal excretion. The 10 L seawater volume during spawning period showed dense amount of fecal was produced.



Figure 4.17: Dense amount of fecal excretion were released on top surface of sediment.

Table 4.12: Amount of fecal excretion released onto the sediment.

Observation	Amount of fecal excretion	
70 clams (r)	Dense	
35 clams (r)	Moderate	
Pored PET bottle (gd)	Moderate	
Compact sediment (gd)	Moderate	
Oven sediment (s)	Little	
Natural wet sediment (s)	Moderate	
	Spawning	Resting
5 cm sediment thickness	Moderate	Little
3 cm sediment thickness	Little	Little
20 L seawater volume	Little	Moderate
10 L seawater volume	Dense	Little
30 °C seawater temperature	Dense	Dense
24 °C seawater temperature	Moderate	Dense

(s = spawning period, r = resting period, gd = gonadal development period. See Appendix 3 for illustration of scoring on amount of fecal excretion)

4.3.3.6 Protrude of siphon

Protrude of siphon by *S. regularis* can be clearly observed starting from Day 14 onwards of culture (Figure 4.18). Protrude of siphon indicates that the razor clams are in good condition and comfortable with the treatment design. Only two treatments (high stocking density and 5 cm sediment thickness during spawning period) showed more than 50% of razor clams protrude their siphon while the rest of treatments recorded less than 50% (Table 4.13). Some of the clams did not protrude out their siphon from the sediment but their presence can be indicated by the presence of their fecal.

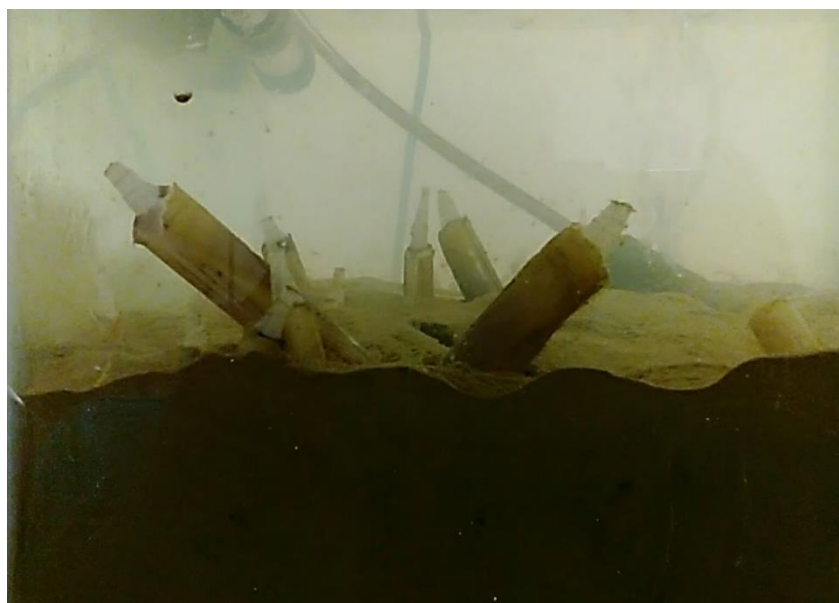


Figure 4.18: Treatment of high stocking density with 70 individuals of *S. regularis* in a tank. After Day 20 of culture, most razor clams buried their body with siphon protruded.

Table 4.13: Observation on protrude of siphon razor clams on Day 14 of culture period less of more than 50% of razor clam individuals.

Observation	Protrude of siphon	
70 clams (r)	> 50%	
35 clams (r)	< 50%	
Pored PET bottle (gd)	< 50%	
Compact sediment (gd)	< 50%	
Oven dried sediment (s)	< 50%	
Natural wet sediment (s)	< 50%	
	Spawning	Resting
5 cm sediment thickness	> 50%	< 50%
3 cm sediment thickness	< 50%	< 50%
20 L seawater volume	< 50%	< 50%
10 L seawater volume	< 50%	< 50%
30 °C seawater temperature	< 50 %	< 50 %
24 °C seawater temperature	< 50 %	< 50 %

(s = spawning period, r = resting period, gd = gonadal development period)

Another qualitative observation recorded were the shell damage of about 1-2 mm and presence of periostracum damage making the shell turned into black colour (Figure 4.19). Only two out of the 31 individuals that died during the whole experiments showed no visible signs of infection due to periostracum damage.

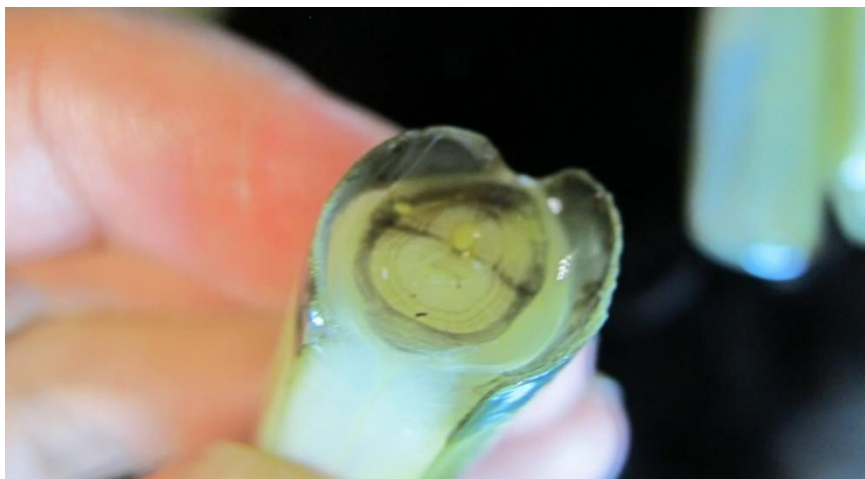


Figure 4.19: Damage of razor clam shell known as periostracum damage.

4.4 Discussion

4.4.1 Mean survival rate of *S. regularis* at the end of culture

In general, 14 out of 20 treatments showed survival rate of more than 50.00% from the initial stocking density of razor clam broodstocks. Results of bivalve broodstock survival rate that ranged 50.00% to 90.00% from stocking density indicate the setting of conditioning system is suitable for many bivalve species (da Costa et al., 2011). Species with high survival can be potential species to be cultured in large scale production (Laing & Spencer, 2006; Tobergte & Curtis, 2013). The survival rate of bivalves or razor clam broodstock are less studied compared with larvae stage. Thus, this survival rate result cannot be compared with other favourable value of survival rate from same species or related species especially between broodstock stage. Most of the reports documented the survival rate of bivalve during their larval stage (Berntsson et al., 1997; Gallagher & Mann, 1986; Shumway et al., 1993; Videan et al., 2006).

Different amount of stocking density of clams in the conditioning tank seems to influence the survival of razor clam broodstocks. Culture with 70 razor clam individuals

recorded higher survival rate and this could be due to high density that could prevent on shell gaping of the clams, thus reduced the energy necessary to keep shells closed due to the weight of neighbours pushing one another (da Costa et al., 2011). However, too high stocking density should be prevented in order to ensure razor clam have optimal feed supply. Thus in this study although high stocking density manage to give high survival, low density was used due to limitation of feed supply and broodstock supply. Moreover, since this study used a close system, it is important to consider the space, nutrient supply and released of waste of each clam that can affect the survival rate and growth rate (Yan et al., 2006).

The best sediment design is to ease the burrowing process of razor clam and also can prevent the shell gaping (García et al., 2011). In Experiment 2 that tested two different sediment compactness, both treatments did not show significant difference in survival rate (Table 4.5). However, clams in compact sediment able to burrow faster compared to less compact sediment. Thus more compact sediment is more preferable. Moreover, conditioning tanks containing PET bottles also gave difficulties in monitoring and cleaning process of the tank.

