Life cycle of Asian clam (*Corbicula fluminea*) from Tumpat, Kelantan

By

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

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DECLARATION

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

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I certify that the Report of this final year project entitled " Lifecycle of Asian Clam (*Corbicula fluminea*) from Tumpat, Kelantan" by Siti Zanaria Binti Mohd Kamari, matric number F14A0366 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Husbandry Science) with Honours,

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ABSTRACT

Asian clam, *Corbicula fluminea* is well known in Kelantan as popular snack. A study of the life cycle of Asian clam (*Corbicula fluminea*) was be documented based on identification stage of life cycle Asian clam (*Corbicula fluminea*). Samples were taken from sg. peng nangka,kg tok uh, Tumpat. The brooding of *Corbicula fluminea* started to develop in Jun 2017. The larvae continued to develop until the maturation stage 4-5days after fertilization. The early stage of *Corbicula fluminea* were trochophore, D-shape veliger, veliconcha and pediveliger. The findings was obtained from dissection *Corbicula fluminea* have been fertilized, cut the innerdemibranch that usually contain the egg and larvae, then observed under light microscope (Rax Vision Y100). From the study, the early stage of life cycle *Corbicula fluminea* is the same with other clams for example *Corbicula australis* and *Corbicula leana.*

Keyword*: Corbicula fluminea*, life cyle, aquaculture, production

Kitaran hidup Etak (*Corbicula fluminea*) dari Tumpat, Kelantan

ABSTRAK

Etak, *Corbicula fluminea* ditemui dan dikenali di negeri Kelantan dan ia adalah makanan popular di Kelantan, Malaysia. Kajian keatas kitaran hayat etak (*Corbicula fluminea*) didokumenkan berdasarkan pemerhatian dan pengenalpastian setiap peringkat kitaran hayat etak. Sampel akan diambil dari sg. peng nangka, kg tok uh, Tumpat. Pembesaran etak mula berkembang di dalam bulan Jun 2017. Larva terus membesar sehingga peringkat kematangan selama 4-5 hari selepas persenyawaaan. Peringkat awal bagi etak adalah bentuk trochophore, Veliger- D, veliconcha dan pediveliger. Penemuan ini diperolehi daripada pembedahan etak yang telah disenyawakan, memotong perut dalaman yang biasanya mengandungi telur dan larva, kemudian diperhatikan di bawah mikroskop cahaya (Rax Vision Y100).Berdasarkan kajian ini, peringkat awal kitaran hidup etak adalah sama dengan kerang lain seperti *Corbicula australis* dan *Corbicula leana*.

Katakunci: *Corbicula fluminea*, kitaran hidup, akuakultur, pengeluaran

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LIST OF ABREVIATIONS

dpf	day post fertilization
g	gram
h	hour
L	liter
mg	miligram
mg/L	Milligram per liter
min	minute
mL	mililiter
mM	milimeter
Moh	Ministry of health
NIS	Non Indigenous invasive species
pH	Potential hydrogen
PNW	Pacific Northwest
Ppt	Part per thousand
µg	microgram
µl	microliter
µm	nanometer
who	world health organization

LIST OF SYMBOL

%	Percent
ºc	Degree Celsius
≈	Approximately equal
>	More than

CHAPTER 1

INTRODUCTION

1.0 RESEARCH BACKGROUND

Asian clam or scientifically known as "*Corbicula fluminea*" (Sousa, et al., 2008) is an invasive aquatic species. In Malaysia, Asian clam is known as "Etak". It has been one of the quaint side food especially among the people of East Coast of Malaysia. In Kelantan, this *C. fluminea* is widely sold smoked and raw from around Pasir Mas and Kota Bharu for past few decades.

Ordinarily, freshwater bivalve is a filter feeder aquatic species. Usually it can be found on lake, streams of all in silt, mud, sand or gravel as they will consume food particle supplied and on a sediment surface of buried beneath the sand of a running water.

Asian Clam or known as "golden clam," is a freshwater bivalve mollusk with a yellow-brown, light brown to black in color with distinctive elevated, evenly spaced concentric ridges on the surface. It is small bivalves, averaging less than 1.5 inches with an oval triangular shape (Elliott & Ermgassen, 2008).

Figure 1.1: Live Asian Clam (*Corbicula fluminea*)

1.1 Overview of Asian clam (*Corbicula fluminea*)

The beginning of non-indigenous invasive species (NIS) in aquatic ecosystems has grown exciting in recent decades (Sousa. et al., 2008). Freshwater ecosystem are being tremendously altered by human activities, resulting in decrease of the native species with a following renewal and disperse of non-indigenous invasive species (Sousa. et al., 2008).

Asian clams (*Corbicula fluminea*) have quick development and dispersal rates, are hermaphroditic, a have variable frequencies of reproduction occurrence during the year (McMahon R. F., 2002). Clam growth and reproduction is depends on temperature and also by food. In this way, comprehension Asian clam life history and growth dynamics in environmental heterogeneous.

Hermaphroditic freshwater clams in the genus *Corbicula* produce non-reductional spermatozoa. Hermaphroditic *Corbicula fluminea* from Saga Prefecture, Japan and *Corbicula fluminea* from Taiwan also produce non-reductional spermatozoa. *Corbicula fluminea* may lack either first or second meiosis, bringing about non-reductional spermatozoa. Along these lines, gynogenetic reproduction happens in the both species; maternal chromosomes are also non-reductional, and spermatozoa activate development of the eggs, but do not contribute to the offspring (Komaru, et al., 1997).

1.2 PROBLEM STATEMENT

Asian clam or "Etak" had been one of the popular snack foods among Kelantan's local and foreign tourist. The issues is now, is it the seller of “Etak” or the Kelantanese get the Corbicula fluminea form any sources for example form rivers in local area or get other source from suppliers in Thailand the country that very near to Kelantan.

This factor would be the main cause wide spreading of NIS in Kelantan. Thus, the study of lifecycle of *Corbicula fluminea* presented will be undertaken to enhance the production of local seed, *Corbicula fluminea*.

1.4 OBJECTIVE

The objective the study is to identify the life cycle of Asian clam (*Corbicula fluminea*)

1.5 SCOPE OF THE STUDY

This study reveals the life cycle of *Corbicula fluminea* for increase the seed production. The scope of this study covers:

1. Aquaculture- *Corbicula fluminea*

1.6 SIGNIFICANCE OF THE STUDY

Corbicula fluminea is widely consumed and favored by most of the local people in Kelantan and as well as of the tourist. This popular snack food is among the local people, however the source of *Corbicula fluminea* still lack from Kelantan itself.

Present study show, production of Asian clam (*Corbicula fluminea*) is low in Malaysia. Therefore, the study of the identify lifecycle of Asian clam will gains high production of Asian clam. Thus, the sector of clams in economic development in Malaysia increase.

