UNIVERSITY OF GHANA, LEGON

INVESTIGATIONS INTO HATCHERY AND NURSERY OPERATIONS FOR THE CULTURE OF THE FRESHWATER PRAWN (*Macrobrachium vollenhovenii*, Herklots 1857) IN GHANA

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DECLARATION

This thesis is the result of research work undertaken by Atsu, Dzidzornu Kwaku Edzordzi. in the Department of Marine and Fisheries Sciences, University of Ghana, under the supervision of Prof. P. K. Ofori-Danson, Prof. F. K. E. Nunoo and Dr. S. Addo.

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ABSTRACT

The study was conducted to demonstrate hatching and larval development of the African River Prawn *Macrobrachium. vollenhovenii Herklots, 1857)* and to establish the spatial and temporal distribution of the prawns and related crustaceans in the Lower Volta River from Torgorme (Akuse) to Ada with focus on the Volta Estuary of Ghana. The ecological survey was undertaken from August 2013 to July 2014 followed with aspects of reproductive biology of the *M. vollenhovenii* from August 2014 to July 2015 and then the hatching and larval development of the from January to December 2016. Fisheries dependent data was collected together with experimental prawn fishing. The species encountered included *Atya gabonensis (*Giebel, 1875*), Macrobrachium macrobrachion,* (Herklots, 1851), *Macrobrachium vollenhovenii* and *Penaeus* spp. Comparatively more *Penaeus* spp. were sampled in the dry months (November to January) in more saline zones while *Macrobrachium* spp. were more in the rainy months (May to July) in the more freshwater zones. The dominant species in the estuary was the *M. vollenhovenii* (72.52%) with male to female ratio of 1:1.3 and non-berried to berried females ratio as 1:1.7. The berried females were obtained throughout the year with increased numbers and larger ones in the rainy season. The size and weight of gonad correlated positively with size of berried females and appeared to be a function of the number rather than the size of eggs. Out of four treatment media (Artificial Sea salt, Freshwater, Seawater and Rock salt) used for the larval development, the Freshwater medium could not support the life of the larvae after 48 hours. The 8th Larval stage (ZVIII) was observed from the 23rd day of culture in both Seawater and Artificial Sea Salt media. Survival rate correlated negatively with stocking densities of 50, 100, 150 and 200 larvae/liter, an indication that thinning is necessary as the larvae grow from one stage to another. Survival rates in the 50 larvae/liter were 36.8%, 30.8% and 25% in the Seawater, Artificial Sea salt and Rock salt

treatments respectively. From the results, *M. Vollenhovenii* larvae could be hatched and developed for culture in the grow-out process to boost prawn culture in Ghana.

DEDICATION

I dedicate this work to my loving wife, Mrs. Edem Negble Atsu, my parents: Late Rev. E. M. Atsu and Madam Awo Fortune Gassor and to my idol friend and brother the Late Mr. Sievers Burghard of Germany. It was their love, dedication and support that saw me through my education up to this stage.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Crustaceans are important food source; species such as lobsters, shrimps, prawns and crabs have become economically key crustaceans with some harvested from the wild stocks and others being farmed (FAO, 2018; 2016; 2014). While some crustacean species are in decline, human intervention has created some support in the production of the crustaceans.

According to FAO (2018), aquaculture production grew globally at 5.8% annually from 2000 to 2016 and the per capita food fish supply increased from 18.5 kg in 2011 to 20.5 kg in 2017 (FAO, 2018). Incidentally, capture fish production remained stagnant; 92.2 million tonnes in 2011 to 90.9 million tonnes in 2016 while aquaculture production in the same period increased from 61.8 million tonnes to 80.0 million tonnes (FAO, 2018). The total aquaculture production in 2016 including 30.1 million tonnes of aquatic plants came up to 110.1 million tonnes (FAO, 2018), aquaculture production indeed can be deemed very important in food fish security.

Out of 59.9 million tonnes of farmed food fish produced in 2011, crustaceans constituted 5.7 million tonnes accounting for 9.5% of global aquaculture production (FAO, 2012). In 2012, crustaceans contributed 9.7% (6.4 million tonnes) and 22.4% by value (FAO, 2014). In 2014 crustacean production was 6.9 million tonnes contributing 9.3% out of the 73.8 million tonnes of total global aquaculture production (FAO, 2016). Production of the crustaceans increased to 7.8 million tonnes constituting 9.6% of the 80.03 million tonnes of farmed food fish produced in 2016 (FAO, 2018). Crustacean production could be crucial in contribution to livelihood and income for people engaged in the aquaculture sector.

The Giant River prawn (*Macrobrachium rosenbergii,* de Man, 1879*)* is the most dominant farmed and well researched freshwater prawn in global perspective (D'Abramo, *et al*., 2003; New, 2005, 2002, 1988; New & Valenti, 2000; New & Singholka, 1985). Other farmed species include the Oriental River prawn (*M. nipponense,* De Haan, 1849) in China and the Monsoon River prawn (*M. malcolmsonii,* Milne-Edwards, 1844) in India (New, 2005). According to FAO (2012), world aquaculture production of crustacean in 2010 consisted of 29.4% freshwater species and 70.6% marine species.

The African River prawn, *Macrobrachium vollenhovenii* (Herklots, 1857) is endemic to the eastern Atlantic, with viable fishery in the West African sub–region (Nwosu & Wolfi, 2006). Willfuhr-Nast, *et al.* (1993) therefore recommended *M. vollenhovenii* for culture, as an alternative to the most commonly cultured *M. rosenbergii* (Bello-Olusoji, 2004, FAO, 2000, Marioghae, 1982). Despite its economic importance and future potentials, very little documented works are available on this prawn in West Africa (Lawal-Are & Owolabi, 2012).

According to Attipoe & Amoah (1989) and Alhassan (2011) the *Macrobrachium vollenhovenii* is the dominant prawn species in Ghana where they constitute a major component of the fishery. Some species of the prawn generally spend part of their life cycle in the brackish water bodies, the juveniles are thus found in the Volta estuary and aligned water bodies (Addai, 2010; Atsu, 2003) where all manner of fishing practices are carried out that threaten the viability of the prawns, coupled with ecological changes brought about by the construction of Volta Hydroelectrical Dam upstream (Gordon, 1999).

Prawns have been farmed using traditional methods mostly in south-east Asia for a long time. First experiments with artificial breeding of *M. rosenbergii* were done in the early 1960s in Malaysia, where it was discovered that the larvae needed brackish water for survival (New, 2002). Industrial-scale rearing processes were perfected in the early 1970s in Hawaii, United

State of America, and other countries (New, 2002). The technologies used in freshwater prawn farming are reported to be basically the same as in marine shrimp farming: hatcheries produce post larvae, which are nursed and transferred into grow-out ponds, where they are grown to marketable size (New, 2002).

According to FAO (2012) some developing countries in Asia and the Pacific (Myanmar and Papua New Guinea), sub-Saharan Africa (Nigeria, Uganda, Kenya, Zambia and Ghana) and South America (Ecuador, Peru and Brazil) have made rapid progress to be recognized as major aquaculture producers in these regions. Nonetheless, their contribution to world aquaculture production remains very small (4.1% by quantity and 3.6% by value). Incidentally, these low-income food-deficit countries, mostly in sub-Saharan Africa and in Asia, are home to 20% of the world's population (1.4 billion people). Aquaculture production from Africa according to FAO (2012) was due to rapid development in freshwater fish farming mainly in Nigeria, Uganda, Zambia, Ghana and Kenya. The production is however, overwhelmingly dominated by finfishes (99.3% by quantity), with only a small fraction from marine shrimps (0.5%) and marine molluscs (0.2%). In Ghana, aquaculture production is dominated by the Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758) (Family Cichlidae) followed by the African Catfishes (*Clarias gariepinus*, Burchell, 1822) (Family Clariidae) and the African bony tongue (*Heterotis niloticus*, Cuvier, 1829) (Family Osteoglossidae) (FAO, 2014) and of late the culture of the marine shrimps (the *Penaeus* spp. – from the family Peneidae) on commercial scale. There is however no record on the culture of the freshwater prawns in Ghana.

The freshwater prawns as well as many other farmed aquatic animals have four life stages: (i) egg (ii) larva (iii) juvenile (iv) adult. There are also three stages in their culture process namely: (i) *the breeding process* to produce fry that develop into larvae (ii) *the nursery process* to produce seeds (post larvae or juveniles) for stocking into production systems (iii)

the *grow-out process* to produce marketable size (D'Abramo *et al*., 2012; Hicks & Pierce II, 2011). The breeding and the nursery stages constitute crucial stages for commercial aquaculture ventures. These stages according to D'Abramo *et al*. (2003), hampered earlier attempts at prawn culture in the United States. The bottleneck was removed when research work established improved hatchery and nursery practices. It is therefore regarded as the prime area to devote attention in promotion of commercial prawn culture (D'Abramo *et al*., 2003; Chowdhury, 1993)

Seed for aquaculture could come from two (2) main sources, namely: (i) *Capture-Based Aquaculture* (CBA) (ii) *Hatchery-Based Aquaculture* (HBA) (Alessandro & Paul**,** 2008). The CBA, where the seeds are taken from the wild, collected at any growth stages deemed fit and subsequently grown in aquaculture facilities to marketable size. The HBA, where the seeds are produced from Aquaculture Hatchery (consisting of broodstocks, eggs, larvae, fingerlings and juveniles).

The CBA does not, however, have full control on the reproduction and breeding cycles of the farmed aquatic organisms. Nonetheless, it provides opportunity for Research Scientists to understand the biology and culture potential of the organism concerned. CBA is also used to culture many aquatic organisms either partly or fully using fry caught from the wild to supplement seeds from HBA to meet the demand, or the quality of the HBA seeds. The system, however, falls short of providing opportunity for seed improvement of the farmed aquatic organisms when used solely. The CBA also has the potential to impoverish the dwindling wild fish stock if practiced in large scale as it competes directly with capture fisheries; it is also difficult to get large number of the desired size at one time. The CBA is therefore considered unsuitable for commercial operators (Lovatelli, 2008).

For sustainable fish farming therefore, HBA is the best source of prawn seeds. Nonetheless the CBA forms the base for the HBA, especially in experimental oriented operations. There are however several methods in the HBA; the method selected depends on the reproductive biology of the species concerned, local environmental conditions and the facilities available. The HBA offers opportunities for manipulations during the early life stages of the farmed organism to obtain the desired flesh quality, growth rate, sex reversal through genetic improvement as in the case of the Genetically Improved Farmed Tilapias – (GIFT). The manipulations can also enhance the disease resistance of the farmed fish (Alam *et al*, 2014; Bisht, 2013).

The berried or ovigerous prawns can be obtained from CBA especially in the tropical climate (New, 2002) where breeding occur all year-round contrary to what pertains in temperate climate where development of the brood stock is crucial for all year round availability of berried prawns. Nonetheless, brood prawn development in culture environment can ensure selective breeding and improved offspring for better growth performance (Belsare, *et. al*., 2007). The challenge of obtaining the berried prawns from the wild for culture is the possibility of losing eggs during capture and transportation of the berried prawns over a long distance. This calls for careful handling during capture and transportation (Lal *et. al*., 2014).

Potential for freshwater prawn culture in Ghana has been reported (Gordon, 1999), but no extensive research had been carried out to demonstrate the successful culture of any of the reported species. New (2005), reported of assistance by the Institute of Aquaculture, Sterling University to some aquaculture scientists in Ghana to develop the culture of *M. vollenhovenii*, but efforts still remain negligible. Development of scientifically proven culture techniques of the prawns in commercial quantity will provide a viable backbone for the sustenance of the local market. This will contribute in raising the livelihood of the riparian communities along

the banks of rivers in which the prawns occur in the country and offset any danger of wild stock extinction.

The availability of fresh and saline waters in Ghana, according to Armah (1993) and Koranteng, *et al*. (2000), especially in the estuaries and lagoons provides suitable environment for hatchery production of the *M. vollenhovenii* on commercial basis. The suitability of the quality of the available water resource should however be investigated. New and Singholka (1985) stated that suitable water intake for *M. rosenbergii* was speculative, there is therefore the need for research on suitable water quality parameters for the species in Ghana. A research into suitable technique for our local setting to address any negative impact on the culture of the species is crucial for any meaningful freshwater prawn culture venture in the country.

1.2 Research Justification

The interest in the culture of the prawns is high, but limited knowledge of the culture technology is one of the main drawbacks for the realization of this dream in most parts of the world (New, 1988) including Ghana (Gordon, 1999; Attipoe, 1989). Research and pilot scheme projects are therefore needed using indigenous species under local environmental conditions to whip up the interest in the culture of the prawns. Adoption of scientifically proven methods and procedures for cultivating the prawns will be very significant in the development of their culture not only in Ghana, but also in the West Africa sub-region. The success in the culture of the prawns will lead to employment opportunities, improve protein consumption, poverty alleviation, enhance rural livelihoods and contribute to foreign exchange earnings in Ghana.

In 2014 the freshwater crustaceans including the prawns available for human consumption from aquaculture constituted 4% of all farmed aquatic species and the marine counterpart species including the shrimps constituted 36% (FAO, 2016). The report released by FAO indicated that out of the 106 million tonnes of farmed aquatic animals produced in 2015,

finfish accounted for 67.8% and the remaining 32.2% are other aquatic animals including the prawns (FAO, 2017). This calls for efforts in developing of farmed non-finfish species.

The production of marine species elsewhere was dominated by *Penaeus vannamei* (Boone, 1931) with substantial amount acclimatized and farmed in freshwater taking over the formally most popular farmed *Penaeus monodon* (Fabricius, 1798) production (FAO, 2016). Dominant among the farmed freshwater crustacean species were the crayfish and the prawns (FAO, 2016). The need therefore to develop the local strain of the crustacean species in Ghana is crucial, not only to match the production elsewhere in the world, but also to address the economic challenges of the country or perhaps offset likely collapse of the prawn fishery.

The adult of *M. vollenhovenii* is an omnivorous detritivore with preference for animal remains (Marioghae, 1982). Mwangi (1984) indicated that this species preyed effectively on frog tadpoles and fry of the tilapias, an indication that *M. vollenhovenii* could be used in polyculture to control excessive breeding of the Tilapias (Marioghae, 1987) as well as to control the invasion of tadpoles in fish ponds. The *M. vollenhovenii* is also noted to be voracious predator of schistosome intermediate aquatic snails *Biophalaria* and *Bulinus* species and could be used to control the population of these snails (Sokolow *et al*, 2014). The larvae of the species cultured elsewhere were observed to have carnivorous tendency and complete the larval life within 16 days (New & Singholka, 1985). At the end of the larval life the prawn developed into post-larval (PL) stage, a miniature adult that crawls rather than swims freely when they assume omnivorous feeding habit. They feed on aquatic insects and their larvae, vegetable matter including algae; other crustaceans, fish, and other animal components (New & Singholk, 1985). Per these characteristics, the *M. vollenhovenii* should be a suitable candidate for culture in Ghana.

The farming of the freshwater prawns is not highly technical or capital intensive as compared to the farming of their marine counterparts (the penaeid shrimps) (Nandlal & Pickering, 2006). It would therefore be an easier and more accessible industry to small-scale farmers that dominate the fishing industry mainly in the rural set-up of Ghana. An experienced tilapia farmer could therefore easily culture the prawns. Depending on the site of the farm and skill of the farmer; the prawns could perhaps be more profitable than the most commonly farmed tilapia in the country currently (D'Abramo *et al.*, 2003).

Ghana is endowed with sufficient water resources and climatic conditions that could supposedly favour the culture of the *M. vollenhovenii* found in the waters of the country. It is therefore necessary to determine the culture conditions of the prawns to harness their benefits to the citizenry. Development of scientifically proven culture techniques of the prawns in commercial quantity is therefore needed to provide a viable backbone for the sustenance of the local industry. This will contribute in raising the livelihood of the riparian communities along the banks of the water bodies in which the prawns are found. The culture of the prawns will as well broaden the farmed aquatic animal base in Ghana for increased diversity of aquaculture produce in the market which at the moment is focused on the finfishes only.

Crucial to aquaculture development is the hatchery that provides seeds for the on-growers and take pressure off the wild stock where fish farmers resort to when fish seeds are not available (Ahmed, 2008). Dependence on the wild stock does not only further worsens the dwindling of the wild stock, but also contributes to poor growth of marketable size of aquaculture produce. Stunted growth of farmed aquatic organisms leads to discouragement and loss of interest and investment. It is therefore crucial to treat hatchery processes as the foundation for any meaningful and sustainable aquaculture development.

Seed production is the base rock and boost to aquaculture development and therefore deserves attention and commitment in efforts to the development aquaculture (Nandlal & Pickering, 2006). Choudhurry (1993) indicated that the major constraint to expansion of prawn farming in the Pacific Island countries and territories was the difficulties in obtaining reliable quality supply of post-larvae for stocking in the grow out facilities. Efforts were therefore made to develop prawn hatchery techniques to address the constraint. Ahmed (2008) also reported that establishment of prawn hatcheries in Bangladesh led to the intensification of prawn culture in that country. He further indicated that out of 81 hatcheries established only 42 were functional at the time of reporting due to lack of technical knowhow and skill manpower. New (1988) attributed the growth of the prawn farming in Thailand and Hawaii to availability of post-larvae from hatcheries. Development of larval production techniques therefore would form the basis for any meaningful commercial culture production of the prawns in Ghana.

Cultivation of indigenous prawn species in this study could give good and sustainable yield devoid of ecological imbalances that could come along with exotic farmed species. The knowledge acquired from the study will add, in no mean dimensions, impetus to the requirements of responsible and sustainable aquaculture as indicated by FAO Code of Conduct for Responsible Fisheries in Article 9.3.1: "*States should conserve genetic diversity and maintain integrity of aquatic communities and ecosystems by appropriate management. In particular, efforts should be undertaken to minimize the harmful effects of introducing non-native species or genetically altered stocks used for aquaculture including culture-based fisheries into waters, especially where there is a significant potential for the spread of such non-native species or genetically altered stocks into waters under the jurisdiction of other States as well as waters under the jurisdiction of the State of origin. States should, whenever possible, promote steps to minimize adverse genetic, disease and other effects of escaped farmed fish on wild stocks"* (FAO, 1995). This study is therefore necessary to serve as the

foundation for sustainable prawn farming in Ghana in commercial scale devoid of any negative impact on the wild stock.

1.3: Research Design

The research was conducted in two phases:

Phase 1:

I. Survey of the crustaceans in the Lower Volta River below the hydroelectrical dam at Akuse with focus on the Volta Estuary at Ada Foah for information on spatial and temporal availability of the wild stock, especially the *M. vollenhovenii*.

II. The reproductive biology of the African River Prawn *M. vollenhovenii* from the Volta Estuary for information on the recruitment pattern and conditions for their survival and reproduction.

Phase 2:

I. Hatchery based aquaculture (HBA) processes to determine the optimal conditions for hatching the eggs by the berried *M. vollenhovenii* females collected from the Volta Estuary.

II. Larval development of the newly hatched *M vollenhovenii* larvae to determine the optimal growth conditions of larvae under culture condition in hatcheries.

1.4 Objectives

The primary objective of the study was to establish the occurrence, spatial and temporal distribution pattern of the crustaceans in the Lower Volta River with focus on the Volta Estuary in Ghana and to investigate the potential hatching and larval development of the African River Prawn *M. vollenhovenii* under HBA for enhanced culture practices in Ghana.

1.4.1 The specific objectives were to investigate:

I. Temporal and spatial distribution of the prawns and closely related crustacean resources in the Volta Estuary.

II. Reproductive biology of the *M. vollenhovenii* females in the Volta Estuary.

III. Hatching process of the berried *M. vollenhovenii* females under culture conditions.

IV. Optimal culture conditions for the larval development of the *M. vollehovenii*.

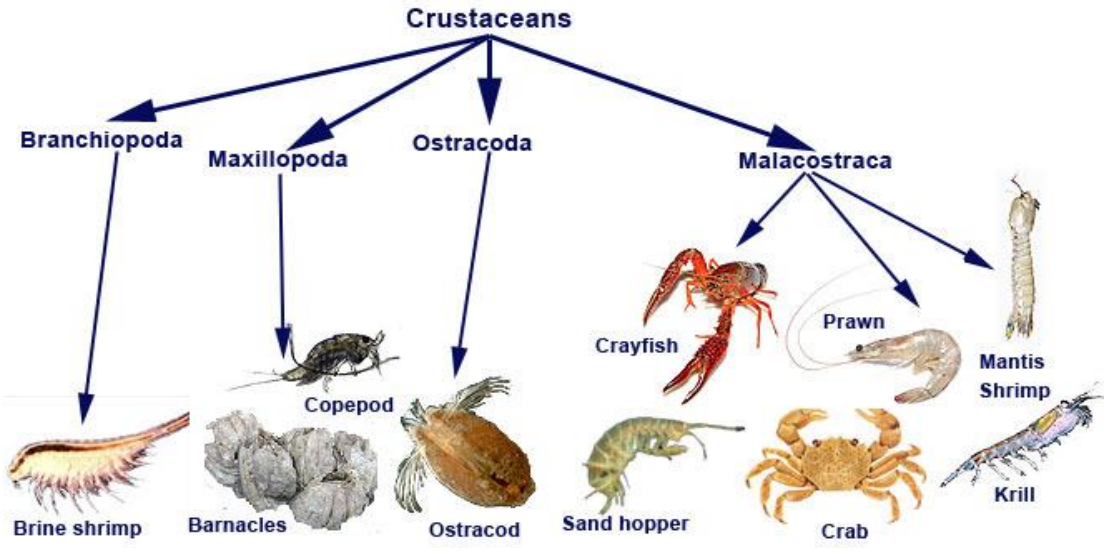
CHAPTER TWO

GENERAL LITERATURE REVIEW

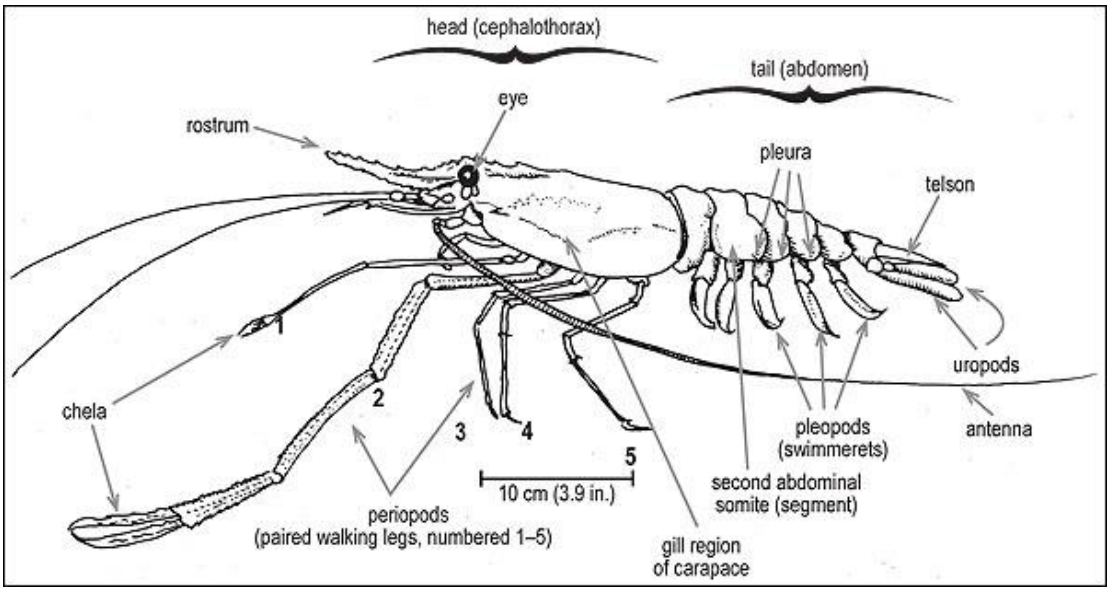
2. 1 Characteristics of Crustaceans for Culture

Crustaceans (Figure 1 A) are a sub-group of arthropods; hard-bodied, exoskeleton animals including organisms such as spiders, scorpions, centipedes, millipedes, insects and crabs. Crustaceans are characterized by segmented body parts and the aquatic species use gills to breathe in their aquatic habitat. There are many aquatic crustaceans with sharp morphological differences including brine shrimps, prawns, crabs, copepods (zooplankton), lobsters, and barnacles (Meyer *et al*., 2009; Poore, 2004; Ruppert *et al*., 2004; Holthuis, 1980).

The species of crustaceans that are important for aquaculture are mostly in the Class Malacostraca (New & Singholka, 1985) (Figure 1 A). Members of this class (Figure 1 B) have 19 segments (1-5 are the head region, 6-13 for the thorax, and 14-19 for the abdomen) and in some, the segments (head and thorax) may be partially fused to form the cephalothorax, or carapace as in the shrimps and lobsters. The major groups of cultured Malacostraca — lobsters, crabs, crayfish, shrimp and prawns are members of the order Decapoda, having five pairs of walking legs (periopods) on segments 9 – 13 (Figure 1 B). In some species (Marine lobster, crabs, and some prawns) the second pair is clawed or chelated and may be used for capturing food or for defence (Figure 1 B). The penaeid shrimps and palinurid lobsters however do not have chelated appendages (De Grave *et. al*., 2008).). The appendages on the abdomen, called pleopods may be modified for swimming (swimmerets) and/or hold fertilized eggs in the females. Most decapod crustaceans (with exception of crabs) have a fan-shaped tail (comprised of telson and several uropods) (Figure 1 B). The abdomen, equipped with the paddle-like telson serves for locomotion, and it is often used to rapidly escape from potential predators (D'Abrano *et al*., 2012; Fearnley-Whittingstall & Fisher, 2007; Ishmael & New, 2000; Holthuis, 1980).



A: Crustaceans (Courtesy MESA, 2015)



B: External anatomy of Freshwater Prawn (from Hicks & Pierce II, 2011).

Figure 1 Crustaceans and External Morphology of the Prawns

The structural arrangement poses a unique challenge for growth, because these animals shed off their shell in the process of ecdysis or moulting for new and larger one to emerge, which is shed off in subsequent growth process (Plate 1). The moulting in juvenile crustacean is frequent (short intermoult period) with significant weight increases. (moult increment) (Karplus *et al*., 2000).

Plate 1: Empty Shell of *M. vollenhovenii*

The intermoult period gets longer and longer, and the weight gain (as a percentage) declines with increasing growth and size. Under culture conditions, particularly during the larval period mortality is most likely to occur during ecdysis. This is associated with nutrient deficiency referred to as “moult death syndrome” (Ravi Kumar *et al*., 2004); first discovered in hatcheries for marine lobsters caused by vitamin C deficiency, but probably not limited to that species (Fransen & De Grave, 2009). Apart from this challenge for most crustacean culture; the newly moulted ones become prey for others, the non-moulting animals attacking the vulnerable, unprotected soft-shelled newly moulted ones. The cannibalism is most pronounced when dietary requirements are not met or when they are stocked at high densities (Fransen & De Grave, 2009; Gillett, 2008; New, 2002).

The crustacean taxonomist, Tin-Yam Chan, stated that "the terms shrimp and *prawn* have no definite reference to any known taxonomic groups. Although the term shrimp is sometimes applied to smaller species, while *prawn* is more often used for larger forms, there is no clear distinction between both terms and their usage is often confused or even reverses in different countries or regions." (De Grave & Fransen, 2011). In recent aquaculture literature, however,

a distinction has been made between the two groups; the name prawn is used for freshwater forms of Palaemonids and shrimps for Penaeids (Appendix A) (Seabrook, 2012; De Gave *et al*., 2008; Paterson, 2007; Valencia and Campos, 2007; Bauer, 2004; Chan, 1998; Pillay, 1993; Holthuis, 1980). In the Caridea prawns (Freshwater prawns) the pleura of second abdominal segment overlaps both the 1st and 3rd somites or segemants; only the first two pairs of legs are chelated (Appendix A). In the Penaeidea shrimps (Marine shrimps), the pleura of second abdominal segment only overlaps the 3rd somite, and it is overlapped by the first. The shrimps therefore have sequential overlapping body segments; chelate (claw like) on the first three pairs of legs (Appendix B) (Carpenter & De Angelis, 2014).