Normally, experiment on bivalve broodstock conditioning culture try to maintain a long culture duration in order to develop the gonad content and then promote to natural spawning of the clam directly in the water column. However, the duration of conditioning varies among different species. In this study, performance of culture duration was tested until 50 days and resulted in $53.27 \pm 1.97\%$ survival rate. This indicates that *S. regularis* have the potential to be maintained in hatchery in long period throughout the year.

Between two sediment condition, there is no significant different between oven dried sediment and natural wet sediment. Therefore, the wet sediment from natural habitat was chosen as standard design as it is less troublesome in preparation for burrowing activities of

adult clams as long as the sediment is free from detritus and other large macrobenthos (da Costa et al., 2011).

There was no significant difference in *t-test* of mean survival rate between 5 cm and 3 cm sediment thickness during both gonadal periods. The shell length of clams used in this experiment was more than 4 cm and the clams still can burrow itself in sediment with 3 cm thickness although the sediment thickness were less than their shell length. However, in 3 cm sediment thickness, both period of gonadal development showed their foot prints on the sediment with lying of razor clam on or near the sediment surface compare to 5 cm sediment thickness. This indicate the clams were not comfortable during the culture period and struggled to burrow themselves into the sediment. Thus 5 cm sediment thickness was chosen compared to 3 cm sediment thickness. The 5 cm sediment thickness is enough because thicker sediment will give more difficulties during the collection of clams, cleaning process and need to obtain large amount of sediment.

Experiment on seawater volume between two periods gonadal development showed the 10 L seawater volume has lower survival rate compared to 20 L seawater volume. Accumulation of ammonia in the water column can occur from excretion and absorb into the body of clams which can cause illness (Laing & Spencer, 2006). Furthermore, temperature and salinity can easily fluctuated in small water volume compared to large volume of water and thus can affect the survival of clams (Brown & Hartwick, 1988).

Temperature at 30 °C culture gave significantly lower low survival rate compared to at 24 °C for both period of gonadal development. During broodstock culture at 30 °C temperature, there were dead clams inside the sediment instead at 24 °C temperature. Thus, setting of 30 °C could be the maximum temperature for this species. Highest optimal temperature for *S. regularis* culture need to be at more specific value for maximum impact

on the maturation of gonad during conditioning culture. Moreover, in this study during site survey, the mean temperature at sampling area was 26 °C to 30 °C during low tide. Thus, setting on temperature for broodstock culture should avoid higher temperature at prolong duration in order to find the best effect on broodstock conditioning. Regulate the ideal temperature for culture of razor clams in laboratory are very important because temperature has been considered as primary factor in regulating bivalve reproduction and also affect the survival rate the bivalve culture (Utting & Millican, 1997; Chavez-Villalba et al., 2002; Marshall, 2012).

In general, the survival rate and qualitative observations showed that the temperature and volume of seawater influence more on mortality of clams. From this two factors, noticeable dead clams inside the sediment may attract disease infection which could affect the survival rate of clams.

4.4.2 Other factors affecting the survival rate and growth of *S. regularis*

Results of shell length and wet weight of the clam broodstock showed that they are not good indicator for monitoring the performance of culture the broodstock stage (López-Alvarado, 2015). In this study, culture duration was only for 25 days which may be too short to evaluate the growth of clam species (Utting & Millican, 1997; da Costa et al., 2011). Labelling of individual on the adult razor clams also might cause confusing and miss measuring among individuals could occur.

Throughout this study, reduction in of wet weight was observed in all experiments (Table 4.6). According to Fréchette (2010), food availability in bivalve culture can influence the wet weight gain especially for improvement of the gonadal development. In this study amount of food given was only 1% of dry meat razor clams which is not enough for

broodstock conditioning (Bamio, 2011). Management of the microalgae culture is very important for bivalve culture and it represents an essential and demanding part for production of bivalve (Coutteau & Sorgeloos, 1992). In general, the production of essential algae contributed about 30% or more of the entire hatchery management costs (Coutteau & Sorgeloos, 1992).

In term of water system design for broodstock conditioning, circulation and flow system can be used to maintain broodstock in good condition compared to close system (Robinson & Breese, 1984). But this system need to consider seawater resource and waste of high number of microalgae. A close system can overcome reducing number of valuable microalgae used for diet of clams during broodstock conditioning (Utting & Millican, 1997). The negative side of a close culture system is dead clams inside the sediment can cause disease to other clams. Moreover, spawning of broodstock could happened randomly and collection of eggs and sperms are easier in close system (da Costa et al., 2011).

In almost every case, razor clam mortalities in this study were associated with the damage of the periostracum. Although obviously-damaged clams were already excluded in this study, it was later noted that small (1–2 mm) tears in the periostracum could become infected and ultimately resulted in death of the clams (Marshall, 2012).

4.5 Conclusion

Overall, there were 14 out of 20 treatments that showed survival rate of more than 50.00% from the initial stocking density of razor clam broodstocks. Higher stocking density of clams, lower volume of seawater and higher seawater temperature significantly reduced the survival rate of razor clams. However, different sediment compactness, different sediment type and different sediment thickness did not showed any significant different of survival rate of razor

clams. Survival rate of razor clams between spawning and resting period also did not showed any significant trend under sediment thickness, volume seawater and seawater temperature experiments.

Based on the overall findings, the broodstock conditioning culture of *S. regularis* can be set up using a closed system with 50% daily water exchange. The tank must contain 5 cm thickness of wet natural sediment and 20 L of seawater. The suggested rearing temperature is 24 °C or ambient temperature. In order to obtain better growth performance, the razor clams broodstock must be supplied with high quality and sufficient amount of microalgae. Usage of broodstock with shell damage should be avoided since this could lead to mortality.

CHAPTER 5

IMPACT OF BROODSTOCK CONDITIONING TO THE GONADAL CONDITION INDEX, OOCYTE DIAMETER AND PARASITE PREVALENCE

5.1 Introduction

Razor clam is also known as “ambal” in Sarawak and this marine bivalve is one of the main highly prized for human consumption. Until now, supply of this species is based on wild catch and there is no effort to manage the natural resources yet (Rahim, 2011). Further increase in fishing pressure can cause the resources of broodstock razor clams to dwindle in future. Research on the induced spawning technique of *Solen regularis* to produce larvae was performed by Gelian and Rahim (2009) but they faced problem with presence of endoparasites (trematode and nematode) in the clam’s body cavity. Further studies by Rinyod and Rahim (2011) reported on presence of endoparasite in 19 out of 24 months during the experiment on reproductive cycle of razor clam obtained from Asajaya Laut. During the spent and resting period of *S. regularis* reproductive cycle, most clams cannot be sexually differentiated because their gonad has been exhausted and does not develop yet (Rinyod & Rahim, 2011).

Broodstock conditioning is one of the practice in bivalve culture to bring adult bivalve into spawning condition in a hatchery environment (Utting & Millican, 1997). During the conditioning period, physical and nutritional factors are manipulated to initiate gonadal development and gametogenesis in order to control the maturation process and maximise the fecundity of the broodstock. There are several factors being evaluated to reflect the impact of broodstock conditioning to the clams.

The improvement of razor clam broodstock quality after being subjected to the conditioning process can be evaluated via gonadal development (GCI and oocyte diameter improvement) with reduced parasite infestation of broodstock. The increment of GCI which reflects the improvement stages of gametogenic development, thus increase the potential survival of larvae produced (Grant & Tyler, 1983; Darriba et al., 2005). Improvement in survival and viability of razor clam larvae are also depending on the improvement of oocyte diameter. Study by Kraeuter et al. (1981) indicates that survival of large oocytes is better than small oocytes in hard clam *Mercenaria mercenaria*. Presence of ovarian parasite could be removed or reduced by conditioning culture in order to make a successful bivalve production (Park, 2005; Perrigault et al., 2011; Golez et al., 2014; Carrasco et al., 2015).

