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HABITAT CHARACTERISTIC OF *Corbicula fluminea* AT TWO DIFFERENT RIVERS IN SOUTHERN THAILAND

By

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DECLARATION

I declare that this thesis entitled “Habitat Characteristic of *Corbicula fluminea* at Two Different Rivers in Southern Thailand” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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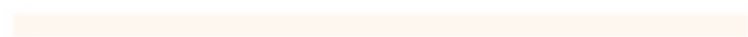
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LIST OF ABBREVIATIONS

SD	Standard Deviation
SL	Maximum Shell Length
SPSS	Statistical Package for the Social Sciences
Zn	Zinc
Cu	Copper
Hg	Mercury
Cd	Cadmium
DO	Dissolved Oxygen
CO ²	Carbon Dioxide
mg/L	Milligram per Litre
m ²	Meter Square
APHA	American Public Health Association
AWWA	American Water Works Association
USEPA	United States Environmental Protection Agency
EPA	Environmental Protection Agency
USDA	United State Department of Agriculture
I ⁻	Iodide
I ₂	Iodine
Na ₂ S ₂ O ₃	Sodium Thiosulphate
NO ₃ ⁻	Nitrate
PO ₄ ³⁻	Phosphate
CaCO ₃	Calcium Carbonate
H ₂ SO ₄	Sulphuric Acid
NaCl	Sodium Chloride

LIST OF SYMBOLS

%	Percentage
°C	Celcius
<	Less Than
>	Greater Than

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Habitat Characteristic of *Corbicula fluminea* at Two Different Rivers in Southern Thailand

ABSTRACT

Corbicula fluminea has been a traditional food for the locals from both Kelantan and in Southern Thailand. However, the ecology of *C. fluminea* has not been fully explored in Malaysia and also in Southern Thailand. *C. fluminea* were sampled in two different rivers at Southern Thailand; Pattani River and Saiburi River. The water quality parameters involved in this study are dissolved oxygen, pH, temperature and the nutrient analysis which is phosphate and nitrate. Water chemistry from both rivers were analysed to determine suitable waters for habitation. Both of this rivers have a suitable pH levels ranging from (6.53-6.7), temperature range (31-34°C) and contain a sufficient dissolved oxygen (DO) in a range of (3.3-8.1 mg/L) for the *C. fluminea* to survive. *C. fluminea* were collected and measured its shell length. This species is most abundant in length size 11-20 mm. The highest density of *C. fluminea* recorded at Pattani River, 369 clams per m² while only 196 per m² at Saiburi River. The over-exploitation by the villagers at Saiburi River has caused the densities of *C. fluminea* differs significantly. The substrate type between these two rivers were also analysed; Pattani River has a sandy loam substrate while the substrate in Saiburi River is a loamy sand. The availability of the phytoplankton were also identified in both rivers and overall there were 29 species from 22 different family in both rivers. This study shows that the densities of *C. fluminea* is likely to be influenced by the water quality rather than substrate types and also the phytoplankton availability.

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Ciri- Ciri Habitat *Corbicula fluminea* di antara Dua Sungai di Selatan Thailand

ABSTRAK

Corbicula fluminea telah menjadi makanan tradisi masyarakat di Kelantan dan di Selatan Thailand sejak dari dahulu. Walau bagaimana pun, ekologi *C. fluminea* masih belum sepenuhnya dikaji di kedua- dua buah negara iaitu Malaysia dan Thailand. *C. fluminea* telah di sampel di dua sungai yang berbesza di Selatan Thailand iaitu Sungai Pattani dan juga Sungai Saiburi. Parameter air yang telah dijalankan di dalam kajian ini termasuklah oksigen larut, pH, suhu dan analisis nutrient iaitu nitrat dan fosfat. Sifat- sifat kimia air telah dianalisis untuk menentukan ciri- ciri air untuk habitat *C. fluminea* yang sesuai. Kedua- dua sungai ini mempunyai taraf pH (6.53-6.7) dan suhu yang sesuai (31-34 °C) serta mempunyai oksigen larut yang mencukupi (3.3-8.1 mg/L) untuk *C. fluminea* terus hidup. *C. fluminea* telah dikutip dan diukur saiz panjang cengkerangnya. Spesies ini paling banyak dalam saiz 11-20 mm. Ketumpatan *C. fluminea* yang telah direkodkan di Sungai Pattani ialah sebanyak 369 kerang per m² manakala hanya 196 per m² di Sungai Saiburi. Eksploitasi berlebihan oleh penduduk kampung di Sungai Saiburi telah menyebabkan terdapat perbezaan besar diantara ketumpatan *C. fluminea* di kedua- dua sungai ini. Jenis substrat di kedua- dua sungai ini juga telah dianalisis dan Sungai Pattani mempunyai substrat jeni lempung berpasir manakala jenis substrat di Sungai Saiburi ialah pasir liat. Ketersediaan fitoplankton juga dikenalpasti dikedua- dua sungai dan secara keseluruhannya, terdapat 29 jenis spesies dari 22 jenis keluarga yang berbeza. Kajian ini menunjukkan bahawa ketumpatan *C. fluminea* mungkin dipengaruhi oleh kualiti air berbanding daripada jenis substrat dan juga ketersediaan fitoplankton.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

C. fluminea is a small clam with an inflated shell, slightly round to triangular in shape. The most distinctive feature is the shell which consist of numerous heavy concentric ridges (Park and O’Foighil, 2000). There are three morphotypes that were classified as *C. fluminea*, *C. fluminalis* and another species for which a specific name was not assigned (being referred as *Corbicula spec*) (Renard *et al.*, 2000). This species is often confused with *C. fluminalis* due to its similarity in colour and texture (Renard *et al.*, 2000). Nowadays, the population of freshwater mollusks is declining (Bogan, 1993). It is afraid that the population of *C. fluminea* or known as Etak is declining too in Malaysia and in Southern Thailand. There are several factors that may be a cause for this species to decline in both Malaysia and Southern Thailand.

The major cause of the declines and extinctions of freshwater bivalves is habitat modification and destruction such as from the construction of dams, canalization, changes in water depth, due to flow changes and changes in fine particle deposition (silt and sand)(Bogan, 2008). Bivalves, including *C. fluminea* depends on the sediment for its habitat and burrowing activities. The sediment also rich in organic matter which is one of the food source for this bivalve.

The over exploitation and harvesting of *C. fluminea* by the villagers may become one of the factor for this species to become less. The locals, especially villagers will harvest this clam and sell it in the market as a source of side income. This clam have been a traditional food and snacks for the locals since a long time ago and the demand for this clams is increasing throughout the year (Zalina, 2014).

The villagers usually harvest or collect this clam by using a clam dredge or a basket. They did not have a standard harvesting size for this clam that will be allowed to harvest. Thus, there is a high potential for the juveniles to be harvested too. This will cause the rate the decreasing rate of survival for the *C. fluminea* juveniles. The lack of ecological information of this species may be the root problem for this to happen.

Despite its originality from Asia (Sousa, 2008), there is no ecological studies about this species for the tropical climate as in Malaysia and Thailand. Most of the research done for this species are in the four season climate zone which have different climate with our country. As this species is known as the world most successive invasive species there are a lot of research to study about their invasion history and activities but very little on their ecological.

1.2 Problem Statement

C. fluminea or known as Etak by the locals is commonly being harvested as a traditional food and snack but there is lack ecological research that has been conducted for this species. The number of this species is declining especially in Kelantan and Southern Thailand but to become worse, there is no scientific information on ecological aspect of this species in Southeast Asia that can be used in order to sustain this species population from declining. Despite this species is declining, they are still heavily harvested without any management regulation in both Malaysia and Thailand

1.3 Objective

1. To characterize the habitat of *C. fluminea* at two different rivers in Southern Thailand.

1.4 Significance of Study

The finding from this study could be used by the local authority to be set as a base ecological information to sustain *C. fluminea* habitat and to prevent this species from declining.

CHAPTER 2

LITERATURE REVIEW

2.1 *C. fluminea* in Thailand

Corbicula fluminea is a freshwater clam commonly can be found in Southeast Asia including in Southern Thailand. The genus *Corbicula* is a member of the subclass Heterodonta, order Veneroida, superfamily Corbiculacea (Sphaeriacea), and family Corbiculidae (Newell, 1969). The bivalve *C. fluminea* is one of the most invasive species in aquatic ecosystems and is well known by its rapid and extensive spread. This is native in Asia and has invaded several ecosystems worldwide in the last 80 years (Sousa, 2008).

This non-indigenous invasive species is able to colonize in new environments because of its rapid growth, earlier sexual maturity, short life span, high fecundity and its association with human activities (Sousa *et al.*, 2008). *C. fluminea* can influence the habitat heterogeneity by modifying the diversity of the macrozoobenthic assemblages favouring sessile crustaceans, gastropods and insects and negatively affecting other bivalve species (Ilarri *et al.*, 2012).

2.2 Shell Morphology

C. fluminea is usually less than an inch in size (25 mm) but its length can be as large as 2.25 in (50 to 65 mm) (McMahon, 2002). The outline of the shell is usually triangular or round and the beak is located centrally along the dorsal side of the clam and is typically inflated (Nedeau *et al.*, 2009). The shape is oval in juveniles and tends to be near triangular in adult specimens (Araujo *et al.*, 1993). These bivalves are

typically light-coloured and their spaced concentric sulcations (ridges) are almost equal in size, lateral teeth on their posterior and anterior sides with many fine serrations. There are two morphs of this species (McMahon, 1991). One is a yellow-green to light yellowish brown morph on its external shell and a white to light blue or purple nacre. Figure 2.1 below shows the two different morphology of this species by its shell colour. The other morph is limited to the southwestern United States (in its US range only) and is dark olive green to black on its periostracum or its external shell and displays a dark royal blue in internal shell (Kramer-Wilt, 2008). Previous studies suggested that the distinct difference of inner shell colour especially for sympatric individuals, derived mainly from their different genetic constitutions and not just environmental conditions (Wang *et al.*, 2014).