1.7 LIMITATION OF THE STUDY

The life cycle of *Corbicula fluminea* can be determined from collected *Corbicula fluminea* at certain area which in Sg. Peng Nangka, Kg Tok Uh, Tumpat Kelantan and then brood them and further with artificial insemination of reproduction *Corbicula fluminea* and lastly observed under microscope of their early life cycle stage, the problem was too little and very few article and journal as sources for any reviews that been referred in this study, thus it become more limited to found and hard to get the references.

Other than that, *Corbicula fluminea* also sensitive and not adaptable to new condition or new environment so the mortality high and it will be delayed the research. The amount of sampling that not enough or dead is limiting factor for longer the period for life cycle of Asian clam.

CHAPTER 2

LITERATURE REVIEW

2.1 MORPHOLOGY OF ASIAN CLAM

The Asian clam, *Corbicula fluminea* is one of the dominant part intrusive species in freshwater amphibian biological systems. The fast development, prior sexual development, short life expectancy, high fruitfulness and its connection with human exercises makes *Corbicula fluminea* a non-indigenous obtrusive animal types likely to populate new conditions. This species, at first spread in Asiatic biological systems (Sousa, et al., 2008).

Invasive species is known to have high tolerant to natural extremes. This species have quick development, early development, short life spans, and lifted fecundity, let fast populace recuperation after decline by refractive, ecological extremes. Broad protection limits offer minimal versatile incentive to intrusive, r-chose species, since populace decreases happen in their flimsy environments paying little respect to level of stress resilience (McMahon, 2002). Two species of this morph was recognize.

Despite controversy about the reproductive mode, *Corbicula fluminea* is usually described as a hermaphroditic species (Sousa, et al., 2008). Fertilization happen inside the paleal cavity and the incubation of young exist in the innerdemibranches. The larvae pass along trocophore, veliger and pediveliger stages, thus entity and liberate as a D-shaped form with straight hinged shells (Ckerman, et al., 1994).

Juvenile at the season of discharge have minor measurements (around 250μm), being totally represented with a well-developed shell, adductor muscles, foot, statocysts, gills and digestive system (McMahon, 2002) After discharge to the water section, adolescents grapple to silt or hard surfaces because of the nearness of an adhesive byssal string following an extremely concise period (a greatest of four days) in the plankton (Ckerman, et al., 1994).

All known morphotypes of *Corbicula* as described recently by Korniushin (2004). Eventually, shape on revise and refine sub-atomic phylogeny utilizing mitochondrial quality arrangement information for Corbiculidae, the movement of discrete conceptive modes in these bivalves, advocate the self-reliant origination of euviviparity and, undoubtedly, matrotrophy (Glaubrecht, et al., 2006).

Filter feeding in *Corbicula fluminea* is a complex phenomenon, requiring a comprehension of how the physiological procedure of bolstering is affected by morphological requirements forced upon gill working and by the worldly impacts of natural factors. Likewise, the versatility in the channel bolstering reaction and the limit with respect to elective encouraging modes add to the accomplishment of *Corbicula fluminea* as an obtrusive species (Way, 1990).

For shell morphology of *Corbicula fluminea*, the outer shell is yellowish brown and the inside is white, while the umbone area is light orange and there is purple flash along the teeth. This type is similar to the yellow type described Ishibashi et al. (2003).

Hermaphrodites (both male and female) was a sexually organ for adult *Corbicula fluminea* that are accomplished of both cross and self – fertilization, thus it takes only one individual to start a population. Fertilization situated in the paleal cavity with larvae brooder in the innerdermibranchs. Juveniles are generally little, around 250 μm, totally shaped with completely created shell to develop to a length of 6 to 10 mm.

2.2 LIFE CYCLE OF ASIAN CLAM

In previous research from Morton (1977), the innerdemibranchs of *Corbicula fluminea* are accommodate themselves for the incubation of fertilized eggs and developing larvae. In *Corbicula fluminea*, the innerdemibranch embrace significantly expand in water tubes due to the less frequent appearance of interlamellar connection as differentiate with the outerdemibranch. At the time of incubation the innerdemibranch is swollen with developing embryos. The inner demibranchs have been eradicate from each of five specimens of each age grouping of *Corbicula fluminea* from Plover Cove every month for two years (Brian, 1977). Eggs and larvae happen within the ctenidia. There was enlightened increase in the mean length of the developing larvae from 150 µm as a post fertilized ovum to 200-220 µm at the time of release as a D-stage larvae. Only female *Corbicula.fluminea* are accomplished of incubate the larvae that are, undoubtedly, their own ova fertilized by the inhalation of sperm into the supra-branchial chamber via the inhalant siphon. Mucus cells in the interlamellar junctions of the ctenidia of *Corbicula fluminea* may nourish the developing larvae. When incubating the adults still continue to grow (Brian, 1977).

This *corbicula*'s lifespan is there intensely variable, the journey of life was from 1 to 5 years. Within the first 3 to 6 months when the shell length reaches 6 to 10 mm, the maturation period occurs, and the number of annual reproductive periods can be intensely variable (McMahon & Bogan, 2001).

This fluctuation in the quantity of regenerative in the end might be interconnected to water temperature and additionally with the amount of nourishment (Cataldo & Boltovskoy, 1999).

The found Asian freshwater clam, *Corbicula fluminea*, present a majority of the life-history attributes that adapt it for life in unchangeable, unsure habitats (McMahon & Bogan, 2001). *Corbicula fluminea* grows speedily, to a limited extent since it has higher filtration and digestion rates than other freshwater bivalve species. A high proportion (85–95%) of no respired assimilation to growth from this species was assign , enabling people to achieve 15– 30 mm in shell length in the principal year of life and 35– 50 mm in the terminal third to fourth year (McMahon & Bogan, 2001).

Corbicula fluminea's of newly released juveniles are small (shell length ≈ 250 μm) but fully formed, with a well-developed bivalve shell, adductor muscles, foot, statocysts, gills, and digestive system (McMahon & Bogan, 2001).

The comparatively short life span, early maturity, high fecundity, bivoltine juvenile release patterns, high growth rates, small juvenile size, and volume for downstream dispersal of *Corbicula fluminea* makes it both highly invasive and conformed for life in unstable lotic habitats subject to uncertain catastrophic environmental disturbance (McMahon & Bogan, 2001).

2.3 REPRODUCTION OF ASIAN CLAM

2.3.1 BROODING BIOLOGY

Byrne et al. (2000), was reported that in most years, initiation of spawning happen in late September/early October develop in embryos being present in the gills from October onwards. Not all the clams in the populations were brooding at the same time, imply reproductive asynchrony in the populations.

Throughout the brooding season, gills exhibiting signs of partial release of juveniles were common. At the end of the breeding season, discontinue embryos in the process of being resorbed were also observed in the gills. These were generally a brown colour and were partially degraded.

Although these clams are among the most important molluscs in freshwater systems, their taxonomy is poorly understood. Recent research indicates that their difficult systematics is due to the triploid chromosome number, hybrid status, clonality and unusual ameiotic breeding systems of many *Corbicula* species (Komaru, et al., 1997).