Therefore, this chapter aims to evaluate the impact of broodstock conditioning culture for 25 days toward GCI, oocyte diameter and parasite prevalence of adult razor clams *S. regularis*. This study compares the quality of razor clam adults that undergone the conditioning process with wild razor clams obtained at the start and at the end of the conditioning period. The experiments were conducted during two different gonadal development stage i.e. spawning and resting periods.

5.2 Materials and Methods

5.2.1 Experimental set up and experiment design

The broodstock conditioning experiments were conducted from May 2016 until December 2016 during Phase 2 as described in Chapter 4 that used two different stages of gonadal development (spawning and resting) and each period involved two trials. The date of sampling and experiment set up between two periods of gonadal development are summarised in Table 5.1. Impacts of broodstock conditioning were evaluated by observing

the gonadal condition index (GCI), oocyte diameter and parasite prevalence between conditioned and unconditioned wild razor clam adult. There were two control treatments for each trial: 1) wild before conditioning and 2) wild after the conditioning. The improvement of razor clam quality that underwent broodstock conditioning was compared with clams obtained from wild before starting the experiment and after the conditioning period.

Table 5.1: Summary of experimental set up and design in each trial and period.

Experiments	Gonadal development period	Wild before conditioning	After 25 days conditioning	Wild after conditioning
Trial 1	Spawning period	3 May 2016 (10)	4 May – 28 May 2016 (6)	24-May 2016 (10)
Trial 2		24 May 2016 (10)	25 May – 18 Jun 2016 (2)	21 Jun 2016 (5)
Trial 1	Resting period	6 Nov 2016 (10)	7 Nov –1 Dec 2016 (6)	7 Dec 2016 (4)
Trial 2		6 Dec 2016 (10)	7 Dec – 31 Dec 2016 (6)	5 Jan 2017 (10)

(n) = number of individuals used for each trial and survived the conditioning culture

5.2.2 Gonadal condition index (GCI)

In each sampling, 10 live specimens of *S. regularis* with size more than 4 cm shell length were used for GCI, oocyte diameter (for female only) and parasite enumeration analysis. The actual number of specimens examined for each trial and each treatment were subjected to the availability of specimens collected and survived clams at the end of conditioning culture (Table 5.1). Total shell length, dried shell weight and total wet weight of each clam were measured using dial calliper (Mitutoyo, 5005-671; 0.02 cm) and digital balance (SHIMADZU, BL 220H; ± 0.000 g), respectively. Total shell length was measured from the two ends of shell at hinge side and total wet weight was measured by placing each clam in a petri dish containing seawater.

The gonad content was removed from each clam and weighed. It was further examined to determine the clam sexes and presence of parasite through microscopic observation (Nikon compound microscope, Eclipse E100) at 40 × magnification. The shells were washed using tap water, dried at 60 °C for three hours and dried shell weight was weighed. Calculation of GCI was performed for each clams using GCI formula as follows: $GCI = \text{Wet weight of gonad tissue} / \text{shell dry weight}$ (Rinyod & Rahim, 2011). The mean value of GCI was calculated for each experiment.

5.2.3 Oocyte diameter

Foot of female *S. regularis* was removed from the body by cutting along the narrow bridge from digestive tract until the end of foot. The foot was mounted on a glass slide or glass petri dish and flattened beneath a cover slip (Leung et al., 2010) in order to make it partially transparent so that the oocyte diameter and encysted metacercariae within the foot were visible for observation under a compound microscope (Leung et al., 2010; O'Connell-Milne, 2015). Oocyte diameter was measured using field of view technique of a compound microscope (Nikon, Eclipse E100) at 40 × and 100 × magnification. Ten largest oocytes were measured per clam and the mean value was calculated for each experiment.

5.2.4 Parasite prevalence

The samples were prepared as described above. Parasite prevalence was based on the number of clams infected with parasite within total samples and expressed as percentage. It was calculated as follows:

$$\text{Parasite prevalence (\%)} = \frac{\text{Number individuals infected}}{\text{Total number individuals}} \times 100$$

5.2.5 Qualitative observation

Throughout the experiments, the appearance of gonad content and presence of endoparasite were also noted during the gonad content observation under stereo microscope (OLYMPUS, SZ51) for the infected razor clams.

5.2.6 Data and statistical analysis

Data in this chapter were analysed using descriptive statistic only due to low number of samples that survived the 25 days conditioning culture and the problem to get a balance ratio of both sexes. Comparison of mean values was made between two treatments (wild before broodstock conditioning vs. broodstock after 25 days conditioning) and (broodstock after 25 days conditioning vs. wild after conditioning). Results of GCI and oocyte diameter were analysed among same period of gonadal development only, while parasite prevalence was compared between the two periods.

5.3 Results

5.3.1 Gonadal condition index (GCI)

After 25 days of broodstock conditioning, all trials in both gonadal development stages showed increase in the mean GCI values compared to both control treatments (wild before conditioning treatment and wild after conditioning treatment). Generally, mean GCI values during spawning period was higher than resting period.

In spawning period (Figure 5.1), mean GCI value showed high improvement for Trial 2 (0.132 to 0.287), while only small increase was observed in Trial 1 (0.134 to 0.136). Between the two trials, higher improvement in mean GCI value was recorded in Trial 2 (wild

before conditioning vs. after conditioning = 0.155; after conditioning vs. wild after conditioning = 0.107) than Trial 1 (0.002 and 0.035, respectively).

In contrast, resting period (Figure 5.2) showed smaller improvement in mean GCI value after broodstock conditioning treatment for Trial 1 (0.123 to 0.187) and Trial 2 (0.000 to 0.020). Between the two trials, higher improvement in mean GCI value was recorded in Trial 1 (wild before conditioning vs. after conditioning = 0.064; after conditioning vs. wild after conditioning = 0.067) than Trial 2 (0.020 and 0.020, respectively).

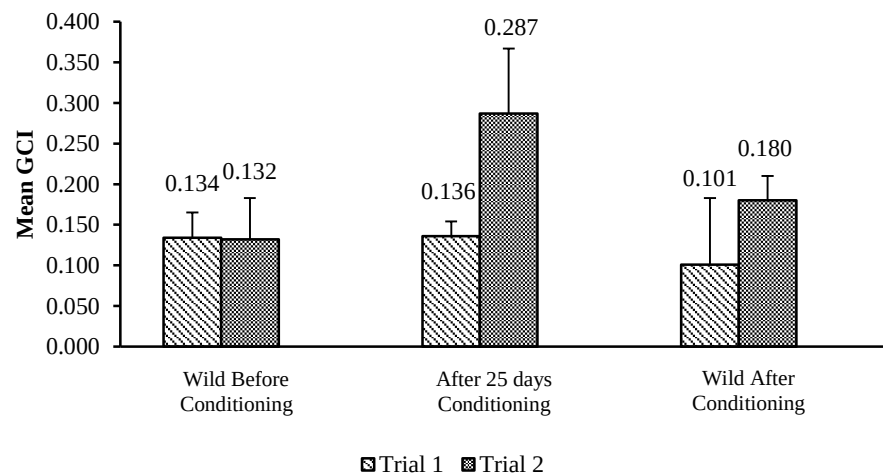


Figure 5.1: Comparison of mean GCI values between before conditioning and after conditioning (laboratory and from wild) during spawning period.