Figure 2.1: Shell Morphology of Two Colour Morphs of *C. fluminea* Clams

(Source: Wang *et al.*, 2014)

2.3 Reproduction and Life Cycle

The genus *C. fluminea* is characterized by various modes of reproduction including planktonic development via a free-swimming larva, intrabranchial brooding of shelled juveniles, tetragonous brooding, and prolonged incubation in the maternal gills, with juveniles reaching 1.3 mm in length with a well-developed hinge (Korniushin & Glaubrecht., 2003).

In general, they reach their sexual maturity within the first year following spawning at a shell length of 10 mm (Ituarte, 1985, Mouthon, 2001). Incubation and spawning occurs in the inner demibranchs of the gills for the mature clams (King *et al.* 1986). After fertilization and early cell divisions, a trocophore is formed which evolves into a straight-hinged stage that then develops into a pediveliger stage (100 to 112 h after spawning) (King *et al.* 1986). The life cycle of the *C. fluminea* is as shown in Figure 2.2. Then, juveniles (length mean \pm SD: $221 \pm 10 \mu\text{m}$) already shelled and with adductor muscles, foot, statocysts, gills, and digestive system fully formed are released out of the gills into the water (King *et al.* 1986, McMahon 2002).

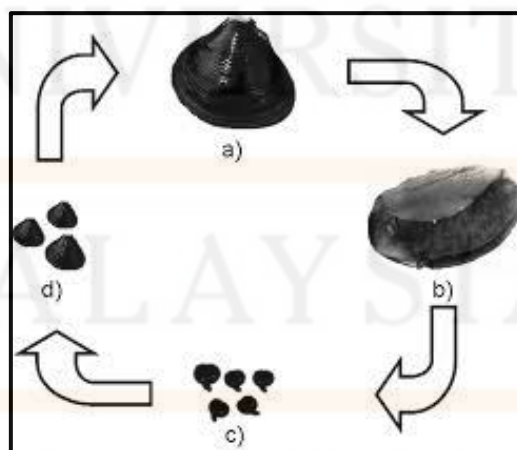


Figure 2.2: The Life Cycle of *C. fluminea* A) Adult Specimen; B) Inner Demibranch With Larvae; C) Small Juveniles Recently Released (With A Completely Developed Foot and With The Common D-Shaped Configuration) and D) Small Adults

(Source: Sousa *et al.*, 2008).

They are released via the excurrent siphon on the adult into the surrounding water. The juveniles will create a mucilaginous byssal thread to either anchor themselves to sediment or other hard surface substrates or use the thread to be carried through the water column by water currents and dispersed long distance (Kramer-Wilt, 2008). This thread can also attach to the body of avian species which enable to transport the juvenile clams to other bodies of water. Juveniles reach maturation within 3 to 6 months and are generally 6 to 10 mm and its variation in shell size can be seen as in Figure 2.3 below.



Figure 2.3: Variation of *C. fluminea* Shell Size from Juvenile to Adult

(Source: Ilarri & Sousa, 2012)

Juveniles are well adapted to the benthic compartment and the species hence lack a planktonic stage (King *et al.* 1986, Phelps 1994). Juveniles are released in large quantities (up to 700 juveniles clam-1 day-1 (Aldridge & McMahon 1978)) but high mortality rates during the first three weeks are common (Britton 1982, King *et al.* 1986). They present a very high growth rate (shell length as large as 29 mm reached within the first year of growth (Aldridge & McMahon 1978)), which due to their higher filtration and assimilation rate compared to other organisms (McMahon 2002).

This species life span is generally 2 to 3 years and during their life span, clams seem to reproduce once or twice a year (Rosa, 2013). The age and life span of North American *C. fluminea* populations have also been estimated from the presence and number of shell growth rings or banding patterns in the periostracum (Sickel, 1973; Sinclair & Isom, 1961, 1963) where each growth ring represent 1 year of life. However, others believed that the growth ring and banding patterns are not accurate to reflect age as there are many other larger specimen that have no distinguishable growth annuli (McMahon, 1983).

2.4 Habitat and Substrate Composition

Freshwater mussels spend their entire life partially to wholly bury in mud, sand or gravel in just about any habitat with permanent water. Mussels occupy a wide variety of habitats (pools, runs and riffles) and substrate types (mixed mud, sand and gravel), but are usually absent or rare in areas of bedrock, shifting sand or deep silt (Gilbert *et al.*, 1994).

C. fluminea is present in habitats ranging from bedrock to deep silt, and can be found in ponds, lakes and streams of all sizes (Neck, 1986). The optimal habitat for *C. fluminea* studied in Texas was sandy-bottomed streams with intermediate flow (Noreña *et al.*, 2015). Some controversy exists regarding *C. fluminea* classification as a freshwater or brackish water species. Some authors include the species in brackish group despite its obvious preference for freshwater (Evans *et al.*, 1977) but the classification as a freshwater species with high tolerance to estuarine habitats has been the most commonly used (Britton, 1982).

The Asian clam occurs both in lentic and lotic habitats (Britton, 1982). Densities of *C. fluminea* increased significantly with increasing depth unlike the other freshwater mussels where its abundance decreased with depth (Noreña *et al.*, 2015). This species is rarely found in intermittent streams or temporary ponds, possibly because this species have a low tolerance of aerial exposure (Byrne *et al.* 1991) and it clearly prefers high-order tributaries (i.e. above second order streams (Karatayev *et al.*, 2005, Schmidlin & Baur 2007).

Habitat for most species mussels was observed in all four habitat types. Haag *et al.*, (2007) stated that, approximately a free-flowing stream (higher current velocity and coarser substrate) had the highest mussel diversity (abundance and species density). Lentic habitats (no flow, fine substrate) were characterized by lower diversity but supported mussel assemblages distinctive from lotic habitats. The preferred substrate of the Asian clam has been argued to be composed of sand and gravel, both typically found in low current water bodies (Vaate & Greijdanus-Klaas 1990).

Densities of *C. fluminea* increased significantly with increasing depth, whereas the abundance of freshwater mussels decreased with depth. Total number of species and individuals collected are influenced by the type of substrates and areas which consist most of cobble contained significantly fewer individuals than areas composed predominantly of gravel (Gilbert *et al.*, 1994.). *C. fluminea* was the most abundant species collected and they were found in a larger number on intermediate substrate sizes unlike densities of other freshwater mussels that are not correlated with bottom type (Haag & Thorp, 1991).

2.5 Effect of Temperature

As this species is native at semi-tropical or Southeast Asia (Morton, 1979), *C. fluminea* was rarely exposed to the extreme temperature. The lower temperature limit for *C. fluminea* is between 0 and 2°C, and the upper temperature limit is 37°C (Ilarri & Sousa, 2012b).

In Mosquito Creek, Florida (USA), a population of *C. fluminea* was found in water of pH 5.6. Although the pH level is said that it is the cause of *C. fluminea* mortality, a later research proved that pH 5.6 is the lowest limit for this clam to survive (Karatayev *et al*, 2007). It was suggested that conchiolin layers in unionid shells might retard shell dissolution in lotic habitats with low pH, but data were lacking to test the hypothesis (Karatayev *et al*, 2007).

2.6 Effect of Oxygen

This species is not significantly affected by the dissolved oxygen as *C. fluminea* shows no capacity to regulate oxygen uptake rate with the concentration reduction of the dissolved oxygen (McMahon, 1979). The freshwater mussel *Pleurobema sintoxia* has been found to be relatively unaffected by hypoxic conditions. The presence of 3 to 10% oxygen at 20°C (0.6–1.8 mg/L) to be adequate for normal oxygen demands (Badman, 1974).

2.7 Feeding Mechanism

Organic matter in streambed is one of a factor that can determine the presence of *C. fluminea* in a given site as this clam is primarily a suspension-feeder filtering

phytoplankton and detritus from the water column at very high rates (Cherry *et al.*, 1980, Silverman *et al.*, 1995). Besides, the *C. fluminea* is a pedal-feeder using the organic matter available in the sediment as a food resource (Hakenkamp & Palmer, 1999) and the sedimentary material is transported to the labial palps by using ciliary tracts on the foot (Thorp & Covich, 1991)

However, there are two specific modes of deposit feeding that have been reported among freshwater bivalves which are pedal feeding and siphon-suction as introduced *C. fluminea* are also very proficient at combining suspension and deposit feeding to meet their energy requirements (Way *et al.*, 1990). It has been observed that juveniles (<1 year old) collect fine organic materials from their substrate by pedal sweep and also by using their cilia to create an anterior suspension feeding current (Yeager *et al.*, 1994). Deposit feeding is a very important food source for bivalves as the mortality will be high when the organic materials in the substrate are not available.

2.8 Food Sources

Phytoplankton act as an important biological indicator for the water quality. They are important primary producers of the food web in the water but some of them can be harmful to humans and also to other vertebrates releasing toxic substances (hepatotoxins or neurotoxins etc.) into the water (Ariyadej *et al.*, 2004) and thus decrease its aesthetic value by reducing water clarity and creating taste and odour problems (Schmidt & Kannenberg, 1998). Algae are widely present in freshwater environments, such as streams, lakes and rivers. Bacillariophyta (diatoms), Chlorophyta (green algae), and Cyanobacteria are the three major groups of phytoplankton in freshwater ecosystems.

Phytoplankton was considered as a main food source for bivalves and phytoplankton abundance in shallow area are strongly controlled by bivalve grazing (Arapov *et al.*, 2010). Bivalves can also significantly reduce the effect of eutrophication by grazing (Officer *et al.*, 1982).

2.9 Economic Impact of *C. fluminea*

This species has caused millions of dollars' worth of damage to intake pipes used in the power and water industries in USA. The high number of population will clog water intake pipe both when they are alive or dead and the cost of removing them is estimated at about a billion US dollars each year. The juveniles that have a higher chances to be transported by the water current have been a problem to the electrical generating facilities when they attached themselves at the walls and with the massive number of this species will definitely obstructing the water flow. Several nuclear reactors have had to be closed down temporarily in the USA for the removal of *C. fluminea* from the cooling systems (Isom, 1986).

