Asian Clam (*Corbicula fluminea*) Larvae Production in Broodstock Conditioning

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Abstract

Nowadays, the inclining of Asian clam market demand for supplying the seller *Etak* in some areas in Malaysia particularly Kelantan where etak had become the main concern due to the stock availability in the natural population was over-exploited. Thus, an effort to expedite the availability seed of *Etak* must be initiated to conserve the Asian clam population in their habitat. Until now, the information about production larvae is needed to begin the domestication process. The scientific goal aims to evaluate the best time to produce Asian clam larvae to supply the hatchery production process. To answer this question, the evaluation of broodstock conditioning adult *Corbicula fluminea* monthly during a year and this condition was compared into the fine sand sediment as well as no sediment. The results showed that the Asian clam adult pattern released larvae monthly during a year wherein the great number larvae were produced 22464 Ind/L totally in December and the produced larvae in Oct and Nov was almost similar with range 22350-22500 Ind/L. The condition index with fine sand sediment was 3.8419 % higher than the control treatment 3.5750 %. And last, the ingestion rate both treatment was 1.126 ± 0.534 µg/h and control treatment 1.609± 0.434 µg/h.

INTRODUCTION

In recent years, the state of Kelantan area has seen significant advances in Asian clam, *Etak* becomes the commodity species in Malaysia, especially in Kelantan state. This species revealed the great potential to domestication process (Aquaculture) where the information of all life cycles is needed such pattern of production larvae (Rak, 2006). However, until now the insufficient information for this stage becomes the main problem to the aquaculture process (Cheng, 2015). Therefore, the information is needed to monitor the Asian clam reproduction activity to gain valued insight for harvester where the great number of larvae would be released monthly during a year to comply with the rearing process.

The stock availability in nature occurred over-exploitation where this condition causes negative repercussions, such as ecological instability (Lee *et al*., 2013). *Corbicula fluminea* is also one of the indigenous fauna filters and pedal feeders, which found in sandy bottoms of rivers in Kelantan (Rak, 2006). The report from Patrick *et al*. (2017) mentioned that Asian clam preferred habitat with fine or

coarse sand substrates compared to mud or bare concrete. The support research found as well that substrates influence the distribution of this clam (Colwell *et al.*, 2017). The scientific goal aims to monitor the pattern of Asian clam larvae production to supply the hatchery production process.

METHODOLOGY
Place and Time

The experiment was conducted during October 2018-September 2019, and then the hatchery Asian clam larvae production in this study was addressed at the Aquaculture Laboratory, Faculty of Agro-based Industry at UMK Campus Jeli, and the sampling location at around Kampung Kubang Batang, Tumpat (6° 13 32.8 N 102° 15 21.7 S).

Research Material

The tools used include aquariums, aerators, autoclaves, analytical balances, laminar flow, test tubes, petri dishes, glass microfiber filter (GFC, Whatman, pore size 60 and 150 µm) calipers, microalgae, and microscope. Adult Samples are taken from the catch at Kg Kubang Batang River.

The water parameter was evaluated by using DR-2800 spectrophotometer (Hach, Germany) referring to the company manual in the laboratory setting (Filgueira *et al.*, 2013).

Research Design

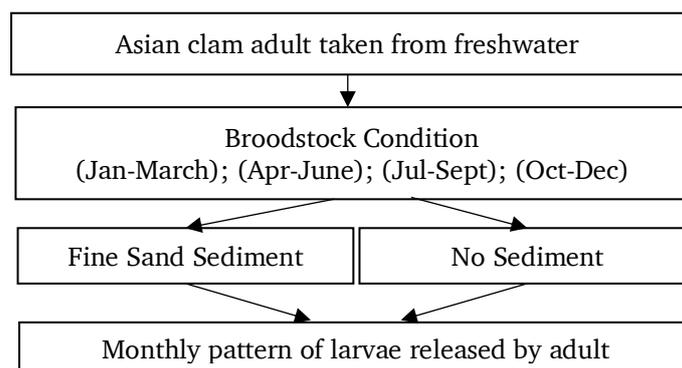The experiment design in this research was explained in Figure 1.