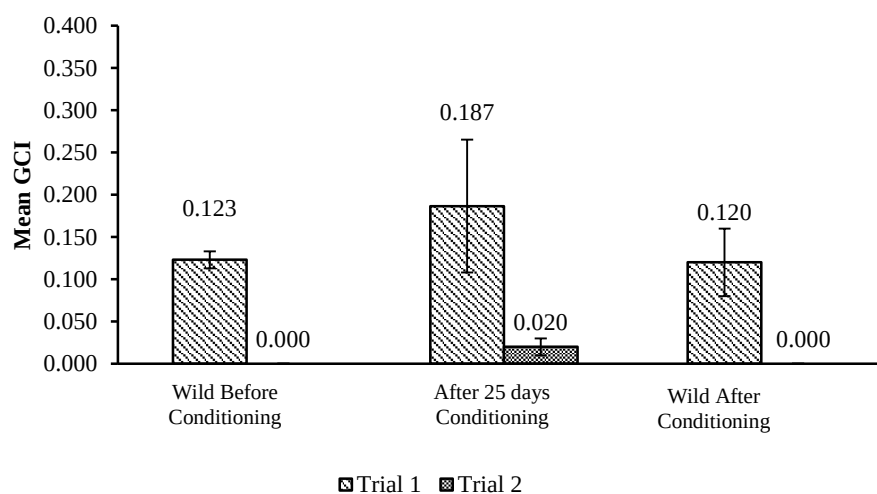


Figure 5.2: Comparison of mean GCI values between before conditioning and after conditioning (laboratory and from wild) during resting period.

5.3.2 Oocyte diameter

Generally, larger values of mean oocyte diameter were observed during spawning period than the resting period but no clear trend was observed on the impact of conditioning in both trials and gonadal development stages.

Trial 1 during spawning period (Figure 5.3) showed reduction in the mean oocyte diameter (35.55 μm to 32.35 μm) after conditioning but increment was observed in Trial 2 (18.70 μm to 28.15 μm). Between the two trials, the conditioned clams of Trial 1 showed increment in the mean oocyte diameter (after conditioning vs. wild after conditioning = 7.03 μm) but Trial 2 showed slight reduction (1.07 μm).

During resting period (Figure 5.4), Trials 1 showed reduction in the mean oocyte diameter (25.42 μm to 24.20 μm) after conditioning but oocyte development was observed in Trial 2 (no oocyte to 28.15 μm). Between the two trials, the conditioned clams of Trial 1 showed reduction in the mean oocyte diameter (after conditioning vs. wild after conditioning = -1.44 μm) but Trial 2 showed oocyte development (10.00 μm).

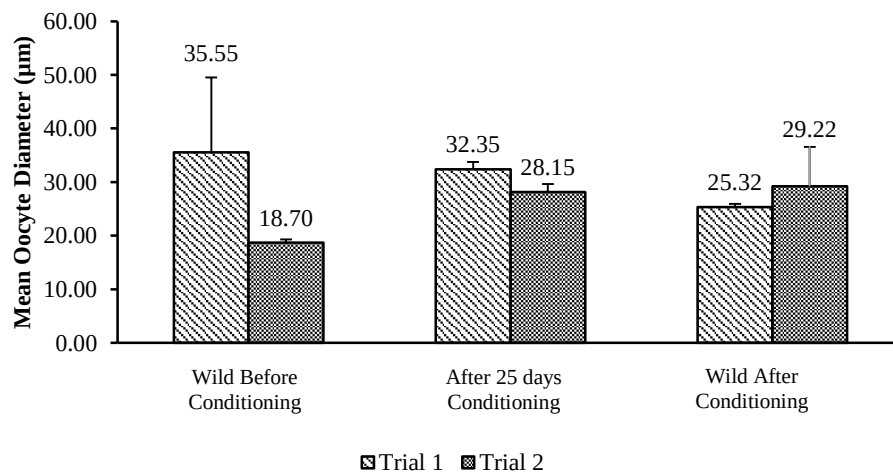


Figure 5.3: Value of mean oocyte diameter during spawning period between before conditioning, after conditioning and from wild.

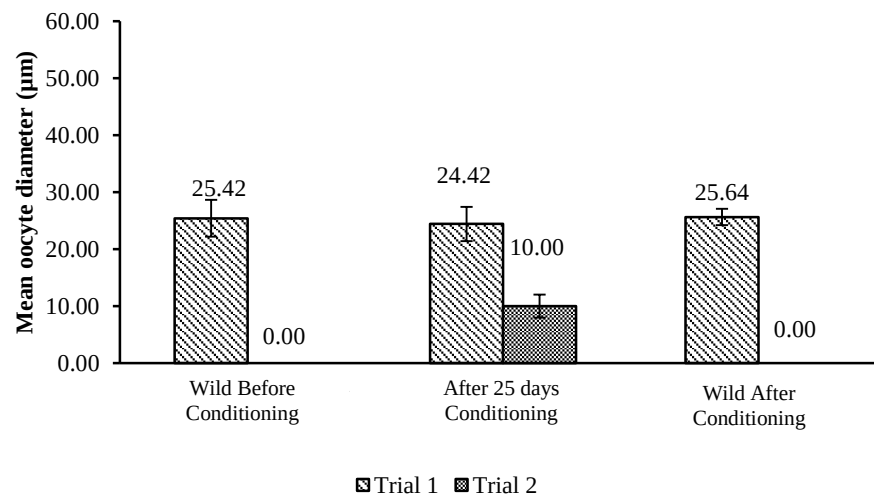


Figure 5.4: Value of mean oocyte diameter during resting period between before conditioning, after conditioning and from wild.

5.3.3 Parasite Prevalence

In general, both gonadal development stages showed absent or reduction of parasite prevalence after 25 days of conditioning (Figure 5.5 and Figure 5.6). During spawning period, small amount of parasite was still presence in Trial 1(9.01%) and total elimination of parasite was observed in Trial 2 after underwent the broodstock conditioning. During resting period, Trial 1 showed total elimination of parasite after underwent conditioning for 25 days compared to the wild that still recorded 50.00% of parasite prevalence. However, all razor clams used in Trial 2 were free from parasite infestation.

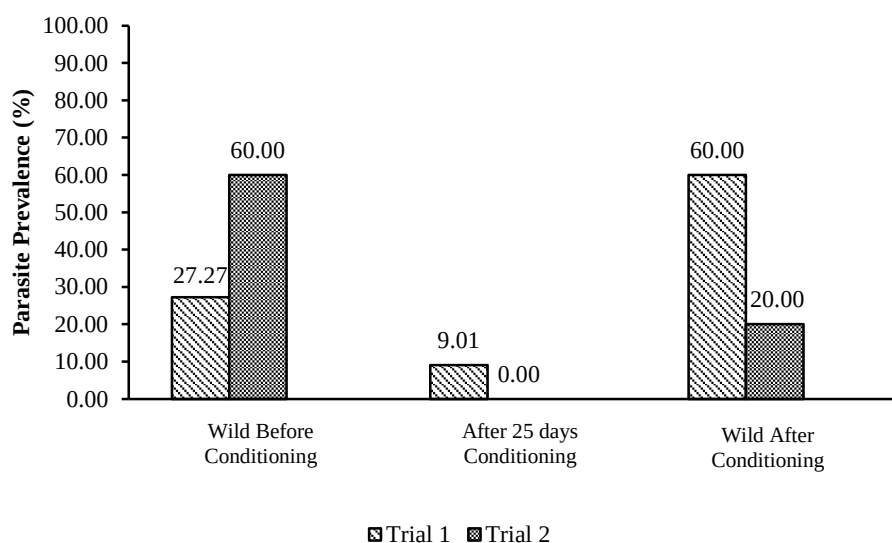


Figure 5.5: Comparison of parasite prevalence (%) values between before conditioning and after conditioning (laboratory and from wild) during spawning period.