3.0 Environmental Impact of *C. fluminea*

C. fluminea has one of the highest filtration rates per biomass, compared to sphaeriids and unionids (McMahon, 1991). The major impact from this species will be on reduction of planktonic communities (Cohen *et al.*, 1984; Lauritsen, 1986b; Leff *et al.*, 1990). Next, *C. fluminea* is a source of food to fishes, than might induce accumulation in higher trophic levels of accumulated heavy metals (García & Protogino, 2005). The other species, *C. fluminalis* can effectively bio accumulate heavy metals such as Zn, Cu, Hg or Cd (Pourang, 1996). However a clear relationship

between feeding habits and bioaccumulation of Cd, Cu and Zn it is not clearly confirmed (Villar *et al.*, 2005).

C. fluminea is known to alter benthic substrata and competes with native species of bivalves for food and space. Several studies have shown that the filter feeding of *C. fluminea* had resulted in a significant removal of suspended matter from the water column. *C. fluminea* preferentially invades sites where native mussel communities are already in decline by anthropogenic ecosystem disturbances (Strayer, 1999) by outcompeting them for space and food (Aldridge and Muller, 2001). However, their impact on the native mussel is weaker than zebra mussels *Dreissena polymorpha* (Strayer, 1999).

3.1 Water Quality Parameters

Water quality is one of the parameters that influence *C. fluminea* and is important for a healthy ecosystem. In order to survive, some basic conditions must be met by the aquatic life or the population will become stress. When the specific conditions failed to meet their need, they will die. Thus, it is important to carry out various water quality parameters in order to identify the health level of a river so that it is safe for human and also for the aquatic life. In order to develop a water quality index, there are several parameters that need to be considered such as physical and chemical parameters (DID, 2009).

3.1.1 pH

pH is a measure of the acidity or alkalinity of water. It is usually measured by using colorimetric test where litmus paper changes colour with increased acidity or alkalinity. The photosynthesis that occurs in the stream may result in varies pH value (Mccaffrey, 1997).

3.1.2 Dissolved Oxygen (DO)

Dissolved oxygen is the amount of oxygen in the water which indicates the health of the water bodies. Dissolved oxygen content in water reflects the physical and biological processes that happen in water and is influenced by aquatic vegetation. Oxygen is needed by fish and other aquatic organisms, and levels of dissolved oxygen will determine the ability of ponds and other water bodies to support aquatic life. Oxygen dissolves in water at very low concentrations measured in parts ppm or mg/L. The process of the green plants and algae using the sunlight to convert water and carbon dioxide (CO²) to oxygen and carbohydrates or photosynthesis contributes to the amount of oxygen in water.

Dissolved oxygen level can vary during the day and night. This is because the during the day, the dissolve oxygen concentration will increase due to the photosynthesis while at night, oxygen will be removed from the water through respiration the process whereby plants and animals consume oxygen and release carbon dioxide as they convert organic material to energy. Besides, dissolved oxygen can also be significantly affected by the temperature of the water bodies where the warmer the water, the less amount of oxygen it can hold (Sallenave, 2012).

3.2 Nitrate and Phosphate

Every living organisms need nutrient to sustain growth and life function. Water bodies require some nutrients to be healthy but the excessive of nutrient content in water bodies can be harmful. A water bodies can be polluted if they received excessive amount of nutrient that will enhance algae growth. Nitrate, a compound containing nitrogen, can exist in the atmosphere or as a dissolved gas in water, and at elevated levels can have harmful effects on humans and animals. Nitrates in water can cause severe illness in infants and domestic animals (Minnesota Pollution Control Agency, 2008)

Phosphates, the inorganic form, are preferred for plant growth, but other forms can be used when phosphates are unavailable. Phosphate will stimulate the growth of plankton and aquatic plants which provide food for larger organisms, including zooplankton, fish, humans, and other mammals. Plankton represents the base of the food chain. The increasing amount of phosphate will cause an increase in the fish population and overall biological diversity of the system. But as the phosphate loading continues and there is a build-up of phosphate in the lake or surface water ecosystem, the aging process of lake or surface water ecosystem will be accelerated.

The excessive nutrient in water will cause an imbalance in the "production versus consumption" of living material (biomass) in an ecosystem. This happen when the amount of phytoplankton or vegetation that act as a primary producer will be higher than the number of consumer. This phenomenon is likely to be problematic because overproduction can lead to a variety of problems ranging from anoxic waters (through decomposition) to toxic algal blooms and decrease in diversity, food supply, and habitat destruction.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

Pattani River is located in southern Thailand, Thailand. It originates in Betong district, Yala Province and empties into the Gulf of Thailand at the town of Pattani. Within Yala Province the river forms the Bang Lang Reservoir. The river is 214 kilometres long. Saiburi River is one of the river in the Southern Thailand that is located in the east coast of the Southern Thailand. Figure 3.1, 3.2 and 3.3 show the location of both study area.

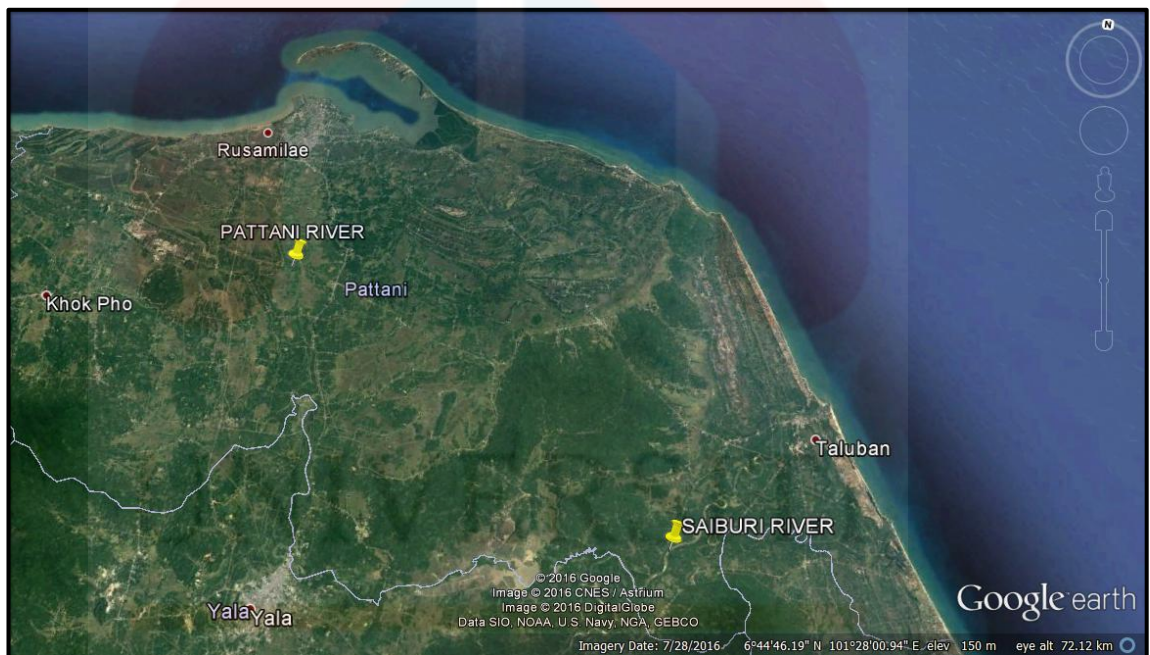


Figure 3.1: Two Different Rivers As The Sampling Sites; Pattani River That Located at Nong Chik District and Saiburi River That Located at Teluk Deraman District

(Source: Google Earth, 2016)

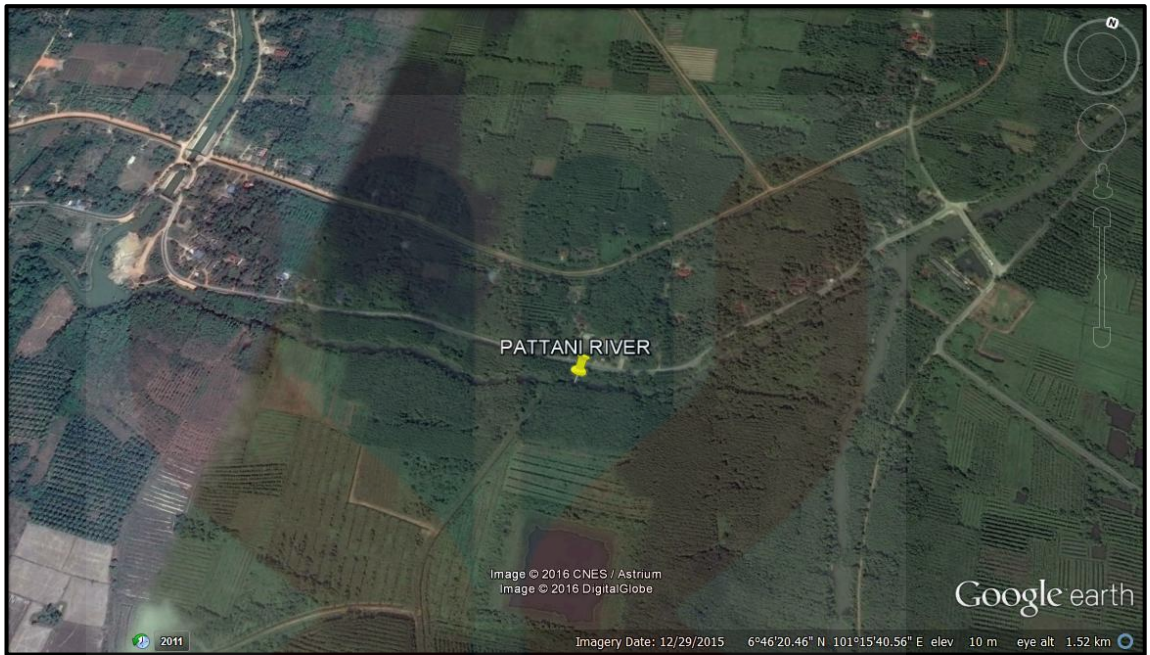


Figure 3.2 Sampling Site at Pattani River
(Source: Google Earth, 2016).



Figure 3.3 Sampling Site at Saiburi River
(Source: Google Earth, 2016).