In this species, non-reductional biflagellate sperm with somatic DNA levels contribute all of the embryonic genome. North American *Corbicular fluminea* also have biflagellate sperm. In *Corbicula fluminea*, the velum is shed in the gill or shortly after release. An abundance of mucous cells lining the interlamellar junction epithelium is also reported for *Corbicula fluminea* (Byrne, et al., 2000).

According to Byrne et al. (2000), the product of these cells may maintain the enlarge embryos. Alternatively, function to oblige passage of juveniles out of the gills from the mucus secreted by these cells. Mucous cells in the gills of post-release juvenile *Corbicula fluminea* producemucous threadswhich suspend small clams in the water column and assist downstream drifting. This juvenile dispersal mechanism enhances colonization of new areas by these invasive clams.

The veliger larvae brooded by *Corbicula fluminea* appear to be less reduced. By comparison, *Corbicula australis* enlarge along a alter veliger larva with a non-functional velum which cant' be used for swimming or seize molecule. Detailed differentiation of the structure of the larvae of these two and that of the other brooding *Corbicula* species will undoubtedly provide important insights into the evolutionary changes in development associated with the colonisation of freshwater by these clams. In the absence of good adult shell characters, larval morphology may assist in discerning the phylogenetic relationships among the species (Byrne, et al., 2000).

This size difference was also noted for *Corbicula fluminea* by Brian (1977) who suggested that the embryos must use extraembryonic nutrition. Most *Corbicula fluminea* populations have biannual reproduction with distinct spring and autumn breeding periods.

In the study of Byrne et al. (2000), molecular data show that *Corbicula australis* is clearly nested within aclade of *Corbicula* together with other freshwater lineages including *Corbicula fluminea, Corbicula sandai* and *Corbicula leana.* In this investigation, *Corbicula australis* was collected from two population in upper Nepean River, Douglas Park (34° 11' 715" S; 150° 42' 757" E) and Menangle (34° 07' 357" S; 150° 44' 521" E). Seasonal gonad were collected from both sites in March, May, August and October. The reproduction condition of each specimen was assessed by examination of the visceral mass and the gills were examined for the existence of embryos. This investigation to determine the brooding cycle of *Corbicula australis* to provide baseline data to assess the reproductive response of the clams to planned experimental releases from upstream impoundments.

According to Glaubrecht et al. (2006), they recently discovered Asian clam vast spectrum of reproductive strategies. These include sexually reproducing species with both sexes and assorted other uncomman reproductive chacteristics, enraged from oviparity and ovoviviparity to euviviparity. Also a variety of genetic structures, such as polyploidy, androgenesis and clonality, with unthinkingly assorted hereditary collaboration among clones of Corbicula (Glaubrecht, et al., 2006). Corbiculidae been summarized the three reproductive mode oviparity, ovoviviparity and euviviparity are exhibited. They also found to be endobranchous which that being ovoviviparous,at least until the stage of juveniles with straight-hinged shells (D-shaped) but before that it was incubate their young in their innerdemibranchs (Glaubrecht, et al., 2006).

2.3.2 Androgenesis and Oogenesis

Corbicula fluminea is self-fertilizing enable single individuals to begin new populations. Within 3 to 6 months at a shell length of 6–10 mm maturation occurs, so spring-born juveniles can engage in fall reproduction. Utmost life span is extremely variable, enraged from 1 to 4 years, within which early maturity and bivoltine reproduction enable the individuals to engage in one to seven reproductive accomplishment.

Its good reproductive potential and growth rate allow it to reach high densities after invading a new habitat or re-establish dense populations. If metabolic rates was high it allow rapid burrowing and suspended of silt was tolerant (McMahon & Bogan, 2001).

The class Corbicula accommondate sexually duplicating species with two particular genders (korniushin & Glaubrecht, 2003) and in addition bisexuals, at any rate some of which recreate through androgenesis (Komaru, et al., 1997).

The actualize of androgenetic propagation is totally demonstrate in Corbicula. After treatment by a biflagellate sperm that contains a full supplement of atomic chromosomes, the oocyte launches the whole maternal atomic genome as two polar bodies however protect the mitochondria. Creating incipient organisms are agonized inside the bisexual mother's gills, where they probably maintain the healthful advantages. Incipient organisms are found inside the gametogenic follicles before gamete discharge, proposing that self-treatment is a typical method of multiplication (Hedtke, et al., 2008).

The study by Chung (2007), the level of individual female clams 40.0– 45.0 mm in shell length that were at first sexual development was more than 56.3%, and for clams more than 50.0 mm in shell length, it was 100%. Shell length at half of sexual development (rate of sexual development) was 41.0 mm. Accordingly, female mollusks were resolved to be sexually develop at two years old. In this examination, Specimens of *Meretrix lusoria* were gathered month to month by digging to water depths of 10–20 m at the intertidal and subtidal zones of Simpo, on the west coast of Korea, for two years from January 2002 to December 2003. The purpose behind this investigation is to portray vitellogenesis amid oogenesis, the conceptive cycle, and the size at sexual development for *Meretrix lusoria* utilizing cytological, histological, and morphometric methods. Results will be utilized for enhanced fisheries administration of this species (Chung, 2007).

2. 4 PHYSIOLOGICAL ADAPTATIONS OF ASIAN CLAM

Corbicula fluminea is extremely tolerant of turbidity (Way, 1990), *Corbicula fluminea* have higher metabolic rates (McMahon & Bogan, 2001), and Capacities for quick tunneling and resistance of suspended opening permit *Corbicula fluminea* to colonize littler, more factor lotic living spaces. *Corbicula fluminea* for the most part less tolerant of ecological anxiety. *Corbicula fluminea* give off an impression of being less tolerant of hoisted temperatures, hypoxia, emersion, low pH, and low calcium fixation. *Corbicula fluminea* have inadequately created capacities with regards to respiratory temperature acclimation to such an extent that the oxygen take-up rates of people are moderately unaffected by their earlier long haul temperature encounter (McMahon & Bogan, 2001). It isn't realized what controls the arrival of the hatchlings in *Corbicula fluminea* however temperature may assume a critical part (Morton, 1977).

Inspection of (Table 5.2) reveals that in all perspectives of view other than adjustment related direct with life in aggravation inclined, variable-stream living spaces *Corbicula fluminea* for the most part less tolerant of natural anxiety seem, by all accounts, to be less tolerant of raised temperatures (McMahon & Bogan, 2001).

Table 5.2: Major physiological protection and limit adjustments the nonindigenous *Corbicula fluminea*, based on information in McMahon & Bogan (2001).