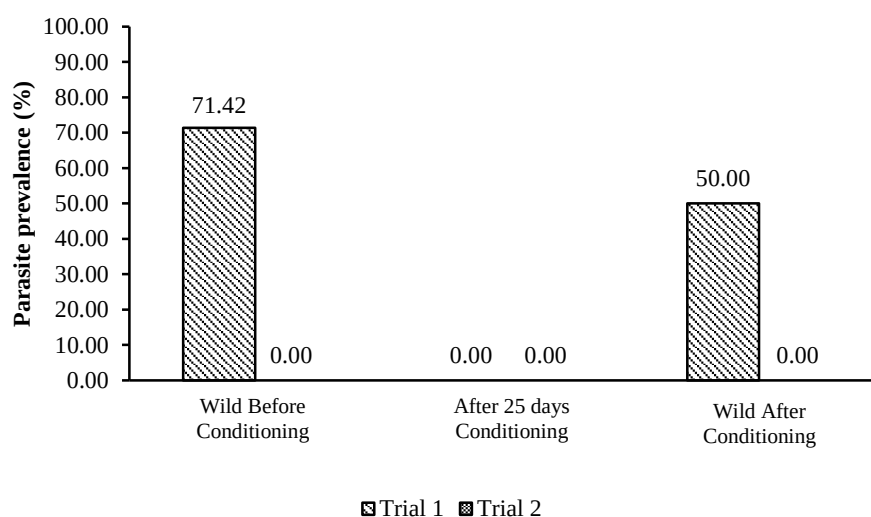


Figure 5.6: Comparison of parasite prevalence (%) values between before conditioning and after conditioning (laboratory and from wild) during resting period.

5.3.4 Qualitative observation

A dissected wild razor clam showed presence of gonad content along the digestive tract until end of its foot during the spawning period (Figure 5.7), while gonad content was almost lacking during resting period (Figure 5.8). During spawning period, the presence of gonad content can be seen clearly with naked eye. However, it was difficult to differentiate the

content between gonad and parasite during spent-resting period (Figure 5.9). The foot of razor clams needs to be flattened on petri dish in order to differentiate between gonad cell and parasite after being stripped out.

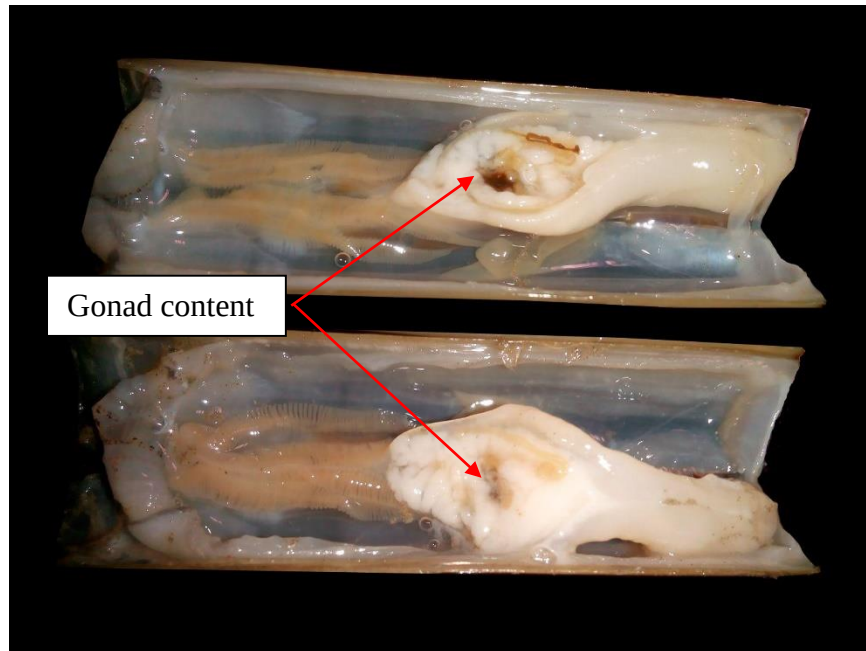


Figure 5.7: Razor clams with full gonad content until end of the foot that were obtained during spawning period in May 2016.

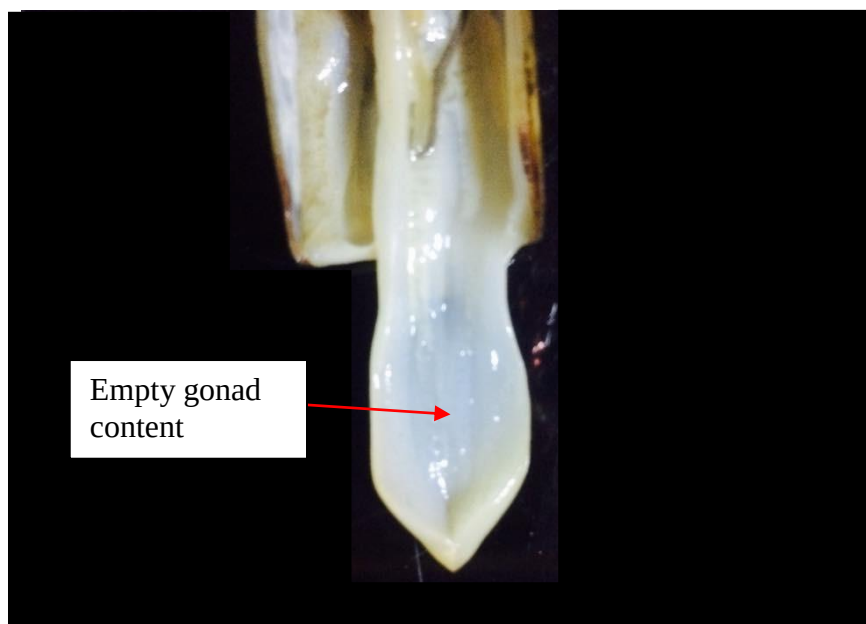


Figure 5.8: Razor clam without any gonad content during resting period in December 2016.

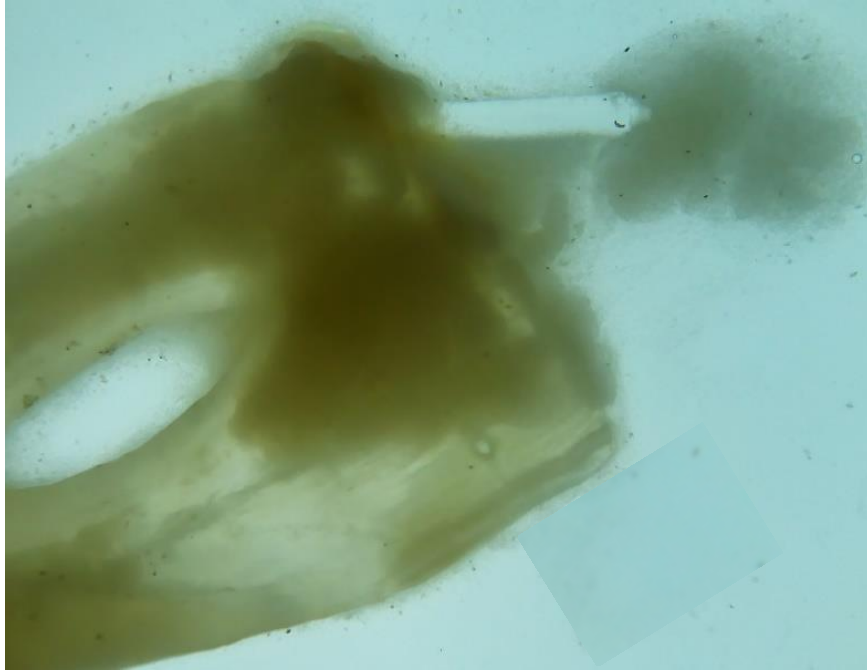


Figure 5.9: Thin layer of foot flattened to examine the presence of oocyte and to confirm the sex of razor clams during spent-resting period.