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3.2 Data Collection

There are 2 rivers that were selected for sampling. For the in-situ water quality measurement, the test were carried out in field while for the lab analysis the water samples were collected in a plastic bottle and brought back to the lab. Water pH and temperature were tested in the field while for the DO, nitrate and phosphate, the samples were fixed with the reagents as soon as possible to prevent oxygen levels from shifting due to agitation or atmospheric contact. Sediment and *C. fluminea* samples were preserved in ice while phytoplankton samples were preserved by 5% formalin before transportation back to the laboratory for further analysis.

3.3 Field Sampling and In- Situ Measurement

3.3.1 Water Sampling

Water samples were collected in glass bottles for DO while the samples for the nutrient analysis were collected in the 500 mL PTFE plastic bottles. The collection method of water samples were according to the Standard Method (APHA, AWWA and WEF, 1998). For the DO sample, the samples were first fixed with reagent and then stored in the ice box and the water samples for nitrate and phosphate were preserved in ice box as well.

3.3.2 Temperature

The temperature of each location were measured in situ by using a mercury-in- glass thermometer according to APHA standard, method 2550 (APHA, 1998). The temperature of each stations were recorded according to the station numbers.

3.3.3 pH

The pH of each location was tested in situ by using pH meter according to APHA standard, method 4500-H⁺ B (APHA, 1999). The pH of each stations were recorded according to the station numbers.

3.3.4 Substrate Sampling

Surface sediment were sampled in this study. Surface sediment is considered to range from zero to 6 inches in depth while a shallow aqueous layer is considered to range from 0 to 24 inches in depth (USEPA, 1994).

The sample were collected by using a metal scoop for about 1 to 6 inches depth and then put in the plastic bag. The samples were kept in the icebox during transportation to the laboratory for sediment analysis, method using pipette method (Kilmer & Alexander, 1949) and stored in the fridge at 4°C.

3.3.5 *C. fluminea* Sampling

C. fluminea were collected at 5 different points at each rivers. The points were randomly selected to prevent bias. The *C. fluminea* were collected by using a clam dredge by dragging the dredge approximately 1 m measured by the quadrat. For each stations, there were 5 replication made. Every *C. fluminea* that remained in the dredge were counted and then put in the plastic bag to be brought back to the laboratory for further analysis.

3.3.6 Phytoplankton Collection

As for the collection of phytoplankton, approximately 300 litres of river water were filtered through the phytoplankton net (40 μ m). The water sample were stored in a 50 mL PTFE plastic bottles and then preserved by 5% formalin solution before brought to the laboratory for further analysis.

3.4 Laboratory Analysis

The analysis of dissolved oxygen, nitrate and phosphate were carried out by using the standard method (APHA, 1992). For each parameter sampling, they have their specific method for preservation and analysis.

3.4.1 DO

The concentration of dissolved oxygen were determined using Azide Modification Method where the oxygen in the water sample oxidized iodide ion (I⁻) to iodine (I₂) quantitatively. The amount of iodine generated were then determined by titration with a standard thiosulfate (Na₂S₂O₃) solution. The endpoint were determined by using starch as a visual indicator. The amount of the standard thiosulfate (Na₂S₂O₃) solution titrated indicated the same amount of oxygen in the samples (Environmental Chemistry of Boston Harbor, 2006)

3.4.2 Nitrate

Nitrate test was conducted according to the Standard Method EPA Method No. 352.1 (EPA, 1971). A series of nitrate solution were prepared (2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L and 10 mg/L) and placed in the tube rack including the blank sample too. Sulphuric acid (H_2SO_4), sodium chloride (NaCl) and brucine sulphate were pipetted into the samples. Then, the tube rack were placed in 100°C water bath for 25 minutes and after removed from heat, the tube were then placed in the cold water bath until reach thermal equilibrium. The absorbance of NO_3^- was determined using GENESYS 10S Series spectrophotometer at 410 nm. Calibration curve was obtained by plotting the absorbance of the standard against mg NO_3^-/L to determine the concentration of the nitrate in each samples.

3.4.3 Phosphate

The experimental method for total phosphate follows the Standard Method APHA 4500-PE Ascorbic Acid Method. A series of phosphate solutions were prepared (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L and 30 mg/L). Phenolphthalein and sulphuric acid (H_2SO_4) were then added into the series of samples. Then, a combined reagent of sulphuric acid, ammonium molybdate solution, potassium antimonyl tartrate solution and ascorbic acid were added too into the samples. GENESYS 10S Series spectrophotometer was used to read the absorbance of PO_4^{3-} at 880 nm. Calibration curve was obtained by plotting the absorbance of the standard against mg $\text{PO}_4^{3-}/\text{L}$ to determine the concentration of the nitrate in each samples.

3.4.4 Sediment Analysis

The sediment were put in a plastic tray and a large clump of the sediment were break down into a smaller size. The sediment were air-dried for approximately 3 days at the place that does not exposed to the direct sunlight. Upon dried, the sediment were sieve through sieve size no. 10 (2 mm) and weight for approximately 20 g for each samples. The experimental analysis for the particle size distribution follows the Pipette Method (Kilmer & Alexander, 1949) to determine the percentage of the sand, silt and clay of each sample. Based on the percentage, the type of soil for each sample were determined by using United States Department of Agriculture (USDA) particle size distribution and textural classification chart (Kilmer & Alexander, 1949) as presented in Table 3.1 and Figure 3.4.

Table 3.1: Particle Size Distribution

Type of soil	Size
Very coarse sand	2.0-1.0 mm
Coarse sand	1.0-0.5 mm
Medium sand	0.5-0.25 mm
Fine sand	0.25-0.10 mm
Very fine sand	0.10-0.05 mm
Silt	0.05-0.002 mm
Clay	< 0.002 mm

(Source: *Soil Survey Manual Introduction*, 1966)

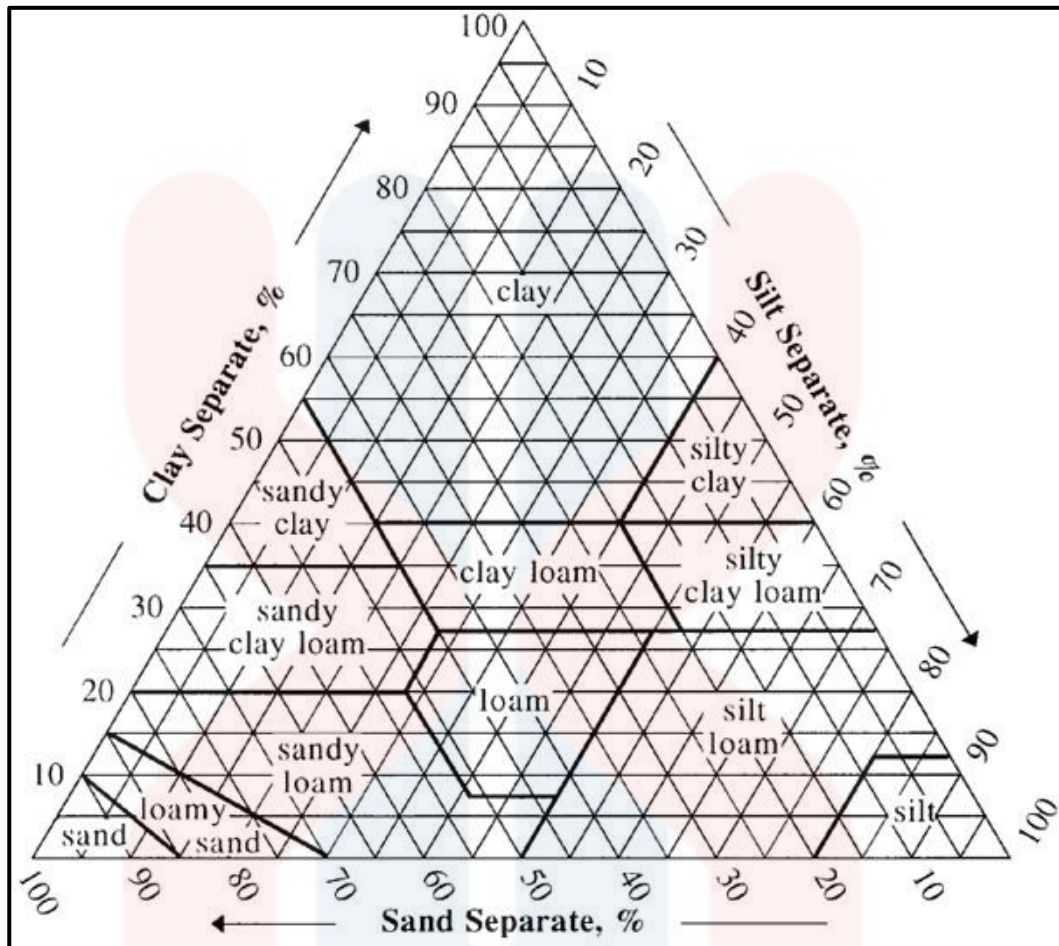


Figure 3.4: The textural classification chart triangle provided by USDA

(Source: *Soil Survey Manual Introduction*, 1966)

3.4.5 *C. fluminea* Measurement

C. fluminea were measured by using a Vernier calliper for its length and height. The colour of the internal and external shell of the *C. fluminea* were also observed to identify the shell morphology.

3.5 Phytoplankton Identification

For phytoplankton samples, 1 mL of the water sample were observed under the microscope with 4x or the 10x magnification by using Sedwick-Rafter Cell to

identify phytoplankton samples. The identified samples were then scored for absence (-) and presence (√) in the different water sources for each category. The number of particular alga in the mount was also noted. Identification was through comparative morphology and description using relevant text books, manuals and articles (Trégouboff & Maurice, 1957; Compère, 1977; Nguetsop *et al.*, 2007; Bellinger & Siegee, 2010). Algae were classified according to algaebase.org.

3.6 Data Analysis

Mann Whitney Test was performed to identify the significance level of the water parameters, densities of *C. fluminea*, and also the length size distribution of this species between Pattani River and Saiburi River. All data analysis were performed by using Statistical Package for the Social Sciences software (SPSS).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter discusses results of *C. fluminea*, sediment and water quality parameters and also phytoplankton in both Pattani and Saiburi rivers. Table and graph were used to describe and analyse data.