Physiological adaptation	*Corbicula fluminea*
Temperature tolerance	Cannot tolerate >36°C or <2°C
Metabolic rate	High
Respiratory response to temperature	No temperature regulation of O2 uptake rate
Capacity for respiratory temperature acclimation	Little capacity for respiratory temperature acclimation
Capacity to regulate oxygen uptake rate with progressive hypoxia	No regulators of O2 consumption with progressive hypoxia
Hemolymph osmotic concentration	High, 65 mosmols·L–1
Emersion tolerance	Intermediate emersion tolerance, 36 days at 20°C in high relative humidity
Lower Ph limit	Intermediate, ≈5.6
Turbidity tolerance	Very high

CHAPTER 3

MATERIAL AND METHOD

3.1 SAMPLING ORIGIN AND COLLECTION

A total 30 samples of Corbicula fluminea was roughly collected from "Sg. Peng Nangka,Kg Tok Uh, Tumpat, Kelantan (60 07' 59" N 1020 09' 9" E) with a metal hand scooper. Besides, to collect the clams, around 50 L of encompassing water were be collected from stream. The specimen were brought to the aquaculture lab, University Malaysia Kelantan in great condition and was been set to the tank than continue the experiment promptly.

Asian clams will be originally found using a vertical tow net that scooped sediment into the net. A basket dredge was made using a Sunnex® stainless steel wire meshed basket of 260 mm open diameter, 300 mm height and 180 mm bottom diameter, all surfaces had a diagonal mesh of 5mm (Minchin, 2014)

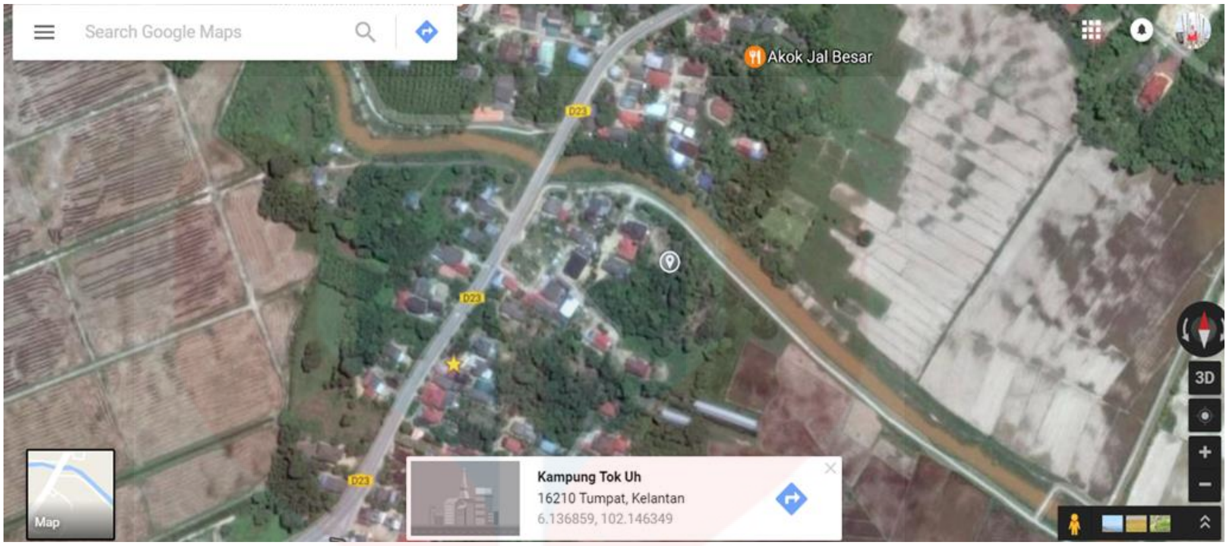


Figure 3.1: The location of sampling collected from Sg. Peng Nangka, Kg. Tok Uh, Tumpat, Kelantan, 6136859, 102 146349.

3.2 EXPERIMENTAL PREPARATION

After transportation to the laboratory, *Corbicula fluminea* was removed from plastic bucket. Asian clam was kept in tank container with supplement oxygen. As the water evaporated from these containers it was add fresh de-chlorinated water every few day. 2 m of aquarium tank with circulated water was provided. Inside the aquarium there is tray and inside the tray was put sand and 1% and 2% potted soils as there are organic matter.

The *Corbicula fluminea* was feed with on microscopic plants and animal (phytoplankton). For adult *Corbicula fluminea* was be given crushed brine shrimp and algae pellets as their food. If use mortar and pestle than grind the brine shrimp and algae pellets into a really fine powder. This would probably aid in feeding veliger, and it was need small amount. It would be placed a small number about 1 ml of macrophytes in water as the veliger might eat a film off these plants as well.

3.3 SPAWNING ASIAN CLAM

3.3.1 GONAD INDUCTION

The accomplishment of the technique depends on spawning occurrence, time for response and occasions of brood stock mortality. After spawning, gametes are fertilized and a progression of planktonic larval stages is refined in bring hatching tanks and raceways. Embryonic development goes on for roughly 12 h until the hatch of free-swimming and non-feeding trochophore larvae. Following 24 h post-treatment larvae morph transform into veliger arrange, influenced clear by the nearness of calcium to carbonate shells and velum. Veliger hatchling are bolstered with live or safeguarded phytoplankton and should likewise obtain harmonious zooxanthellae. The last stage is the pediveliger arrange at roughly 1 week post-preparation, when settlement happens and transformation is soon achieved (Mies & Sumida, 2012).

Thermal cycling for liberated veliger, the heat used was about 28 ˚C to 35 ˚C. About 30 *Corbicula fluminea* was taken from broodstock tank and put them in the 1 L beaker with the plain water inside the beaker and the temperature was measured about 28 ˚C to 35 ˚C by used thermometer. Background for the beaker was black board in color, the reason black board was used is to improve the vision for liberation of gamete.

The *Corbicula fluminea* was then being observed and cultured algae was pipette into the water to stimulate the clam to extend their siphon. After 2 h observation, any substances that being disposed out from their siphon was collected and transferred into centrifuges tube. After that, the *Corbicula fluminea* was transferred into another beaker which is the temperature was low, another word in cold water and the degree Celsius was about 4 ˚C to 7˚C.

3.3.2 ARTIFICIAL INSEMINATION

To initiate them to release veliger. About 30 adults of *Corbicula fluminea* will be removed from their aerated tank and placed them in the small tank on the counter top at room temperature. The water is just de-chlorinated well water. To achieve this it was retrieve water from our faucet and then was let it sit on the counter for 2 days and allowed the chlorine to diffuse out of the water. The pH is neutral (7-8), the salinity is about 0.15 ppt, the oxygen >9mg/L.

After allowing the clams to acclimate to room temperature for 2 d it will be remove 2 adult clams at random and place them on a petri dish under a dissecting microscope. A forceps will be used and a dissecting needle to pry open these two adults. The locate of the visceral mass will be found of the clam and puncture this and flush out their gonads with water so that the gonads pooled into the bottom of the petri dish. A clean disposable plastic pipette then will be used and suck up the gonads it will be remove from the two adults *Corbicula fluminea*.