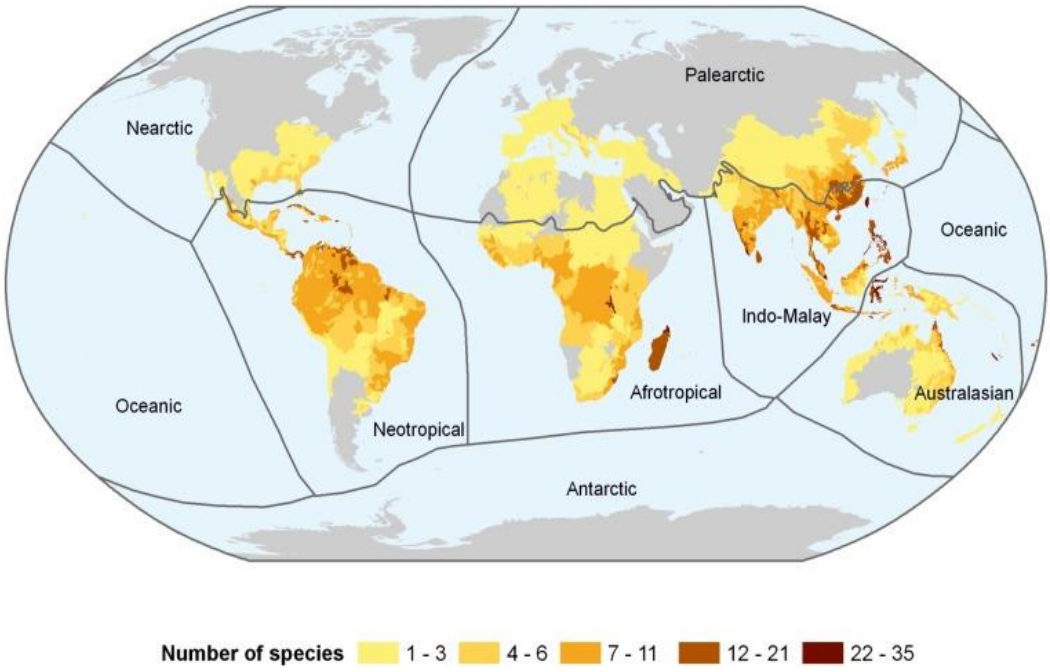
About 200 species of the prawns belonging to genus, *Macrobrachium* have been identified (Valencia & Campos, 2007); almost all of which live in freshwater or at least spend part of their life in freshwater (New, 2002).

2.2: *The Most Commonly Farmed Taxonomic Groups of the freshwater Prawns*

The freshwater prawns are a group of the most common freshwater crustaceans dominated by the Atyidae and Palimonidae (Appendix B) (De Grave *et al*., 2008; Bauer, 2004). They occur in a vast range of habitats from torrential mountain streams to sluggish oligohaline waters (Seabrook, 2012). Freshwater prawn species belong to eight families and 59 genera; the family Palaemonidae with 276 species is second largest after Atyidae with more marine and brackish water species known than the freshwater taxa. The genus *Macrobrachium* is the most dominant numerically in the family and are mostly restricted to brackish and fresh water bodies (De Grave *et al.* (2008). Holthuis (1980) listed 61 species of the Palaemonidae as either of commercial interest or forming an important component of subsistence fishing and culture especially in developing countries.

2.3: *Distribution of the Freshwater Prawns*

Species of *Macrobrachium* (Bate, 1815) are distributed throughout the tropical and subtropical zones of the world (De Grave *et al*., 2008) (Figure 2). They are found in rivers, lakes, brackishwater, irrigation ditches, canals, ponds and estuaries. Most species require brackish water at the initial stages of their life-cycle and are therefore found in waters that are directly or indirectly connected to the sea (Kaplan, 2010). Nonetheless, some complete their life cycle in inland saline and freshwater lakes (Alhassan, 2011; Marioghae, 1987; Prah, 1977); some prefer rivers containing clear water, while others prefer highly turbid waters (Holthuis, 1980).

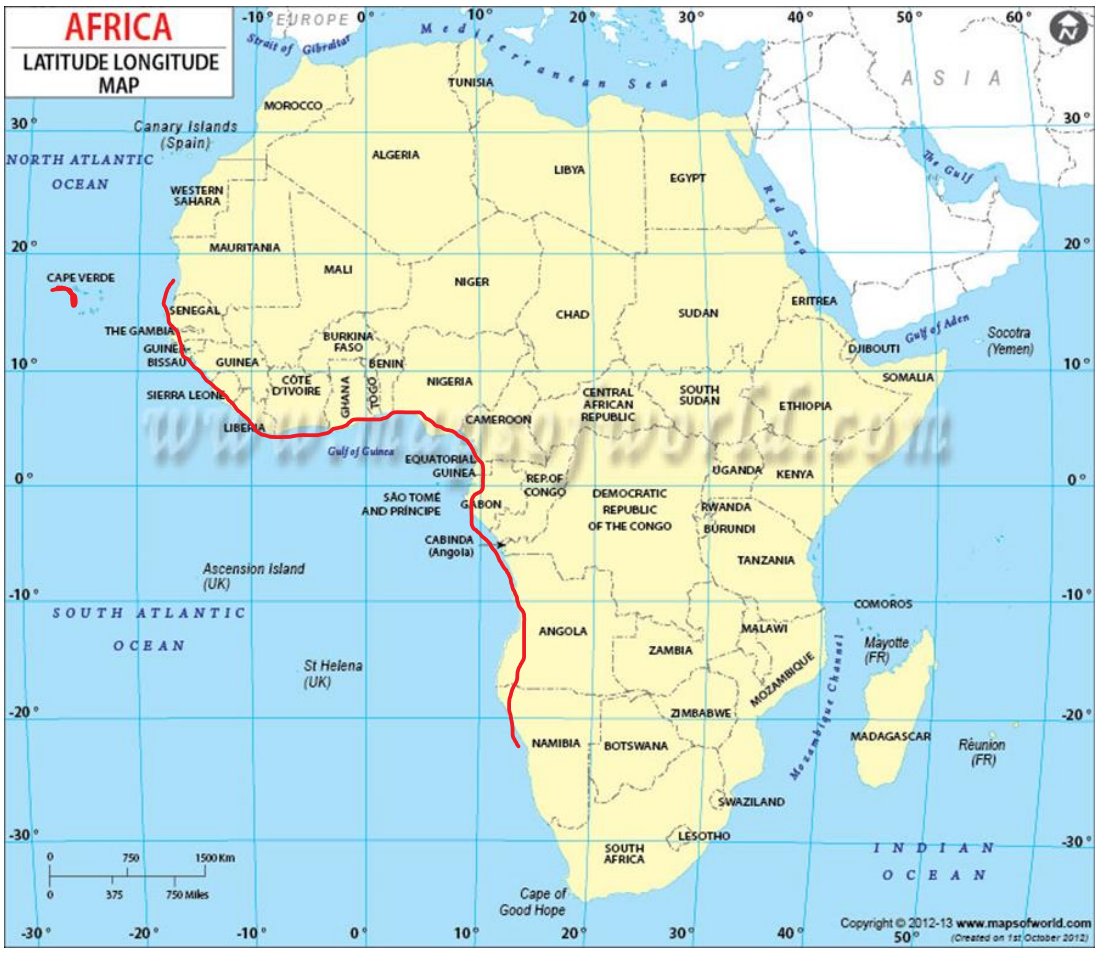


Adapted from De Grave *et al*. (2008)

Figure 2 : Global Diversity and distribution of Freshwater Prawns

The maximum size and growth rate vary among the prawns; the largest identified according to Carpenter & De Angelis (2014) include; the giant river prawns, *M. rosenbergii* (De Man, 1879): 26 – 34 cm total length; *M. americanum* (Bate, 1896): up to 23.5 cm total length (García-Guaerrezo & Hendrick 2009); *M. carcinus* (Linnaeus,1758) up to 30 cm total length (Kutty & Valenti 2009); *M. malcolmsonii* (Milne-Edwards, 1844); *M. choprai* (Tiwan, 1949)

and *M. vollenhovenii* (Herklots 1857): up to 18.9 cm total length (Carpenter & De Angelis, 2014). The *M. americanum* (Cauque river prawn) is found naturally in watersheds of Americas; *M. carcinus* (painted river prawn) is found in watersheds connected to the Atlantic Ocean; *M. choprai* (Ganges river prawn) found in the Ganges and Brahmaputra river systems; *M. lar* (Fabricius, 1798) (Monkey river prawn) found in East Africa to the Marquesas Island of the Pacific Ocean; *M. malcolmsonii* (Monsoon river prawn) found in waters of Bangladesh, India and Pakistan; *M. rosenbergii* (Giant river prawn) found in South and Southeast Asian area, Northern Oceanic and in the Western Pacific Island; *M. vollenhovenii* (Africa river prawn) found in Africa and naturally distributed in West Africa from Senegal to Angola and Cape Verde (Figure 3) (New, 2002, Pillay, 1993, Marioghae, 1987; Mwangi, 1984).



: Location of Africa River Prawn *Macrobrachium vollenhovenii: Map by* Bill Spicer (2017)

Figure 3: Distribution of *M. vollenhovenii* in the West Africa Sub-region

The Palaemonids have highly diversified reproductive and growth patterns (Sastry, 1983; Hartnoll, 1982; Powell, 1982). They therefore occupy a very important niche in the ecosystem in which they inhabit, either as preys or predators (Anderson, 1985; Fresi *et al*., 1984; Bell and Coull, 1978; Welsh, 1975).

The species of the *Macrobrachium* are mostly restricted to specific geographic regions (Anker, 2005). In the Eastern Atlantic, the African River Prawn, *Macrobrachium vollenhovenii*, is one of the endemic species with appreciable fisheries in the West Africa Sub-region (Powell, 1976; 1977; 1982; Nwosu and Wolfi, 2006) (Appendix D)

2.4: *Ecology and Life History*

According to Lara & Wehrtmann (2009), the species of *Macrobrachium* that complete their larval development entirely in freshwaters produce large but fewer eggs while those that need brackish water for the larval development produce relatively small but numerous eggs. The former according to Chowdhury (1993) are mostly not of commercial importance.

The freshwater prawns like their marine counterpart, the *Penaeus*, have four life stages, *viz*, eggs, larva, juvenile and adult, which is characteristic of crustaceans. The number of moults in their lifespan, duration of the moults and intermoults are not fixed, but rather depend on the environmental conditions, especially temperature and food availability (Chowdhury, 1993).

The fecundity is generally reported to depend on the size of the female. Mature female of 50 – 100 g laid 50,000 – 100,000 eggs, but a female at first maturity lay 5,000 – 20,000 eggs. The *M. rosenbergii* for instance, is reported to lay 80 000 to 100 000 eggs (New, 2002). Deekae & Abowei (2010) reported of 180 – 5,800 eggs per Brackish River Prawn, *M. macrobrachion* female from Luubara Creek Ogoni Land, Niger Delta in Nigeria indicating

strong correlation between the number of eggs and body size. Studies by Bhuiyan *et al.* (2007) on *M. dayanum* in Bangladesh as well showed that small size of 4.3 cm spawned 67 eggs and those of size 7.1 cm produced 95 eggs on the average. Kingdom & Erondu (2013) also indicated 11,402 eggs for size 6.70 cm and 56,000 eggs for 11.40 cm class size of females. These authors further indicated that relative fecundity increased with length of the egg-bearing female. Fecundity could therefore be attributed to species variation, size and environmental factors. Mating is reported to be all year round; intensity nonetheless, depends on the season with peak just before onset of rainy season (Ishmael and New, 2000; Karplus, *et al*., 2000).

Macrobrachium naturally breed all year round (D'Abramo *et al*., 2006; Choudhurry, 1993) with some seasonality. The female prawn, with mature gonads mate just after moulting with a hard-shell male. During mating the male deposits the spermatophore between the walking legs of the female. The female releases the eggs a few hours after mating. The eggs are fertilized as they are extruded from the gonophore of the female. The eggs are later transferred to the brood chamber, between the first three pleura of the female where they are held by a thin membrane and kept aerated by vigorous movement of the abdominal appendages. The incubation takes 21 days in the natural habitat at temperatures not below 28^0C. The female can lay eggs twice in a month (D'Abramo, *et al*., 2006; Nandlal & Pickering, 2005, New, 2002; Chowdhurry, 1993). Hatching takes place normally in the night (New, 2002).

The larvae are carnivorous, feeding on zooplankton, small insect and larvae of other aquatic invertebrates (Yamasaki-Granados *et al*., 2013; Murthy *et al*., 2008). In culture, their feed can be supplemented by high protein, locally made feed (Pereira de Barros & Valenti, 2003). The larvae take 22 - 40 days to metamorphose into post-larvae under culture conditions; the duration however depends on quantity and quality of food, water temperature and water

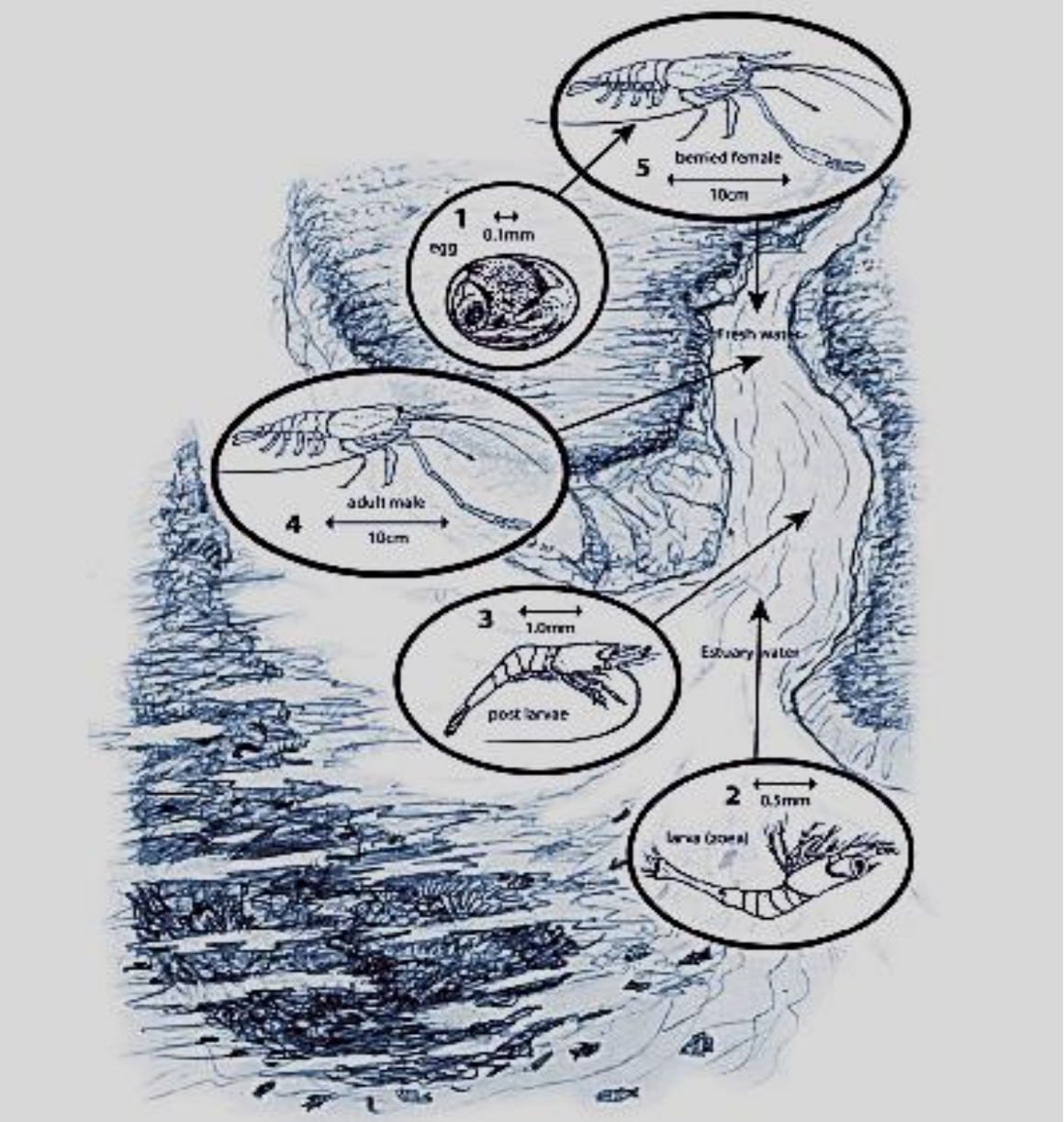
quality, light, genetics of the stock used and skill of the operator (New, 2002). The survival rate according to Nandlal & Pickering (2005) could be as high as 80% and as low as 10% based on these factors. Careful attention to all aspects of hatchery management is therefore essential to achieve success in the hatchery. During this period, the larva passes through eleven distinct stages in most species (D'Abramo *et al*., 2006; Nandlal & Pickering, 2005; New, 2002; Chowdhurry, 1993).

The live cycle of the freshwater prawns starts with the brood prawns (Appendix F), although spawning behaviour and larval development are quite variable. For *Macrobrachium* species, females generally become reproductively mature within 6 months of growth (D'Abramo *et al*., 2003). Mating occurs between hard-shelled males and soft-shelled females; females that just completed a prenuptial moult. The male deposits a gelatinous mass of sperm underneath the female body, between her fourth pair of walking legs. Eggs are laid within a few hours after mating and are fertilized by the sperm deposited by the male during mating outside of the female's body (D'Abramo *et al*., 2003). The eggs are then transferred to the abdominal (tail) region, into a "brood chamber," where they are kept aerated and cleaned by the movement of the abdominal swimming appendages. The eggs remain attached to the abdomen until they are hatched. The bright-orange colour of newly spawned eggs gradually changes to orange, then brown, and finally gray (Appendix F) about 2 to 3 days before hatching (Anderson, 1985).

After hatching, the larvae swim upside down with tail first. In most species that require brackish water in their life cycle, they need the brackish water within approximately 48 hours after hatching if they are hatched in freshwater (Nandlal & Pickering, 2006; Sandiffer, 1976). In natural conditions they migrate to brackish water with a salinity of 10 - 14‰. The larvae are aggressive sight feeders and feed almost continuously, primarily on small zooplankton,

worms, and larval stages of other aquatic invertebrates (D'Abramo *et al*., 2003; Sandiffer, 1976). The larvae undergo 11 moults in most species, each representing a different metamorphosis. Following the last moult, the larvae transform into post-larvae (PLs). Transformation from newly hatched larvae to post-larvae requires 15 - 40 days, depending on food quantity and quality, temperature, and a variety of other water quality variables (New, 2005; New & Valenti, 2000, New & Singholka, 1985).

After metamorphosis to post-larvae, the prawns resemble miniature adult prawns (Figure 4), about 7 to 10 mm long and weighing 6 to 9 mg depending on the water condition and species. At attainment of post-larval status the prawns change from planktonic life style to bottom dwelling, crawling individuals (D'Abramo *et al*., 2012)). When they do swim, they move like adults with the dorsal side (back) uppermost and head-forward direction. Post-larvae can tolerate a range of salinities and migrate to freshwater upon transformation (Nandlal & Pickering, 2005; Chowdhury, 1993; Sandiffer, 1976).



(Courtesy of Nandlal & Pickering, 2005)

Figure 4: Life Cycle of the Freshwater Prawns *Macrobrachium* Species

The food for the larvae include includes larval and adult insects, algae, mollusks, worms, fish, and faeces of fish and other aquatic animals. At high densities, or under conditions of food limitations, prawns become cannibalistic (New & Valenti, 2000, New & Singholka, 1985). The Post-larvae are translucent and may have a light-orange-pink head. As they change to the juvenile stage, they take on the bluish to brownish colour of the adult. Juveniles are intermediate in size between post-larvae and adults; however, no standard definition for the juvenile stage exists (Nandlal & Pickering, 2005; Chowdhury, 1993).

Marioghae (1987), reported of the following salinity and dissolved oxygen tolerance for some fresh water prawn species: *Palaemon maculates* 1– 30‰, *Palaemon elegans* 2‰ to marine conditions, *Palaemontes africanus* from freshwater conditions to 20‰, *Nematoplalaemon hastatus* 3‰ to marine conditions, *Macrobrachium vollenhovenii* from freshwater conditions to 27‰, *Macrobrachium macrobrachion* freshwater conditions to 10‰, *Macrobrachium felicinum* from freshwater conditions to 2‰, *Macrobrachium dux* from freshwater conditions to 2‰ (Padlan, 1982).

The post larvae (7 mm -10 mm; 6 mg - 9 mg) of the freshwater prawns, *Macrobrachium* species can tolerate a wide range of salinities and migrate to fresh water upon transformation (Brown *et al*., 2010). The adult sizes recorded include *Palaemon maculates* - 43 mm, *Palaemontes africanus* - 31 mm, *Macrobrachium vollenhovenii* - 189 mm, *Macrobrachium macrobrachion* - 138 mm, *Macrobrachium felicinum* - 88 mm, *Macrobrachium equidens* - 96 mm, *Nematoplalaemon hastatus* -75 mm (Nwosu & Wolfi, 2006; Jimoh *et al.*, 2012; Marioghae, 1987; Powell, 1982; Holthuis, 1980).

2.5 Freshwater Prawn Fisheries

In Ghana, several species of the prawn are found in freshwater bodies, most especially along the banks of the Volta River where they constitute a major component of the fisheries (Attipoe & Amoah, 1989). The most dominant species in Ghana is the *M. vollenhovenii (*African river prawn*)* (Alhassan, 2011 Attipoe and Amoah., 1989; Rutherford, 1971).

The *Macrobrachium* species could therefore be fished out in lagoons, creeks, streams, rivers and estuaries with dip netting, fixed bag netting, push or drag netting and traps (Rahman, 2001; Bardach *et al*., 1976). The adults are mostly found in turbid sandy freshwater bodies (Maciel *et al*, 2011; Barko & Hrabik, 2004; Robison & McAllister, 2011) in both moderate and slow flow rates (Barko & Herzog, 2003). They aggregate at banks of rivers and streams

during in floods when plant and animal materials are available for foraging (Truesdale & Mermilloid, 1979). Hobb (2001) reported that the prawns receive reproductive cues from floods and use the flooded terrestrial habitat for their reproductive activities. The freshwater prawns therefore inhabit both lotic and lentic water bodies; in the open, fringes and vegetated banks of these water bodies (Hall *et al*. (2011; Robison & MaAllieter, 2011; Bouchard & Robison, 1980). The prawns therefore constitute a major fishery of the people in the riverine areas in support of their livelihood.

2.6 Global Prawn Culture Potential and Practices

Prawns have been farmed using traditional methods in south-east Asia for a long time. The traditional method involved stocking and holding young prawns in ponds, tanks, rice fields and other productions systems until they are matured for harvest (Sukumaran & Muthukumaran, 2004; Miao & Ge, 2002). The prawns can also be combined in polyculture with finfishes in freshwater culture systems (Miao & Ge, 2002). Farmers with some experience in Tilapia and other freshwater finfish farming could easily culture the prawns (Nandlal & Pickering, 2006; Costello, 1971). The freshwater prawns are territorial and are therefore stocked at low densities in grow-out facilities without excessive pressure on the environment. The prawn farming could therefore be regarded as environmentally friendly and sustainable (Nandlal & Pickering, 2006; Sukumaran & Muthukumaran, 2004; Pereira de Barros & Valenti, 2003). The giant river prawn, *Macrobrachium rosenbergii* forms the basis for intensive prawn farming since the 1960s (De Grave, *et al*., 2008; FAO, 2005). Some other species commonly farmed include oriental river prawn *Macrobrachium nipponense* in China, the monsoon river prawn *Macrobrachium malconsoni* in India. Several other species are also available in the freshwater aquarium trade (FAO, 2005; Werner, 2003; FAO, 2002; 2001; FAO, 2000); culture potential of a number of the prawns is under research considerations.

First experiments with artificial breeding of *M. rosenbergii* were done in the early 1960s in Malaysia, where it was discovered that the larvae needed brackish water for survival (Chowdhury, 1993). Industrial-scale rearing processes however were perfected in the early 1970s in Hawaii (New, 2002). The technologies used in freshwater prawn farming are basically the same as in marine shrimp farming; hatcheries produce post-larvae, which are grown and acclimated in nurseries before being transferred into grow-out ponds, where the prawns are grown to marketable size (New, 2002). The farming of the prawns is nonetheless not as technically demanding and capital intensive as farming of the marine shrimps. It is therefore more accessible system for small-scale operators (Nandlal & Pickering, 2006; D'Abramo, 2003).

According to Choudhurry (1993), the earlier breakthrough in hatchery production was achieved using "green water" (plankton laden water) as food for the larvae. The green water system is however, being replaced by circulatory system that provides cleaner water. Three hatchery systems: i) flow through system ii) recirculating static iii) recirculating dynamic (New & Valenti, 2000) are being used to produce quality and reliable post-larvae mainly in the Asian countries. The larval development of caridean crustaceans that may have potential for aquaculture is highly variable and is one of several critical components to realizing commercial-scale farming. (De Grave *et al*., 2008).

2.7 Prawn Species for Culture in the West Africa Sub-region

The *M. vollenhovenii* commonly found in Ghana has the maximum total length of 189 mm (Holthuis, 1980) in the same size class as *M. americanum* (Bate, 1896), *M. carcinus* (Linnaeus, 1758) and *M. malcolmsonii* (Edwards, 1844). It is hardy in many ways and survives in waters with dissolved oxygen as low as 1‰. Experiment by Mwangi (1984) in Kenya proved experimentally that the species can tolerate salinities ranging from freshwater

condition up to 22‰. This species may therefore be cultivated in a wide range of salinities. *M. vollenhovenii* appears to be quite safe in ponds with pH below 6, even if the water is badly fouled; this species (like many members of the genus) can climb out and survive out of water for about 12 minutes (Marioghae, 1982) and could be farmed successfully in the West African sub-region as an alternative to species farmed in other parts of the world (Kingdom and Erondu, 2013; FAO 2000; Willfuhr-Nast *et al*., 1993).

M. vollenhovenii like others is omnivorous detritivore with preference for animal remains (Marioghae, 1982). Mwangi (1984) showed that this species preyed effectively on frog tadpoles and the fry of *Tilapia zilli* and *Oreochromis niloticus* and could be used to control excessive breeding of the tilapias in culture facilities (Marioghae, 1987). The larvae of the species cultured elsewhere were observed to complete the larval life in 16 days (New & Singholka, 1985). At the end of the larval life the prawn developed into post-larval stage, a miniature adult that crawls rather than swims freely.

There were several unreported and undocumented attempts to culture the *M. vollehnovenii* in Ghana. The documented ones included the work of Prah in 1982 and 1977 when he undertook feasibility study of culture of the prawn in a small reservoir along the Accra-Tema Motorway in Ghana (Prah, 1982; 1977). The latest recorded work was in 2005 when a team from Stirling University assisted aquaculture scientists in the culture of the prawn in Ghana (New, 2005). There was no work however on the hatchery of the prawn published in the country.

2.8 Prawn Culture Systems

Well established prawn culture systems involve three phases: i) breeding, ii) nursery iii) grow out. The first two (breeding and nursery) are normally referred to as hatchery phase and form the basis for any viable commercial or large-scale aquaculture operations (SRAC, 2006; Nandlal and Pickering 2005). The hatchery phase of any farmed organism starts with development of the parental stock (brood stock) to ensure sustained production of adequate

and desired quality seed (Mohanta, 2000). According to New (2002), the brood stocks with eggs could also be easily obtained from the wild in the tropics and kept in hatcheries until the eggs are hatched. Many hatcheries therefore use this method to produce post-larvae (Mohanta, 2000; Hsiech *et al*., 1989; Chowdhury, 1979). Dependence, however, on the wild brood stocks may affect planning and management of the hatchery due to seasonal fluctuations and quality assurance of the offspring in any prawn culture anywhere (Mohanta, 2000). The collection of brood stock from the wild referred to as Capture-Based Aquaculture is however ideal for aquatic species under investigation to perfect their culture process and for small scale fish farming in extensive and semi-intensive systems (FAO, 2004).

The freshwater prawns are suitable for cultivation in tropical and subtropical climates and grow within temperature range of 22 – 32^0C (Nandlal & Pickering, 2006; New, 2005). They are hardy and have ability to adapt to various types of fresh and brackish-water conditions. They thrive in waters with dissolved oxygen range of 3 – 7‰; pH 7 – 8.5 and can tolerate nitrogenous compound up to 0.3 ppm (New, 2002). It accepts pelleted feed and has omnivorous feeding habit. Although hardy, Nandlal & Pickering (2006) reported that they are sensitive to water quality and require proper water quality management to ensure successful culture. The authors further indicated that the prawns share culture technology with the tilapias apart from being more sensitive to water quality parameters than the tilapias. It is therefore crucial for prospective prawn farmers to investigate their culture requirements and devote time and attention at every stage in the culture process to succeed.