4.2 Density and Distribution

A total of 5 points with 5 trials were sampled for *C. fluminea* and the total number of individuals collected at Pattani River was higher than Saiburi River. A total of 618 individuals were collected within 5 trials which is ($n=93$, $n=114$, $n=104$, $n=167$ and $n=140$) in each trials respectively at Pattani River. During sampling in Saiburi River, a total of 329 individuals were collected ($n=44$, $n=28$, $n=97$, $n=64$, $n=96$) for each trial respectively as shown in Figure 4.1.

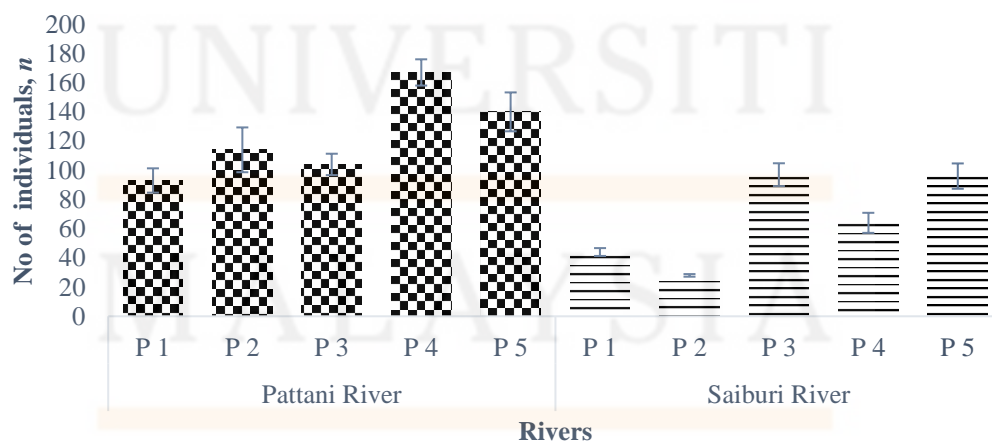


Figure 4.1: Total Number of Individuals Collected at Both Pattani River and Saiburi River

(Mean \pm SD)

During the sampling, *C. fluminalis* found in the dredge during the collection of *C. fluminea* were separated. These two species can be easily differentiate by looking at their shell shape where *C. fluminalis* have a tall triangular shell, widely spaced shell sulcations, and a maximum shell length (SL) of 54 to 60 mm (McMahon, 1983) while *C. fluminea* have a small, rounded shell (Sinclair & Isom, 1961, 1963; Aldridge & McMahon, 1978) with narrowly spaced sulcations (Sinclair & Isom, 1961, 1963).

The population survey of *C. fluminea* has never be done before at both river. As there is no previous study done, the determination of the population declination cannot be done at these two rivers. However, this two study area were both heavily stressed by harvester in the past and also in current time (Zalina, 2014).

The mean density of *C. fluminea* at Pattani River is 369 clams/m² while the mean of clam density at Saiburi River is 196 clams/m² as shown in Table 4.1. The significant different in mean density ($P < 0.05$) of this clam between the two rivers is from the routine harvesting activities by the villagers. As for the Pattani River, harvesting activities at the river is lower because the location of the study area is not easily assessed by the villagers compared to the study area in Saiburi River.

Villagers usually use a basket or a clam dredge to collect the clams at a shallow water. The depth of the study area at Pattani River is approximately 50 to 130 cm which has limit the harvesting activities by the locals. In the other hand, the study area at Saiburi River is very shallow and can be easily assess by the villagers. The depth of the river is only in the range 20 to 60 cm which enables the villagers to use either basket or clam dredge to collect the clams.

Table 4.1: Comparison of Densities for *C. fluminea* between Pattani River and Saiburi River.

Station	No of clam measured	Density per m ²	Station	No of clam measured	Density per m ²
Pattani River, n=618			Saiburi River, n=329		
1	93±8.3	278	1	44±2.7	131
2	114±15.4	340	2	28±0.9	84
3	104±7.3	310	3	97±7.8	290
4	167±9	499	4	64±6.9	191
5	140±13.3	418	5	96±8.7	280
Mean	123.6	369	Mean	65.8	196

Next, the other factor that contribute to the different densities between the two rivers is the water current. Most of the bivalves including *C. fluminea* tend to burrow themselves in the sediment. But, for a juveniles the chances for them to be carried away by the water current is higher than a less small individuals. This is based on study by (McMahon, 1983) where he claimed that the smaller clams have a high probability to be transported along the water current to the downstream.

4.3 Shell Length Frequency

Figure 4.2 shows comparison of length frequency distribution of *C. fluminea* between Pattani and Saiburi River. Based on the result, the range of clams collected were 7.0 to 23.4 mm and 8.9 to 19.5 mm respectively with the majority of the clam collected were in size class 11 to 20 mm. Meanwhile, clams from 11 to 15 mm is found to have a highest length frequency distribution (Pattani, $n=320$; Saiburi, $n=203$) followed by the clams from 16 to 20 mm size (Pattani, $n=251$; Saiburi, $n=123$). 25 clams were collected in the class size of 6 to 10 mm and 22 clams from the class size

of 21 to 25 mm at Pattani River while only 3 clams for the class size of 6 to 10 mm at Saiburi River and no clam collected in the class size of 20 to 23 mm. For the class size of 1 to 5 mm and 26 to 30 mm, no clam were found at both rivers.

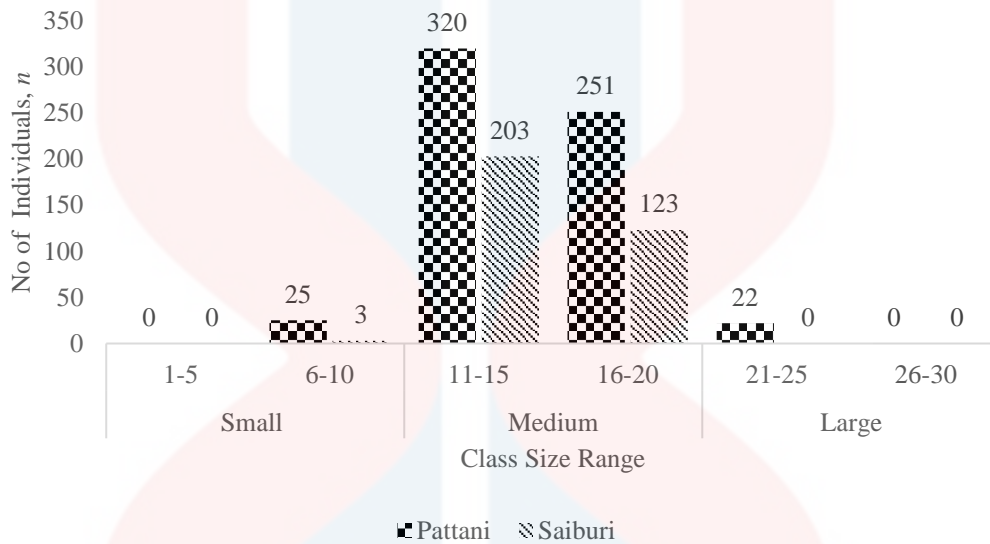


Figure 4.2: Comparison of Length Frequency Distribution of *C. fluminea* between Pattani and Saiburi River

The structure of the population was analysed according the following size classes (shell length): small individuals (1 to 10 mm), medium individuals (11 to 20 mm) and large individuals (21 to 30 mm) (Reyna *et al.*, 2013) . As for the sample in Pattani River, 92.4% ($n=570$) of the sample collected were categorized in medium size which sizes range from 11 to 20 mm and the other 4% ($n=25$) were categorized in the small size. The sample collected at Saiburi River were also consist of 99% ($n=326$) of medium size clams and only 1% ($n=3$) from the small size class. Clams from the large size class only found at Pattani River for 3.6% ($n=22$) while no clams from this class found at Saiburi River.

Even though the number of individuals for each class size varies between Pattani and Saiburi Rivers, both of the *C. fluminea* collected at the two study area does

not show a significant different for shell length ($P > 0.05$), Pattani (14.9 ± 2.8) and Saiburi (14.8 ± 1.9) as shown in Table 4.2

Table 4.2: Comparison Size Range and Mean Shell Length of *C. fluminea* between Pattani River and Saiburi River

Sites	Pattani River	Saiburi River
Size range (mm)	7.0-23.4	8.9-19.5
Mean shell length \pm SD (mm)	14.9 ± 2.8	14.8 ± 1.9
Total number of individuals	618	329

The frequency of distribution of clams at both rivers varied across the range of size classes and they show the similar trend in the size class distribution. The trend of the size classes started with a very low number of clams in 1 to 10 mm size class followed by a significant increase in an 11 to 15 mm size classes. The distribution of clams fell gradually in 16 to 20 mm size class to 21 to 25 mm size class and reach the lowest for the 26 to 30 mm size class.

The observed differentials in frequencies between size classes may be a result of a variety of factors. Juveniles when first being released into the water, they will immediately attach to the bottom substrate by secretion of adhesive substances on the foot tip (Heinsohn, 1958; Sinclair & Isom, 1961, 1963). However, the newly released larval stages would be carried downstream away and transported passively on water currents and they are incapable of swimming (McMahon, 1983) which may explain the drop in the clams number between 1 to 5 mm and 6 to 10 mm class size. As the number of small size clams found in Saiburi River is relatively smaller than Pattani

River (Figure 4.2), it can be assumed that they smaller clams were transported along the water current to the downstream (Sousa, 2008).

For the medium sized clams, they have a highest frequency distribution in both rivers. The abundant of this clam size ranging from 11 to 15 mm and 16 to 20 mm size class is because this species have a high reproduction potential and also a hermaphroditic (McMahon 1999; McMahon & Bogan 2001). Besides the high reproduction, *C. fluminea* also have a physical characteristic that give them higher capacity for locomotion and efficient burrowing. This burrowing activity help them to avoid predators either fish and also from human and usually, they will burrow themselves 10 to 15 cm in the sediment.