With the plastic pipette (that is now full of Asian clam gonads) it was be return to the small tank that is holding all the other live adult Asian clams and slowly approach clams that had their inhaling siphon visible and very gently release a small amount of the gonads from my pipette into the inhaling siphons of several of the live adults in the container.

It was repeated this until it used up all the gonads that was flushed out into the petri dish. This technique seems to cause some sort of response in the live clams and within in 24 h they were seen actively releasing veliger.

It was repeat the technique again. It tried to release the gonads into as many siphoning adults as possible and it may help to increase the water temp to 23°C.

To specify eggs and developed fertilized larval forms (hereafter referred to as veliger), the gills was dissected of approximately 30 clams. Clams between 11 mm and 19 mm will be dissected occasionally when the target size class was not met wholly. Squash mounted and examined the ctenidia under 100X magnification light microscopy, Leica Dm500 (Denton, et al., 2012).

Bivalve brood stock are for the most part submitted to a molding procedure before instigated bringing forth with the guideline of advancing egg creation. Recommended methods incorporate the standard expansion of refined microalgae, increment in photoperiod and keeping physical-chemical vacillations at the very least (Mies & Sumida, 2012).

3.4 OBSERVATION ON RELEASNG THE LIFE CYCLE/ D-SHAPE

Slide preparation was begins with fixation of the specimen first. *Corbicula fluminea* was fixed in formalin solution with ratio 1:9 with distilled water 90 ml and 10 ml formalin into centrifuge tube. About 24 h the specimen was stored at room temperature. From the clam, the body of the species gave a transverse area will be cut of the instinctive mass, which oblige the gonad, renal gland and stomach (digestive) related tract, and segments of the gill and mantle. The activities of dissection was done under dissection microscope, Leica Dm500. The gill part was being cut and put under slide. Used 10x, 40x, 100x magnification for the preparation on microscope slide was be examine, to observed the stage of lifecycle development that appear. 1 drop (1 ml) methylene blue being used to get the visually on the specimens clearly.

CHAPTER 4

RESULT

4.1 Early Growth

Within each clam the young were at the same stage of development in (Table 4.1) indicating that spawning, fertilization and embryogenesis were synchronous. In each sample collected during the study, some clams contained clutches of non-shelled early embryos while others brooded advanced shelled juveniles. Early embryos were encountered infrequently, presumably due to the short duration of this developmental stage.

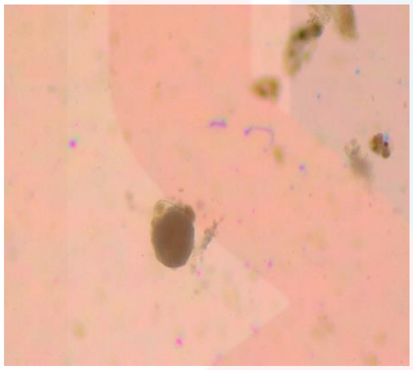
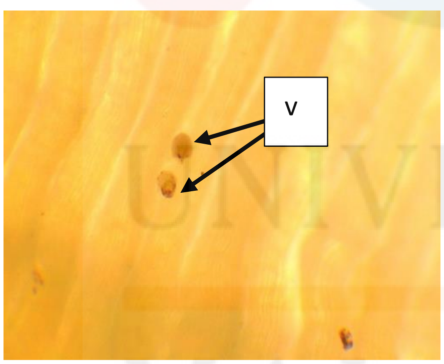
The most common stages encountered in the gills of *Corbicula fluminea* were veliger larvae and D-shelled pediveligers (Figure 4.1-4.9), The veligers had a prominent lobe-like velum which was vestigially ciliated and was not used for swimming or particle capture (Figure 4.2- 4.4).

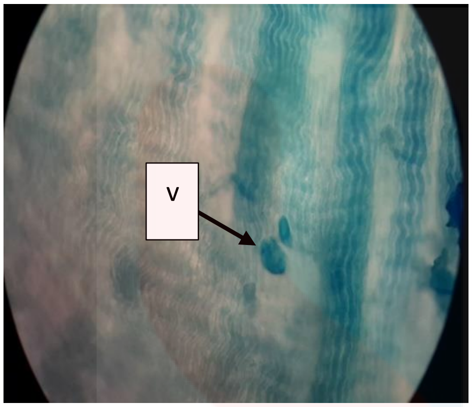
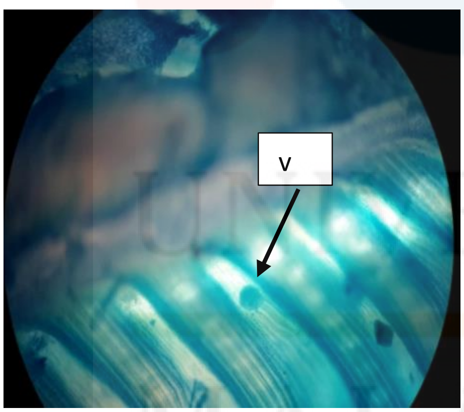
The first is a short trochophore stage. The improvement of a velum and emission of a larval shell prompt a D-formed veliger, which is the principal unmistakable planktonic hatchling. Later a moment larval shell is emitted and this veliconcha is the last commit free-swimming veliger.

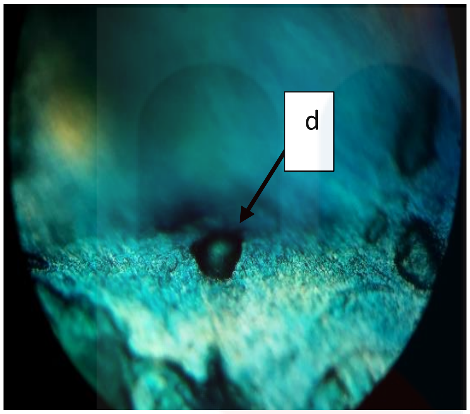
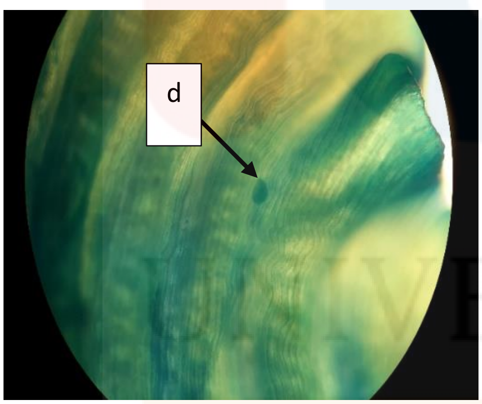
Then again, the last larval stage, the pediveliger, can either swim utilizing its velum or slither utilizing its foot. Pediveligers select substrates on which they "settle" by discharging byssal strings and experience transformation to wind up plainly plantigrade mussels. The emission of the grown-up shell and change in development pivot prompt the merged heteromyarian shape.