Although the hatchery phase of the prawn culture requires brackish water, hatcheries do not necessary need to be located at the coast, they can be sited inland (New, 2002). Every hatchery nonetheless, requires site specification for optimum production and cost effectiveness (Nandlal & Pickering, 2005). The requirements for hatcheries and indoor nurseries are similar and can be operated as such (New, 2002). Factors to be considered in establishing prawn

hatcheries include quality freshwater supply, access to brine, and availability of electricity; adequate drainage system and availability of skilled and non-skilled labour (Chowdhury, 1993). The success of prawn hatchery, according to Chowdhury (1993) depends on good water quality and that problems caused by poor water quality could hardly be addressed successfully. Reliable quality water supply therefore plays a crucial role in sustained production of prawn seeds (Nandlal & Pickering, 2005; News, 2002).

The prawn hatchery systems so far described include: Flow Through system (Correia *et al*., 2000); Recirculating Static and Recirculating Dynamic systems (New & Valenti, 2000) have been used to produce prawn seeds in hatcheries. The plan and design of the hatchery nonetheless depend on the production targets, weather conditions and other localized conditions including finance. There is therefore no prototype hatchery (New, 2002; Chowdhury, 1993). The earliest system developed through research in the 1960s by Takuji Fujimura was the "green water", a variant of the flow-through system (New, 2002; Chowdhury, 1993,). This system was supplemented by the clear water technique that uses biological filtration in Recirculating Dynamic or Recirculating Static systems (New, 2002; Chowdhury, 1993;).

Prawn hatcheries need some level of shading that could be provided using local materials or roofing sheets; different types, shapes and volumes of water holding facilities are reportedly used in the prawn hatcheries with success (Nandlal & Pickering, 2005; D'Abramo *et al*., 2003; New, 2002; Chowdhury, 1993). Sukumaran & Muthukumaran (2004), however, reported of outdoor system that reduces water exchange rate, electricity and feed cost with cut down in culture period by 20%. Prawn hatcheries therefore do not necessarily need complex system to achieve success. The water holding facilities being used in the hatcheries should have smooth surface and the inside corners rounded off to ensure proper cleaning, better water circulation and prevention of aggregation of the larvae in the corners that may lead to injuries

and mortalities (New, 2002; Chowdhury, 1993). According to Nandlal & Pickering (2005) and Chowdhury (1993) conical shaped bottomed tanks are more efficient for hatching of eggs and early growth stages of larvae. Both hatching and at least larval development at the early stages can take place in the same holding facilities (Nandlal & Pickering, 2005; New, 2002; Chowdhury, 1993).

Generally, some treatment is required to make water suitable for hatching of eggs and larval rearing in the hatcheries. D'Abamo *et al*. (2003) and New (2002) recommended salinity of water in hatching tanks to vary from 0 - 5‰ at 25 – 31^{0}C and pH 7.0 – 7.2 with dissolved oxygen not less than 5 mg/L (New, 2002). Although, eggs could hatch in purely freshwater, slight salinity, according to Law *et al*. (2001) resulted in better egg hatchability. The eggs are observed to hatch mostly in the night; the newly hatched larvae are noted to be attracted to light (D'Abramo *et al.*, 2003; New, 2002; Chowdhury, 1993). Nandlal & Pickering (2005) recommended that salinity of the larval water should be increased daily at 3‰ rate until the 12‰ required by the larvae is reached; Prior to this the brood prawns should be removed soon after the eggs are hatched.

It is generally reported that the larvae are reared through the eleven (11) stages into post-larvae within 22 – 42 days depending on temperature, food quality and availability, volume and quality of water, stocking density, genetics of the prawn involved and management skill of the operator (Nandlal and Pickering, 2005; Chowdhury, 1970). New & Singholka (1985), however reported of *M. vollenhovenii* completing the larval stage in 16 days. The challenges in culture of the prawn larvae are to create conditions that mimic natural environment or to provide critical conditions necessary for survival. The prawns in the natural environment live in low densities and are free to move to areas of suitable water quality and desired food resources. New (2002) therefore recommended that larvae could be stocked at 60 – 100 individuals/L if they are to be grown to PL in one tank; 500 individuals/L initially and later

thin to 50 individuals/L into several tanks after about 10 days when they reached the sixth larval stage in a two-stage rearing method or stock at 100 individuals/L in 35 – 45 cm depth of water and gradually increase the depth to 70 – 90 cm (this is an improvement in the first method and avoids stress in the thinning method, which involves collection and transfer of the larvae from one tank into another). Survival of the larvae depends on conditions created in the hatchery and varies from 10 to over 80% (Yamasaki-Granados *et al*., 2013; Murthy *et al*., 2008; Nandlal & Pickering, 2005; Murthy *et al*., 2004; Choudhury, 1979; Murthy & Satheesha, 1998).

In the natural environment, the larvae are inclined to carnivorous feeding habit that gradually changes with time to omnivorous tendency as they grow (D'Abramo *et al*., 2003, New, 2002, New and Singholka, 1985). In culture, the larvae can be fed inert formulated feed (Murthy *et al*., 2008). Some researchers, however, indicated that there is no nutritionally complete formulated diet currently available for successful larval culture, live food is therefore, required (D'Abramo *et al*., 2003; New, 2002) and can be produced according to the age of the larvae. The protein levels in the inert formulated feed for the larvae suggested by Sukumaran and Muthukumaran (2004) should vary from 27 – 35% with higher levels, but not exceeding 40%, for juveniles. A wide variety of feeds including nauplii and flaked adult of brine shrimp (*Artemia* spp.), freshwater cladoceran (*Moina* spp.), fish eggs and flesh, flesh of molluscs, worms, egg custards and commercial feeds, according to Murthy (1998) and New (2002) and other researchers, are used by prawn hatchery operators. The prawn larvae can therefore be fed with live, organic and inert food materials with varying levels of success. Dependence on feed materials that are not readily available and expensive makes prawn culture unsustainable, expensive and non-profitable (Murthy *et al*., 2008). Pereira de Barros & Valenti (2003) investigated acceptance and varying sizes of different types of food materials for *M. rosenbergii* larvae and came to the conclusion that total replacement of live

feed by organic and inert feed materials might be possible in stages 7 to 11 (ZVII to ZXI). The need for live feed according to their work is crucial at least in the first-six stages (ZI - ZVI) stages of growth; they however did not establish any difference in growth with regards to varying particle size of inert feed materials.

CHAPTER THREE

SPATIAL AND TEMPORAL DISTRIBUTION OF FRESHWATER PRAWNS IN THE LOWER VOLTA RIVER IN GHANA

3.1 Introduction

The freshwater prawns, *Macrobrachium* species are widely distributed throughout tropical and subtropical zones of the world and constitute a large proportion of macro invertebrates of high economic value in almost every aquatic ecosystem (Jimoh *et al.,* 2012). Brackish water is requirement for some of these species in the larval stages of their life cycle and are therefore found in lagoons and estuaries directly or indirectly connected with the sea, although some complete their life cycle in inland saline or freshwater lakes. Some species prefer clear-water while others are found in turbid conditions (New, 2002; King, 1995; New & Singholka 1985, 1982). *Macrobrachium vollenhovenii* for instance is naturally found in West Africa as *M. lar* is native to East Africa to the Marquesas Islands of the Pacific (De Grave *et al*, 2008).

In the Volta Estuary of Ghana, *Macrobrachium* spp. are found together with the marine *Paenaeus* spp, especially at higher salinity zone. The juveniles of the freshwater prawns were therefore reported to occur in the Volta estuary and aligned water bodies (Addai, 2010; Darpaah, 2009; Atsu, 2003; Dankwa, 2002 & 1998). Other researchers reported of similar trend in a number of estuaries and opened lagoons in other areas (D'Abramo & Brunson, 1996; Padlan, 1982; Gosner, 1978).

The number of species in the Volta Estuary may dwindle due to ecological changes brought about by the construction of Hydroelectrical dams on the Volta River system upstream and fishing pressure (Alhassan, 2007; Gordon 1999; Attipoe and Amoah, 1989) as well as other anthropogenic activities. Disregard to fisheries laws and regulations in Ghana by the fishers in the fishing industry may further worsen the conditions of the prawn resource to its extinction. The need for prudent steps to prevent possible loss of the resource is therefore

crucial for sustainability and maintenance of the livelihood of people who depend on the prawn fishery.

Unfortunately, much work has not been done on the freshwater prawns in the estuary. This study is therefore aimed at collecting data on the occurrence of the crustaceans in the lower portion of the Volta River with focus on the Volta Estuary in Ghana to establish their occurrence and for informed management decisions.

3.1.1 Objectives of the Study

The main objective of the study is to determine the occurrence and the distribution of the freshwater prawns in the Lower Volta River of Ghana below the Kpong hydroelectrical dam at Akuse with focus on the Volta Estuary.

The specific objectives were to determine the:

I. Occurrence of the freshwater prawns and related crustaceans in the Lower Volta River with focus on the Volta Estuary.

II. The spatial distribution of the *Macrobrachium* and related species in the Lower Volta River below the hydroelectrical dam at Akuse.

III. Determine the dominant *Macrobrachium* species in the Volta Estuary.

Hypotheses

Ho: The freshwater prawns in the Volta Estuary and its aligned water bodies are evenly distributed temporally and spatially.

Ha: The distribution of the freshwater prawns in the Volta Estuary and its aligned water bodies are based on the seasons and location.

Ho: There is no dominance in the freshwater prawns found in the Volta Estuary.

Ha: The Africa River Prawn *M. vollenhovenii* is the dominant freshwater prawn in the Volta Estuary.

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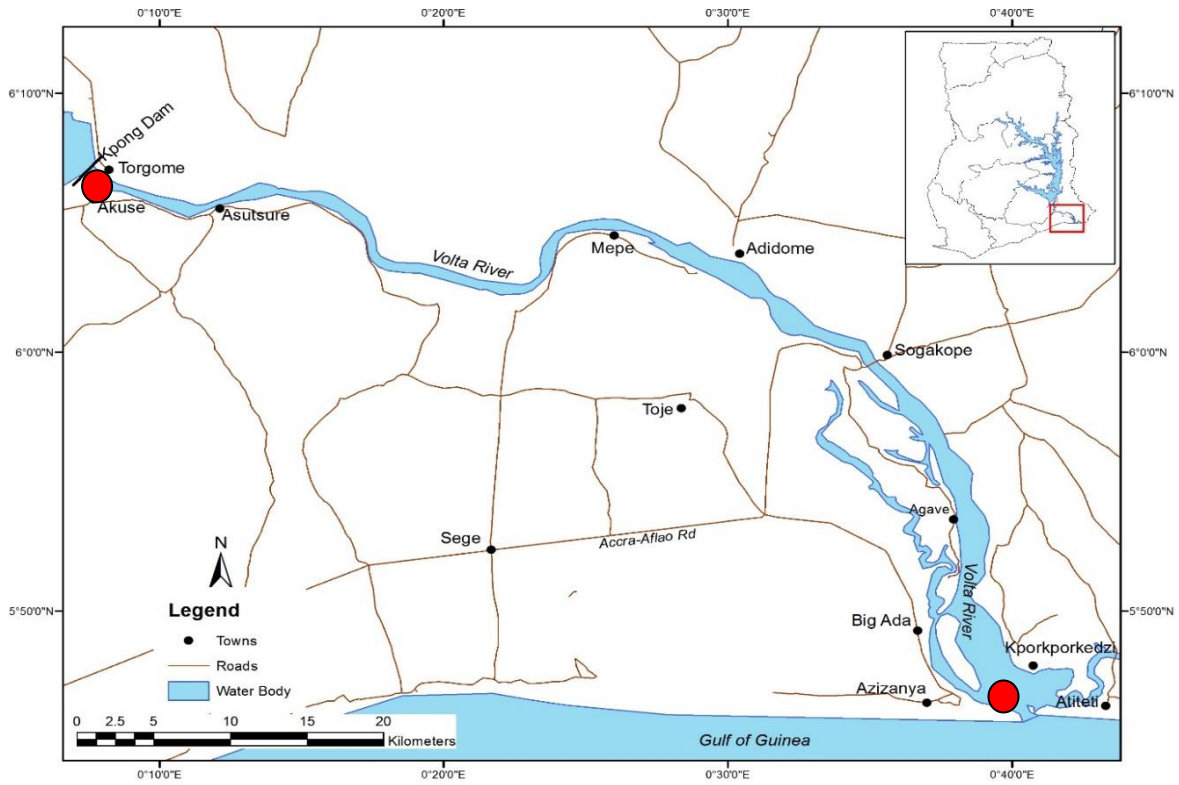
3.2: Materials and Method

3.2.1 Study Area

The study was conducted in the lower Volta River in Ghana at two key stations namely: i) the Estuary at Ada Foah (5º47'N 0º38'E) in the Greater Accra Region ii) Torgome (6º7'12'N 0º7'30'E) in the Volta Region (Figure 5). Torgome is separated from Akuse by River Volta in the Eastern Region where the Kpong hydroelectrical dam is installed. The study focused on the estuary due to the fact that the feasibility survey prior to the start of the study indicated that the prawns were mainly harvested in and around the estuary and sold in the main Ada market at Kase (Figure 6) about 2 hours' drive east of the capital city Accra on the main Accra-Aflao road. The Volta River empties into the Gulf of Guinea at Ada Foah (Figure 6), a coastal town of Ghana and the capital of Ada East District of Greater Accra Region in Ghana. Ada Foah is about a kilometer by road from the tradition capital of Ada, Big Ada (Figure 6); both Ada Foah and Big Ada are along the estuarine environment of the Volta River. Other aquatic fish commonly found in the area include the Volta Clam, *Galatea paradoxa*, crustaceans and finfishes (both marine and freshwater species) all of which are sent to the Kase market on Tuesdays and Fridays (the market days).

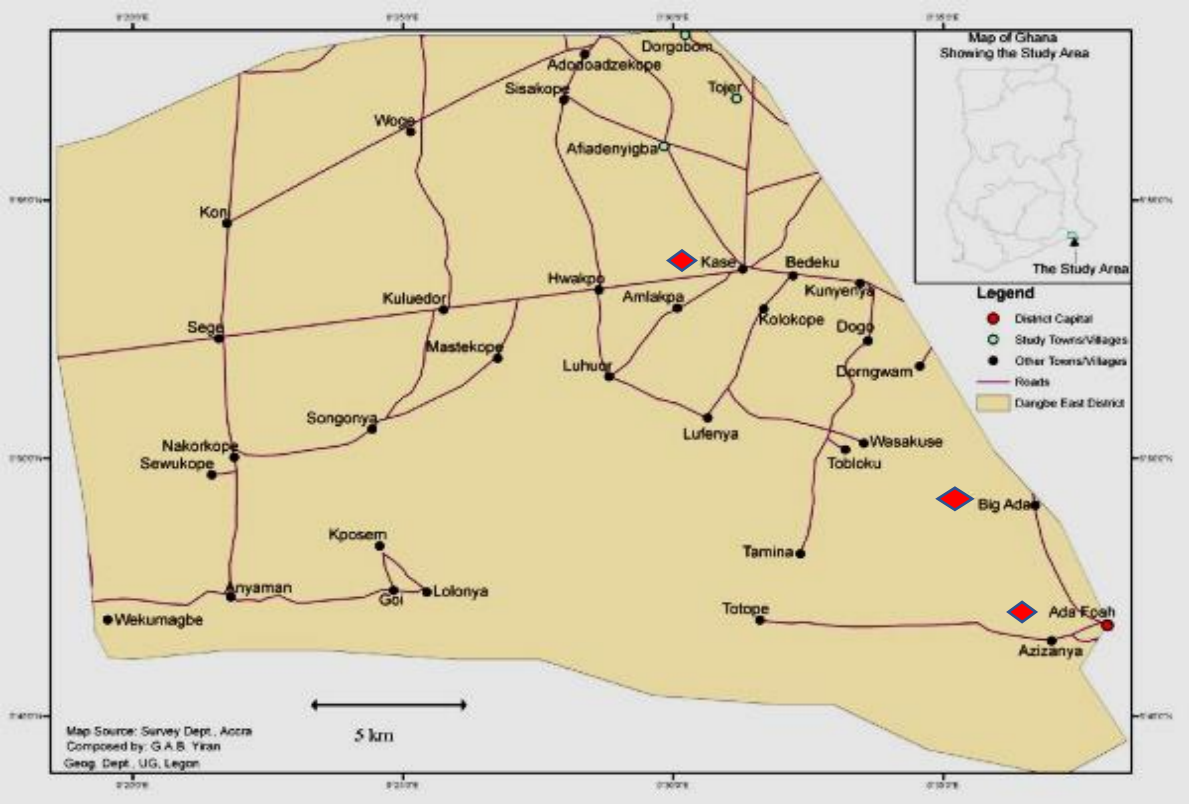
The Ada area is within the coastal savanna zone and the main occupation of the citizens are farming and fishing. There are numerous tourist sites such as forts, estuary, holiday chalets, and beautiful beaches along the bank of the Volta River and that portion of the Gulf of Guinea which attract tourists from all walks of life.

Torgome (Figure 5), on the other hand is a small farming and fishing village below the Kpong hydroelectrical dam at Akuse in the purely freshwater zone of the River Volta. Torgome is located north of the Volta Estuary, among the aquatic organisms fished in this area are the freshwater prawns (*Macrobrachium* species) and crayfish (*Atya* species) in addition to variety of finfishes.



● - Focal Sampling Stations

Figure 5: Map of the Lower Volta River in Ghana showing the study area



◆ : Locations of Activities during the study

Figure 6: Map of Ada East District in Ghana (Ayi *et al.*, 2010)

3.2.2 Field Work

Data collection was undertaken from the Volta Estuary to Torgome in 12 months (from August 2013 to July 2014). Activities involved interaction with local prawn fishers through interviews, procurement of prawn samples from fishers and experimental fishing during the study period with help of prawn fishers. These sampling station were used based on results of the interactions with the prawn fishers and prawn mongers.

At the Volta Estuary, three zones (Zone A, B & C) were demarcated at one km intervals based on the salinity regime: Zone A; from the mouth of the estuary, Zone B; middle portion and Zone C; the freshwater end of the study area (Figure 7).

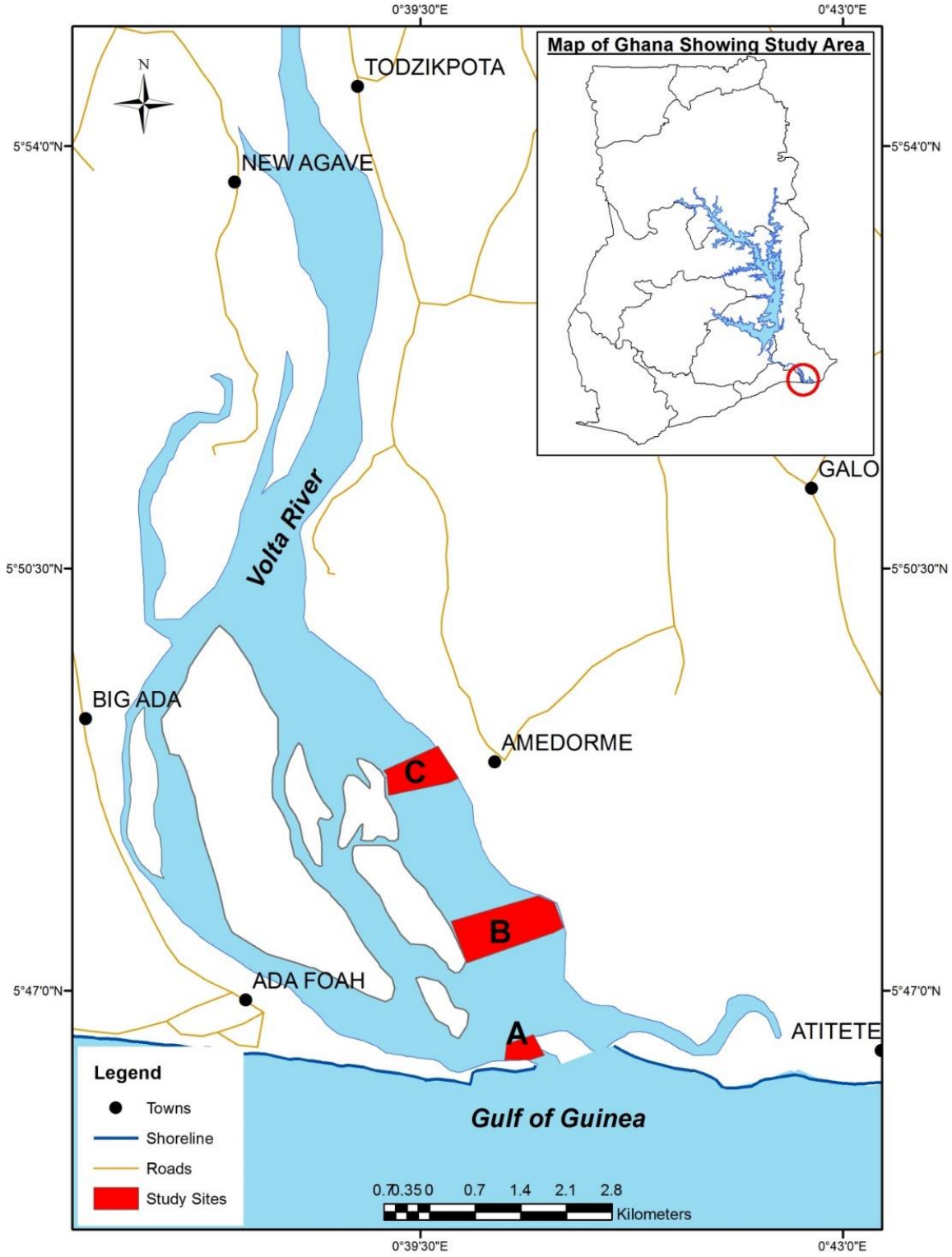


Figure 7 Map of Volta Estuary of Ghana showing the Sampling Zones

Two types fishing gears were used to collect the prawn samples: drag nets and basket prawn traps. The experimental fishing was carried out at each of the three zones using mainly two types of drag nets (Plates 2); fine size mesh net (square mesh size 0.6-0.7cm) (Plate 2 A) and seine fishing net of stretched mesh size mesh size of 2.5 cm (Plate 2 B). The length of the seine fishing net was 100 m and depth 5 m and was used by hired fishers at depth range of 1 to over 6 m in all the three zones.

Basket prawn traps (Plates 3 A & C and Figure 8) were used mainly in Zones B and C where the wave action is low; the traps were made from strips of bamboo, cane/sticks and palm branches woven into conical baskets with an opening at the wider end (Plate 3 A and C). The total average length of the basket traps ranged from 40 to 45 cm with the wider end 14 to 16 cm in diameter. The openings varied from 3.5 to 5 cm in diameter (Figure 8).

The fine mesh net was used in Zone A and part of Zone B at depth ranging from 1 to 1.2 m from the mouth of the estuary to about 2 km upstream.

The traps were set from about 15 hours GMT and retrieved the following morning between 6.00 and 9.00 hours GMT. The smaller size traps were deployed at shallow parts of the river and the bigger ones at mid-water at depths ranging 3 to over 6 meters. The traps were fitted with floats for easy location and were baited with coconut, palm nut, palm kernel oil cake (Plate 3.2 B), fish, animal and plant materials.

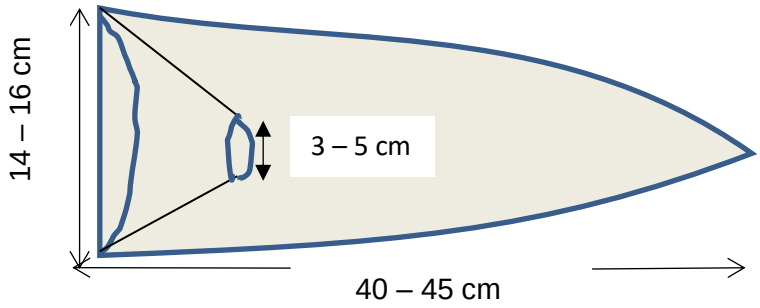
Plate 2: The Drag Nets used in the study at the Volta Estuary

A: Freshwater basket Prawn Trap

B: Palm kernel oil cake as prawn bait

C: Prawn Fisher inspecting prawns trap in the Volta Estusry

Plate 3: Basket Prawn Trap and Bait



Schematic Sketch of Freshwater Basket Prawn Traps

Figure 8: Sampling Zones in the Volta Estuary of Ghana

At Torgome, similar fishing methods as done in the estuary for the prawns were undertaken using the traps mainly (Plate 4).

Plate 4: Prawn Fishing at Torgome

3.2.3. Identification and Morphometric Measurement

The samples were transported on ice in ice-chest to the laboratory at Department of Marine and Fisheries Sciences, University of Ghana, Accra. They were sorted into species, size classes and sex in the case of *M. vollenhovenii* which was the focus of the study. Identification of species was done using identification guides by Powell (1982) and Rutherford (1971) following the keys presented in Appendix B. Total length measurements were done using Fish Measuring Board and a Vernier calipers. An electronic balance (Philip Harris No A20002, Ashby Park, China) was used for weight measurement to the nearest 0.1 g (Appendix C).

3.2.4: Water Quality Parameters

Water quality parameters such as; pH, DO, Salinity, Conductivity, Total Dissolved Solutes (TDS) were measured in-situ using electronic meter, HANNA HI9829 (HANNA Instruments Inc. USA, in-situ. Nutrients; phosphate, nitrate, sulphate were measured in the laboratory using Hatch equipment, DR 2800 according to HACH (2005) procedures.

3.2.5 Summary and Analysis of Data:

Data was summarized using tables and graphs in Microsoft Excel 2007and 2010 editions.

The nonparametric Kruskal-Wallis test (One-way Analysis of Variance), Student's t-test (Sokal and Rohlf, 1987) and Chi Square tests were used for test of hypothesis. The significance threshold was set at $p<0.05$.

For species diversity, Shannon-Weiner's Diversity Index (H') (1949), Pielou Index of Evenness (J') (1966) and Margalef's Richness Index (d') (1958) were used according to the following formulae:

Shanon Weiner's Diversity Index; $H' = nLogn - \frac{\sum fiLogfi}{n}$ (Shannon,1948).

where n = the total number of individuals present & fi = the number of occurrence of the ith term

Peilou's Index; J' = $\frac{H'}{Hmax}$ (Pielou, 1966)

Where H' = Shanon Weiner's index & Hmax = Log K, K = number of species present.

Margalef's Index; $d' = S - \frac{1}{Ln(N)}$ (Margalef, 1958)

Where S = number of Species present & N = the total number of individuals present.

Principal Component Analysis (PCA) (Pearson, *1901),* a non-parametric statistical technique was used to examine the interrelations among the set of variables in order to identify the underlying structure of the variables.

Correlation was performed on the number of crustaceans encountered and salinity in the sampling zones to establish the impact of salinity on distribution pattern of the crustacean in the study area.

3.3 Results

3.3.1 Occurrence and Distribution of Crustaceans Encountered in the Study Area

The crustacean species encountered during the study included *Macrobrachium vollenhovenii* (Herklots,1857), *Macrobrachium macrobrachion* (Herkots, 1851), *Atya gabonesis* (Giebel, 1875) and *Penaeus* species (Table 1).

Table 1: Crustacean species encountered in Lower Volta River (Torgorme to the Estuary at Ada).

Taxon	Species			
	1	2	3	4
Class	Malacostraca	Malacostraca	Malacostraca	Malacostraca
Order	Decapoda	Decapoda	Decapoda	Decapoda
Infraorder	Caridea	Caridea	Caridea	Penaeidea
Family	Palaemonidae	Palaemonidae	Atyidae	Penaeidae
Genus	Macrobrachium	Macrobrachium	Atya	Penaeus
Species	*M. vollenhovenii* (Herklots, 1857	*M. macrobrachion* (Herklots, 1851)	*A. gabonensis* (Giebel, 1875)	Not identified to species level
English Name	African River Prawn	Brackish River Prawn	Gabon shrimp	

Figure 9 shows the proportions of the three genera of the crustacean species (*Macrobrachium*, *Atya* and *Penaeus*) encountered in the Volta Estuary. The *Macrobrachium* species was the most dominant, recording 73% (86,634) and *Penaeus* species recording 27% (32,787); *Atya* species encountered was 37 individuals with negligible proportion compared to the *Macrobrachim* and *Penaeus*. The genera *Macrobrachium* and *Penaeus* were therefore more common in the sample from the estuary and its environs. Between the two genera, the

Macrobrachium was more dominant (*M* = 7219.5 ± 3029.3) than the *Penaeus* (*M* = 2732.3 228.5); *t*(22) = 4.96, *p* <0.05.