In female razor clam after the conditioning, high number of oocyte cells with larger diameter was observed during spawning period (Figure 5.10), but this trend changed to low number of small oocyte cells during resting period (Figure 5.11). Throughout this study, the endoparasites were presence only in female razor clams within the oocyte compared to sperm of male razor clams during spawning period (Figure 5.12).

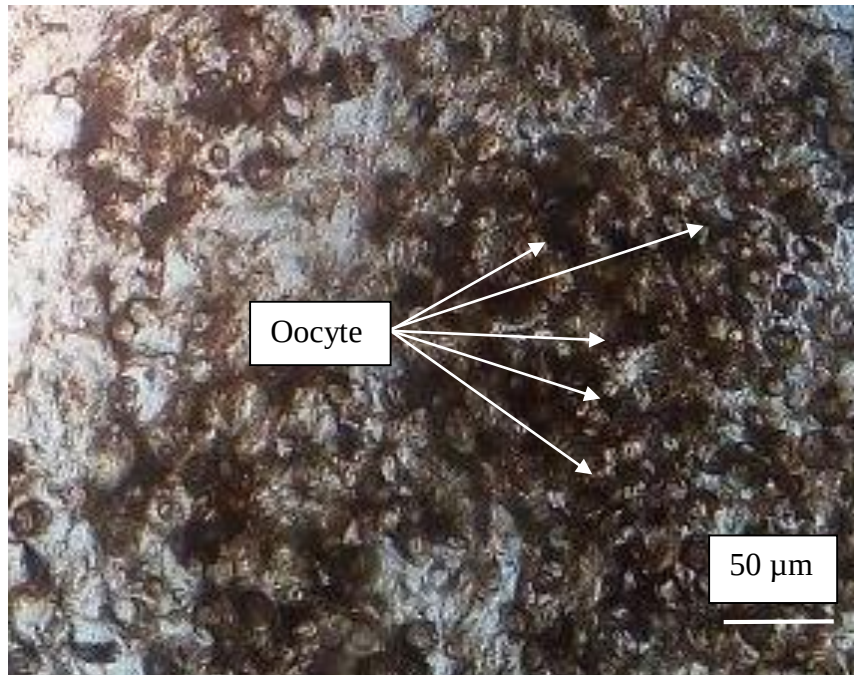


Figure 5.10: Large oocyte diameter and higher number of cells in conditioned broodstock during spawning period (40× magnification).

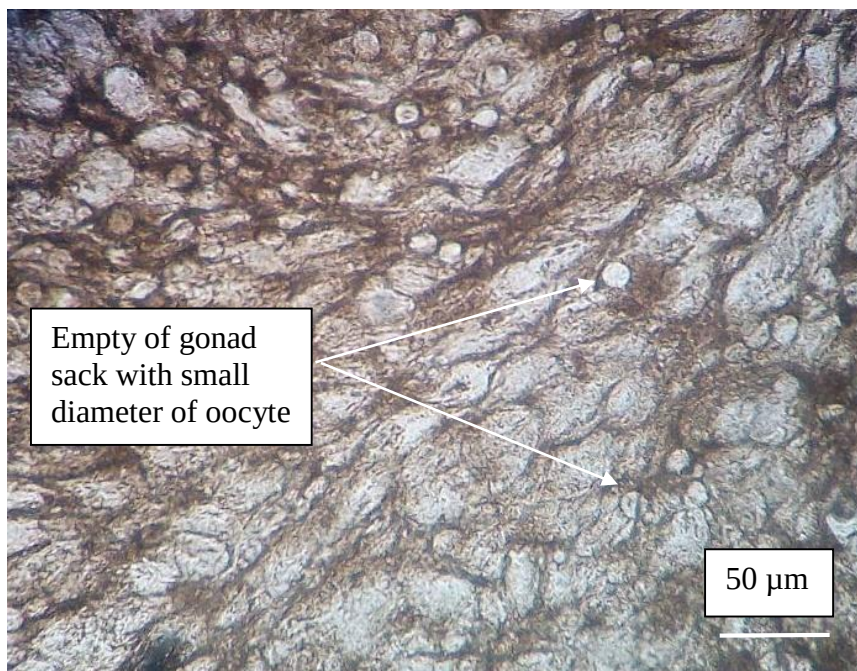


Figure 5.11: Small number of oocyte cell and presence of empty gonad sack with small oocyte diameter during resting period after the broodstock conditioning (40× magnification).

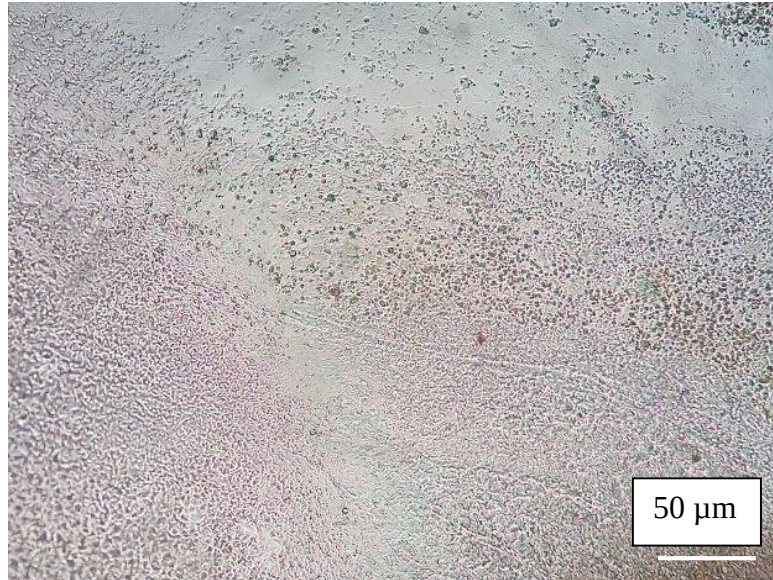


Figure 5.12: Sperms of wild razor clam that are clear without any parasite prevalence. (40 × magnification)

Infested razor clams contained endoparasite that were enclosed in a capsule and attached within the gonad content. The parasites were packed inside capsules in form of cyst before being release into water column (Figure 5.13). These cysts can be observed at high magnification (more than 40 ×) under compound microscope. Heavily infested razor clams showed reduce number of gonad cell.

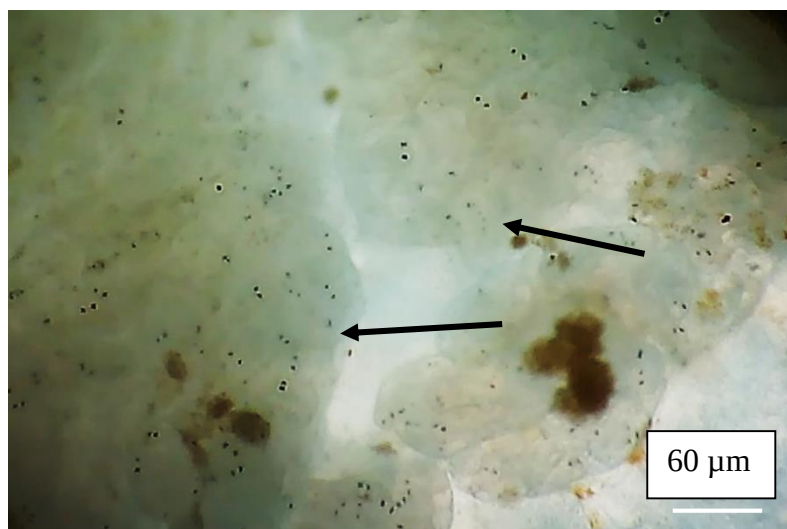


Figure 5.13: Parasites in the form of cyst (indicated by arrow) attached within the gonad content.