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┌─────────────────────────────────────────────────────┐
│        Asian clam adult taken from freshwater        │
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│              Broodstock Condition                    │
│ (Jan-March); (Apr-June); (Jul-Sept); (Oct-Dec)       │
└─────────────────────────────────────────────────────┘
          ┌───────────────┴───────────────┐
          ▼                               ▼
┌─────────────────────┐       ┌─────────────────────┐
│  Fine Sand Sediment │       │     No Sediment     │
└─────────────────────┘       └─────────────────────┘
          └───────────────┬───────────────┘
                          ▼
┌─────────────────────────────────────────────────────┐
│        Monthly pattern of larvae released by adult   │
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Figure 1.   Experiment design of Asian clam production.

Work Procedures
Sampling and Handling the Sample

In the present research, the adult Asian clams were taken from Kg Kubang Batang River by a random sampling method with shell length standard size >10 mm, which is indicated ready for the spawning process. The taking sample process has utilized the grab for taking benthos (Filgueira *et al.*, 2013) then after that, the samples were cleaned, next to measuring the clam using the ruler, and last the sample should be selected with the needed standard size.

Broodstock Conditioning

Conditioning is the process of inducing gametogenesis, or the ripening of gonads, to make clams ready for spawning. This process was crucial due to if the conditioning unsuccessful, the larvae could not be released with a great number (Rajagopal *et al.*, 2000). In the present research, 90 adult *Corbicula fluminea* taken from the freshwater was brought into three tanks for broodstock condition (30 clams for each treatment). This condition was divided into 2 treatments such as control (no sediment) and fine sand sediment with sediment standard size 0.25-0.125 mm. Afterward, make sure the water quality conditions (temperature and dissolved oxygen (DO) levels) were in

good condition by DO meter, pH and salinity used pH meter, and for ammonia using a spectrophotometer at 650 nm (Filgueira *et al.*, 2013). Before the clam was entered into the tank, it was estimated shell length, shell height, and shell width. The last, the broodstock conditioning was given feed 100 micros let freshwater microalgae two times a week. Afterward, the Asian clam was settled 24-36 h to release larvae. This process was monthly replication during a year could be seen as an example in Figure 2 Dec-time.

Figure 2.   Broodstock conditioning system of Asian clam with no sediment (Dec-time).

Condition index (CI) was determined according to formula given in Scarlet *et al.* (2015) as:

$$CI = \frac{DmW}{DsW} x100\%$$

Note:
DmW   = dry meat weight (g)
DsW   = dry shell weight (g)

The measuring process for condition index, the all adult from tank broodstock conditioning was taken then it measured the dry meat weight the clam and dry shell weight using a digital scale. This process was replicated monthly for a year. The dry meat process was conducted by a drying oven with a temperature of around 60-70 °C.

The clearance rate is that the volume of water is completely cleared of particles per unit of time. When all particles presented to the gill are cleared from suspension, then the clearance rate is the same as the filtration rate. For the Asian clam larvae according to Willadino *et al.* (2012), the measurement could be using a 10 % number of feeding on an adult. To measure microalgae density the following formula could be used:

$$MD = \frac{\bar{x} \text{ cells}}{\text{square}} \times \text{dilution factor} \times 10^4$$

Note:
MD   = microalgae density
$\bar{x}$ cells   = the average number of cells

The measuring process of larvae number was done by taking 1 L water from larvae tank, then put on the hemocytometer using an automatic pipette (Utting and Spencer, 1991) (Figure 3).
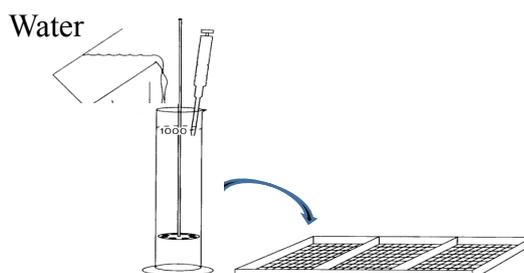

Figure 3.   Method for estimating numbers of larvae.

The ingestion growth rate (IGR) was estimated according to Willadino *et al.* (2012):

$$IGR = (C0 - C1) \times \text{water volume} \times h$$

Note:
C0 = initial food concentration
C1 = final food concentration
h = exposure time

Data Analysis

Data analysis was performed by analysis of variance (ANOVA) by using Turkey's Honestly Significant Difference post hoc test.