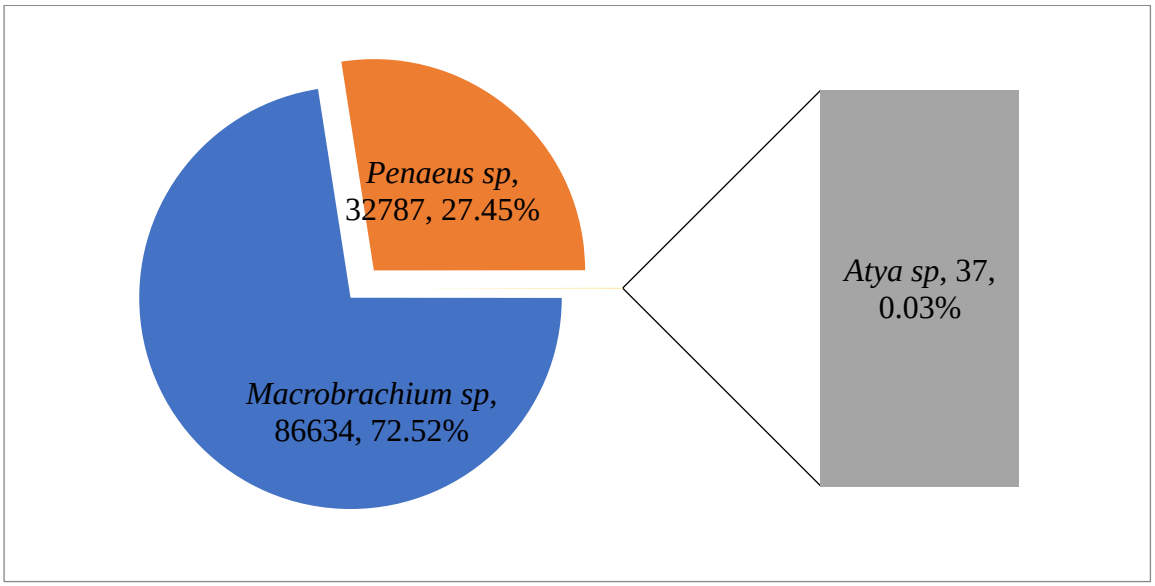


Figure 9: **Relative Abundance of the caridean crustaceans encountered in the Volta Estuary**

Figure 10 portrays the protional presentation of the two species of the genus *Macrobrachium: M. macrobrahion and M. vollenhobenii* observed in the Volta Estuary durung the study. The dominant one was *M. vollenhovenii* (75%) and *M. macrobrachium (25%).* Chi-square test for homogeneity showed the number of *M. vollenhovenii* samples was significantly higher than the number of *M. macrobrachion* sampled (χ^2 = 1.34, p<0.05).

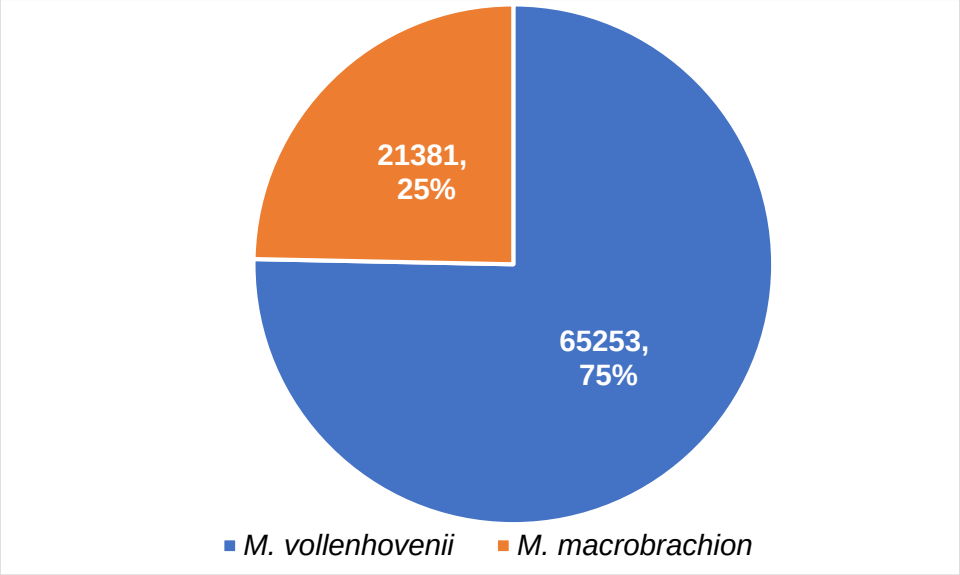


Figure 10: Species of the Genus *Macrobrachium* Encountered in the Volta Estuary

In Figure 11, all the three genera (*Atya, Macrobrachium and Penaeus*) were present in Zone B; *Macrobrachium* and *Penaeus* species occurred in Zone A, while *Macrobrachium* and *Atya* species occurred in Zone C.

In Zone A (Figure 11) located at the confluence of the estuary, the dominant crustacean was the *Penaeus* (82%). The lesser occurring *Macrobrachium* species recorded 18%. In Zone B, located after Zone A into the river, about 2 km from the confluence of the river and the sea *Macrobrachium* species was the dominant species (82%) and the *Penaeus* species (18%), a reverse of the pattern in Zone A (Figure 11). The occurrence of *Atya* species in Zone B was minimal (less than 1%). Zone C located at the upper reaches of the river (about 3 km from the confluence of the river and the sea was dominated by *Macrobrachium* species and a few number (20 individuals) of *Atya* species with negligible proportion. The *Penaeus* species was completely absent in this zone.

Two sample t-test assuming equal variances affirmed the dominance of the *Penaeus* (M = 2009.42, SD = 208.29) as compared to *Macrobrachium* species (M = 423.333, SD = 15.11);

$t(22) = 7.55$, $p<0.05$ in Zone A. In Zone B, a t-test clearly confirmed the dominance of the *Macrobrachium* ($M = 3196.00$, $SD = 302.53$) over the *Penaeus* ($M = 722.83$, $SD = 40.90$); $t(22) = 8.10$, $p<0.05$.

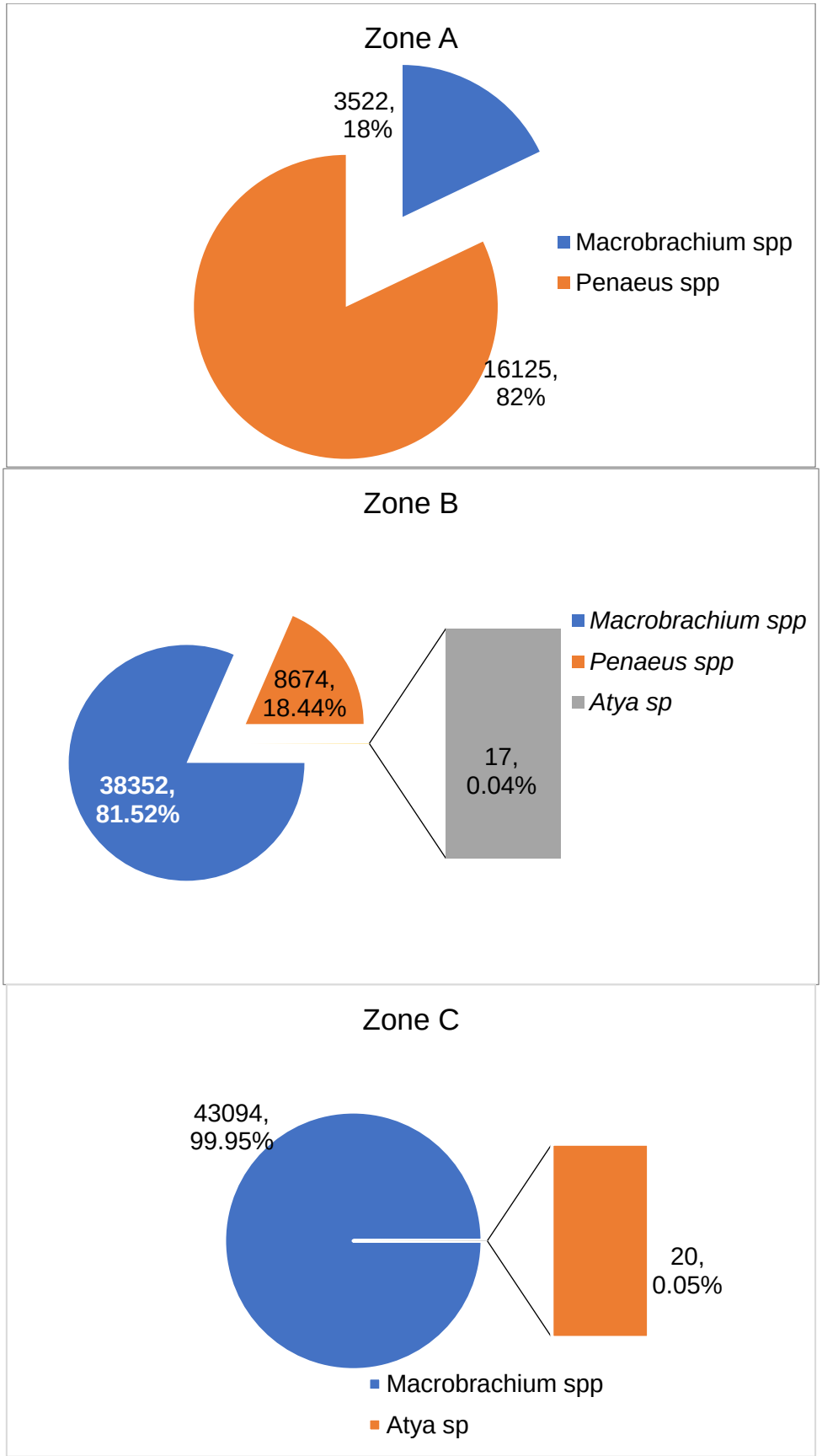


Figure 11: Relative Abundance of the Crustaceans in the Zones at the Volta Estuary

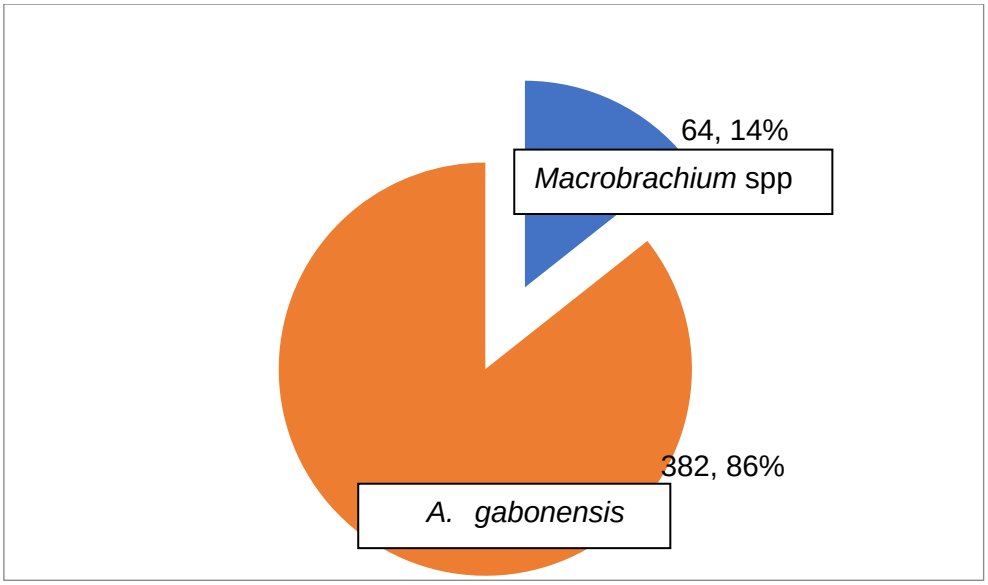
From Table 2, Zone B (H' = 21) has the highest species diversity followed by Zone A (H' = 0.20), Torgome (H' = 0.18) came as the third highest in species diversity and Zone C the least (H' = 0.002). In terms of Species Evenness Zone C (J' = 0.66) had the highest followed by Torgome (J' = 0.60), Zone B (J' = 0.44) and Zone C (J' = 0.007). Zone B (d' = 0.91) was the richest in the species encountered followed by Torgome (d' = 0.16), Zone A (d'= 0.10) and Zone C (0.09) was the least.

Table 2: Diversity Indices of Crustacean Encountered in Lower Volta River

Index	Zone A	Zone B	Zone C	Torgome
Number	19,674	44,206	43,114	446
H'	0.20	0.21	0.002	0.18
J'	0.66	0.44	0.007	0.60
d'	0.10	0.19	0.09	0.16

H'= Shanon Weiner's Diversity Index
J' = Peilou's Index (Evenness)
d' = Margalef's Index (Species richness)

At Torgome, Figure 12, only two genera were encountered; *Atya* and *Macrobrachium*, the *Penaues* was completely absent. This zone was purely freshwater (salinity; 0 – 0.5‰) very close to the Kpong hydroelectrical dam at Akuse. Here, the *Atya*, 86% (382) was more abundant in the sample than *Macrobrachium* 14% (64). Chi-square test of homogeneity indicated significant difference between the number of *Atya* species sampled from the number of *Macrobrachium* species ($\chi^2 = 3.07$, $p<0.05$).



M: *Macrobrachium* species; A: *Atya* species

Figure 12: Relative Abundance of Freshwater Crustaceans encountered at Torgome

In Figure 13, the *Penaeus* species were more abundant in the months of November to January (dry months of the year). The number declined from May to July and started rising from August. The *Macrobrachium* species were more abundant in the months of June and July, the highest rainy months of the year; declined in August and picked up from September. From October through to July, the number of *Macrobrachium* occurrence was fluctuating.

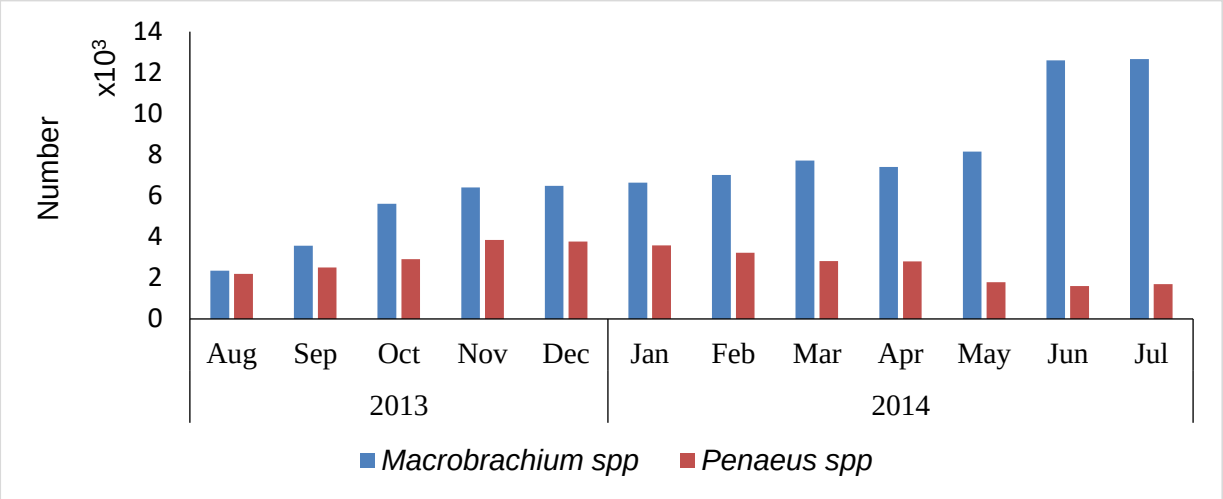


Figure 13: Monthly Occurrence of *Macrobrachium* and *Penaeus* species in the Volta Estuary

3.3.2 Distribution of the Crustacean Species Encountered by Size and Body Weight.

The *Macrobrachium* species increased in mean total length from 10.6±2.0 cm in Zone A, 12.0±1.7 cm in B and 12.7 cm in Zone C (Figure 14). Similar trend was observed in *Atya* speces, the total length was 3.20±1.2 cm in Zone B and increased to 3.50±1.7 cm in Zone C. The *Penaeus* species rather showed decrease from less saline zone to high salinity zone; those sampled from Zone B were smaller (8.70±1.6 cm) than those from Zone A (9.80±1.1 cm) (Figure 14,). ANOVA test conducted to determine the significance of these size changes in the zones indicated no significance: $F(2:2) = 281.30$), $p = 0.003$. The marginal increment in size from Zone A through to Zone C are not significanly different statistically ($p<0.05$)

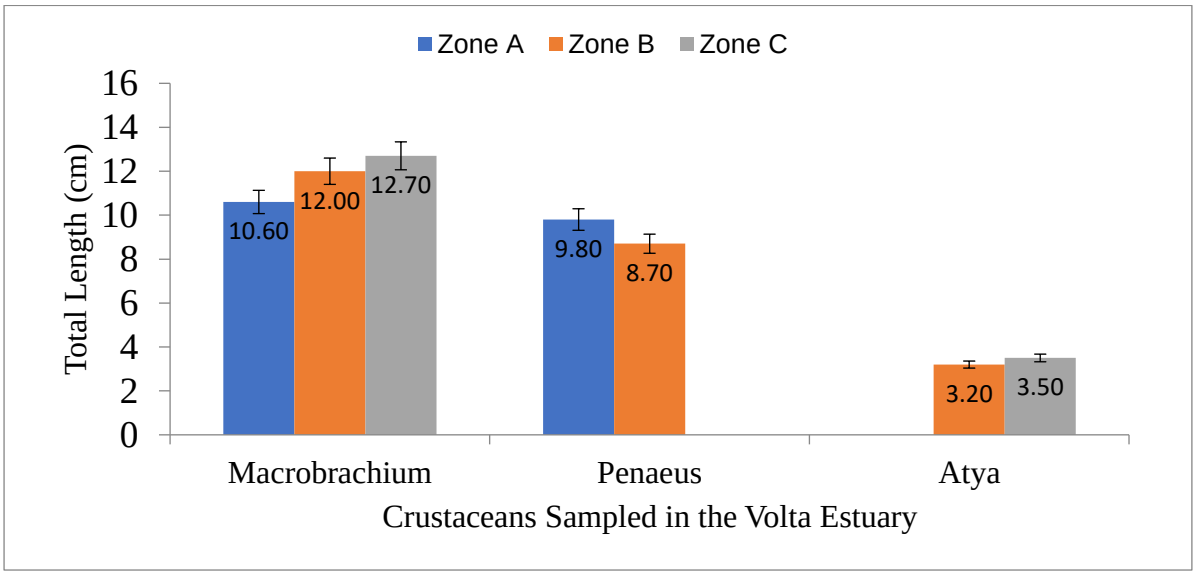


Figure 14: Spatial Distribution of the Mean Size of the Crustaceans Sampled in the Volta Estuary

Relative smaller size (2.6 – 3.5 cm total length) of *M. vollenhovenii* (Figure 15 A) were most abundant in the sample constituting 23.05% and larger size class 5.6 – 7.5 cm total length constituting 18.11%. The smallest sized group 1.5 – 2.5 cm total length constituted 12%. Generally, there was decline in proportion of the size groups from 2.6 – 3.5 to 5.6 – 6.5 cm.

For *M. macrobrachion (*Figure 15 B), 2.6 – 5.5 cm total length size groups made up 43.53% of the sample and larger size group (6.6 – 7.5 cm total length) made up 15.85%. At 5.6 – 6.5 cm total length class size, *M. macrocrobrahcion* composition was 13.21.

Only two size classes were observed in the case of *A. gabonensis* (2.5 – 3.5 and 3.6 – 4.5 cm total length) (Figure 15 C). The larger composition, 51.19% was observed in the size class of 2.6 – 3.5 cm total length and lesser composition of 48.81% was observed in 3.6 – 4.5 cm total length size class. Naturally, the Atya species are naturally stout and shorter than the *M. vollenhovenii* and *M. macrobrachion (*Appendix B).

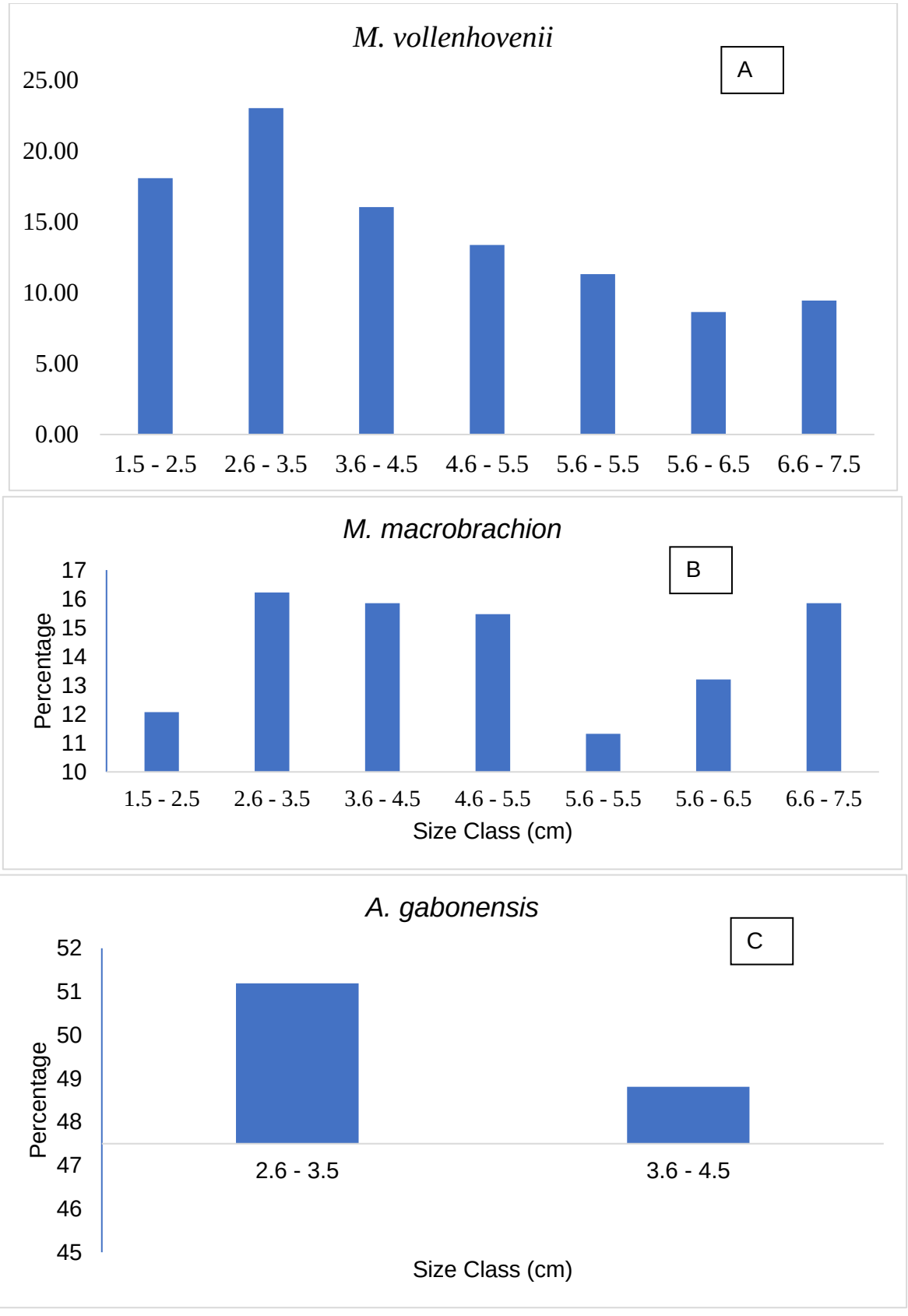


Figure 15: Size Classes of the three Freshwater Crustaceans Encountered in the Lower Volta River

From Figure16, comparatively large (12.7 – 13.3 cm total length) *Macrobrachium* species were encountered from April to June and small sized ones (10.5 -11.1 cm) in December to February. There was no clear seasonal pattern in the class size of *Penaeus* species encountered, but most of the small ones (6.5 – 9.0 cm total length) occurred from December to February).

There was no encounter with *Atya* species in January and March; the size of those encountered varied from 3.4 – 4.9 cm with larger ones (4.8 to 4.8 cm total length) in June and July.

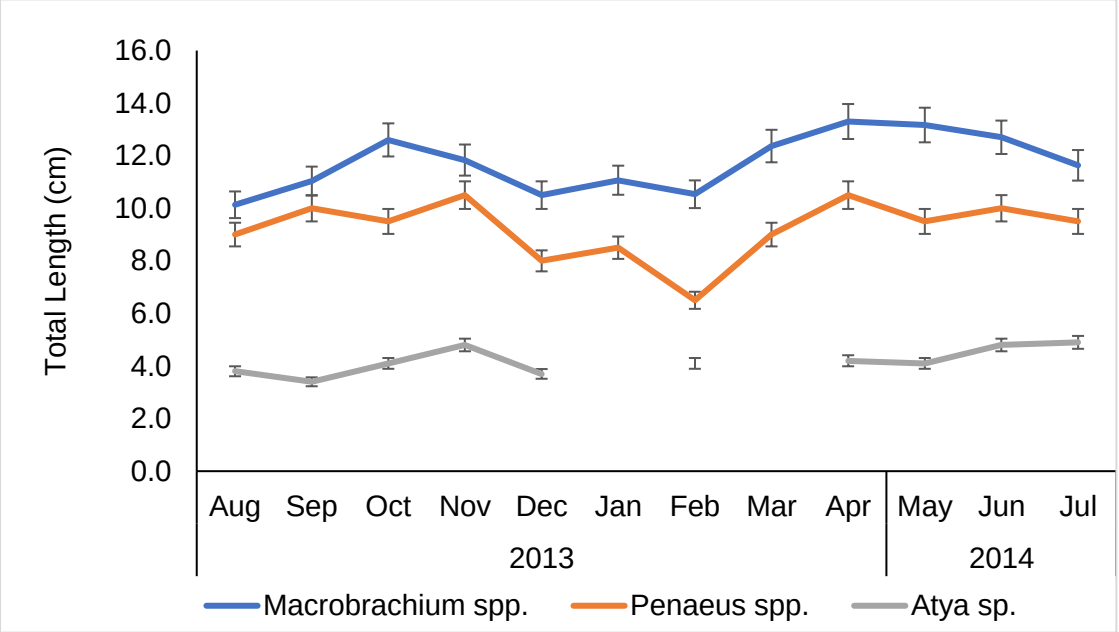


Figure 16: Monthly Variation in Mean Total Length of Crustacean Species Encountered in the Lower Volta River (with Standard Error Bars)

3.3.3 *Water Quality Parameters and Distribution of the Crustaceans*

The dendrogram (Figure 17) classified the zones into two statistically different clusters out of the three originally demarcated zones. Zone C being more of freshwater stood out in one group statistically different from Zones A and B in another group (Figure 17); Zone A and B therefore had more similar characteristics (conductivity, salinity, turbidity, depth (Table 3)) different from Zone C.