The sampling result from both rivers show that the distribution of clam from size class 21 to 25 mm and 26 to 30 mm is very poor. There are a significant gap between the total numbers of clams in medium size compared to the large size. The dramatic decline in large size of clams is because of the routinely harvesting activity by the villagers to be sold and consumed and the clam of a larger size are preferably collected rather than a medium and small size clams.

4.4 Substrate Type

Table 4.3 shows the comparison percentage of substrate types between Pattani River and Saiburi River. Based on the result, 84% of the sediment at Pattani River was classified as fine sand while the percentage of a fine sand at Saiburi River is 87%. Pattani and Saiburi rivers are both have a clay percentage which is 16% and 12% at respectively. The result of the sediment analysis shows that there is no silt found in the substrate at Pattani River while only 1% of silt found in Saiburi River.

Figure 4.3 is used to compare the percentage of substrate types between Pattani River and Saiburi River. Based on the texture triangle, soil analysed in Pattani River is a sandy loam while the substrate in Saiburi River is a loamy sand.

Table 4.3: Comparison Percentage of Substrate Types between Pattani River and Saiburi River

Substrate Type (%)	Pattani River	Saiburi River
Sand	84	87
Clay	16	12
Silt	0	1
Total (%)	100	100

Type of substrate is one of the important factor for a *C. fluminea* habitat however, this species can also live in a wide variety of substrates type including bare rock, loose gravel, sand, and even silts and muds (Home & Mcintosh, 1979). In spite of that, their most preferred habitat is fine or coarse sand substrata rather than mud or bare concrete (Sickel & Burbanck, 1974). Furthermore, substrate type is also important for the juveniles of *C. fluminea* and other infauna bivalve to attach their byssal thread and preventing them to be transported along with the water current. The juveniles rely on the temporary attachment of the byssal thread to the substrate rather than burrowing themselves like an adult does.

Both rivers have a sandy substrate but substrate at Saiburi River composed of more clay and silt percentage than substrate in Pattani River. The structure and porosity of the soil influenced the oxygen in the soil itself. Clay, in general have a smaller pores (micropores) which will prevent the air to move easily thus resulting a lower oxygen level in the soil. *C. fluminea* however, showed low densities on silt (Karatayev *et al.*,

2003, 2005) and achieved the highest population density and greatest success in well-oxygenated substrates such as coarse sand or gravel or sand-gravel mixtures and it is almost always eliminated from areas with reducing sand, mud, or silt sediments of high organic and low oxygen content (McMahon, 1983).

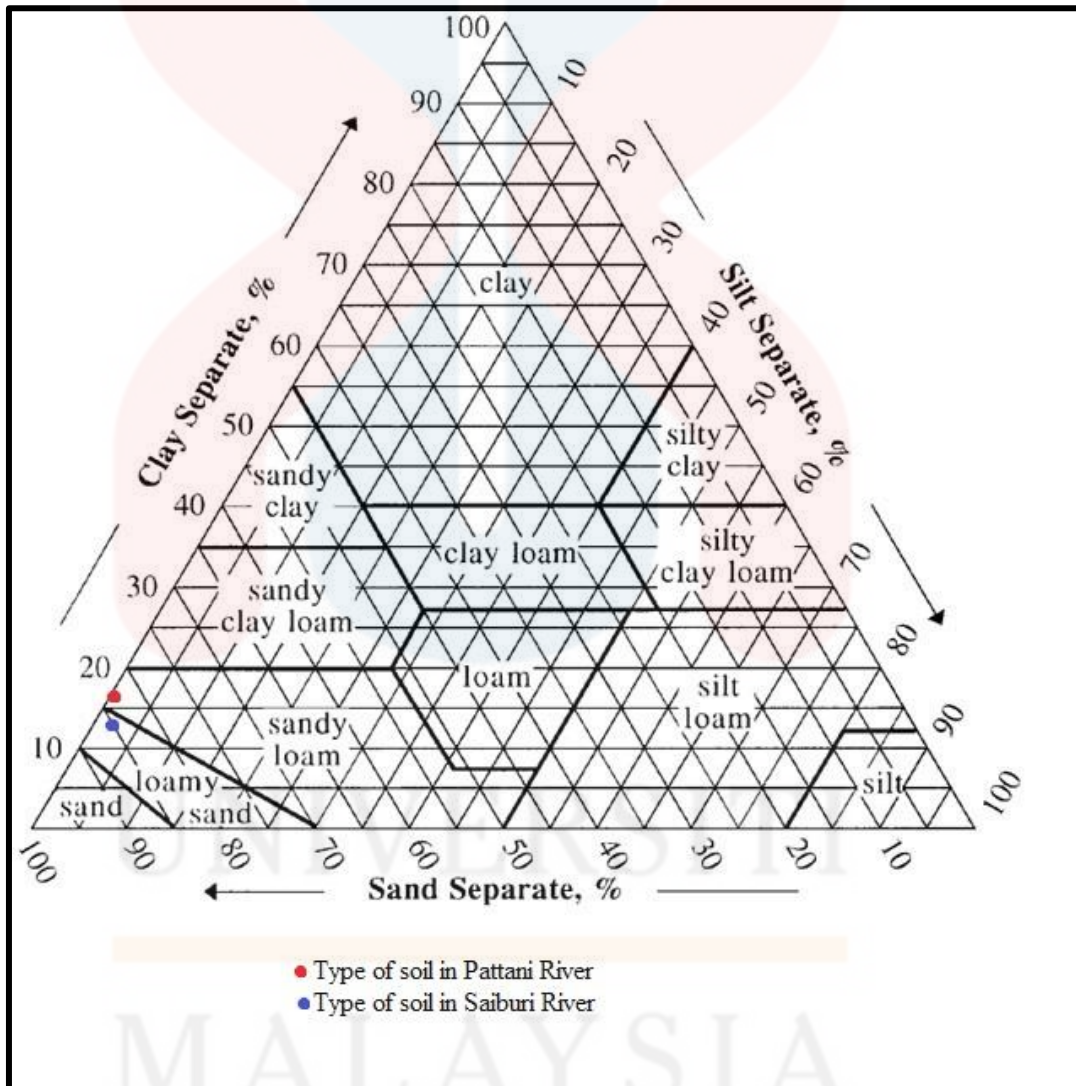


Figure 4.3: Type of Soil in Pattani and Saiburi Rivers Based on Texture Triangle

(Source: *Soil Survey Manual Introduction*, 1966)

The substrate is one of the food source for bivalves as they seem to outgrow the ability to efficiently meet their metabolic needs by collecting food from their substrate (Noreña *et al.*, 2015). As for *C. fluminea* which is a pedal feeder, they will take up the fine particulate from the substrate by using the ciliary tracts on their foot. The availability of the nutrient and organic matter in the substrate will influence the density of *C. fluminea* as this species rely heavily on it as a food source other than phytoplankton.

4.5 Water Quality Parameters

Parameters of pH, temperature, dissolved oxygen, nitrate and also phosphate were measured in both rivers. The results were presented in Table 4.4. For Pattani River, the mean pH value is (6.53 ± 0.06) and (6.7 ± 0.15) for Saiburi River. The temperature at both river remain constant for every three trial which is 31°C in Pattani River and 34°C in Saiburi River. The concentration of dissolved oxygen in both rivers were significantly varies which is $(3.3 \pm 0.10 \text{ mg/L})$ in Pattani River while the concentration of dissolved oxygen is $(8.1 \pm 0.10 \text{ mg/L})$ in Saiburi River. The concentration of nitrate and phosphate in Pattani River were $(0.31 \pm 0.01 \text{ mg/L})$ and $(0.07 \pm 0.01 \text{ mg/L})$ respectively. In Saiburi River, the concentration of nitrate was $(0.1 \pm 0.07 \text{ mg/L})$ and $(0.1 \pm 0.07 \text{ mg/L})$ for phosphate. The data for water quality parameters are as in Table 4.4 below.

Table 4.4: Water Quality Parameters at Pattani River and Saiburi River (Mean \pm SD)

Parameters	Pattani River	Saiburi River
pH	6.53 \pm 0.06	6.7 \pm 0.15
Temperature (C°)	31	34
Dissolved oxygen (DO) (mg/L)	3.3 \pm 0.10	8.1 \pm 0.10
Nitrate (mg/L)	0.31 \pm 0.07	0.1 \pm 0.07
Phosphate (mg/L)	0.07 \pm 0.01	0.5 \pm 0.10

4.5.1 pH

The pH values recorded show that there are no significance different ($P > 0.05$) between these two rivers. These two rivers has a pH value that is normal for a freshwater lakes, ponds and streams which have a pH of 6 to 8 depending on the surrounding soil and bedrock. pH of water is important to determine the solubility and biological availability of chemical constituents such as nutrients (phosphorus, nitrogen, and carbon) and heavy metals that can be utilized by aquatic life.

4.5.2 Temperature

The high significant differences in temperature ($P < 0.05$) between the two rivers is because the sampling time where the sampling time at Pattani River is during early of the day while the sampling for Saiburi River is at noon. Temperature of an aquatic habitat is important as it is use to determines which organisms will thrive and which will diminish in numbers and size because each organism have their own

thermal death point. As for this species, the known lower temperature limit is at 2°C while its upper temperature limit is in range 36 °C to 37 °C (Ilarri & Sousa, 2012b). Besides, there are also a range of temperature that contribute to optimal abundance or for reproduction to occur which is minimum of 15 °C and maximum between 36 °C to 37 °C (McMahon, 1999; Rajagopal *et al.*, 2000)

4.5.3 Dissolved Oxygen (DO)

Dissolved oxygen (DO) can affect the solubility and availability of nutrients, which can be released from sediments under conditions of low dissolved oxygen (Hunt & Christiansen, 2000). The statistical analysis shows that there is no significant of DO concentration between this two rivers ($P > 0.05$). However, the mean concentration of these two rivers differ significantly which in Pattani as much as 3.3mg/L and 8.7 mg/L in Saiburi River as shown in Table 4.5 as dissolve oxygen in Pattani River is relatively lower than in Saiburi River.