RESULTS AND DISCUSSION

Broodstock Condition System of Asian Clam (*Corbicula fluminea*) Condition Index (CI)

The condition index of Asian clam in two treatments (with fine sand sediment and no sediment) could be seen as following (Table 1).

Table 1.   The condition index of Asian clam with two treatments during a year.

Time	Condition Index (%)		CI (%)
	Fine Sand Sediment	Without Sediment	
Jan	3.7098	3.4056	
Feb	3.7397	3.3265	3.7171
Mar	3.7019	3.3968	
Apr	3.7254	3.4331	
May	3.8353	3.5508	3.7559
Jun	3.7069	3.5194	
Jul	3.8298	3.4732	
Aug	3.8218	3.6072	3.8227
Sep	3.8165	3.6235	
Oct	3.9366	3.8389	
Nov	4.0308	3.8186	4.0720
Dec	4.2485	3.7108	
Average	3.8419 ± 0.1625	3.5587 ± 0.1662	

Table 1 depicts that the condition index of *Corbicula fluminea* with fine sand sediment was 3.8419 % higher, while control treatment 3.5750 %. It means the Asian clam on the sediment treatment readier for the spawning stage. However, as general, both condition indexes indicated available to spawning. If we compare to the previous study by Cataldo *et al.* (2001), the CI was 2.839-6.854%. We could see as well that the CI based on three months classified, in Oct-Dec was the highest with 4.0720 % compared to other months. According to the present research, we could conclude that in this range month the *C. fluminea* would release the juvenile with a great number.

At this stage, adult clams are prepared for spawning by maintaining the tank's condition index, water parameter, and clearance rate. According to the present results, the condition index (CI) of *C. fluminea* in the experiment was in the range of 3.5750-3.8419 %. These data indicated that *C. fluminea* were ready to reproduce when compared with previous studies. The CI for *Corbicula fluminea* reported by Cataldo *et al.* (2001) ranged between 2.839-6.854%. This experiment reveals that the value of CI analysis was carried out to estimate the reproductive activity of a particular species indirectly or to evaluate the readiness of *C. fluminea* adult for the spawning process. In this present study could indicate the readiness of the *C. fluminea* for realizing the larvae.

According to Rajagopal *et al.* (2000), the reproductive intensity of bivalves varies with age and size since younger individuals experience rapid growth and invest little to no energy on reproduction. On the other hand, energy allocation transitions gradually from somatic growth and maintenance to reproduction when size increases. Furthermore, poor or stressful

environmental conditions may lead to a low CI for the clam (McMahon, 2002). CI has also been linked to several factors such as food availability, temperature, salinity, and chemical contaminants (Filgueira *et al.*, 2013).

These water parameters may be significant at local spatial scales, resulting in population diversity in nearby localities (Filgueira *et al.*, 2013). However, the more considerable CI variability between populations may be better explained by significant spatial scale impacts such as primary productivity than local effects (Costil *et al.*, 2005; Filgueira *et al.*, 2013).

Besides, limited food sources might be the biological explanation of this adverse effect of stocking density on CI given that there is a direct relationship between food availability and bivalve CI (Filgueira *et al.*, 2013). The grazing activity of bivalves, which may exert top-down control on the population of phytoplankton in their habitat, maybe the reason for this observation.

Water Parameters

In this following Table 2 could be seen the water quality parameters condition in the broodstock system. The given Table 2 reveals about the value water quality parameters on *Corbicula fluminea* broodstock condition, the first parameters were temperature on range 25- 26 °C when pH was 6.9, DO was 5.9 – 6.2 mg/L, salinity was 0.02 ± 0.0022 ppt as well as Ammonia was 0.0858-0.1009 mg/L. Temperature becomes the most critical parameter for this condition, according to the previous study where the optimum temperature was 26-27 °C.

Table 2.   Water quality parameters on the Asian clam broodstock condition system.