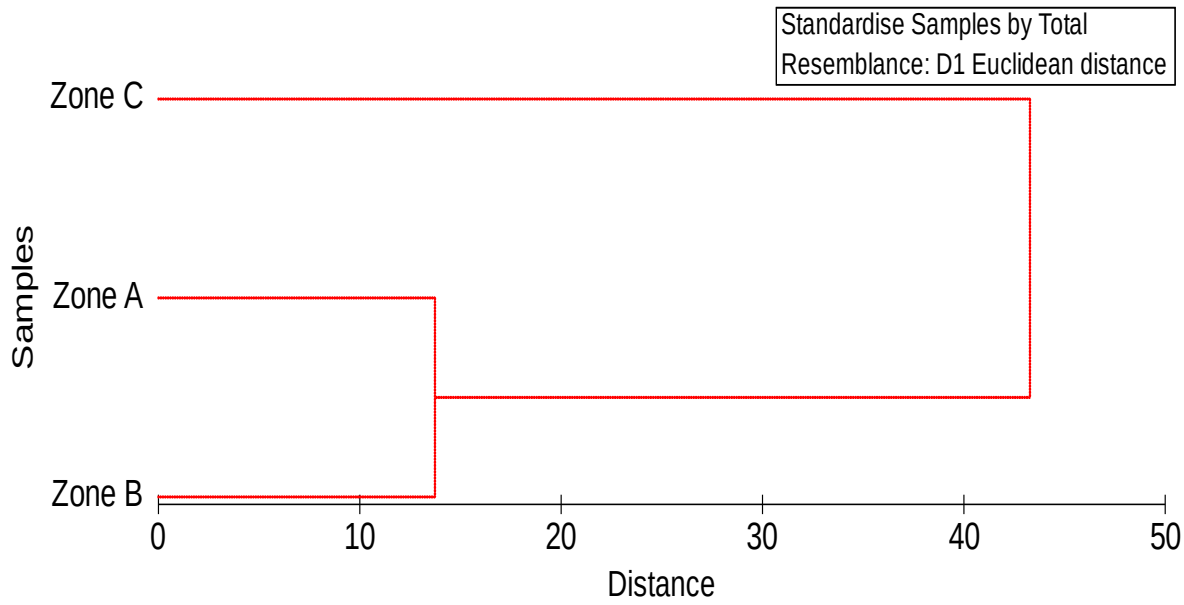


Figure 17: Dendrogram Cluster of the Sampling Zones in the Volta Estuary

Table 3 is the correlation of the principal components (PCs) with the original variables measured in the zones giving rise to the observed classification of the zones in the estuary. The PC 1, contributing 93.5% of the observed classification correlated with salinity (0.50), conductively (0.56) and sulphate (0.59), and PC 2 contributing 6.5% correlated with conductivity (0.60) and sulphate (0.63). The contributions of PC 1 and PC 2 added up to 100% of the correlation of the PCs with the original variables in the demarcated zones in the study area. PC 3 depicted correlation with conductivity (0.60) and turbidity (0.72); PC 4 correlated strongly with Nitrate (0.90) while PC 5 correlated strongly with dissolved Oxygen (0.95). The contributions of PC 3, PC 4 and PC 5 however was negligible and did not play much role in the observed distribution pattern of the crustaceans in the zones.

Table 3: Coefficients in the Linear Combinations of Variables Making Up the Principal Components

Variable	PC1	PC2	PC3	PC4	PC5
DO (mg/l)	0.011	-0.062	-0.042	-0.134	0.954
pH	0.002	-0.024	-0.008	-0.211	0.107
Temperature	-0.010	0.021	0.038	0.243	0.232
Salinity	**0.505**	0.160	0.002	0.007	-0.001
Conductivity	**0.561**	**-0.598**	0.519	0.041	-0.022
Turbidity	0.170	**-0.419**	-0.724	0.147	-0.084
NO_3^-	0.007	0.095	0.057	0.896	0.122
PO_4^{3-}	-0.001	-0.042	-0.164	0.194	0.021
SO_4^{2-}	**-0.587**	**-0.628**	0.126	0.047	-0.011
Depth	**0.239**	-0.178	-0.395	-0.110	0.048
PC	Eigenvalues	%Variation	%Variation		
1	9.11	93.5	93.5		
2	0.634	6.5	100		

The dendrogram (Figure 18) of eight water quality parameters assessed were classified into three groups: depth, turbidity and total dissolved solids in one group; temperature, pH and Dissolved oxygen forming the second group; conductivity and salinity in the third group. In the first group, depth and turbidity were more associated with the same Euclidean distance different from total dissolved solids; in the second group temperature and pH had the same Euclidean distance statistically different from dissolved oxygen which had strong loading in PC 5 without any contribution to correction between the PCs and the parameters. The third group made up of salinity and conductivity had the same Euclidean distance and were both

well loaded onto PC 1. The parameters linked by the red line had no statistical difference but were different from those linked with the black line at $p<0.05$.

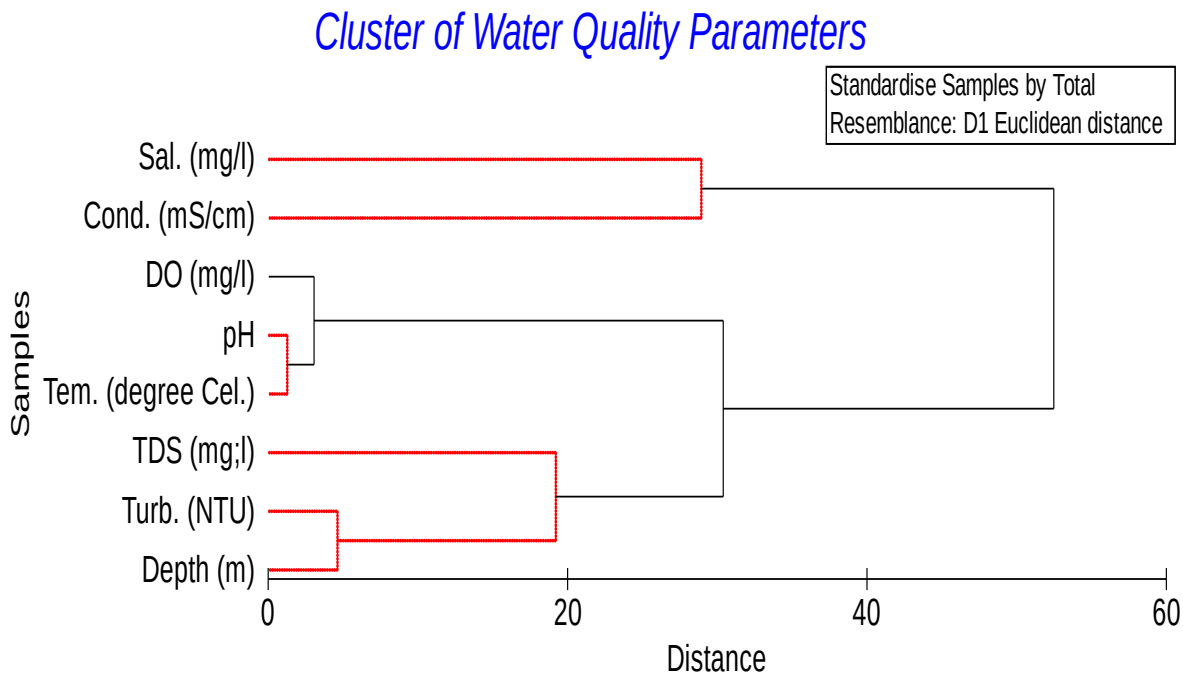


Figure 18: Grouping of Water Quality Parameters Assessed in the Volta Estuary

Table 4 illustrated the correlation of the principal water parameters indicated in the PCA (Figure 18). All the parameters assessed were strongly correlated. Depth correlated positively with salinity (0.865), conductivity (0.866), turbidity (0.881) and negatively corrected with temperature (-0.909). Salinity correlated positively with conductivity (0.998), turbidity (0.966) and negatively with temperature (-0.990). Temperature correlated negatively with conductivity (-0.987) and turbidity (-0.974). Conductivity and turbidity correlated positively (0.998).

Table 4: Correlation among Principal Water Quality Parameters Assessed in the Volta Estuary

	Depth	Salinity	Temperature	Conductivity	Turbidity
Depth	1				
Salinity	0.865	1			
Temperature	-0.909	-0.990	1		
Conductivity	0.866	0.998	-0.987	1	
Turbidity	0.881	0.966	-0.974	0.961	1

There is weak negative correlation of *Macrobrachium* and *Penaeus* species with salinity in Zone A (Figure 19); *(*y = -1.3343x + 463.81; R^2 = 0.0057) and (y = -38.448x + 2916.5; R^2 = 0.0224) for the *Macrobrahium* and *Peneaus* species respectively.

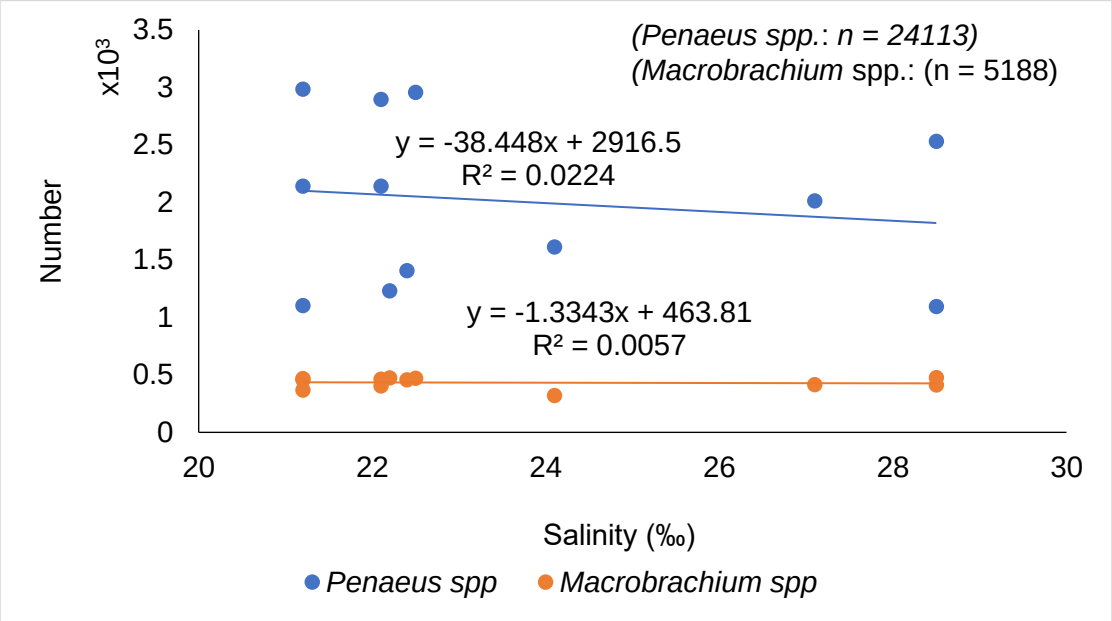
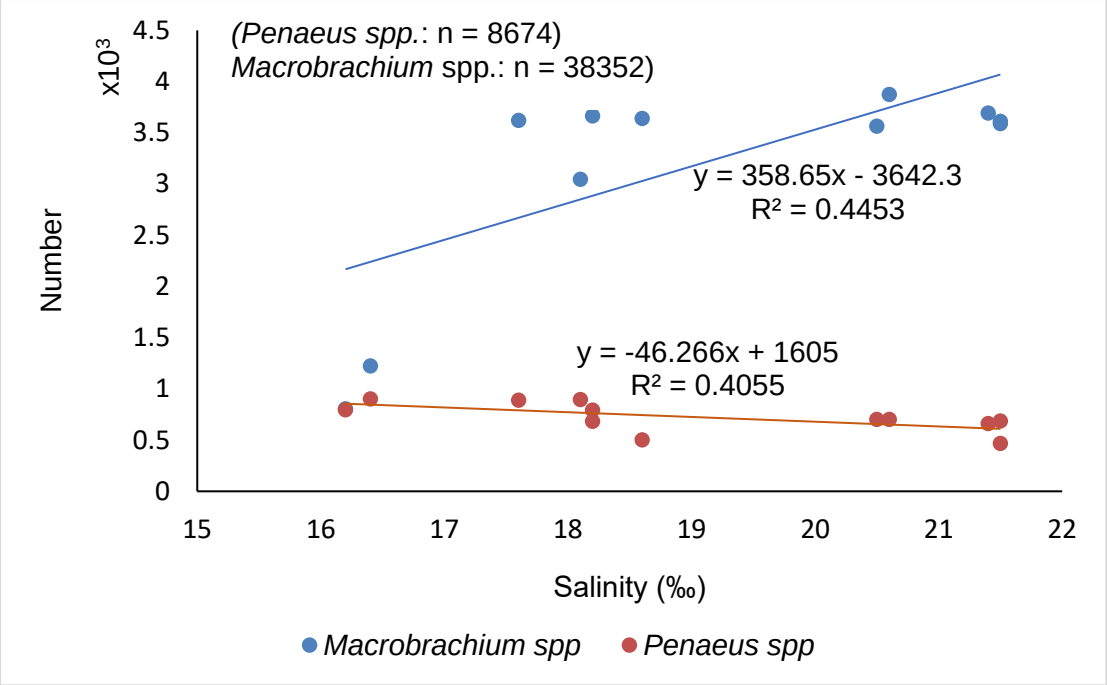


Figure 19: Relationship between Occurrence of the Crustaceans and Salinity in Zone A in the Volta Estuary

In Zone B (Figure 20), the occurrence of the *Penaeus* species correlated negatively with salinity (-43.2x – 1605; R^2 = 0.4055) while the *Macrobrachium* species correlated positively (y = 358.65x – 3642.3; R2 = 0.4055) with salinity.



M: *Macrobrachium* species (prawns): P: *Penaeus* species (shrimps)

Figure 20: Relationship between Occurrence of the Crustaceans and Salinity in Zone B in the Volta Estuary

3.4 Discussion

3.4.*1 Spatial and Temporal Distribution of the Crustaceans in the Lower Volta River with Focus on the Volta Estuary*.

The species of freshwater crustaceans encountered in the Lower Volta River from Torgome to the Volta Estuary included: *Atya gabeonsis* and two species of the genus *Macrobrachim* (*M. macrobrachion* and *M. vollenhovenii* at the ratio of 1:3 (25% *M. macrobrachion* and 75% *M. vollenhovenii).* This confirmed the findings of Attipoe, *et al*. (1989) and Rutherford (1971) that *M. vollenhovenii* is the most dominant *Macrobrachium* species in most of the waters in Ghana. The dominance was not only observed in the total sample, but also in the monthly samples. The *M. volloenhovenii* therefore constitute more commercially viable species of the freshwater prawns in Ghana. In the sample from Torgome, the Gabon shrimps (*Atya gabonensis*) was the most dominant (86% and the *Macrobrachium* 14%). The *Atya gabonensis* is however not popular in the country as compared to the *Macrobrachium* species and are therefore of less commercial importance. They appeared to be mostly confined to localized habitats as the areas around the hydroelectrical turbines where there might be good circulation by the turbines, rocky substrate resulting in clear water and vegetative materials (De Grave & Mantelatto, 2013; Obande & Kusemiju, 2008; Hobbs & Hart, 1982). The presence of *M. vollenhovenii* in the samples from Torgome, north of the Volta Estuary, further indicated the popularity of the *M. vollenhovenii* in Lower Volta River of Ghana.

In the Volta Estuary, three genera of the swimming crustaceans were encountered: *Atya*, *Macrobrachium* and *Penaeus*. The Ma*crobrachium* and *Penaeus* species were found in saline water zones of the estuary. This indicates that the *Macrobrachium* species in the Volta Estuary requires saline water as part of their life cycle. This is in line with the reports that there are two groups of the freshwater *Macrobrachium* species; those that need saline water in the early part of their life cycle and those that complete their life cycle in freshwater (Kaplan, 2010;

Holthuis, 1980). The *Macrobrachium* species encountered in this study are therefore those that need saline water in part of their life cycle. The presence of the marine *Penaeus* species in the brackish water also attest to the fact that the juveniles of the shrimp also need brackish medium for survival. This was further strengthened by the fact that the results indicated negative correlation with salinity in the two demarcated saline zones in which they occurred during the study.

The occurrence of the three genera: *Atya*, *Macrobrachium* and *Penaeus* were significantly different ($p<0.05$) in each of the three demarcated Zones. The observed gradient distribution pattern could be attributed to salinity tolerance of the various genus. The dominant genus in Zone A was the *Penaeus* (82%); Zone B and C were dominated by the *Macrobrachium* species, 82% in Zone B and over 99% in Zone C. The high percentage (82%) of *Penaeus* in Zone A with average salinity of 23‰ and low percentage (18%) in Zone B with average salinity of (19‰) is an indication of their tolerance to high salinity compared to the *Macrobrachium* species. The occurrence of *Macrobrachium* genus on the other hand were less in Zone A (18%) and high in Zone B (82%) and Zone C, over 99%, shows their preference to freshwater condition. The number of *Atya* also increased from Zone B (17 individuals) to Zone C (20 individuals) in the estuary samples (from high saline (19‰) to low saline water (4‰) and recorded the greatest number (382 individuals – 86%) in the Torgome sampling station with freshwater condition (salinity of 0.5‰). This suggests that the *Atya* species though freshwater species might tolerate saline environment to some extent. Large proportion of the *Penaeus* in Zone A could also be due to return of juveniles to the sea adding to new entrance of younger ones into the brackish water condition. The result also showed the transition pattern of the *Macrobrachium* from saline to freshwater as they mature with significantly increased size differences ($p<0.05$); in Zone A, the average total length was

10.60 cm; 12.00 cm in Zone B and 12.70 cm in Zone C with average salinities of 23‰, 19‰ and 4‰ for Zones A, B, and C respectively.

Smaller size (average 8.70 cm total length) *Penaeus* group were encountered in Zone B as compared to those encountered in Zone A (average 9.80 cm total length); the size increment was however not statistically significant ($p<0.05$) though could be a demonstration of tolerance of low salinity by those at more early developmental stage than the more developed larger and older ones that might also be gradually adopting to sea level salinity where the rest of their life cycle will be completed (Hartnoll, 1982; Holthuis, 1980).

The juveniles of both *Macrobrachium* and *Penaeus* are benthic organisms and are mostly found in the demersal zone of the water. While the shrimps were mostly sampled at relatively shallow water in the estuary, the prawns were mostly sampled in deeper water. This observation is also supported by their turbidity tolerance levels. The number of prawns sampled increased with depth while number shrimps decreases with depth and subsequent turbidity.

From the diversity indices; Zone B located between Zones A and C has the most species diversity (0.21) as compared to Zone A (0.20) and Zone C (0.002); conditions in Zone B might be favourable to all the three genera of crustaceans encountered in the study accounting for the representation of each genus in the zone. Although Zone A has the most evenly distribution of the two genera, *Penaeus* and *Macrobrachium*, (Zone A: 0.66; Zone B: 0.44; Zone C: 0.007), Zone B was the most richer in the crustaceans encountered (Zone A: 0.10, Zone B: 0.19; Zone C: 0.09). The swimming crustaceans encountered might prefer the salinity of the zone that was averaged 19‰.

On monthly occurrence of the crustaceans in Zone A, the *Penaeus* sample recorded low values as compared to *Macrobrachium* species from May to July and started picking up from August

and reached the peaks in November to January; the decline started again from February. This trend appeared to follow the tidal regime in the area coupled with the rains; high tides normally starts from October to January in the estuary pushing a lot of saline water into the river. The *Macrobrachium* occurrence on the other hand recorded high values from May to July that coincided with the major rains in the area (Nkrumah, *et al*., 2014), a clear indication of freshwater preference of the *Macrobrachium*.

The low records of *Macrobrachium* and *Penaeus* species in August and September could be attributed to cold weather mainly in the Southern and Middle Belts of Ghana including the study area (Southern Belt) (GMA, 2017).

3.4.2 Water Quality and the Distribution of the Caridean Crustaceans

The distribution of the crustaceans encountered might be influenced by water quality parameters such as salinity, conductivity, turbidity, depth and total dissolved solids since the crustaceans are benthic organisms. These parameters might have acted in conjunction to have influenced the observed occurrence of the crustaceans. The *Macrobrachium* species might belong to the groups that live in saline and turbid waters as reported by Marioghae (1987) and Holthuis (1980) in Nigeria and South-East Asia respectively.

CHAPTER FOUR
ASPECTS OF REPRODUCTIVE BIOLOGY OF *M. Vollenhovenii*

4.1 introduction

The Africa River Prawn. *Macrobrachum vollenhovenii* (Herklost, 1857) is endemic to the Eastern Atlantic with substantial fisheries in West Africa (Kingdom and Erondu, 2013). Adult size, growth rate, fecundity, ability to breed in captivity, and good adaptability to captive environmental factors such as dissolved oxygen, pH and salinity make it highly culturable and therefore regarded as a potential species for culture in the sub-region (Gordon, 1991; Marioghae, 1987). It has been established as the largest species of the freshwater prawns in Ghanaian water bodies (Attipoe and Amoah, 1989).

Sex ratio (male to female) of the *M. vollenhovenii* is reported as 1:1 (Mariogae & Ayinla, 1995; Marioghae, 1982). Work by Kingdom & Erondu (2013) had more male than female in the ratio (1:0.38) in the Niger Delta of Nigeria, the same trend 1:0.91 was reported by Mwangi (1984) in Kenya. In other species; the Brackishwater River Prawn *M. macrobrachion* was reported of male to female ratio of 1:1.4); 1:1.2 by Kavu (1985). For Indo-Pacific freshhwater prawn *M. equidens*, Maciel, *et al*. (2011) reported of male to female ratio as 1:1.6. The male to female ratio is therefore not static but varied based on prevailing conditions of the prawns.

Reproductive activity reported elsewhere indicated the presence of brood prawn all year round with peak periods influenced by environmental factors such as food availability and temperature (Olele, *et al*., 2012). Egg bearing female, the brood prawn (berried) could therefore be available at any time of the year especially in the tropics (Kingdom & Allison, 2011).

Unfortunately, no record is available on the reproductive biology *M. vollenhovenii* in Ghana, most especially in the Volta Estuary its environs.

This study is therefore aimed at collecting data on the aspects of the reproductive biology of the most common freshwater prawn (*M. vollenhovenii*) in the lower portion of the Volta Estuary in Ghana for the development of hatchery procedures of the prawn to enhance its culture in Ghana.

Objectives

The primary objective of the study was to determine aspects of the reproductive biology of the *M. vollenhovenii* in the Volta Estuary.

Specific Objectives

Were to determine:

I. Temporal occurrence of the brood prawns in the Volta Estuary
II. The fecundity of the brood prawn in relation to size of the brood prawns in the Volta Estuary
III. The male to female ratio of the prawns in the Volta Estuary

Hypothesis:

- *Ho:* There is no seasonality in the occurrence of brood *M. vollenhovenii* in the Volta Estuary of Ghana.

  Ha: The brood *M. vollenhovenii* are seasonal in their occurrence in the Volta Estuary.

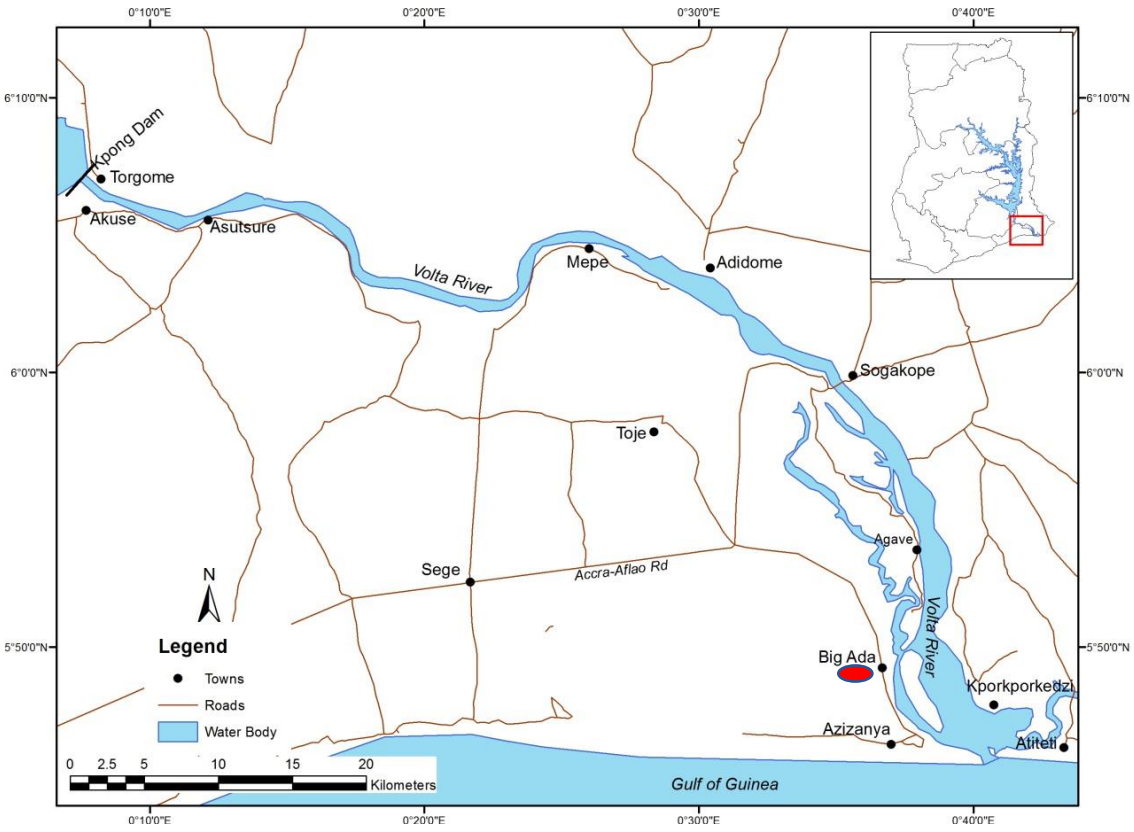
- *Ho:* Fecundity of *M. vollenhovenii* does not depends on the size of the berried prawn.

  Ha: The fecundity is influenced by the size of berried *M. vollenhovenii* in the Volta Estuary.

4.2 Materials and Method

4.2.1 *Study Site*

Studies on aspects of the reproductive biology was conducted on the dominant species of the freshwater prawns; *Macrobrachium vollenhovenii*. Monthly samples were collected with the help of fishermen for 12 months; from November 2013 to October 2014. The collection was done at a prawn landing site (Kpomkpor) at Big Ada ((5º.46'42.3;5'N & 0º.40'21.03'E) in the Ada East District of Ghana (Figure 21). The prawn fishers and mongers that patronage the landing site mostly come from the small fishing villages located on the banks and islands of the portion of the Lower Volta River leading to the estuary. The prawns are normally fished all the days of the week and stored in large collection basket traps of varying dimensions and kept in the river until they are needed.



: Sampling Site

Figure 21: Map of the Lower Volta in Ghana showing the Sampling Site for *M. vollenhovenii*

The landing site was observed during a reconnaissance survey (from the Estuary to Torgome) as one of the main landing site along the lower Volta River from Kpong to the Estuary at Ada. The fishers and fish mongers normally come very early (5.30 – 7.30 hours GMT) to the landing site on Tuesdays and Fridays which are the market days of the main Ada Market at Kase, about 20 minutes' drive from the landing site.

4.2.2: *Acquisition of Prawn Samples*

The prawn samples were collected with the help of fishers using basket prawn traps. The berried prawn samples were collected through special arrangement with the prawn fishers: the samples were fished and kept in designated collection baskets; they were handled and kept carefully to prevent eggs loss from rough handling. The prawn samples were made up of *M. vollenhovenii* and *M. macrobrachion*; the samples were therefore sorted into various species; size classes, sex, berried and non-berried in case of *M. vollenhovenii*.

The non-berried female and males were transported in separate groups and berried females with good mass of eggs were placed individually into separate small plastic containers and placed on ice in ice-chest and transported to the laboratory at the Department of Marine and Fisheries Sciences, University of Ghana, Accra.

4.2.3: *Species Identification*

Identification of species was done with the help of identification guides of Carpenter & De Angelis (2014); Powell (982) and Rutherford (1971) (Appendix B). In the laboratory: total length measurements (from the tip of the rostrum to the tip of telson – Figure 22) were done using measuring board and Vernier calipers to the nearest millimeter. An electronic balance was used for weight measurement to the nearest 0.1 g.