5.4 Discussion

5.4.1 Impact of broodstock conditioning

Overall, all trials for both gonadal development stages showed clear improvement trend in mean GCI value and reduction of parasite prevalence after 25 days of broodstock conditioning. However, a clear impact was not observed on the mean oocyte diameter.

Improvement of mean GCI in conditioned clams was higher in spawning period than resting period. This suggests that conditioning culture can improve the broodstock quality and prolong their spawning ability outside of their normal season. However, this need further study on induced spawning trial. In the current study, the amount of feed supplied to broodstocks is below the optimal level and this should be improved in future study since nutrition level is very important towards the bivalve reproductive cycle (Elisabeth et al., 2008; Golez et al., 2011; Marshall, 2012; Argüello-Guevara et al., 2013).

In this study, the mean oocyte diameter of wild razor clams *S. regularis* obtained from Asajaya Laut ranged from 18.70 to 35.55 μm . However, previous study at same place by Rinyod (2012) reported a bigger mean oocyte diameter range from 23.82 to 39.71 μm . The recent mean oocyte diameter was lower compared to previous study due to different technique with different precision was used in oocyte measurement in which Rinyod (2012) used histology method and microscope camera software, while this study just flattened the razor clam foot on slide and estimated the size via field of view calculation. Moreover, the sampling interval is fortnightly for two years and involved more samples Rinyod, (2012) than this study. Mean oocyte diameter values can be affected by number of female clams analysed whereby this study only used 69 wild female clams. In this experiment the culture duration in laboratory conditioning was only 25 days and this may not be enough for razor

clam to show any development progress in term of increase and reduction of oocyte size and GCI value.

The presence of endoparasites (trematode and nematode) within the clams's body cavity of *S. regularis* had been reported in Sarawak (Gelian & Rahim (2009). In the current experiment, parasite number was successfully reduced or removed from *S. regularis* after 25 days of conditioning. The absence of gonadal parasite is one of prerequisite factor in order to achieve a successful bivalve production (Park, 2005; Perrigault et al., 2011; Golez et al., 2014; Carrasco et al., 2015). It is possible that during the 25 days of culture period, the endoparasites are flushed out from razor clam body after they entered its planktonic state in the water column (Sanil & Vijayan, 2011). By the water exchange process during broodstock conditioning, it can break the parasitic production cycle (Bower et al., 1994; Poulin et al., 2005; Petersen & Garcia-ortega, 2008; Bamio, 2011; Golez et al., 2014; Gosling, 2015; O'Connell-Milne, 2015;). Furthermore, spontaneous spawning of razor clams that release the sperms and eggs during the conditioning could also lead to reduction of parasites.

5.4.2 Qualitative observation

Gonad of conditioned razor clams can be easily distinguished between oocyte and parasites since it contains fewer parasites within the gonad content compared to the freshly collected wild razor clams. The measurement of oocyte diameter also had become easier because the oocyte had increased in size and lack or low amount of parasite in the gonad makes the oocyte more visible under the compound microscope.

Under a stereo microscope, the parasite looks almost similar colour as the gonad especially during resting period. Thus, during the gonad content stripping process for GCI analysis, parasites may be accidently included with the gonad tissue. This may cause small

error to the GCI value, thus confirmation of parasite infestation need to be done with a compound microscope (40×) prior to gonad stripping for artificial fertilisation purpose. Usage of wild broodstocks that are heavily infested with parasites for induced spawning may lead to failure in obtaining viable gametes. Thus, broodstock conditioning culture can be considered as one of the important precaution steps for bivalve culture.

5.4.3 Rearing factors influencing the mean GCI and oocyte diameter

The improvement of bivalve gonad is mainly controlled by interaction of both endogenous and exogenous factors. Among the exogenous factors, temperature and food supply seem to be the most important (Thompson et al., 1996). Results had shown that the mean GCI of conditioned clams was higher than the wild clams after being fed daily with microalgae at 1% of the *S. regularis* mean dry meat. However, this amount is below the suggested optimum value which is 3% of the mean dry meat weight of the adult per day for bivalve promoting the development of GCI (da Costa et al., 2012).

The temperature of *S. regularis* natural habitat ranged from 26 - 30°C but during the broodstock conditioning culture, the temperature was set nearly consistent for 24 hours at air-conditioned room temperature (24°C). Since temperature is a factor that can influence organism growth, GCI and oocyte diameter, future study should try to use a fluctuating ambient rearing temperature.

5.5 Conclusion

After being subjected to 25 days of broodstock conditioning, some trend of improvement on mean GCI value and reduction or removal of parasite prevalence were observed in *S. regularis* broodstock. Improvement of mean GCI was recorded in both spawning and resting

periods, but the mean oocyte diameter did not show any specific trend in size increment. Parasite prevalence in conditioned razor clams was successfully lowered or eliminated compared to wild clams in both gonadal development stages. In other words, the food safety of conditioned clams is further enhanced for human consumption. Thus, the broodstock conditioning of *S. regularis* can be considered as an approach to improve the quality of razor clam broodstocks in future aquaculture industry.

CHAPTER 6

GENERAL CONCLUSION AND RECOMMENDATIONS

Broodstock conditioning is one of the important techniques in bivalve culture. This technique involve in mimicking the natural environment inside a laboratory setting to reduce broodstock stress (Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; Argüello-Guevara et al., 2013). Broodstock conditioning technique is important to improve the gonadal development and remove or reduce the ovarian parasite prevalence of adult bivalves (Utting, 1992; Utting & Millican, 1997; Matias et al., 2009; Argüello-Guevara et al., 2013). In order set up broodstock conditioning culture, study on design set up give best results of survival rate were done on broodstocks of razor clam. Evaluation of impact on broodstocks razor clam toward GCI improvement, oocyte diameter and reduce the parasite prevalence also were studied. Therefore, this study related on established of broodstock conditioning culture technique for *S. regularis* by evaluating the broodstock survival and growth in Chapter 4 then evaluated the impacts of broodstock conditioning towards the clams in Chapter 5.

In Chapter 4, broodstocks of *S. regularis* were tested with different treatments of stocking density, sediment compactness, duration of culture and sediment type during Phase 1. At this phase, the experiments were developing the standard culture design then used in Phase 2 as control treatment. In Phase 2, the experiments of sediment thickness, seawater volume and seawater temperature were tested between two periods of gonadal development (resting period and spawning period) also with standard design (control treatment).

Overall, 14 out of 20 treatments showed more than 50.00% survival rate from the initial stocking density of razor clam broodstocks except for treatments with higher stocking

density of clams, lower volume of seawater and higher seawater temperature that significantly gave lower survival rate. However different sediment compactness, sediment type and sediment thickness did not showed any significant difference of survival rate of razor clams. Survival rate of razor clams between spawning and resting period did not showed any significant pattern thus survival of razor clam was depending on the treatment design not on the period of the gonadal development stage. Generally, there were loss of wet weight and gain in shell length of razor clams after the broodstock conditioning culture may due to low quantity of food supply given.