Corbicula fluminea (Table 4.1) is for the most part clarify as an androgynous animal groups. The preparation occur inside the paleal cavity and hatchlings are brooded in branchial water tubes. At the point when *Corbicula fluminea* adolescents are discharged, they have little measurements (around 250 µm) however totally framed with a very much created shell, adductor muscles, foot, statocysts, gills and stomach related framework and have the typical D-stage arrangement (McMahon, 2002). After the water section discharge, adolescents grapple to residue, vegetation or hard surfaces because of the nearness of an adhesive byssal string. These adolescents can likewise be re-suspended by turbulent streams and scattered for long separations, chiefly in the downstream heading. The development time frame happens inside the initial 3 to a half year when the shell length achieves 6 to10 mm. The life expectancy of this species is to a great degree variable, extending from 1 to 5 years, with regular bivoltine adolescent discharge design (Sousa, et al., 2008)

Table 4.1: Early growth of *Corbicula fluminea*

Figure	Stage
 Figure 4.1: Trochopore 100x magnification	Trochopore
 Figure 4.3: Veliconcha inside innerdemibranch 100× magnification V=veliconcha	Larvae veliconcha

 Figure 4.3: Veliconcha inside innerdemibranch 100× magnification V=veliconcha	Larvae veliconcha
 Figure 4.4: Veliconcha inside innerdemibranch 100× magnification V=veliconcha	Larvae veliconcha

 Figure 4.5: D-shaped veliger 100× magnification d= D-shape juvenile	D-shaped veliger
 Figure 4.6: D-shaped veliger inside innerdemibranch 100× magnification d=D-shape juvenile	D-shaped veliger

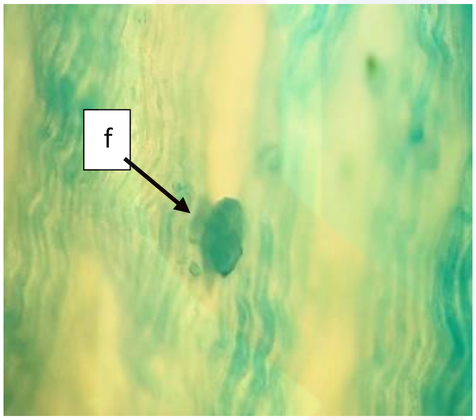
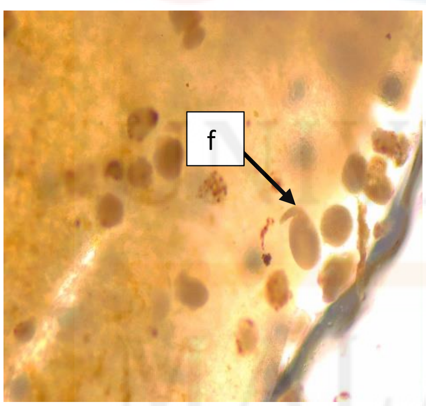
Figure 4.7: Pediveliger 100× magnification f=foot	Pediveliger The juveniles have a well-developed foot.
Figure 4.8: Pediveliger 100× magnification f=foot	Pediveliger The juveniles have a well-developed foot.

 Figure 4.9: Pediveliger 100× magnification f=foot	Pediveliger, The juveniles have a well-developed foot.

CHAPTER 5

DISCUSSION

Corbicula fluminea is the most generally dispersed Asian clam species and is profoundly obtrusive. *Corbicula fluminea* recreates independent from anyone else preparation and broods it's young in the innerdemibranchs (Brian, 1977). Their spermatozoa have two flagella (Ishibashi, et al., 2003).

Corbicula fluminea was also introduced in Japan. According to Nakai and Matsuda (2000), *Corbicula leana* (Prime, 2004) is an indigenous species to Japan, has been replaced by *Corbicula fluminea* in some regions of Japan (Ishibashi, et al., 2003).Most bisexual bivalves are protandrous (that is, spermatozoa are delivered in the life cycle before eggs) (Pigneur, et al., 2012). Be that as it may, hermaphroditic types of Corbicula are protogynous: female gametes are delivered before the sperm. Cross-preparation between bisexual people is recommended by the nearness of mucous fibers containing sperm associating their siphons (Pigneur, et al., 2012).

Based on the result, the trochophore, which is free swimming, turns into a veliger with the improvement of the velum, a larval organ of encouraging and motion. The ownership of a velum consequently describes all resulting larval stages as veligers. Inside 2-9 days post preparation (dpf), the creating veliger hatchlings emit an unornamented and D-shaped shell from shell gland. The hatchlings are alluded to as D-shaped or straight-hinged veligers (70- 160 pm in height; bivalve range = 50-175 μm, <1-7 dpf.

Afterward, however inside 7-9 dpf, a moment, more ornamented larval shell, it has the articulated umbonal region close to the hinges and is round or clam like in profile. This umboned veliger or veliconcha (120-280 pm in height; bivalve range = 8 1-305 pm, 2-24 dpf. Is the last veliger arrange that is free swimming and is ordinarily found in the plankton.

While there is extensive development in the veliconcha, a few new organs frameworks additionally create. One of these is the foot and its improvement prompts an adjustment in conduct and in addition the new name of pediveliger (167 - <300 μm in height; > 10 dpf; bivalve range= >160-350 μm, 6-40 dpf. Alternate changes, which are identified with encouraging, include the gill filaments that frame in the mantle cavity. The gill fibers don't achieve development until after transformation, which takes after this stage.

From previous study Ckerman (1994), the foot (and related byssal device) is all around created in the pediveliger and is utilized for swimming close to the base and in addition creeping on surfaces. These practices separate the pediveliger from prior veliger larvae.

Self-fertilization has likewise been recommended in *Corbicula* (Ikematsu and Yamane, 1977; Kraemer et al., 1986): incipient organisms were found in the instinctive mass, inside the gametogenic follicles, while most developing embryos are typically brooded in the gills in bisexuals (Glaubrecht, et al., 2006).

Table 5.1: Summary of the life-history attributes, *Corbicula fluminea* (McMahon, 2002)

Life history trait	*Corbicula fluminea*
Life span (years)	1–4
Age at maturity (years)	0.25–0.75
Reproductive mode	Hermaphroditic (self-fertilizing)
Growth rate	Rapid throughout life
Fecundity (no. young per average adult per breeding season)	35 000 per hermaphroditic individual
Juvenile size at release	Small, 250 μm
Relative juvenile survivorship	Extremely low
Relative adult survivorship	Low, 2–41% per year
Degree of iteroparity	Moderately iteroparous, 1–7 reproductive periods
Reproductive efforts per year	Two (spring and fall)
Assimilated energy respired (%)	11–42%
Non-respired energy allocated to growth (%)	58–71% depending on cohort and season
Non respired energy allocated to reproduction (%)	5–15% depending on cohort and season
Turnover time in days (= mean standing crop biomass : biomass produced per day)	73–91 depending on cohort
Habitat stability	Stable to unstable

The introduced Asian freshwater clam, *Corbicula fluminea*, shows a greater part of the life-history characteristics (Table 5.1) that adjust it for life in flimsy, unusual territories (McMahon R. F., 2002). *Corbicula fluminea* grows rapidly, to some degree since it has higher filtration and osmosis rates than other freshwater bivalve species. Just a moderately little extent of its osmosis (29%) is committed to respiration, the greater part (71%) being dispensed to development and proliferation. This species assigns a high extent (85– 95%) of no breathed absorption to development, enabling people to achieve 15– 30 mm in shell length in the primary year of life and 35– 50 mm in the terminal third to fourth year (McMahon, 2002).