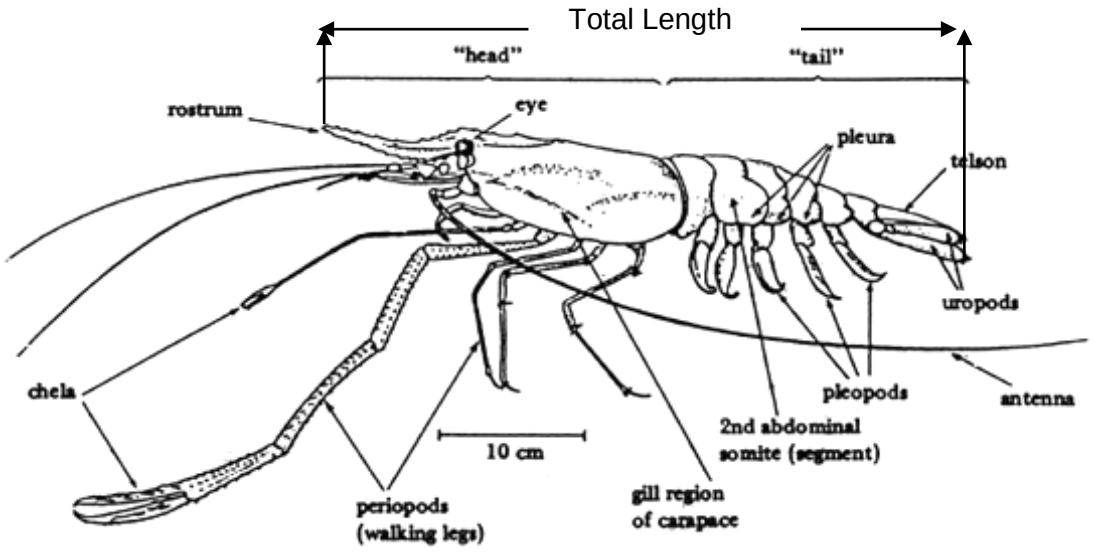


Figure 22: Length Measurements of the Freshwater Prawns (Picture by New, 2002)

4.2.4: *Sex and Fecundity Determination*

Sex of the prawns was determined by visual inspection of the base of the fourth and fifth pair of periopods as described by Kingdom & Erondu (2013) (Appendix B).

A total of 30 berried females randomly selected from each bunch of the monthly samples were used to determine the fecundity. The fecundity was determined by dislodging the eggs from the prawns with help of forceps. Absolute Fecundity - total number of eggs in the gonad was determined as suggested by Bagenal (1978). The total gonad was weighed to nearest 0.1 g and subsamples were taken from each gonad and the number of eggs countered with the aid of OSK Fish Egg Counter (OGAWA SEIKI CO. Ltd Japan).

The absolute fecudity (F) was then determined using the formula:

$F = \frac{W}{w} x\ N$ (King, 1995)

where: F = Absolute Fecundity; W = gonad weight (g); w = weight of subsample of eggs (g); N = number of eggs countered in the subsample.

Relative Fecundity was determined using the formula $RF = \frac{F}{W}$

Where: F = absolute fecundity; W = weight of the berried prawn (g).

The same sample of 30 berried females used in the fecundity determination were used to determine Gonadosomatic Index (GSI) for the monthly samples using the formula $GSI = 100\left(\frac{G}{F}\right)$ (King, 1995)

where: G = Weight of Gonad (g) and F = Weight of Berried Female (g).

Egg dimensions were determined with the help of microscope fitted with ocular micrometer.

4.4.5: *Summary and Analysis of Data*

Pie charts were used to illustrate the percentage proportion of the males, non-berried and berried prawns. Scatter plots were used for the monthly occurrence of the various groups of the prawns; tables were used for the sex, non-berried and berried prawn ratios and testeted with Chi-square for significant differences. Fecundity against total length, body weight and gonad weight were plotted using linear regression techniques and tested with Chi-square for significant differences; the same process was used for the egg dimensions against the size and weight of the berried prawns.

Histograms were used for the percentage proportions of occurrences of the male, non-berried and berried prawns and the size classes of the prawns. Ttest was then used for the test of significant differences and correlations among the various parameters. All significances were determined at $p<0.05$.

4.3 Results

3.3.1: *Relative Occurrence of Macrobrachium vollenhovenii Broods*

A total of 3190 brood *M. vollenhovenii* were obtained from the study area in 12 months (August 2014 to July 2015) for the reproductive studies. This number comprised of 1366 males and 1824 females with observed Male:Female ratio of 1:1.3, which significantly deviated from the expected ratio of 1:1 ($\chi^2 = 5$; df = 1; $p<0.05$). The observed ratio resulted in 44% males and 56% female of the total number prawns sexed (Figure 23 A). In the total pool of male, non-berried female and berried female; the proportion of 43%, 36% and 21% respectively was recorded (Figure 23 B) respectively. Out of the total number of female sexed, 676 were with eggs (Berried prawns) and 1148 were non-berried, this gave a ratio of 1:1.7and by proportion, 54% were non-berried and 46% berried (Figure 23 C). The observed ratio deviated from the expected 1:1 ($\chi^2 = 2.1$; df = 1; $p<0.05$).

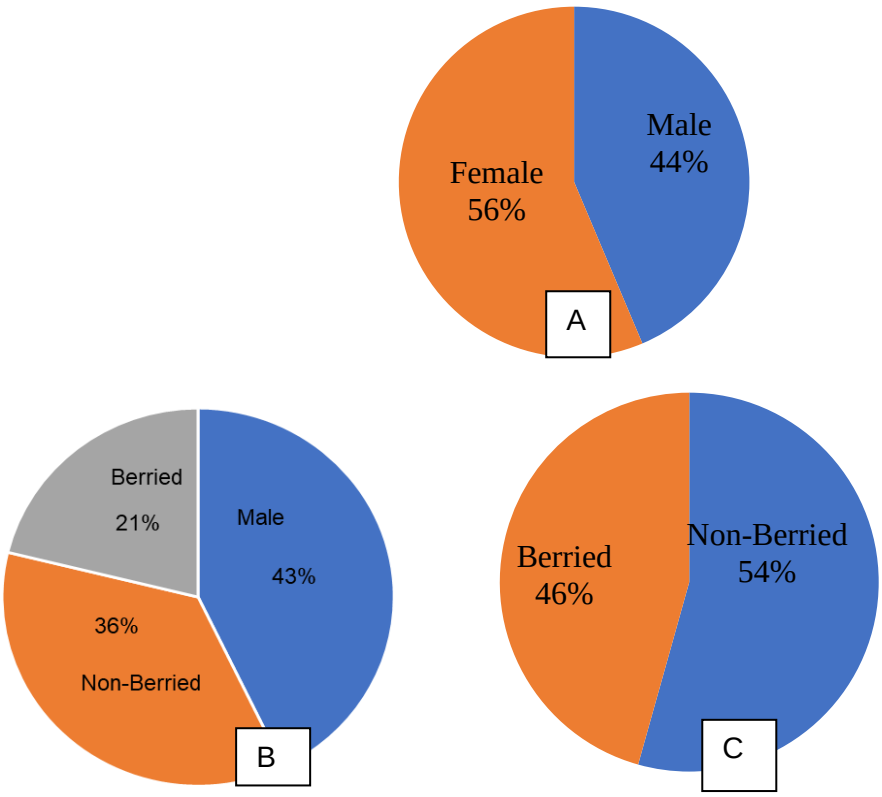


Figure 23: Percentage Occurrence of Male, Berried and Non-Berried Female of *M. vollenhovenii* encountered in the Volt Estuary

4.3.2: Temporal Occurrence and Variations of Male, Berried and Non-Berried Female Prawns

Figure 24 shows that the number of females sexed in each month were higher than the number of males; the female therefore dominated the monthly number of the prawns sampled. The number of both male and female prawns sexed was low in August and December: 56 and 65 males; 100 and 92 females in August and December respectively. The highest number of both sexes of the prawn sampled were in February: 135 males and 179 females.

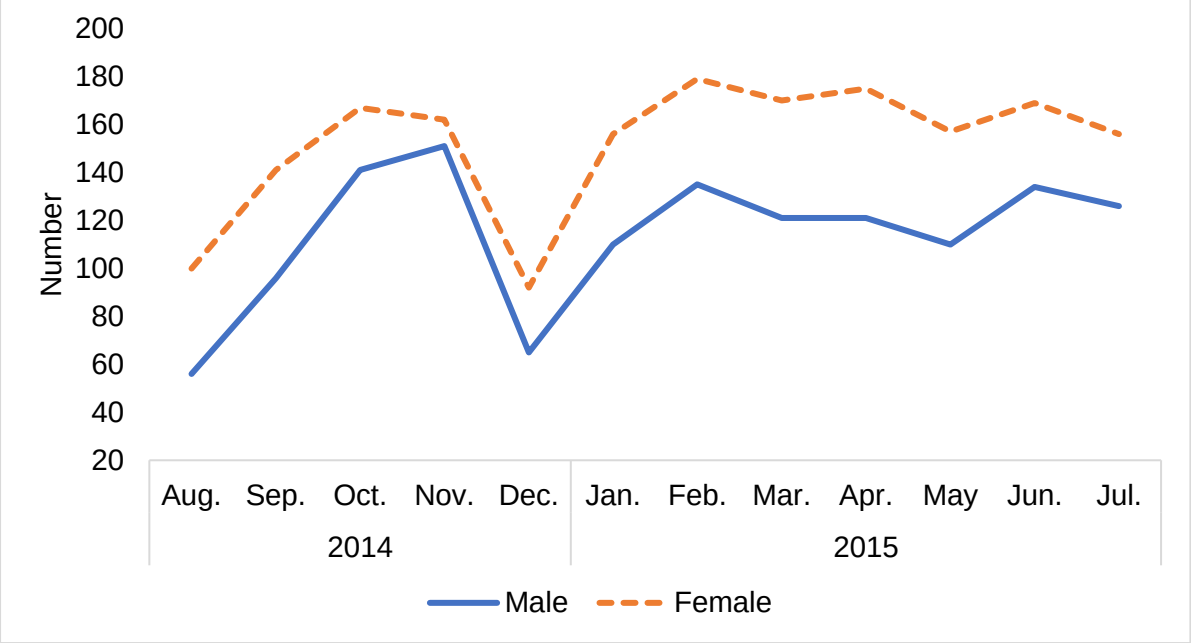


Figure 24: Monthly Sexual Variations of Prawns Sampled in the Volta Estuary During the Study

Figure 25 incates that the non-berried (females without eggs, comprising premature and spent females) were more than the berried prawns in the monthly samples. Both berried and non-berried prawns were sampled through out the year with seasonal variations. The ocurrence of the berried and non-berried prawns were low in August (42 berried and 58 non-berried) and December (36 berried and 56 non-berried). The highest number of berried prawns sampled was 71 in Februardy and 116 non-berried in November.

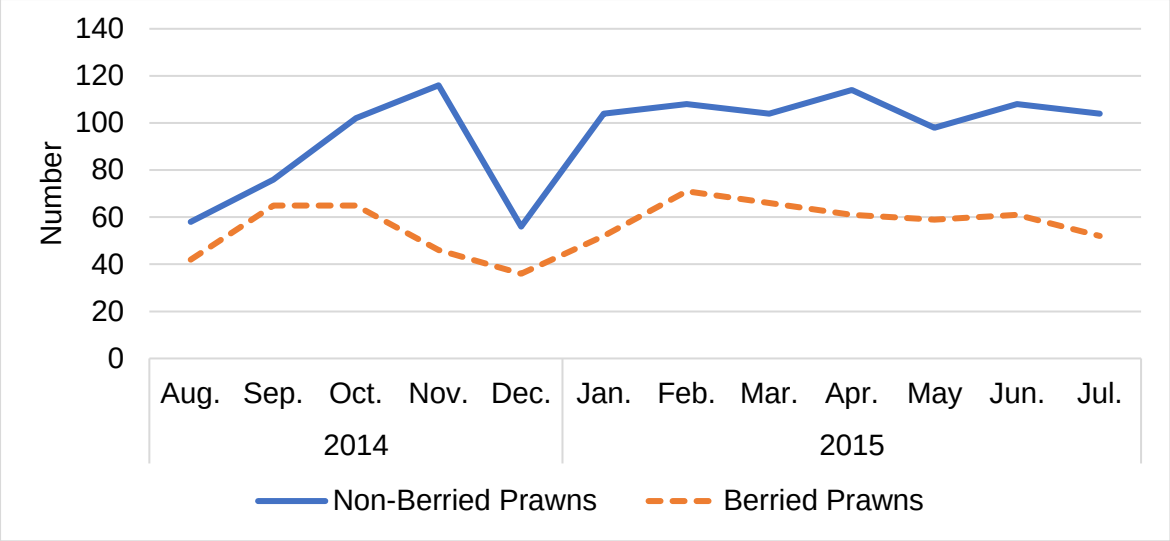


Figure 25: Monthly Variations of Berried and Non-Berried Prawns Sampled the Volta Estuary During the Study

In Table 5, the females were more than males throughout the months of the study: the highest ratio of male to female was observed in August (1:1.79) and the lowest ratio was observed in November (1:1.07). The overall male to female ratio was 1:1.34 which is significantly different from the 1:1 ratio (X^2 = 5.10, p<0.05).

Table 5: Variations in Male to Female Sex Ratio of *M. vollenhovenii* in the Volta Estuary

Year	Month	Total	Male	Female	M:F	X^2
	Aug.	156	56	100	**1:1.79**	0.01
	Sep.	237	96	141	1:1.47	0.01
2014	Oct.	308	141	167	1:1.18	0.14*
	Nov.	313	151	162	**1:1.07**	0.53*
	Dec.	157	65	92	1:1.42	0.03
	Jan.	266	110	156	1:1.42	0.01
	Feb.	314	135	179	1:1.33	0.01
	Mar.	291	121	170	1:1.40	0.01
2015	Apr.	296	121	175	1:1.45	0.01
	May	267	110	157	1:1.43	0.01
	Jun.	303	134	169	1:1.26	0.04
	Jul.	282	126	156	**1:1.24**	0.07*
	Total	3190	1366	1824	**1:1.34**	5.10*

M:F:- Male to Female Ratio; *:- Significant (p<0.05)

Table 6 is the monthly differences in the number of non-berried and berried. The highest sex ratio 1:2.52 was observed in November and the lowest 1:1.17 in September. The overall ratio of berried to female prawns sampled was 1:1.72; significantly different from the normal 1:1 ratio (X^2 = 2.15, df = 1, p<0.05))

Table 6: Monthly Variations in Berried and Non-Berrie Females in Volta Estuary

Year	Months	Total	Non-Berried Female	Berried	F:B	X^2
	Aug.	100	58	42	**1:1.38**	0.11*
	Sep.	141	76	65	**1:1.17**	0.35*
2014	Oct.	167	102	65	1:1.57	0.01
	Nov.	162	116	46	**1:2.52**	3.80*
	Dec.	92	56	36	1:1.56	0.04
	Jan.	156	104	52	**1:2.00**	3.13*
	Feb.	179	108	71	1:1.52	0.01
	Mar.	170	104	66	1:1.58	0.01
2015	Apr.	175	114	61	**1:1.87**	6.16*
	May	157	98	59	1:1.66	0.01
	Jun.	169	108	61	1:1.77	0.01
	Jul.	156	104	52	**1:2.00**	3.14*
	Total	1824	1148	676	**1:1.72**	2.15*

B:F :- Berried to Female Ratio; *:- Signinificant (p<0.05)

4.3.3: Size Class Distribution of Male, Berried and Non-berried Females

Figure 26 shows the modal size class at 6 – 8 cm total length (47.3% and frequency of 262), the 8 – 10 cm group (33.8%) was the second dorminant. The 6 – 10 cm size therefore made up of 81.1% of the total *M vollenhovenii* brood sampled. The lowest proportion of the size class was oberved in the 14 – 16 cm class (0.02% with frequency of 1), this class was only the males without any representation of females in the randomly selected sample used in the analysis. The mean total length of the prawns sampled was 10 cm with 3.74 cm standard

deviation, standard error1.53 and cofficient of variation 0.37. The largest size (male) was 15 cm and the smallest 5 cm giving the size range of 10 cm.

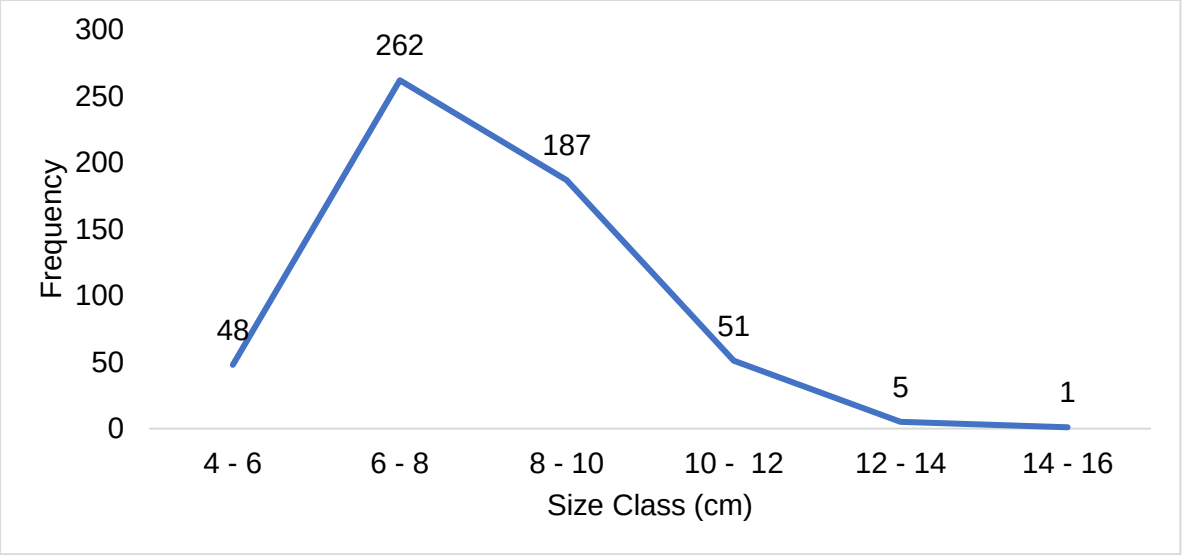


Figure 26: Frequency Distribution of the Various Size Class of *M. vollenhovenii* Sampled in the Volta Estuary During the Study

In Figure 27 and Table 7, the male prawns were generally larger in size; mean total length male:10.29±3.86; non-berried female: 7.99±2.25 and berried female 8±2.05. In the 4 – 6 cm size class, the proportion of berried prawn were the highest (14.3%) followed by the non-berried (8.0%) and the males (6.8%) with the lowest propportion. The same was observed in the 6.1 – 8.0 cm size group (berried prawns: 56.2%, non-berried prawns: 52.3% and males: 39.6%). The trend was reversed in the 8 - 10 cm group: male highest (37.2%) followed by the non-berrid (34.2%) and the berried (24.8); this trend was repeated in the 10 – 12 cm size group: male 14%; non-berried 5.5% and berried 4.8% . No representations of the females in the 14.1 - 16.0 cm size classes. T-test indicated that the proportion of the males in the larger class groups were significantly higher than the non-berried prawns females $t(10) = 0.61$, $p<0.05$ and berried prawns $t(10) = 0.23$. $p<0.05$. The non-berried prawn were also observed to be more of the larger size group than the berried prawns $t(10) = 0.07$, $p<0.05$.

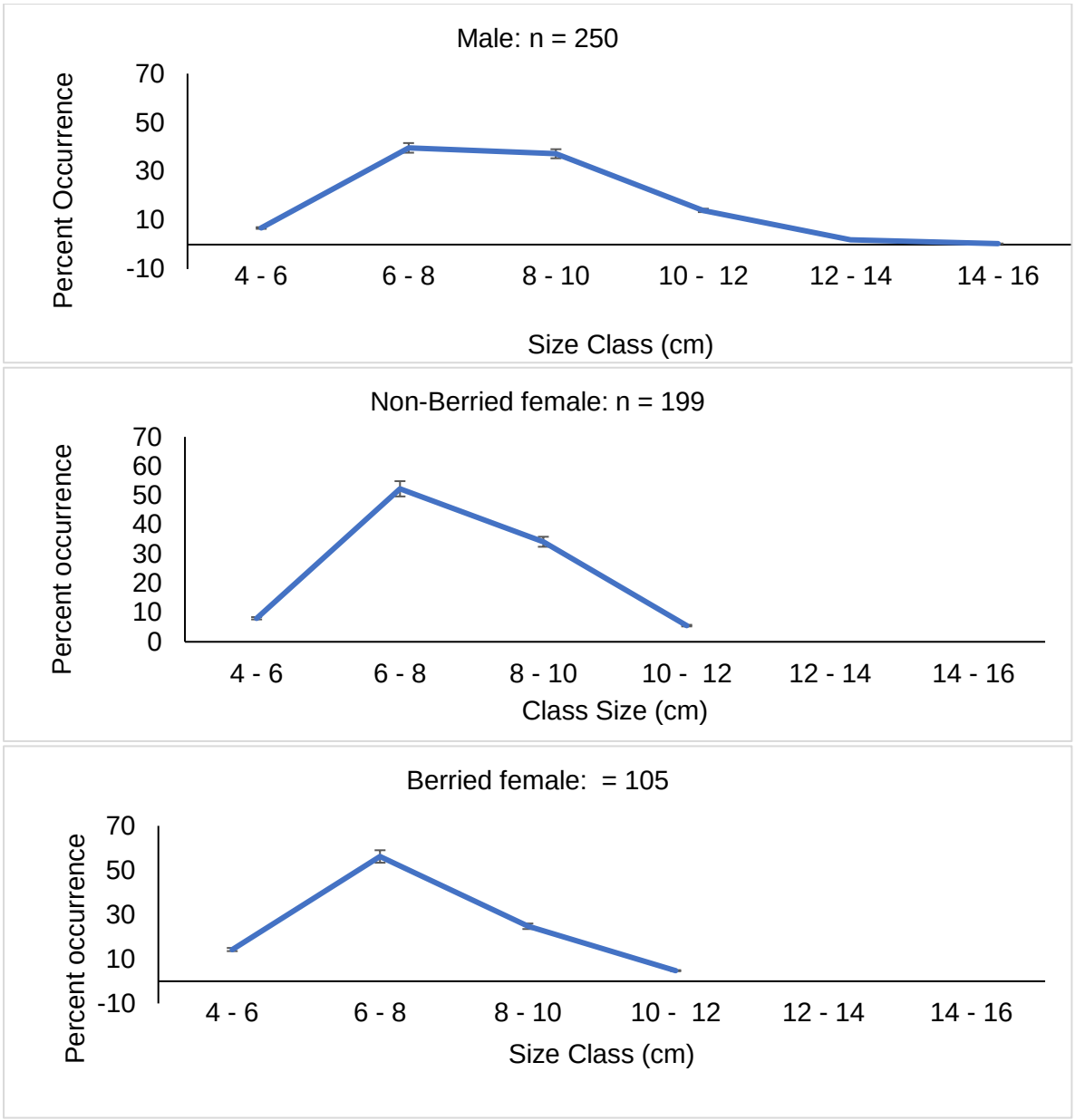


Figure 27: Size Class of Male, Berried and Non-Berried Female Prawns in the Volta Estuary (with Standard Error Bars)

Table 7: Descriptive Statistics of Size Class (cm) of Male, Non-Berried and Berried Prawns in the Volta Estuary

Statistics	Male	Non-Berried	Berried
Mean	10.29	7.99	8.00
Standard Error	1.57	1.12	1.04
Median	9.95	7.95	7.98
Standard Deviation	3.86	2.25	2.09
Variance	14.88	5.04	4.32
Coefficient of Variation	0.36	0.28	0.26
Modal Class	6 - 8	6 – 8	6 - 8

4.3.4: Relationships of Gonad Weight and Fecundity with Total Length and Weight of the Berried *M. vollenhovenii* Females

Figure 28 indicates positive correlation between the totol length and gonad weight of the berried prawns ($y = 0.4068x – 2.6339$; $R^2 = 0.49$). The correlation coefficient deviated ($n = 122$, $df = 16$, $\chi^2 = 0.70$; $p < 0.05$) from the expected correlaton of 1, a positive perfect correlation.

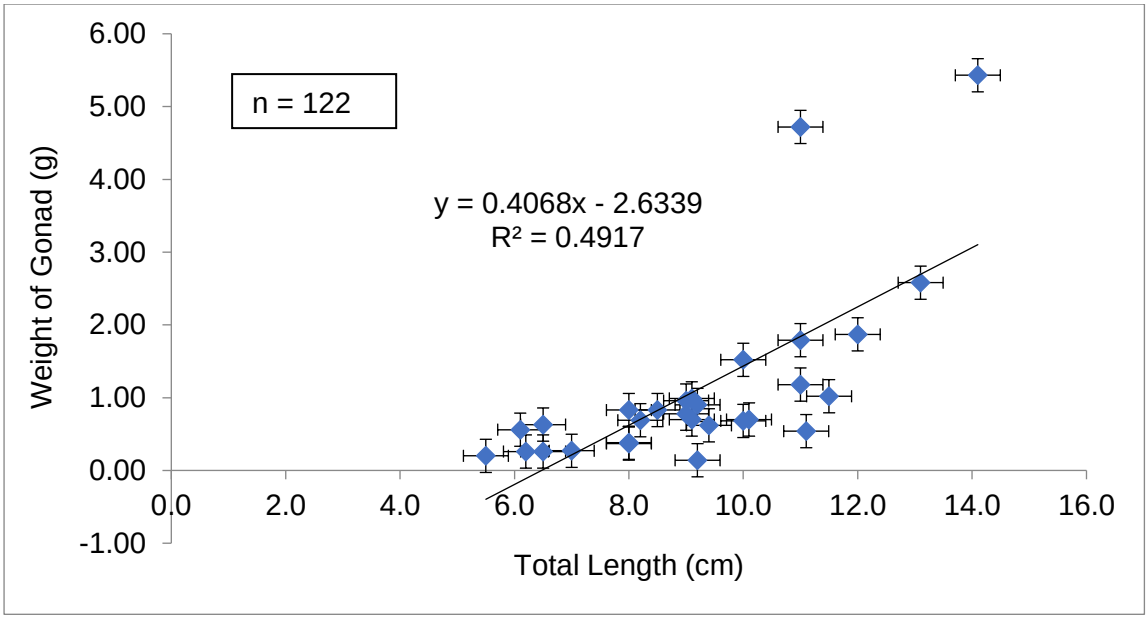


Figure 28: Relationship between the Total Length of Berried prawns and the Gonad Weight of *M. vollenhovenii* in the Volta Estuary (with Standard Error Bars)

Figure 29 also shows positive correlation between the body weight of the berried prawn and gonad weight with (0.1036x -0.154) R^2 value as 0.62 which deviates from the expected perfect correlation of 1 (n = 122, df = 16, $\chi^2 = 0.82$; $p = 0.05$).

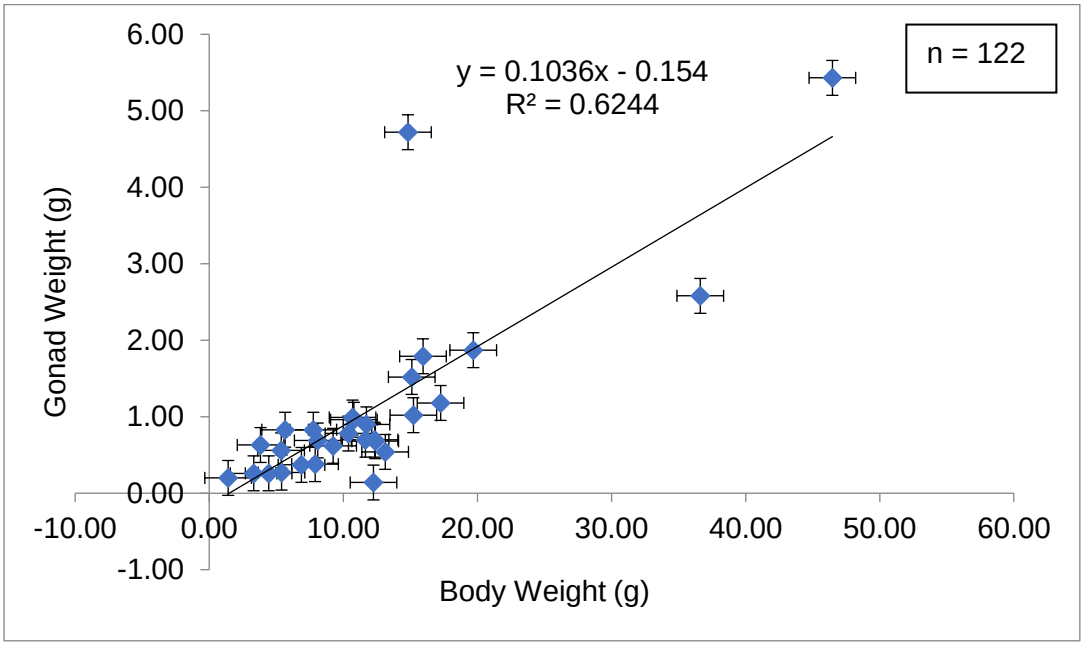


Figure 29: Relationship between Gonad Weight and Weight of Berried *M. vollehovenii* encountered in the Volta Estuary (with Standard Error Bars)

In Figure 30, the total length of the berried prawn used in fecundity ranged from 6.1 – 13 cm. At total length of 6.1 – 7, the absulute number of eggs counted was 6,292 on average gonads used in the counting of eggs; 7.1 – 8, gave 6,398 eggs countered on average; 8.1 – 9 size class produced 15,600 eggs on average; 9.1 – 10 produced 15,680 eggs on average; 10.1 – 11 size class on average produced 15,820 eggs; 11.1 - 12 size class gave 25,485 eggs on average; 12.1 – 13 cm size class produced 39,966 eggs. The cofficient of determinaton (R^2 = 0.84) indicates that 84% of the increases in the absolute fecundity is explained by increases in the total length.