After broodstock conditioning there were some trend of improvement on GCI value, oocyte diameter and reduce or remove parasite prevalence of *S. regularis* broodstock especially during spawning period. After conditioning, gonad of razor clam undergoes conditioning experiment can be studied easily and distinguish between size of oocyte compare razor clam from wild natural habitat. Amount of 1% of mean dry meat of clams used in this experiment was not enough to improve the GCI quality of razor clams.

In conclusion, this study had some important information on technique of broodstock conditioning of *S. regularis* in laboratory scale based on survival, growth and qualitative observation results. *S. regularis* shows high potential on aquaculture sector with high stocking density survived, the design culture is natural material used, can culture in longer period, easy to monitor with low cost for the culture design made up. There also small improvement of GCI and oocyte diameter during resting period and this species can be consistently produced throughout the year. This can help in artificial induced spawning or razor clams to produce good quality of razor clam. Broodstock conditioning also helps to remove the endoparasite within the gonad content then this help in artificial induced spawning and food safety for human consumption. This technique could be useful for future

research on culture of razor clam in larger laboratory scale. Finally, the overall information gathered in the presence study allowed the development of a successful *S. regularis* production.

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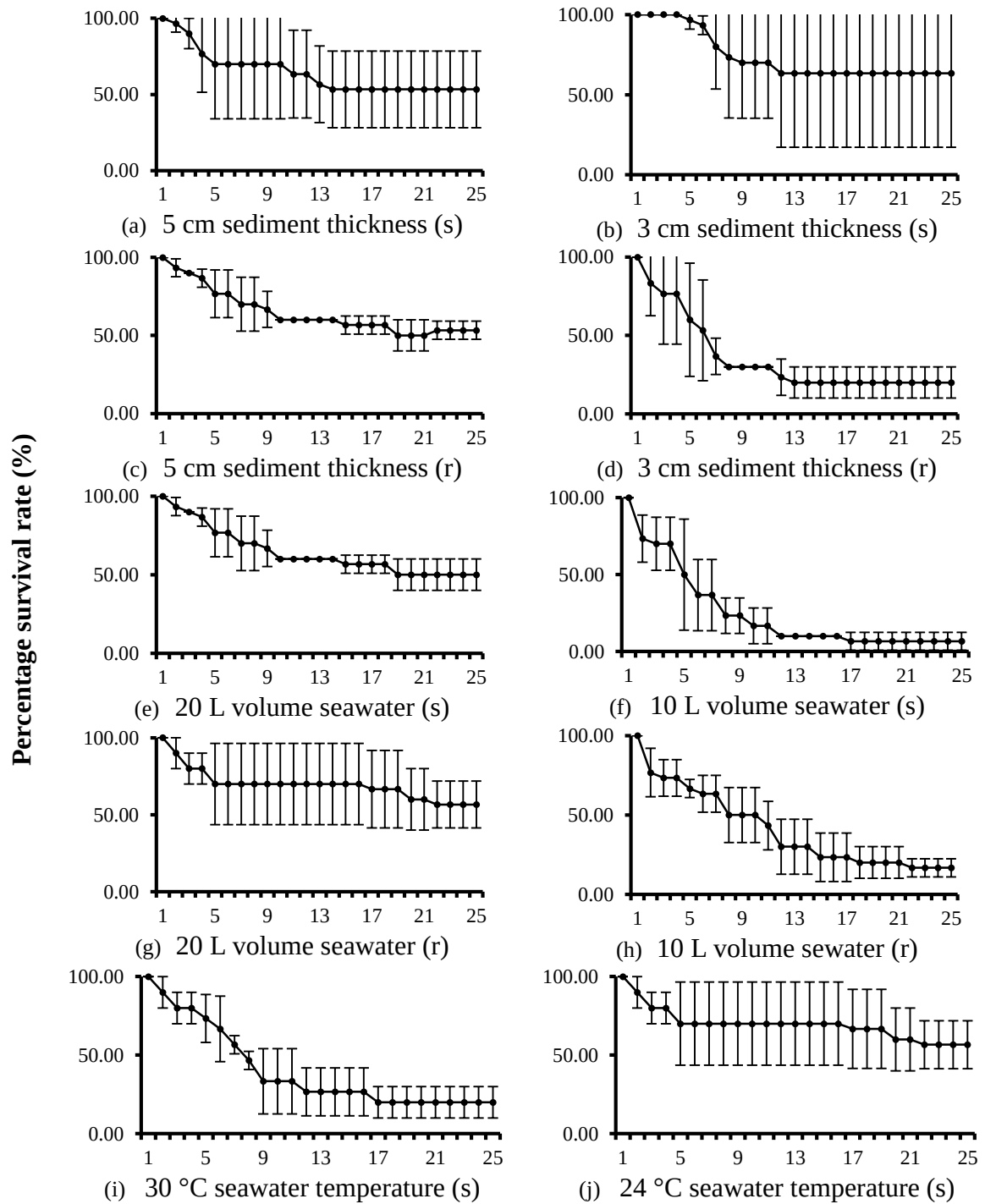
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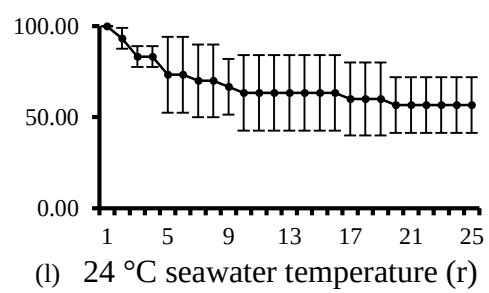
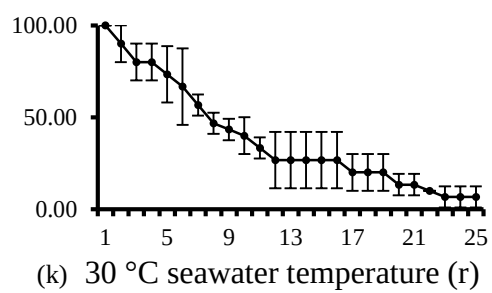
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APPENDICES

Appendix 1: Graph mean percentage survival (%) each day for all treatment with standard deviation. (r) indicate experiment were run during resting season (s) indicate experiments were run during spawning season.





Appendix 2: Example of daily recording sheet survival of clams and algae left.

Masa ternakan: _____

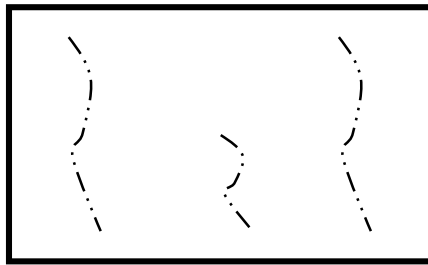
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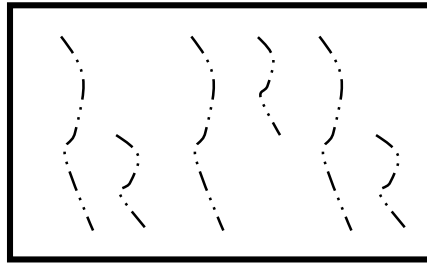
Test design: _____

Day	Date	Temp (°C)	Sali (PSU)	Death				Diet		Water exchnge	Note
				No Individu	Individu (labeled)	Wet Weight	Length	Iso	Cheto		
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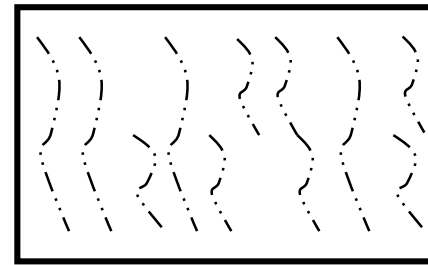
Appendix 3: Illustration amount of fecal between little, moderate and dense on sediment surface.



little



moderate



dense