Thus, *Corbicula fluminea* has the net production efficiencies recorded for any freshwater bivalve, reflected by short turnover times of 73–91 days (Table 5.1). Recently discharged adolescents of *Corbicula fluminea* are little (shell length ≈ 250 μm) however totally shaped, with an all-around created bivalved shell, adductor muscles, foot, statocysts, gills, and stomach related framework. A comparatively low level of non-respired absorption in *Corbicula fluminea* is designated to multiplication (5–15%, comparable to that exhausted by unionoideans); nonetheless, its hoisted digestion rates enable higher supreme vitality assignment to proliferation than in other freshwater bivalves. Fertility is high, assessed at 68 678 adolescents normal adult 1 year. (McMahon, 2002). Juvenile survivorship is still low and death rates stay high for the duration of grown-up life. Low adult survivorship prompts masses overpowered by young people and adolescent individuals (McMahon & Bogan, 2001), a normal for species categories adjusted to unsteady environments (McMahon, 2002).

Preparation and fetus improvement by means of shell arrangement happens inside female gill chambers. Trochopore hatchlings will kick the bucket if expel shape the gill. Differentiate species through shape, pH, SL, hinge length and shell feature like presence and absence of striate was primary characteristics. Nearness or nonattendance of a foot and velum was the second trademark.

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

Within the experiment, brooding tank have higher reading of ammonia it was effect aviability of oxygen decrease and the environment condition become hazardous and toxicity. The worse environment will affect feed taken to *Corbicula fluminea* thus their become stress and lead to died. But within the brooding period, if the value of temperature increase it was affect *Corbicula fluminea* to ate with large quantity cause the metabolism increase.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

For the conclusion, the lifecycle of the *Corbicula fluminea* was identified with every stage of early growth. The evident was provided in result with some description about each of specimen. The experiment was run in the laboratory condition, thus in laboratory conditions, the environmental temperature was provided with (24 °C–25 °C), development of *Corbicula fluminea* takes 4–5 days.

For the recommendation, any further research about Asian clam especially *Corbicula fluminea* should be continue to researcher with more effective procedures with good biosecurity in laboratory environment, which any harmful that come from surrounding will come and affected mortality of Asian clam. A modern and comprehensive technologies and skillful people should be used for better outcome in the future. Most of information documented about Asian clam was poorly known. The studies in this field will reveal more knowledge and profit to people.

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APPENDICES

Figure A.1: Sampling Asian clam (*Corbicula fluminea*) at Tumpat, Kelantan

Figure A.2: Sampling Asian clam (*Corbicula fluminea*) at Tumpat, Kelantan

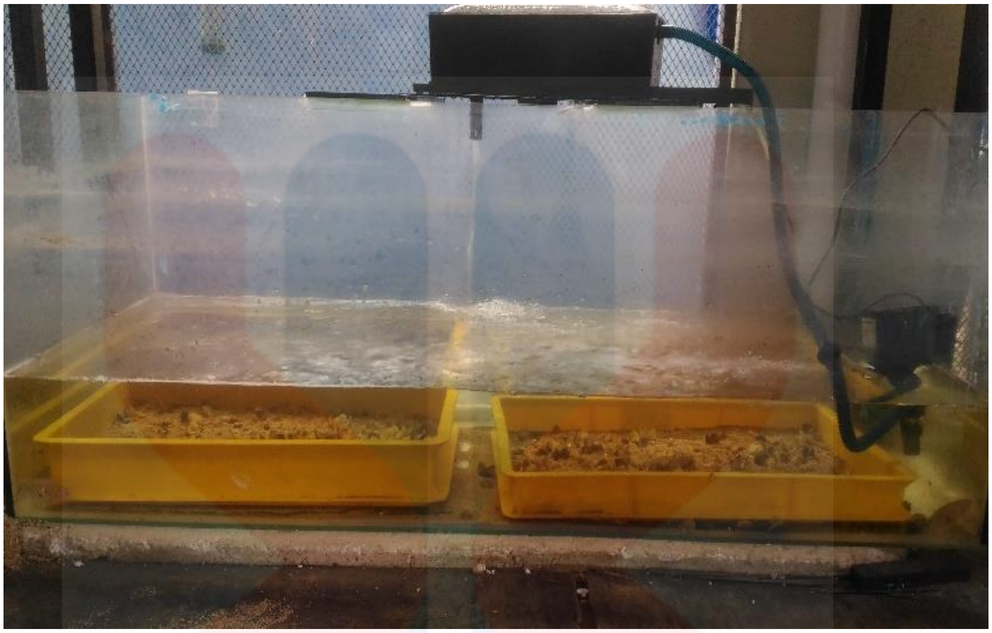

Figure A.3: Brooding tank with circulated air system

Figure A.4: Dissection Asian clam (*Corbicula fluminea*)

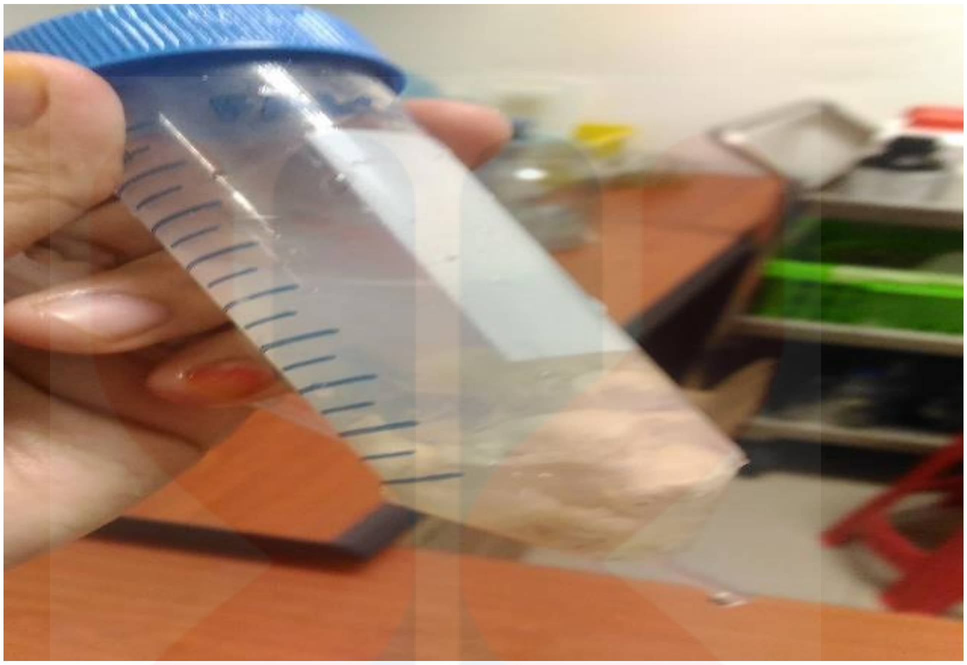

Figure A.5: Fixation Asian clam (*Corbicula fluminea*)