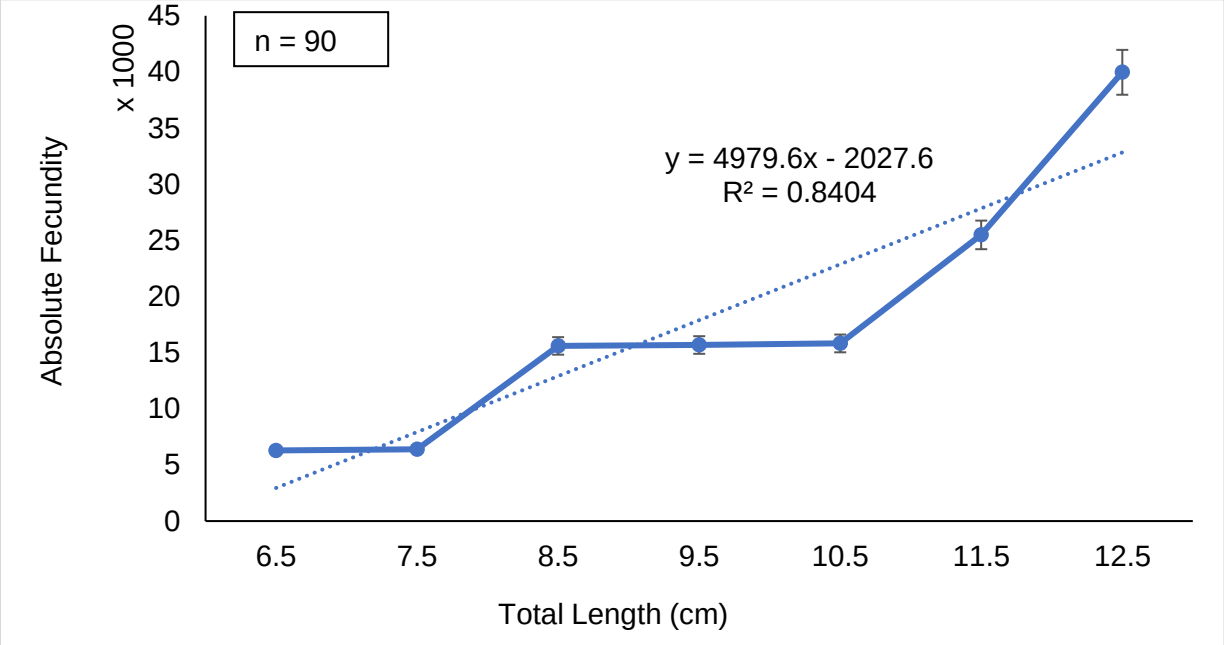


Figure 30: Relation between Absolute Fecundity and Total Length of Berried *M. vollenhovenii* in the Volta Estuary (with Standard Error Bars)

The number of eggs per centimer (cm) is significantly more pronounced (R^2 = 0.92) than the number of eggs per gramme (g) (R^2 = 0.88) (Figure 31). The total length ranged from 6.1 to 13 cm and the body weight of the berried prawn after extraction of eggs ranged from 5.4 to

14.6 g. As 92% increase in the number eggs was due to increases in the total length, 88% increases in the number of eggs was due to increases in weight of the berried prawns.

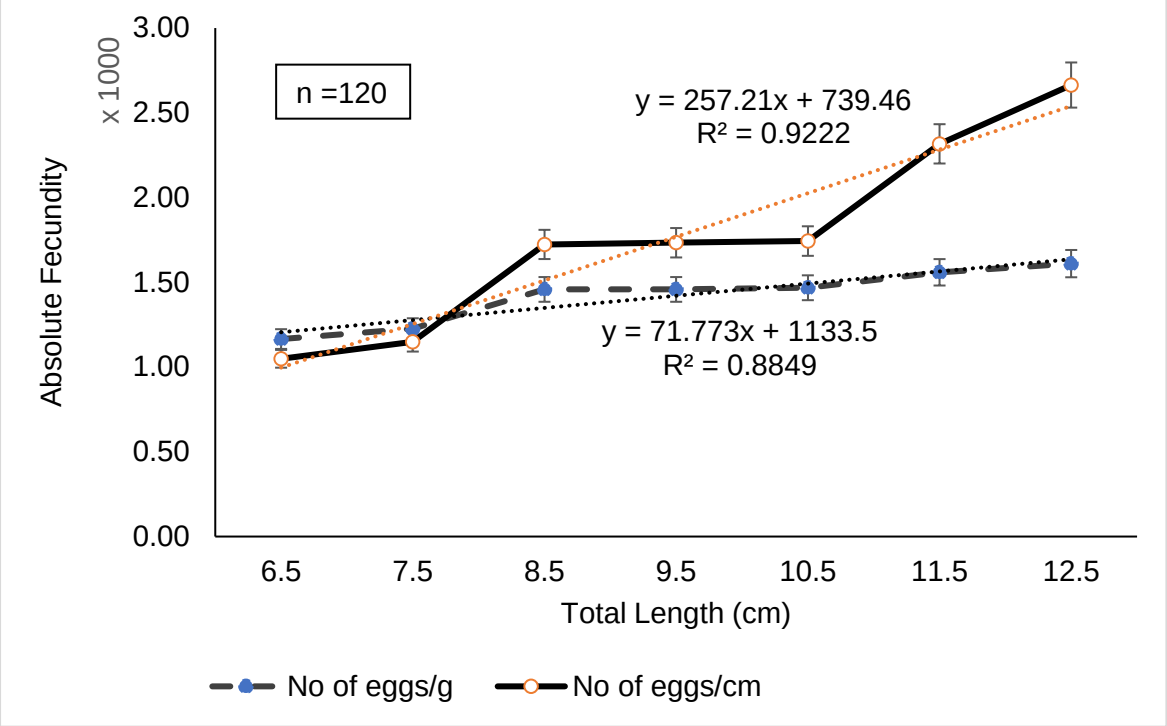


Figure 31: Correlation of Relative Fecundity with Total Length of *M. vollenhovenii* in the Volta Estuary (with Standard Error Bars)

4.3.5: Relationship between Egg Size and Total Length

Figure 32 shows that the egg long axis and short axis of *M. vollenhovenii* varied slightly with size of the berried prawn; the egg long axis varied from 0.35 to 0.37 mm and short axis from 0.25 to 0.26 mm against the total length which varied from 5.5 to 13.1 cm. The correlation equaction: $y = 0.0003x + 0.3561$; $R^2 = 0.1594$) for total length against egg long axis and $y = 0.0001 + 0.255$; $R^2 = 0.0246$ for the total length against egg short axis. A t-test for the relationship; total length ($M = 8.58$, $SD = 2.15$) and short axis ($M = 0.26$, $SD = 0.004$) showed no significant correlation; $t(32) = 15.95$, $p<0.05$; no significant correlation also for the total length ($M = 8.58$, $SD = 2.15$) and long axis ($M = 0.36$, $SD = 0.004$; $t(32)= 15.76$, $p<0.05$).

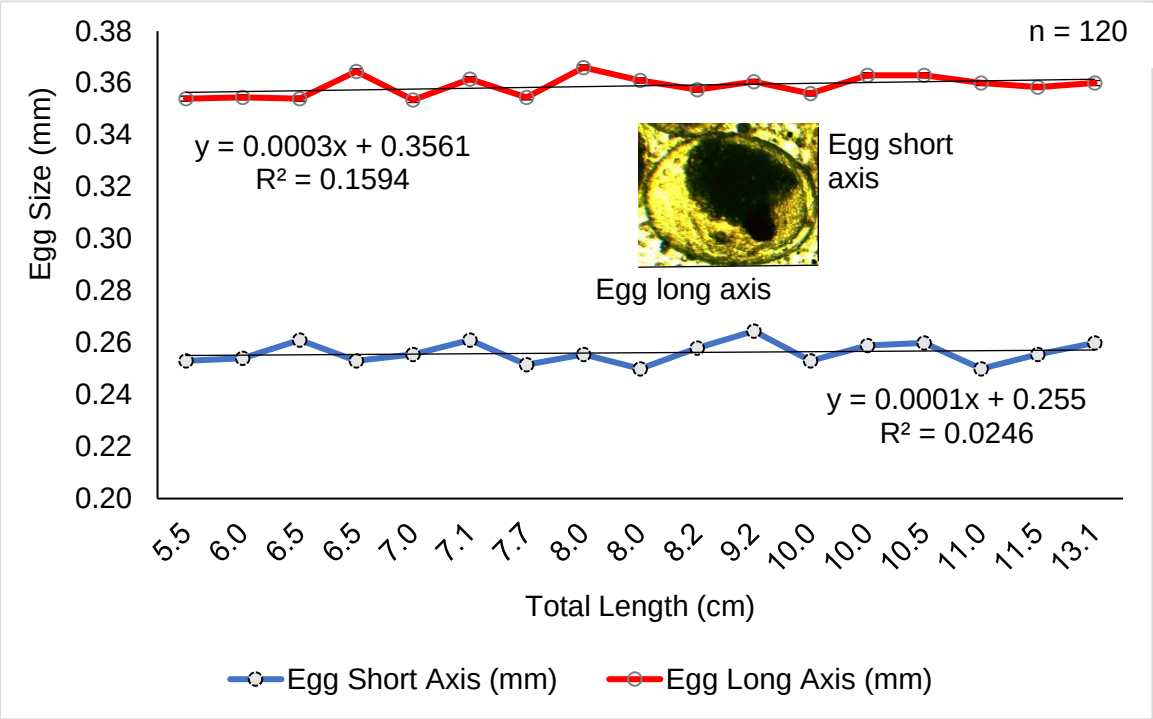


Figure 32: Relationship between Total Length and Egg Size of *M. vollenhovenii* in the Volta Estuary

Table 8 gives details of dimensions of the prawn egg. The eggs mature with changes in colour from bright-orange colour of newly spawned eggs gradually to orange and finally dark gray about 2 to 3 days before hatching – Appendix F (Anderson, 1985). The colour changes with slight increase in egg size: short axis varied from 23 - 27 mm at bright orange colouration stage; 0.25 – 0.27 mm orange colouration stage and 0.25 - 0.29 mm at the final dark gray colouration stage. The variation of the egg long axis was also at narrow range from 0.31 – 0.35 mm - (bright orange colouration stage); 0.31 – 0.35 mm (orange colouration stage) and 0.33 – 0.39 mm (dark gray colouration stage).

Table 8: Size of eggs with maturation stages of *M. vollenhovenii* encountered in the Volta Estuary

	n = 120; (mm)					
Maturation	→					
	BRIGHT ORANGE		ORANGE		DARK GRAY	
	Short axis	Long axis	Short axis	Long axis	Short axis	Long axis
Mean (mm)	0.25	0.34	0.26	0.35	0.27	0.37
Standard Deviation	0.02	0.02	0.01	0.02	0.02	0.02
Minimum (mm)	0.23	0.32	0.25	0.33	0.25	0.35
Maximum (mm)	0.27	0.36	0.27	0.37	0.29	0.39

4.4 Discussion

4.4.1: *Relative Occurrence of Macrobrachium vollenhovenii Broods*

Out of 3190 prawns sampled, 1366 were males and 1824 were females giving male to female ratio as 1:1.3, which deviates significantly ($\chi^2 = 5.00$; df = 1; $p<0.05$) from the expected ratio of 1:1 observed by Marioghae (1982) and Mariogae & Ayinla (1995). The observation of more females in the sample did not also support the findings of Kingdom and Erondu (2013) who observed male to female ratio as 1:0.38 with the male dominating of the *M. vollenhovenii* in the Lower Taylor Creek in Nigeria. Sethi *et al*. (2014) however observed male to female of 1:2.8 of the Monkey River Prawn, *M. lar* in Rangat River, Andaman Islands in India. The implication of more female over male is an indication of sustainability of the prawn through effective reproduction leading to recruits.

The dominance of the females in the samples is a clear indictation that effective reproductive activities are more likey to occur throughout the year. This is buttressed with 46% of berried females with significant difference ($p<0.05$) between berried and non-berried prawns observed in 6 months (August, September, November, January, April and July) out of the 12 months showed a good measure of reproductive activities in the estuary. This could be attributed to good environmental conditions for the prawns and availability of food items for the prawns.

4.4.2: *Temporal Distribution of the Male, Berried and Non-berried Females*

The male, non-berried and berried *M. vollenhovenii* were available throughout the year with signs of seasonality. This is supported by the findings of Sethi, *et al*. (2014), Anyanwu *et al*. (2014), Kingdom & Erondu (2013) and New (2002). The availability of berried prawns all year round is a big boost not only to the perpetuation of the prawn breeds, but also to aquaculture. Prawn seeds for grow out in aquaculture could be obtained from the wild in two forms (i) the post larvae (PL) (ii) the berried prawns. The advantage of using the berried

female from the wild over the post larvae is that larvae of the same size and the required number can be obtained at any time. It is therefore encouraging to observe the presence of berried prawns in all the months of the year in good numbers. The results of the study have therefore indicated either of these scenarios and gives impetus to sustainable prawn resource in the estuary and the culture of *M. vollenhovenii* in the country.

4.4.3: *Size Distribution of Brood prawns*

The brood *M. vollenhovenii* was dominated by size group of total length 6 - 7 cm (47.3%) followed by 8 – 10 cm size group (33.8%). This two groups (8 – 10 cm) together constituted 81.1% of the total *M. vollenhovenii* brood. This is an indication that the more active reproductive size ranged from 8 – 10 cm. The least proportion of the size group was observed at 14 – 16 cm size group, meaning the larger the prawn the less reproductive active they become. The largest of the *M. vollenhovenii* so far caught was of the range 18.2 – 19.5 cm (Powell, 1998; Holthuis, 1980) as compared to 16 cm encountered in this study, perhaps more years of sampling might discover larger ones.

Segregating the male, non-berried and berried prawns, there were more berried females in the 4 – 6 cm size group than the male and non-berried. This further support the fact that at that size the prawns are not only sexually mature but are very active reproductively. Spawning at tender age (small size) as observed might also be attributed to efforts to perpetuate their breed due to some unhealth conditions that might force the organisms to reproduce at early age to keep their strains in a highly pressurized environment. The proportion of berried females reduced from 8 – 10 cm size group to 10 – 13 cm, this might imply that sexual activities slow down as the female prawns mature with increase in size.

4.2.4: *Relationships of Gonad Weight and Fecundity with Total length and Weight of Berried Females*

The assertion that the gonad increases with the size of the berried prawn is supported by the finding that more number of eggs were obtained at 10 – 13 cm total length. From size 6.1 – 8 cm, the number of eggs countered were about 5,000 and increased to about 15,000 at size 8.1 – 11 cm. From size 11.1 – 13 cm the number of eggs further increased from 15, 000 to more than 39,000. This trend was also observed by several researchers in the *Macrobrachium* species (Sethi *et al*., 2014; Kingdom & Erondu, 2013; Almeida *et al*., 2010; Lara & Wehrtmann, 2009). The implication might be fewer number of berried prawns can be used for hatchery production of the prawn larvae at sizes 10 cm and above. The benefits of this observation among other things might include few holding facilities, lower energy requirement for aeration, lower feed requirement and other essential hatchery equipment with subsequent reduction in labour, thus making hatchery operations more manageable and profitable.

The increases of relative fecundity were more pronounced for increases size class (length) than the weight. This suggested that weight differences could not be a factor in increasing number of eggs produced.

4.4.5: *Relationship of Egg Size with Total Length and Gonad Weight*

Analysis of the egg size indicated that the size of the egg was not proportional to the size of the berried prawn giving rise to weak correlation between the total length and egg long axis ($y = 0.0003x + 0.3561$; $R^2 = 0.1594$) and short axis ($y = 0.0001 + 0.246$; $R^2 = 0.0246$). This suggested that differences in the weight of gonad might be a function of the number of eggs and rather than the size of eggs. Larger berried prawns with heavy gonad might therefore bear

a lot more eggs rather than a few large sized eggs. The implication is that large berried *Macrobrachium vollenhovenii* female could produce numerous larvae in the natural set up as well as under culture conditions. Taking large berried females for hatching operations will therefore be more advantageous to hatchery operators.

Colour changes (from bright orange to orange and then to dark gray) of the eggs was matched with maturation of the embryo that increased in size from one stage to another. This observation might be common in most of the *Macrobrachium* species since most authors reported of similar changes in colour of the eggs (Nandlal & Pickering, 2005. New, 2002). The increases of size of the embryo was 8% along the short axis and 8.8% along the long. The marginal increases could be an indication that no new features of the embryo was developed during the developmental stages, from the time the eggs are laid to the time when the eggs are matured for hatching into larvae.

CHAPTER FIVE

EXPERIMENTAL HATCHING AND LARVAL DEVELOPMENT OF THE AFRICA RIVER PRAWN (*Macrobracium vollenhovenii, Herklots, 1857)* IN PLASTIC TANKS.

5.1 Introduction

The prawn hatchery process begins with the berried or ovigerous females that may be obtained from the wild or from an established prawn farm (Nandlal & Pickering, 2005; New, 2002). In tropical waters in particular, where berried prawns are available all year round, the brood prawns could be obtained from the wild and kept in hatchery facilities until the eggs are hatched (New, 2002). The hatchery process involves hatching of the eggs, larval development and the nursing of the larvae to post larval (PLs) or juveniles stage for onward rearing in production facilities to marketable or table size stage. Three stages are therefore identified in the production of the prawn: (i) hatching of eggs, (ii) nursing the larvae (larval development) (iii) grow-out stages. The hatching and nursing processes in particular require considerable training of the personnel involved and labour; it is capital intensive (Chowdhury *et al*., 1993; Dopkin, 1971). It is therefore apparent that growers could better obtain the prawn seeds (PLs) from established hatcheries. Furthermore, growers who wish to raise their own prawn seed stock need the requisite skills in the hatchery and nursery operations if they are to succeed in large scale production that require large numbers of the prawn seed (Nandlal & Pickering, 2005).

Challenges in the hatchery process include maintaining good water quality, suitable salinity and feed materials. According to Chowdhury *et al*. (1993): *no amount of remedial measures can completely overcome the problem caused by poor water quality*. Several methods according to Yamasaki-Granados *et al*. (2013) have therefore been used particularly in Malaysia and other Asian countries to ensure good water quality; these included filtration and

treatment with chemicals such as Copper Sulphate (125 mg/l), Calcium hydroxide (20 mg/l), Formaldehyde (1 x 10^{-5} ‰) and Iodine (dissolved at 1.75%).

The berried prawns (brood stock) are also treated with Copper Sulphate (0.2-0.3 ppm) (Nandlal & Pickering, 2005; New, 2002) and Formaldehyde (20 - 30 ppm) in the laboratory or hatchery before they are placed in the hatching units (Nandlal & Pickering, 2005; New, 2002). Water flushing by changing water in the holding units from 20 – 80% by volume on daily or every other day was another method to control and ensure good water quality (Nandlal & Pickering, 2005; New, 2002; Yamasaki-Granados *et al*., 2013). The larval stages; depending on temperature, feed and water quality take 22 – 35 days take eleven stages after hatching to reach PL stage for *M. rosenbergii* (Nandlal & Pickering, 2005). This may however not be true for all *Macrobrachium* species. Roy *et al.* (2005) reported of nine stages for complete larval development in *M. gangeicum* in 26 days with the 10th stage considered to be the post larva measuring 4.5 – 5 mm. In *M. australiense*, the larval stage was completed in only 6 days with three larval stages (Fielder, 1970).

It has been established that most species of the prawns need saline water at early developmental stages (Ratanak, 2011; Nandlal & Pickering, 2006; D'Abramo *et. al*., 2006; New. 2002). Among the various salinities used in culture of the prawn larvae, salinity at 12‰ has been reported of better performance (Lawal-Are, 2012; Bello-Olusoji, 2004; New, 2002).

Live feed is reported as crucial in the growth of the prawn larvae (Pickering, 2005; New, 2002; Nandlal & Chowdhury *et al*., 1993). The Brine shrimps (*Artemia* species) has been the principal and most commonly used live feed, but its use could present some challenges due to cost and non-availability. Several attempts are therefore being made to replace the Brine shrimps (*Artemia)* feed with other live and inert feed materials (Gomes *et al*., 2013; Yamasaki-Granados *et al*., 2013; Murthy *et al*., 2008; Roy *et al*., 2005; Atkinson, 1977; Ling,

1969). Most of the attempts in the use of other live feed involve the green water rich in plankton.

With regards to the holding facility, no particular type or size is prescribed for hatching the eggs and the culture of the prawn larvae; containers of any type and size; round, square or rectangular tanks can therefore be used (New, 2002). For experimental purposes, aquaria, ordinary plastic basins and earthenware pots could be ideal culture facilities (New, 2002). Conical bottom and round edge containers are nonetheless, recommended for good water circulation and ease of cleaning to ensure good water quality (New, 2002).

The hatchery systems proposed and used include: (i) Flow through system (Correia *et al*., 2000); (ii) Recirculating static (iii) Recirculating dynamic (New & Valenti, 2000); in the production of *M. rosenbergii* and other minor farmed species. Chowdhury *et al*. (1993) on the other hand proposed flow through system with clean water and green water systems.

According to Nandlal & Pickering (2005), in countries where the prawn culture industry is well developed, there are wide varieties of hatchery designs depending on financial resources, production target and other requirements. The recirculating dynamic system that involves changing of water was however indicated to offer opportunity to control water quality and conserve water and energy; reduce demand for seawater or brine. It was reported that for *M. rosenbergii*, rock salt as source of saline water is unsuitable and that the sea water and the reconstituted salt from seawater work fairly well (Kotamee *et al*, 2014; Alam *et al*., 2003).

The need to establish good water quality, suitable feed materials and source of saline water is therefore crucial in the development of a hatchery for *M. vollenhovenii* in Ghana. Unfortunately, there is no information on the hatchery requirements, hatching of eggs and culture of the prawn larvae available in Ghana. There are however reports of work in Nigeria (Marioghae & Ayinla, 1995; Willfuhr-Nast *et al*., 1993), Cameroon (Makobu *et al*., 2014),

Senegal and Cote d'Ivoire in the West Africa Sub-region where *M. vollenhovenii* is endemic. In Ghana, some attempts were made in the past without any appreciable successful record (New, 2005; Attipoe and Amoah, 1989, Prah, 1982, 1977). This study is therefore deemed as ground breaking for the hatchery operations of the freshwater prawns in Ghana.

5.1.2 *Objective*

The primary objectives of the study were to establish suitable conditions for hatching operations of *M. vollenhovenii* through laboratory trials and to establish successful hatching and larval development procedures for the *M. vollenhovenii* larvae in Ghana.

Specific Objectives:

The specific objectives were to determine the optimal:

i. quality of the hatching medium for berried *M. vollenhovenii*.
ii. stocking density of the newly hatched larvae of *M. vollenhovenii*.
iii. medium for the survival and growth of the larvae of *M. vollenhovenii***.**

5.1.3 *Hypothesis:*

1. *Ho:* The number of *M. vollenhovenii* larvae hatched does not depend on the quality of the culture medium.
   Ha: The number of *M. vollenhovenii* larvae hatched depends on the quality of hatching medium.
2. *Ho*: Growth performance of *M. vollenhovenii* larvae does not depend on the source of salinity.
   Ha: Growth performance of *M. vollenhovenii* larvae is influenced by the source of salinity.
3. *Ho:* Stocking density of *M. vollenhovenii* larvae has nothing to do with growth performance and survival of the larvae..
   Ha: Growth performance *M. vollenhovenii* larvae depends on the stocking density of the larvae

5.2 Materials and Methods

5.2.1 *Brood Prawn Sampling Site*

Samples of live berried prawns also known as ovigerous prawns were collected with the help of local fishers using basket prawn traps at Big Ada (Figure 33), (5º46'42.35'N & 0º.40'21.03'E) in Ghana. Following the result of the biological study of the prawns in the previous research work, the samples were collected once a month for three months; January, June and November in 2016 to cover dry and rainy seasons of the year.

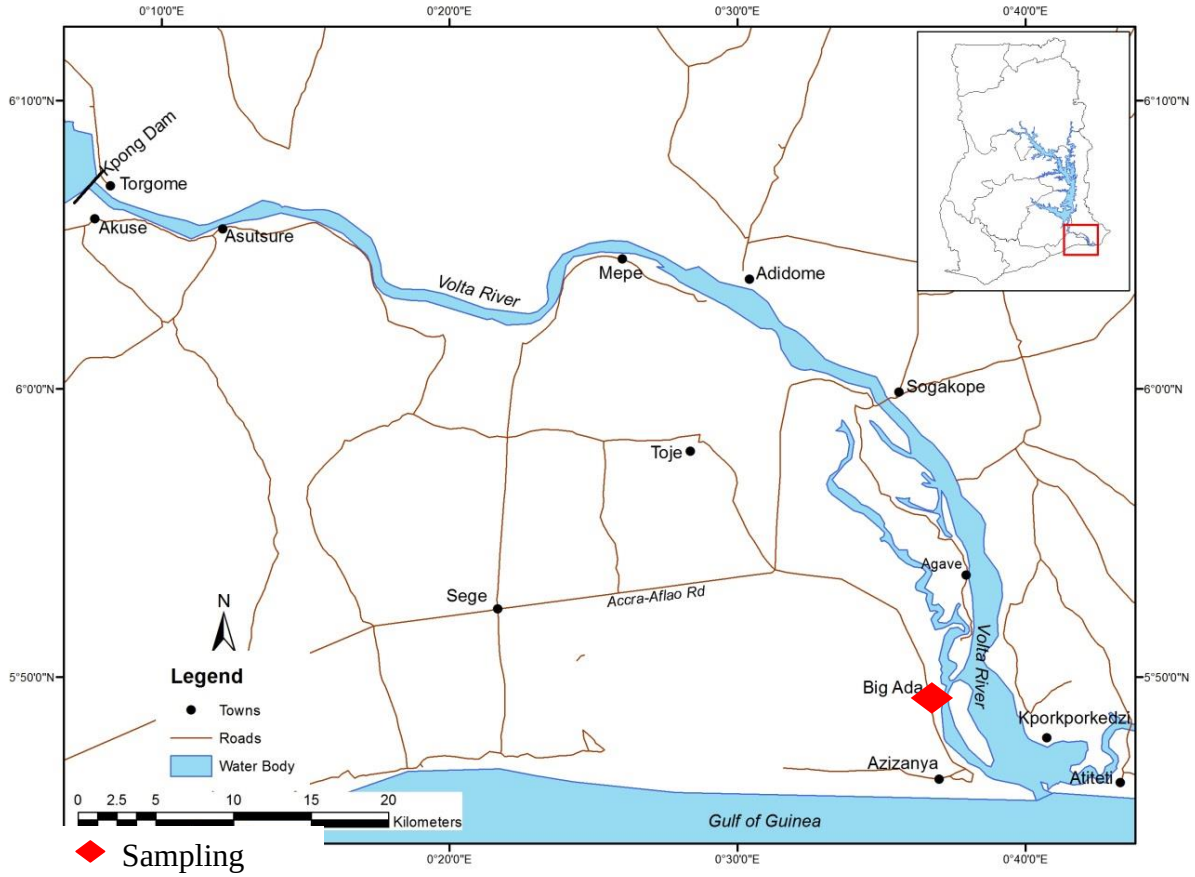


Figure 33: Map of the Lower Volta River Showing the Sampling Site

The landing site at Ada was selected during the earlier studies of Addai (2010) and Atsu (2003) and regarded as the main landing site for the prawns along the lower Volt River from Akosombo to the Estuary at Ada. Prawn mongers along this stretch of the Volta River

therefore troop to Kase on the markets days (Tuesdays and Fridays) to purchase the prawn for processing and retail business. Ada is about 90 minutes' drive east of the harbour city Tema of Ghana. The prawns are fished and stored in basket traps by the fishers and sold out at the landing site, which is about 20 minutes' drive from the market, very early on the market days. The live prawn samples were therefore purchased at about 6-7 hours GMT on the Kase market the days. The landing site served as a mini-market where all type of materials are traded on the Kase market days.