Low concentration of dissolve oxygen in Pattani River is may be from the higher amount of algae growth that is caused by excessive nutrient (Phelps, 1994; Wittman *et al.*, 2008). The dissolve oxygen in the water might get consumed by the process of die- off and decomposition of algae (Minnesota Pollution Control Agency, 2008). Decreased DO levels may also be a sign of too many bacteria and an excess amount of biological oxygen demand (Barnes *et al.*, 1998). This is because, organic waste is decomposed by bacteria where the bacteria will remove dissolved oxygen from the water when they breathe. The increasing amount of organic waste will result in a drop in dissolved oxygen as the bacteria will use up the oxygen available.

The higher concentration of dissolved oxygen in Saiburi River may be due to its fast moving stream compared to Pattani River as the rushing water is aerated by bubbles (Kalff, 2002). As for the slow, stagnant waters, oxygen can only enter the top layer of water, and deeper water like in Pattani River is often low in DO concentration as the organic matter will be decomposed by bacteria at the bottom of the river.

A well-oxygenated water body can dissolve phosphorus (phosphate) and iron combine to form an iron-phosphate precipitate that settles to the bottom. This chemical process is very important because phosphorus will be removed from the water column but only with the presence of oxygen. The failure of this process to occur will lead to an eutrophication which the water body will be dominated by abundance number of aquatic plants.

4.5.4 Standard Calibration Curve

A standard calibration curve of nitrate and phosphate were carried out at various concentrations starting from 2, 4, 6, 8 and 10 mg/L for nitrate and 5, 10, 15, 20, 25 and 30 mg/L for phosphate. The data are presented in Table 4.5 and Table 4.6 for both nitrate and phosphate while standard calibration curves were shown in Figure 4.4 and 4.5.

Table 4.5: Concentration and Average Absorbance Reading for Nitrate

Concentration (mg/L)	Absorbance Reading at 410 nm (nm)
0	0
2	0.069
4	0.176
6	0.255
8	0.33
10	0.41

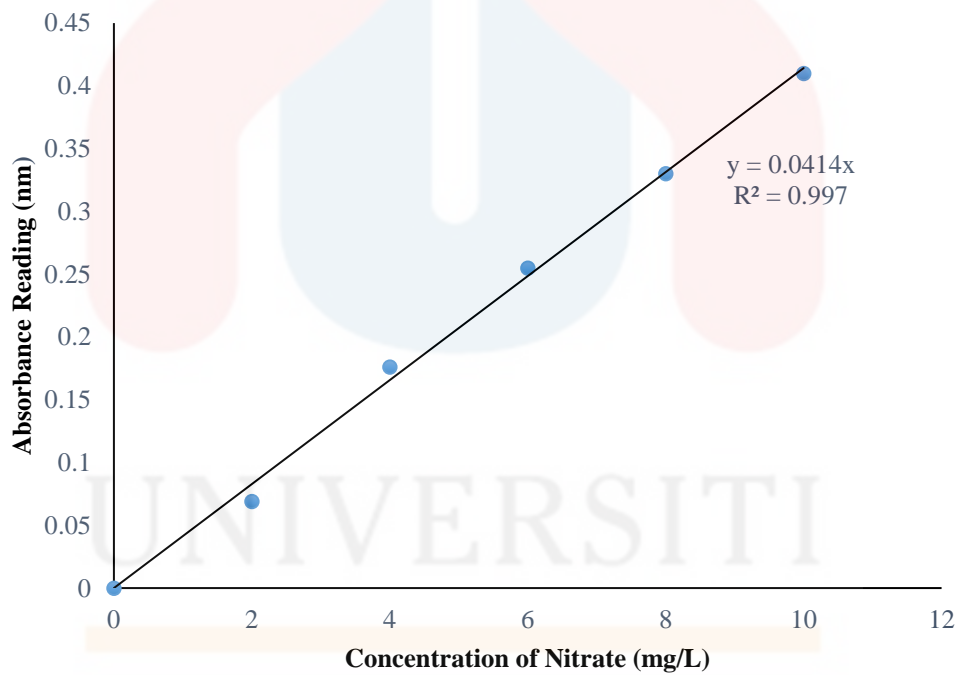
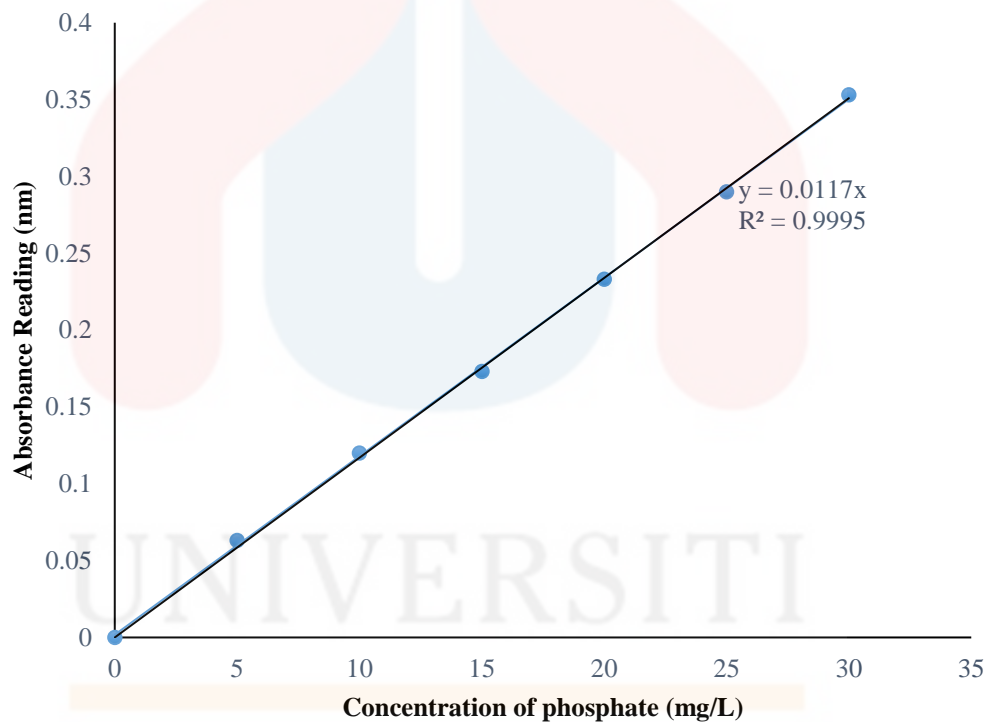


Figure 4.4: Standard Calibration Curves of Nitrate

Table 4.6: Concentration and Average Absorbance Reading for Phosphate

Concentration (mg/L)	Absorbance Reading at 880 nm (nm)
5	0.063
10	0.12
15	0.173
20	0.233
25	0.29
30	0.353

**Figure 4.5:** Standard Calibration Curves of Phosphate

Calibration curve is the most essential stage in measurement procedure where the relationship between the output of the measurement system and the accepted values of calibration standard is established. Referring to the Figure 4.4 and Figure 4.5, the

standard calibration curve were both plotted to be linear on the concentration against the standard solution concentration. The graph trend explain that the absorbance reading increase as the concentration of the nitrate and phosphate increase as they were directly proportional to each other. The correlation coefficient of both nitrate and phosphate indicate a strong correlation as the R^2 value is near to 1 (Figure 4.4 and Figure 4.5)

4.5.5 Phosphate and Nitrate

The concentration of phosphate in these two rivers are significantly differ from each other ($P < 0.05$). The amount of phosphate is one of the nutrient for the algae growth. However, only a small amount of phosphate is needed by them. The increasing phosphate concentration in the river water at Saiburi River cause the phosphate content that is not used by the plant to accumulate. The study area at Saiburi River does not have any aquatic plant. Thus resulting he phosphate concentration too increase as there is no plant to utilize the phosphate in the river. In the other hand, there are a lot of aquatic plant can be found at the study area at Pattani River. The utilization of the phosphate in the water by the plant may result in a lower concentration of phosphate in that river (Minnesota Pollution Control Agency, 2008).

A statistical analysis shows that the concentration of nitrate in both rivers is not significant ($P < 0.05$). Although the differences is not significant, but a higher concentration of nitrate in Pattani River may be a result from a higher densities of *C. fluminea* compared to Saiburi River. This may be because the excretion process by *C. fluminea* consist of soluble ammonium, nitrite, nitrate and phosphate, and insoluble faeces. Besides, the study area at Pattani River is located nearby an agricultural site.

The probability of the fertilizer runoff from the agricultural activities can also contribute to the higher concentration of nitrate in the river.

The amounts of phosphates and nitrates can varies due to the rainfall which washed the farm soils into nearby waterways. These two nutrients will stimulate the growth of plankton and aquatic plants which provide food for aquatic animals. However, the excessive amount of phosphate will then cause the depletion of dissolved oxygen because of the decaying process by the aquatic plants (Minnesota Pollution Control Agency, 2008).. The high phosphate concentration in Saiburi River might due to the irrigation water becomes enriched in phosphorus from fertilizer and soil phosphorus through repeated use of the water in surface irrigation systems and then released into the stream (Yolthantham, 2007).

4.6 Habitat Characteristic for *C. fluminea*

Overall, the water quality at both rivers are in the suitable level for the growth of *C. fluminea*. This species, is likely can be very tolerance to the extreme temperature have no problem to live in a warmer water bodies such as in Saiburi River. The pH level in both rivers were also in the normal range for the aquatic life in a water bodies for their optimum habitat. The lower pH value will result in the deformation of the *C. fluminea* shells which will later cause the high level of calcium carbonate (CaCO_3) in the water bodies. The lower dissolved oxygen level recorded in Pattani River may result from the process of plants die-off. However, previous study showed that *C. fluminea* can survive the lowest dissolved oxygen of 0.6 mg/L (Badman, 1974). The nutrient content of phosphate and nitrate in the water bodies is sufficient for the growth of *C. fluminea* as well as for the other aquatic life. Both of these rivers can provide

nutrient to be siphoned by *C. fluminea* and thus helps to enhance the growth and survival. The sediment type of these two rivers were mainly consist of fine sand so it can be said that *C. fluminea* most favourable habitat is from sand. The abundance of phytoplankton in these two rivers were also one of the factor that enables this species to survive.