Parameters	Control	Fine sand	Optimum	Reference
Temperature (°C)	25.75 ± 0.650	25.97 ±5	26-27	McMahon 2002; Rajagopal *et al.* 2000
pH	6.95 ±0.088	6.98 ± 0.030	6-7	McMahon 2002
DO (mg/L)	5.90 ± 0.460	6.27 ± 0.327	>3	McMahon 2002
Salinity (ppt)	0.02 ± 0.002	0.02 ± 0.003	14-17	McMahon 2002
Ammonia (mg/L)	0.10 ± 0.014	0.08 ± 0.012	<0.2	McMahon 2002

The water quality parameters impact broodstock conditioning because this experiment was conducted in a closed water system. The water parameters in the broodstock conditioning system are detailed in Table 2 where all parameter was categorized and support to broodstock condition. McMahon (2002) stated that the most important environmental factor in bivalve reproduction is temperature, which also determines the success of broodstock conditioning. To the gametogenic activity of bivalves to occur, they require water temperatures of at least 15°C. The right temperature is vital to kickstart multiple stages of reproduction and this is important for *C. fluminea* since it has a bivoltine reproductive cycle that responds to temperature regimes in its habitats such as rivers, lakes, and reservoirs (Mouthon and Parghentanian, 2004).

Initial spawning often takes place in spring when the temperature is at least 16-18°C for ten degree-days. The reproduction will seize once temperatures exceed 27-28°C (McMahon, 2002; Mouthon, 2001) which will resume the temperature is optimum again (Mouthon and Parghentanian, 2004). Besides that, salinity also plays an essential role in bivalve mollusks' reproductive development. When their environments are hyper or hyposaline, shells will close as a typical response to protect internal organs from their surroundings.

The salinity in the present study was in the optimal range for broodstock conditioning. Besides, dissolved oxygen (DO) was controlled by supplying aerator to maintain good water quality even

though macrobenthic organisms generally tolerates DO levels (Miller *et al.*, 2002; Sousa *et al.*, 2008). Nonetheless, low DO levels have been said to cause acidosis in aquatic animals as a result of hypercapnia due to the lowered pH. Ammonia could also affect the mortality in adults when its level is in excess. Therefore, ammonia levels were monitored closely in this experiment. In terms of pH, *C. fluminea* can tolerate as low as 5.4. If pH is more significant than 6.5, this species can survive in an environment with calcium levels of 6 mg Ca/L (Sousa *et al.*, 2008).

Clearance Rate

In this following Table 3 could be seen the clearance rate value of Asian clam when in the broodstock condition system.

Table 3.   Clearance rate of Asian clam in broodstock system condition.

Parameters	No Sediment	Fine Sand Sediment
Average of cells/mm²	250 ± 25	242 ± 14
Dilution factor (mL)	50	50
Viable Density (cell/mL)	125 ± 12.5 millions	120 ± 7.2 millions
Viable Density in tank (cell/mL)	10.4 ± 1.4 billions	10.05 ± 6.004 billions

The given Table 3 reveals that viable density in the tank of Asian clam broodstock was 10.4 ± 1.4 billion for control when 242 ± 14 billion for fine sand sediment. The average of cells/mm² on both treatments was 242-250 cells/mm² and viable density in cell/mL was 125 ± 12.5 million higher a quarter than fine sand sediment.

Last but not least, the clearance rate in broodstock condition was in the range of 9.3 - 10.4 billion cells/mL. This data indicated that food availability (microalgae) is sufficient for adult *C. fluminea* during broodstock conditioning. According to McMahon and Bogan (2001), suspension-feeding rates of *C. fluminea* can be high and varies between 300-2500 L/h. Factors that influence clearance rates of bivalves include body mass, temperature, food quality and food quantity . Clearance rates have been shown to scale allometrically in the form of bivalve dry tissue weight (Cheng, 2015), which appears to improve with increasing *C. fluminea* dry weight (Cheng, 2015). Several studies have also demonstrated the effects of food quality and quantity on the clearance rate of *C. fluminea*. Cheng (2015) reported that the concentration of particle size that is smaller than 53 μm declined downstream, where it was highly populated with *C. fluminea*. Moreover, it was discovered that *C. fluminea* filters particles that are less than 1 μm. The same study recorded the presence of pseudo feces when diets included particles larger than 16 μm which was an indication of wasteful feeding. In conclusion, *C. fluminea* feed most efficiently on particles within the sizes of 1-53 μm.