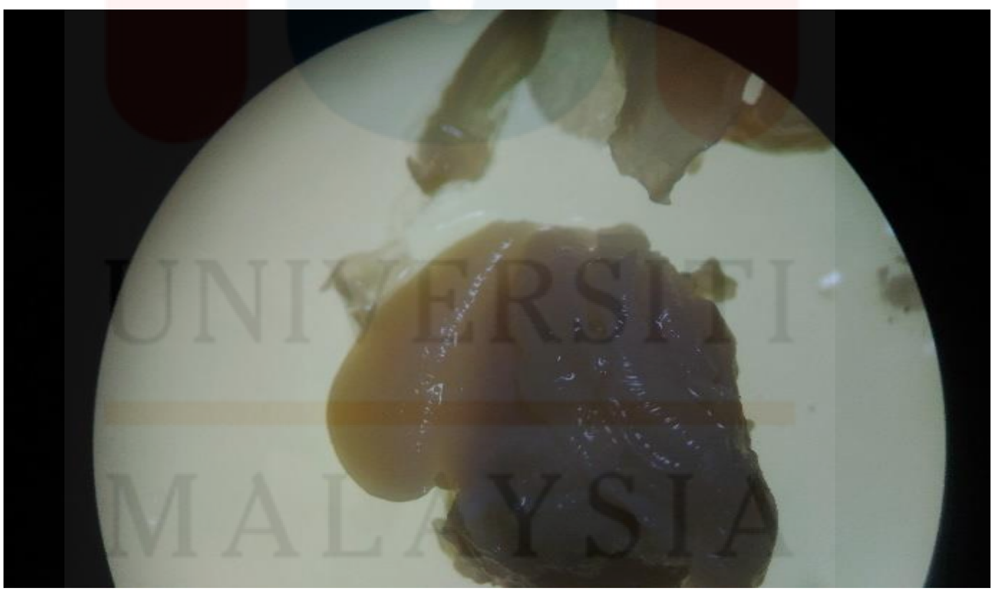

Figure A.6: Asian clam after 24 H fixating ready to dissect under dissection microscope (Rax Vision)

Figure A.7: Dissection of Asian clam (*Corbicula fluminea*)

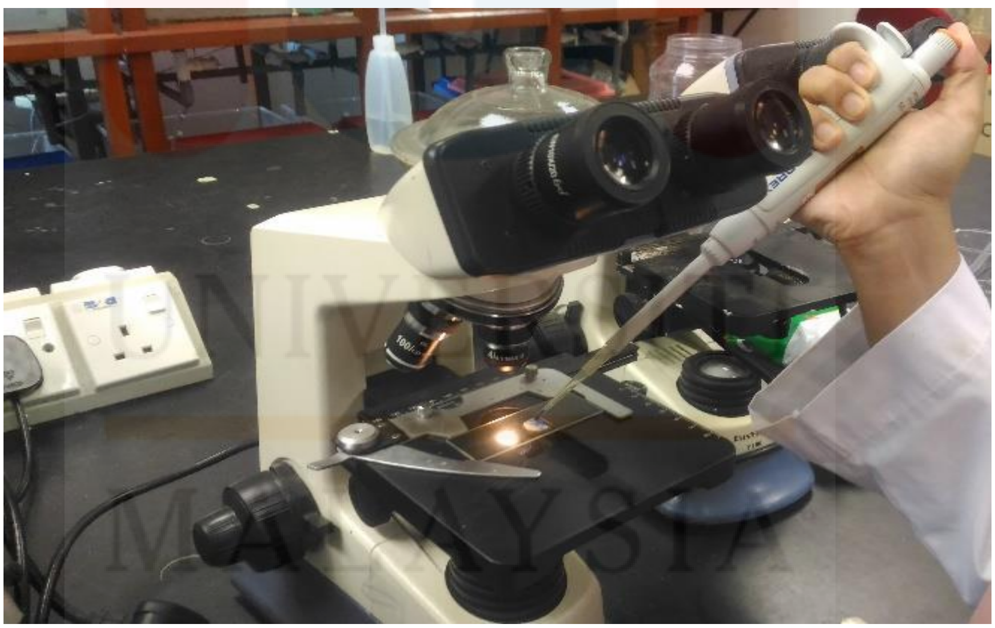

Figure A.8: The specimen (Gills with inner demibranchs) put in slide and being observe under light microscope (Rax Vision Y100)

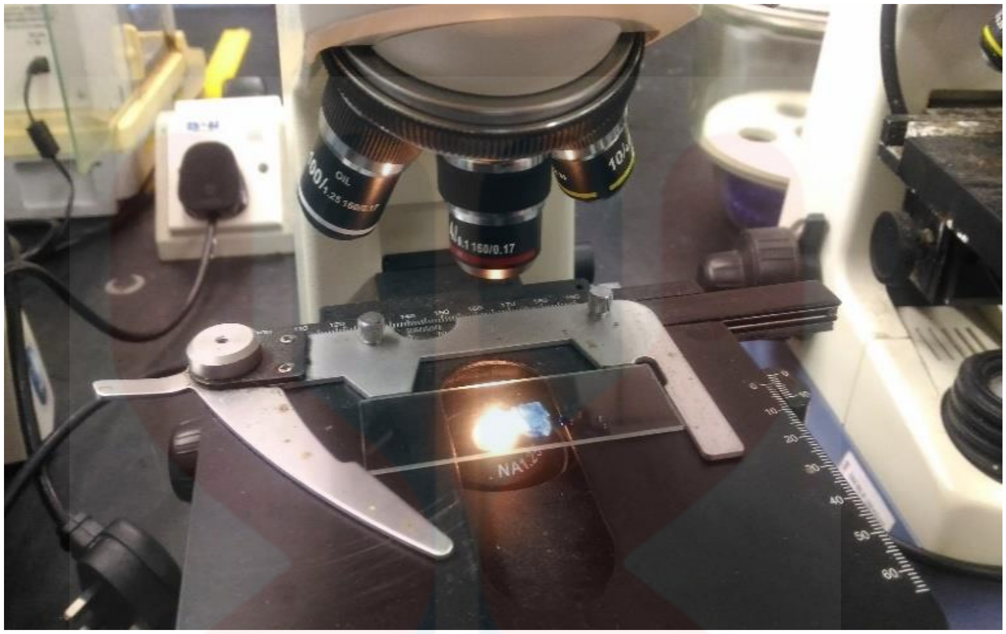

Figure A.9: The observation early stage of Asian clam (*Corbicula fluminea*) under light microscope (Rax Vision Y100)