4.7 Phytoplankton Availability

Overall, a total of 29 species of phytoplankton were identified in these two rivers but only 15 phytoplankton species found in Pattani River while Saiburi River shows the occurrence of 21 species of phytoplankton. These two rivers have a slight differences in term of similarity of phytoplankton species. The differences of species of phytoplankton in both rivers may due to the water quality parameters especially the nutrient content which is nitrate and also phosphate. This nutrient in the water bodies will enhance the growth of the phytoplankton and thus will increase the availability of food source to *C. fluminea*. The identified species of phytoplankton was demonstrated in Table 4.7.

The occurrence of diverse species of phytoplankton in these two rivers may be one of the factor that contribute to the densities of the *C. fluminea*. High amount of phytoplankton, results a higher availability of food source for this species and thus will enhance their growth. The phytoplankton available in the water bodies can also act as an indicator for the water quality.

Table 4.7 Comparison of phytoplankton species availability between Pattani River and Saiburi River.

Species	Family	Phylum	Pattani River	Saiburi River
<i>Asterionella formosa</i>	Fragilariaceae	Ochrophyta	-	√
<i>Botryococcus braunii</i>	Botryococcaceae	Chlorophyta	√	-
<i>Ceratium hirundinella</i>	Ceratiaceae	Miozoa	-	√
<i>Chroococcus</i>	Chroococcaceae	Cyanobacteria	-	√
<i>Closterium</i>	Closteriaceae	Charophyta	√	√
<i>Coelastrum</i>	Scenedesmaceae	Chlorophyta	√	-
<i>Cosmarium</i>	Desmidiaceae	Charophyta	√	√
<i>Cymbella</i>	Cymbellaceae	Bacillariophyta	-	√
<i>Draparnaldia</i>	Chaetophoraceae	Chlorophyta	√	-
<i>Eudorina elegans</i>	Volvocaceae	Chlorophyta	√	-
<i>Fragilaria</i>	Fragilariaceae	Bacillariophyta	√	√
<i>Gonium formosum</i>	Goniaceae	Chlorophyta	√	-
<i>Haematococcus</i>	Haematococcaceae	Chlorophyta	√	-
<i>Hydrodictyaceae</i>	Hydrodictyaceae	Chlorophyta	√	-
<i>Lyngbya</i>	Oscillatoriaceae	Cyanobacteria	√	√
<i>Melosira varians</i>	Melosiraceae	Bacillariophyta	-	√
<i>Nitzschia</i> sp.	Bacillariaceae	Bacillariophyta	-	√
<i>Oscillatoria</i> sp.	Oscillatoriaceae	Cyanobacteria	√	√
<i>Pediastrum duplex</i>	Hydrodictyaceae	Chlorophyta	√	-
<i>Phacus</i>	Euglenaceae	Euglenozoa	√	-
<i>Pinnularia</i> sp.	Pinnulariaceae	Bacillariophyta	√	-
<i>Rhizoclonium</i>	Cladophoraceae	Chlorophyta	√	-
<i>Scenedesmus</i>	Scenedesmaceae	Chlorophyta	√	-
<i>Spirogyra</i> sp.	Sphaerocystidaceae	Chlorophyta	-	√
<i>Staurastrum</i>	Desmidiaceae	Charophyta	√	-
<i>Surirella</i> sp.	Surirellaceae	Bacillariophyta	-	√
<i>Tetraedron</i> sp.	Hydrodictyaceae	Chlorophyta	√	-
<i>Trachelomonas</i> sp.	Thalassionemataceae	Bacillariophyta	√	-
<i>Volvox</i>	Volvocaceae	Chlorophyta	√	√
Total Species			15	21
Overall total species			29	

4.8 Statistical Analysis

Table 4.8: Mann-Whitney Test for Densities of *C. fluminea* between Pattani River and Saiburi River

	DENSITY
Mann-Whitney U	124.500
Wilcoxon W	449.500
Z	-3.658
Asymp. Sig. (2-tailed)	.000

a. Grouping Variable: RIVER

The above Table 4.8 show the Mann-Whitney test for densities of *C. fluminea* between Pattani River and Saiburi River. The data were significantly differ from each other as ($P < 0.05$).

Table 4.9: Mann-Whitney Test for Shell Length of *C. fluminea* between Pattani River and Saiburi River

	LENGTH
Mann-Whitney U	102178.500
Wilcoxon W	157124.500
Z	-.107
Asymp. Sig. (2-tailed)	.915

a. Grouping Variable: RIVER

The above Table 4.9 shows the Mann-Whitney test for shell length of *C. fluminea* between Pattani River and Saiburi River. The analysis shows that the data were not significant from each other as ($P > 0.05$).

Table 5.0: Mann-Whitney Test for Water Quality Parameters between Pattani River and Saiburi River

	DO	PH	TEMP	PHOSPHATE	NITRATE
Mann-Whitney U	.000	.500	.000	.000	.000
Wilcoxon W	6.000	6.500	6.000	6.000	6.000
Z	-1.964	-1.798	-2.236	-2.023	-1.964
Asymp. Sig. (2-tailed)	.050	.072	.025	.043	.050
Exact Sig. [2*(1-tailed Sig.)]	.100 ^b	.100 ^b	.100 ^b	.100 ^b	.100 ^b

a. Grouping Variable: RIVER

b. Not corrected for ties.

The above Table 5.0 shows the Mann-Whitney test for water quality parameters between Pattani River and Saiburi River. The statistical analysis for the DO and nitrate is not significant because the DO and nitrate both have the p value ($P > 0.05$). For the pH of these two rivers, there are no significance in the data value because ($P > 0.05$). Both temperature and phosphate show a significant different as the ($P > 0.05$)

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The habitat of *C. fluminea* in Pattani River and Saiburi River has been characterized. The *C. fluminea*, water and sediment were sampled and analysed and the phytoplankton species in the water bodies have been identified.

The result of density and distribution of *C. fluminea* at the two rivers were not fully influenced by the water quality, sediment types and also availability of phytoplankton because of the harvesting activities by the villagers. Both of this rivers have the most abundance number of individuals of *C. fluminea* in medium size and least in the larger size.

The sediment types of Pattani River was classified as sandy loam while at Saiburi River as loamy sand. However, this two type of soil compost of mostly sand substrate that is the most preferable by *C. fluminea*. This species, need a well oxygenated sandy substrate for its burrowing activities and for feeding.

C. fluminea can tolerate a high temperature exposure in Pattani and Saiburi rivers and they also have a high tolerance towards a cold temperature based from the previous study in Europe. Besides, this species can also tolerate with such a low concentration of dissolved oxygen like in Pattani River. The pH of these two rivers were normal for a freshwater water bodies thus enable this species to inhabit however, a lower pH will degrade *C. fluminea* shells and may cause mortality event.

The nutrient content in both rivers were also influenced by the surrounding area such as agricultural sites and residential. Effluent and discharge from human activities will affect the nutrient content in the water bodies. However, the *C. fluminea*

itself can altered the nutrient in the water bodies by the excretion and filtering process. The high amount of nutrient in water bodies will enhance plant growth thus might cause an eutrophication. When the plant dies, the decomposition process will utilize dissolved oxygen and then will cause a depletion of oxygen.

The availability of the phytoplankton in these two rivers is important for the *C. fluminea* because they act as a food source for this species. This species is known as a deposit feeder as well as a pedal feeder which enables them to siphon the nutrient and phytoplankton in the water bodies at high rates.

5.2 Recommendations

There are several recommendation can be made in order to improve the accuracy of this study, the suggestion is as below:

- i. Increase the number of sampling station at each rivers.
- ii. Include another water quality parameters such as salinity and conductivity in order to gain a more details habitat characteristic information for this species.
- iii. Include analysis for organic materials in the sediment as *C. fluminea* feed on the organic materials in the sediment too beside the phytoplankton.
- iv. Include the gut content analysis of *C. fluminea* collected to determine its feeding selectivity based on the availability of phytoplankton in the river.
- v. Sampling must be done frequently and continuously so that the ecology of this species can be identified throughout the year.

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APPENDIX A

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Sampling site at Pattani River



The clam dredge used to collect *C. fluminea*



Algal bloom that occur in Pattani River



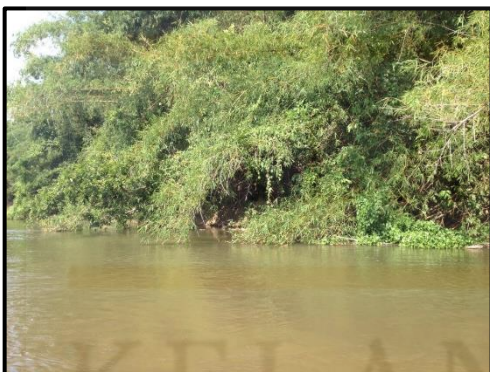
Etok sampling using clam dredge and quadrat



Sampling site at Saiburi River



C. fluminea sampling using clam dredge



The riparian vegetation at the Saiburi River



Sample being fixed and preserved before brought back to laboratory



Two different shell morphology of *C. fluminea*



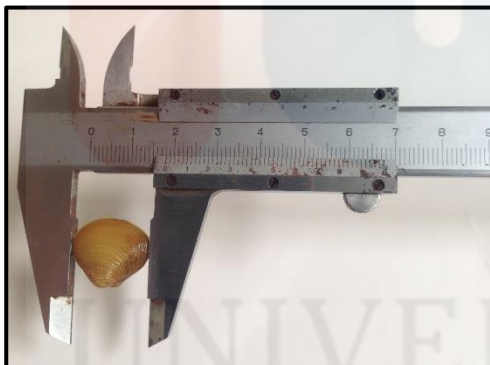
Preparation of calibration curve for nitrate



Variation of *c. fluminea* shell sizes



Preparation of calibration curve for phosphate



Measuring the length of *C. fluminea*



Chroococcus under the microscope with 4x magnification



Measuring the height of *C. fluminea*



Volvox under the microscope with 4x magnification