Estimating Numbers of Asian Clam (*Corbicula fluminea*) Production Rate

The number of larvae production rate *C. fluminea* monthly during a year could be seen in the following Table 4.

Table 4.   Asian Clam (*Corbicula fluminea*) larvae production rate during a year.

Month	Number of Larvae				Average (Ind/L)
	Total (Ind/L)	Average (Ind/L)	per individual	per cm2	
Jan	14148	3537 ± 254	472	34	
Feb	14144	3536 ± 117	471	34	14145
Mar	14144	3536 ± 179	471	34	
Apr	18650	4663 ± 216	622	45	
May	18700	4675 ± 404	623	45	18583
Jun	18400	4600 ± 312	613	44	
Jul	16900	4225 ± 226	563	41	
Aug	17000	4250 ± 103	567	41	16883
Sep	16750	4188 ± 123	558	40	
Oct	22500	5625 ± 154	750	54	
Nov	22350	5588 ± 314	745	54	22438
Dec	22464	5616 ± 103	749	54	

The production of larva in Table 4 elucidates that the Asian clam adult pattern released larvae monthly during a year wherein the great number larvae were produced 22464 Ind/L totally in December as the highest, with average 5616 ± 103, per individual 749 and 54 per cm². While in February and March were the lowest larvae produced with 14144 Ind/L, the average 3536 ± 117, per individual 471 and 34 per cm².

Besides that, the produced larvae in Oct and Nov were almost similar to Dec with range 22350-22500, the production larvae in three months (Apr-Jun) was higher 25% from the following three months with approximately 16800 Ind/L.

Ingestion Growth Rate (IGR)

In this following Table 5 could be seen the Asian clam (*C. fluminea*) larvae ingestion growth rate.

Table 5.   The Asian clam larvae ingestion growth rate.

Study	Ingestion Rate
C. fluminea with sediment Present study	1.126 ± 0.534 µg/h
C. fluminea control treatment Present study	1.609 ± 0.434 µg/h
Asian Clam 2019 (Bolam *et al.*, 2019)	2.45 ± 0.83 µg/h

The given Table 5 elaborates the ingestion growth rate of Asian clam on fine sand sediment was 1.126 ± 0.534 µg/h while control treatment 1.609 ± 0.434 µg/h categorized as well. The result in this study was compared to the previous study by Bolam *et al.* (2019) where the ingestion rate was 2.45 ± 0.83 µg/h.

C. fluminea is known as a simultaneous hermaphrodite due to its ability to self-fertilize and spread its sperm through the water column (Denton *et al.*, 2012). Larvae are brooded inside the branchial gills of adult clams upon fertilization. In the present study, the aim is to acquire the best condition for larvae production.

The larva production of the Asian clam adult had two time periods to produce larvae with great number wherein the great number larvae were produced 22464 Ind/L totally in December as the highest and in Oct and Nov was almost similar with Dec with range 22350-22500 Ind/L. The larvae production in these three months could be used to supply the hatchery process. The rate of larvae production in this experiment is in line with a study by McMahon (2002). *C. fluminea* has a high fecundity (25,000 to 75,000 veligers produced during an individual's lifetime) where egg fertilization occurs internally, and the larval clams are brooded on the gill where they transform into juveniles within 4-5 days (Natale *et al.*, 2014).

The investigation support research as well conducted by (Cao *et al.* 2017),

where the spawned *C. fluminea* would reach the peak of production during Oct-Dec in a year. Several factors influence broodstock conditioning, particularly food availability, which appeared to catalyze *C. fluminea* spawning to meet the energetic demand of brooding (Mouthon, 2001). When food is not a limiting factor, gonad development and fecundity are improved, besides increasing the brooding and individual size of developing embryos (Beekey and Karlson, 2003).

Studies have also reported that initial broodstock condition and the gametogenesis phase the adults are in when conditioning begins are responsible for the differences in conditioning and spawning success or larval viability (Ojea *et al.*, 2004; Denton *et al.*, 2012). Besides, temperatures above 16 C are necessary for reproduction to occur besides reducing bivalve metabolism.

CONCLUSION

In conclusion, this study confirmed that the time in season four (Oct-Dec) is used to establish *C. fluminea* seed since a significant number of larvae were produced.

ACKNOWLEDGEMENT

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