5.2.2: *Experimental Fishing For Brood Prawns and Transportation*

The berried prawn samples were trapped overnight, the traps were set normally at about 15 hours GMT and collected the following day at 8 hours GMT by hired prawn fishers in the Volta Estuary with baited basket traps (Plate 5 and Figure 34) with total length varying from 40 to 45 cm with the wider end 14 to 16 cm in diameter; the openings vary from 3.5 to 5 cm in diameter (Figure 37).

Plate 5: Freshwater Basket Prawn Trap used by fishermen in the Lower Volta River

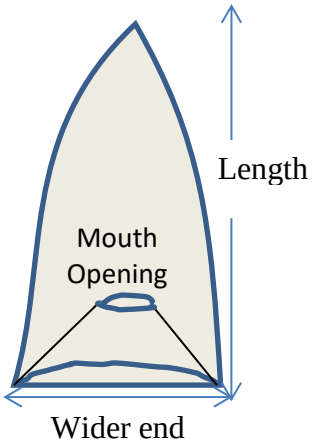


Figure 19: Sketch of Freshwater Basket Prawn trap

The live berried prawn samples were kept separately in PVC pipes of diameter 2.5 cm and length 16 cm long closed at both ends with netting material (Plate 6) as suggested by Nandlal & Pickering (2005) and New (2002. This arrangement was to avoid loss of eggs and injuries of the berried prawns resulting from attacks from each other.

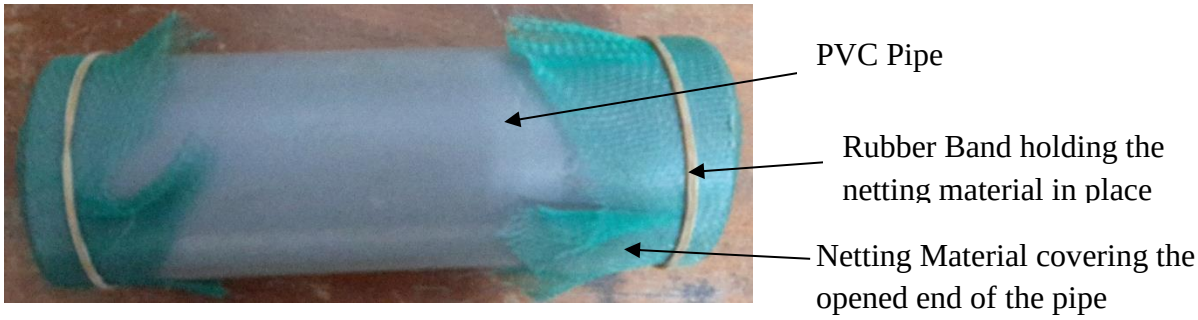


Plate 6: Holding Unit for Berried *M. vollenhovenii* prawns for Ease of Transportation

The PVC pipes were grouped into conically shaped plastic containers, 45 cm long, 30 cm wide at bottom end, 35 cm at opened end and 26 cm deep (Plate 7), filled with water from the river where the prawns had been captured, just to cover the PVC pipes and aerated with 12 V DC 130 W aerator powered by car battery connected to 7 cm^3 head diffuser and transported live to a hatchery at the Department of Marine and Fisheries, University of Ghana.

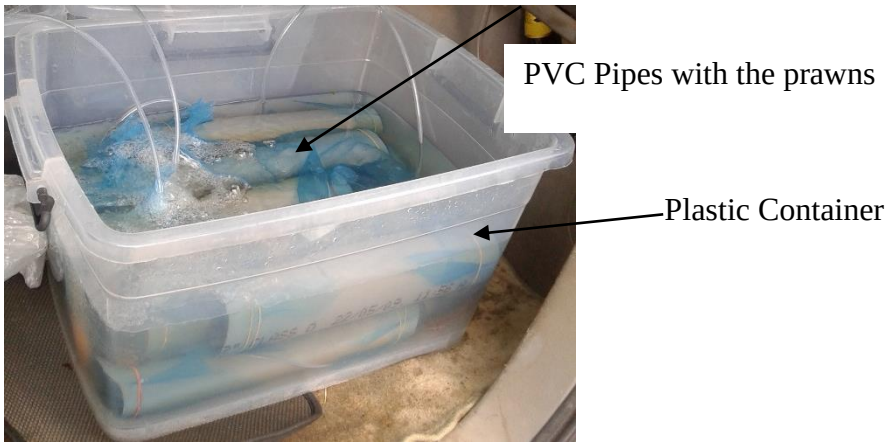


Plate 7: PVC Pipes containing berried prawns with both ends covered in plastic container

5.2.3 *Hatching Process*

The hatching process was preceded by setting up the place for the hatchery where the breeding and the larval development took place. The set up consisted of a shed (10 x 8 x 3 m) with netting walls and roofed with opaque and transparent sheets (Appendix C). Hatchery equipment and materials which were housed in the shed included: hatching tanks, larval rearing tanks (LRTs), broodstock holding tanks, water filters, freshwater storage tank, saltwater storage barrels, mixed water storage tanks, water mixing tanks, air blowers, air tubes and air stones, immersion heaters, stand-by generator (5 and 2 kVA), plastic buckets, basins, containers, siphons and water-hoses (Appendix C).

In the hatchery, the berried *M. vollenhovenii* were identified with the help of identification guides by Yeboah (2012); Powell (1982) and Rutherford (1971). They were acclimatized for 6 hours with aeration while in the PVC pipes in the plastic containers in which they were transported. The water in the transporting containers was changed gradually with seasoned tap water at rate of 30% by volume with aeration (Plate 8) until the water in which they were transported was completely changed.

Plate 8: Acclimatization of the Berried *M. vollenhovenii* in the Hatchery

After acclimatization, the prawns were disinfected in aerated freshwater in plastic containers with formaldehyde at concentration of 30 mg/L for 30 minutes prior to stocking into the

hatching units as described by Nandlal & Pickering (2005), New (2002) and Chowdhuury (1993).

Ten berried prawns from each monthly batch were visually selected carefully with the help of small scoop net based on the presence and pigmentation of egg mass (the larger the egg mass the better). The berried prawns that were obviously active, well pigmented, with no missing appendages or any other damages and carrying large dark gray egg masses were used. The total length of the selected berried prawns ranged from 10.0 cm to 11.8 cm with average total length: 10.0±1.7, 11.2±0.6 and 10.6±1.4 for experiments January, June and September respectively; the weight of the selected berried pawns ranged from: 42.4 to 51.4 g with average weight: 42.4±6.2, 44.6±3.4 and 43.8±7.6 g for experiments January, June and September respectively. The ripeness and maturity of the eggs were also criteria considered in the selection. Berried prawns carrying dark gray eggs were considered to be the best ones as their eggs hatch within 2 to 3 days.

The hatching units consisted of 26.2-litre plastic containers filled with 10 litres of freshwater with salinity of 0‰ and raised gradually to 2‰ within 12 hours (Plate 9).

Plate 9: Prepared Hatching Unit in the Hatchery

The berried prawns were placed individually in each plastic hatching unit; totaling 10 units, with 3 replicates, without feeding until hatching occurred and completed in two to three days.

Careful inspection of the hatching units for observed larvae was undertaken mainly in the mornings. The immature berried prawns were kept in holding units and fed on inert diet made up of fish and prawn flesh at 5% body weight once a day at 17 to 18 hours GMT, the leftover food and metabolite waste were carefully siphoned (07 to 08 and 16 to 17 hours GMT) daily with 2 and 1.8 cm diameter water hose. Water exchange of 20 to 80% by volume was undertaken depending on the condition of the water to avoid deterioration of water quality. The water was thus kept clean and gently aerated. The aerators used were AC Votex Blower No. 127, 360 W with 33 outlets, output 160 L/min and pressure 0.008 Mpa manufactured by Hailev, China; Resume Air Pump, 4.5 W, 4.0 L/min output, pressure 0.012 Mpa with 2 outlets (Model AC-9902 manufactured in China); BOYU Silent Air Pump Model SA 1500 with 2 outlets, 6.0 W, output 3.2 L/min and pressure 0.012 Mpa manufactured in China. Two types of battery powered ones used for emergency included two-1.5 V battery HushTone Battery Air Pump manufactured by Aquatic Edge, China; Car battery powered 12 V 120 W with 15 outlets. The aerators were connected to bar diffusers of dimensions 80 x 1.5 cm and spherical head ones of 7, 5 and 3 cm^3. Feeding of the immature berried female in the holding units ceased when the eggs were matured 2-3 days to hatching as indicated by colour of the eggs. They were then disinfected and transferred to hatching units when their eggs turned dark gray and used for in the hatching process.

The hatching process was carried out in seawater with original salinity of 35 to 37 ‰ diluted with seasoned tap water stored in poly-tank over one week to 2 ‰ and monitored throughout the hatching process. Quality parameters monitored are indicated in Table 9 as recommended by the authors listed. The set up was inspected daily for signs of newly hatched larvae.

Table 9: Water Quality Parameter for the Hatching Medium

Parameter	Mean value	Range	Reference
Salinity (‰)	2	0 – 5‰	Yen & Bart (2008)
Temperature (^{o}C)	27.5±1.2	28 – 32^{o}C	New (2002)
pH	7.8±0.8	7 – 8.5	Soundarapandian *et al*. (2009)
NO_2^{-1} (mgl^{-1})	0.03±0.06	<2	Ayoola (2009)
NH_4^{+} (mg^{-l})	0.04±0.04	10	Ayoola (2009)
DO (mg^{-l})	4.8.4±0.8	5 - 7‰	Ayoola (2009)

5.2.4: *Preparation of Larval Culture Treatment Media*

Table 10 gives details of the four treatment media: (i) (Artificial Sea Salt - ASS (ii) Rock Salt (NaCl) - RS (iii) Freshwater - FW (iv) Seawater -SW used for larval development based on what have been reported by other researchers elsewhere with varying results on the *Macrobrachium* species (The Nhan *et al*., 2010; Alam *et al*., 2005; Chand *et al*., 2015). The salinity of 2‰, in the hatchery units from where the larvae were hatched was gradually increased to 3‰ within 24 hours and further 5‰ and then to 12‰ within 48 hours as described by Niass & Fal, 2017; Chand *et al*., 2015). The salinity of 12‰ used in the larval development was based on the recommendation by several authors including Nandlal & Pickering (2005) and New (2002).

The Salinity of the treatments media were determined by mixing portions of the treatment media with freshwater to get the required salinity using the formula: $C_1V_1 = C_2V_2$ as described by Nandlal & Pickering (2005).

Where: C_1; the salinity of the stock treatment; C_2 the salinity of targeted treatment medium; V_1, volume of original stock treatment medium and V_2 volume of targeted treatment medium.

Table 10: Details of Treatment Media

Treatment	Code	Medium Composition	Prepared Medium	Salinity (‰)
Artificial Sea Salt produced by Pharmacos Ltd	ASS	$MgCL_2$ - 6H2O NaCl $NaSO_4$ $NaHCO_3$, etc.	Dissolved in seasoned tap water (5 - 12‰)	Not applied
Freshwater	FW	Pure Normal Water	0.0 - 0.5	- 0.5
Rock Salt	RS	NaCl in water	Dissolved in tap water (5 – 12‰)	Not applied
Seawater	SW	Normal Seawater	5 – 12‰	35 -37

Key: ASS:- Artificial Sea Salt; FW:- Freshwater; RS:- Rock Salt; SW:- Seawater

5.2.5: Collection of Hatched Larvae

Hatching occurred mostly overnight and took about two days to complete. The spent-out brood prawns were discarded after hatching was completed and the larvae collected immediately hatching was observed. The collection of larvae was carried out by gently aerating the larval aquarium units to evenly disperse the larvae before samples for enumeration were taken. Enumeration of the larvae was done by visually determining the number of larvae per 10 ml container. The 10 ml unit was then used to determine the stocking densities of 50, 100, 150 and 200 per litre into each of the four treatment units.

5.2.6: Feeding of the Larvae

The larvae were fed with microalgae, also referred to as green water, shell free brine shrimps (*Artemia spp)*, minced fresh fish and fresh prawn from the second day of observed hatching as indicated in Table 11.

Table 11: Type and Quantity of Prawn Larval Feed used during Larval Developmental Stages

Age (days)	Larval stage	Types of Feed and Quantity	
		Particulate feed (g/100 larvae)	Microalgae (green water) (cells/ml)
2	I – II	0.02	1.0×10^5
3 – 6	III – IV	0.02 – 0.03	1.0×10^5
7 – 15	IV – VI	0.03 – 0.04	2.0×10^5
16 – 23	VI – VII	0.05 – 0.07	3.0×10^5
24 - 27	VII - VIII	0.08 – 0.09	4.0×10^5

The green water was prepared in separate 26.2- liter plastic containers filled with freshwater and manured with chicken manure treated with 30 mg/l formaldehyde for 24 hours. The microalgae were sieved into another container initially filled with freshwater and the salinity raised gradually within 12 hours to match that of the larval rearing aquaria (LRA). The microalgae were further treated with the 30 mg/l formaldehyde dose before added to the LRA to ensure good water quality. The minced fish and minced prawn flesh were produced by grinding the fillets of fresh fish and prawn meat in a blender. The dry powdered shell free *Artemia* was produced by INVE Aquaculture Inc., Utah State, USA. All the feed materials were fed into the LRA by sieving with net of mesh size of 0.6 - 2 mm based on the age of the larvae. The feeding was done thrice a day: 07 hours, 12 hours and 17 hours GMT at feeding rates indicated in Table 11. The green water was used for the evening and the inert feed for the day.

Aeration, cleaning of the LRA to remove leftover food and metabolites, and water exchange at 20% to 80% by volume was done daily to maintain good water quality.

5.2.7: Monitoring Physio-Chemical Parameters of Larval Rearing Media

Salinity was measured with refractometer daily; water temperature was measured in the morning (9 to 10 and 14 to 15 hours GMT) and kept within the range of 27 to 30 ^{o}C either with warm or cold water as appropriate. Other parameters, namely dissolved oxygen (DO), pH, conductivity, and total dissolved solids (TDS) were measured in situ daily with electronic meter, Oakton Meter. Weekly determined parameters included Nitrite and Ammonium ions, which were analysed in the laboratory following procedures by HACH (2005).

5.2.8: Absolute Larval Number

- Total Larval Number (TLN), according to Nandlal & Pickering (2005) was estimated by counting the number of larvae in a 10 ml test tube visually. The sample in the 10 ml test tube were taken after properly aerating the hatching tank to uniformly disperse the larvae. The total number of larvae per berried prawn was then estimated using the relation:

$$TLN = \frac{Vht}{Vm} x\, n \qquad \text{(King, 1995)}$$

Where: TLN is the total number of larvae

Vht is the volume of the hatching tank.

Vm is the 10 ml volune.

n is the average number of larvae visually counted in 10 ml.

5.2.5.2: Relative Larval Number (RLN)

- Relative Number of Larvae (RLF) (number of larvae produced per unit length or gramme of berried prawn) was determined using the relation:

$RLN = \frac{TLN}{TL}$ (Relative Fecundity by Length) (King, 1995)

Where: TLN is total number of larvae.

TL total length (cm) of the berried prawn.

$RLN = \frac{TLN}{Bwt}$ (Relative number of larvae by Weight) (King, 1995)

Where: ALN is absolut larval fecundity.

Bwt is the body weight (g) of the berried prawn.

5.2.9: *Larval Survival Rate was determined using the relation*:

$S = \left(\frac{Nf}{Ni}\right) 100$ (Gomes, *et al.*, 2014)

In which:

S = Larval Survival Rate expressed as percentage

Ni = initial number of larvae counted.

Nf = final number of larvae countered

5.2.10: *Growth Rate (GR) of M. vollenhovenii larvae*

This was determined by measureing the initial and final total length against the growth period at each age at time t using the relation:

- $GR = TL2 - TL1/T2 - T1$ (Pereira de Barrosand & Valenti, 2003)

Where: TL_2 is the total length (mm) time t_2

TL_1 is the total length (mm)n at time t_1

5.2.11: *Monitoring the Larval Developmental Stage*

Ten larvae from each treatment were removed daily and kept in sample bottles containing 4% formalin for developmental features. The larvae were observed with the help of ocular

micrometer inserted into the eyepiece of microscope and the larval developmental stages were described as suggested by Lal *et al*, (2014); Nandlal & Pickering (2005) and Chowdhury (1993). Estimated 50 larvae was taken from treatments ASS, FW, RS and SW respectively at three days interval from the 3rd to 27th day to determine the Larval Stage Index (LSI). The LSI was adopted from Makombu (2014); The Nhan *et al.* (2009) using the relation:

$$LSI = \Sigma Si \; x \frac{n}{N}.$$ (Maddox & Manzi, 1976).

Where: Si is the developmental stage of the larvae (i = 1 to 8); n is the number of larvae at stage S*i;* N is number of larvae estimated.

The LSI compares the success of larval development at each stage in the various treatments based on proportion of larvae at particular stage. The evaluation ranged from 1 to 3, indicating poor to excellent performance. The LSI therefore evaluates the quality of the culture medium.

5.2.12: *Statistical Analysis of Data*

Data were recorded, organized and subjected to charts, tables and scatter plots using 2010 Microsoft Excel version. One-way analysis of variance (ANOVA) was performed to determine the treatment effects. Significant differences among treatments were determined by using Duncan's Multiple Range Test (DMRT) at 0.05 confidence level. Relationships among the various parameters such as Absolute Larval Number (ALN), Relative Larval Number (RLN), morphometric parameters of the berried prawns and weight of berried prawns were established by Regression Forecasting–Lsin, Multiple Regression and excel 2010 packages. Chi Square, F-statistics and t-test were also used to test significance ($p<0.05$).

5.3: Results

5.3.1 *Water Quality Parameters*

The water quality parameters (Table 12) were subjected to a test of single factor One-Way analysis of ANOVA, $F(3,24) = 0.05$, F-critical = 3.01, $p = 0.99$; $p<0.05$, indicating no significant differences in the quality parameters of the treatments. The parameters generally fall in line with those recommended by New (2002) and other researchers in the West Africa sub region using the same species.

Table 12: Water Quality Parameters in Larval Developmental Units

	Culture Medium				
Parameter	Artificial	Fresh	Rock	Sea	Recommended (New, 2002)
Volume (l)	10	10	10	10	
Salinity (‰)	12	0.5	12	12	<10
Temperature (^oC)	27.3±1.8	27.8±2.1	27.2±1.6	27.5±1.5	28 - 31
pH	7.8±0.9	7.3±1.9	7.7±1.6	7.8±0.8	7 - 8.5
NO_2^{-1} (mgl^{-1})	0.04±0.02	0.03±0.01	0.04±0.01	0.04±0.01	<0.1
NH_4^+ (mg^{-l})	0.03±0.01	0.03±0.03	0.04±0.02	0.05±0.01	<20
DO (mg^{-l})	5.7±1.1	5.9±0.2	5.5±1.4	5.4±1.1	>5

5.3.2 *Assessment of Hatching Performance of the Berried Prawns*

The Total Length of berried *M. vollehnovenii* used in the hatching process ranged from 8.3 cm to 11.8 cm and body weight from 63.2 g to 51.4 g (Table 13) in the three months: June samples had comparatively more large ones (11.2±0.6 cm total length and 44.6±3.4 g body weight) followed by September samples (10.6±1.4 cm total length and 43.8±7.6 g body weight) and then January (10.0±1.7 cm total length and 42.4±6.2 g body weight). T-test

analysis on relative number of larvae produced showed significant differences between the number of larvae produced per unit length and weight; $t(2) = 93.88$, $p<0.01$.

Table 13: Mean Morphometric Parameters for Replicated Berried Prawns, Mean Larvae Produced after Two Days of Stocking in January, June and September 2016

Month	TL (cm)	Body Wt (g)	Gonad Wt (g)	Larvae		
				Total Number	Number/TL	Number/Bwt
January	10.0±1.7	42.4±6.2	1.6±0.5	34479±1123	3217±800	765±27
June	11.2±0.6	44.6±3.4	1.9±0.3	39577±1811	3463±293	870±73
September	10.6±1.4	43.8±7.6	1.9±0.5	38574±1138	3463±836	870±21

'±' = Standard Deviation; n = number of berried prawns; TL = Total Length (cm) and Bwt = weight (g).

5.3.3 *Relation between pre-larval Gonad Weight, Total Length and Body Weight*

From Figure 35, 93% ($R^2 = 0.93$) of increase in the Gonad weight could be explained by increase in Total Length at the rate of 33.4% with regression equation as $y = 0.334 x – 1.72$. The F-statistics F(8.33) > F-critical shows that this positive correlation between gonad weight and total length relation is significant.

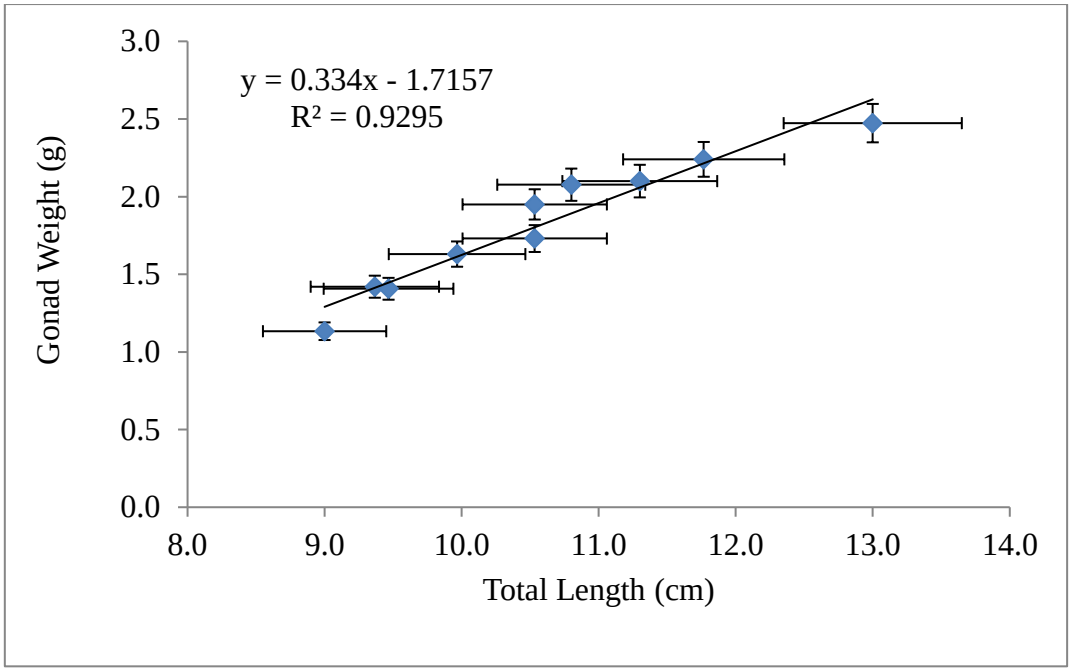


Figure 35: Correlation between Mean pre-larval Gonad Weight and Total length (with Standard Error Bars)

From the correlation equation in Figure 36 shows that 63% (R^2 = 0.63) of increase in gonad weight could be due to increase in wet body weight at the rate of 9% with the correlation equation as y = 0.088x – 2.02. The relation as indicated by F-statistics *F*(103.27) > *F*-critical (39) is significant.

Considering Figure 35 and 36; the Coefficients of Determination (R^2 = 0.93); total length verses gonad weight (Figure 35) and (R^2 = 0.63) gonad weight and body weight (Figure 36) are significantly different (χ^2 = 0.81, df = 1, p<0.05) and deviated from the perfect correlation of 1: χ2 = 0.84, df = 1, p<0.05 (total length/gonad weight) and χ2 = 0.77, df = 1, <0.05 (body weight/gonad weight).

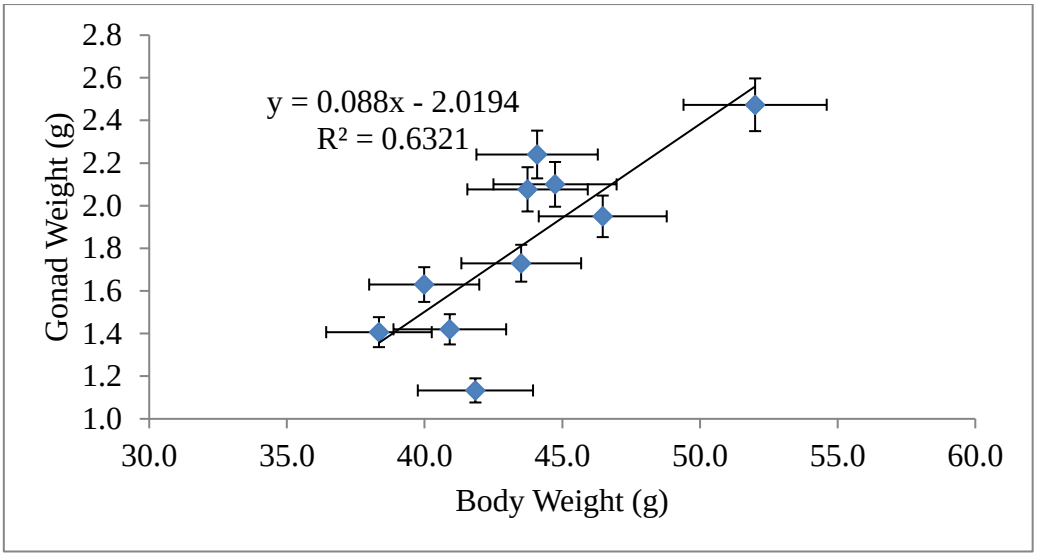


Figure 36: Correlation Between Gonad Weight and Body Weight of the Berried *M. vollenhovenii* used in the Hatching Process (with Standard Error Bars)

5.3.4 *Relation between Total Number of Larvae, Total Length and Body Weight of the Berried Prawn*

In Figure 37, 92% (R^2 = 0.922) of the increase in total number of larvae was determined by the total length of the berried prawn with the regression equation as y = 7590.3x – 34476, which deviated from the perfect correlation of 1 (X^2 = 0.95, df = 1, p>0.05.

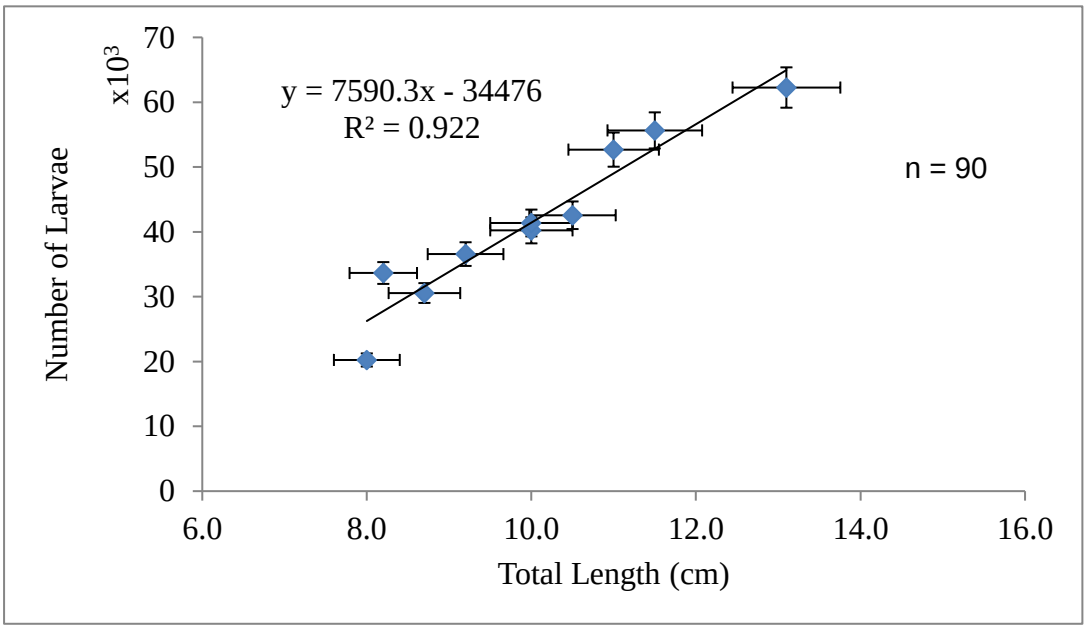


Figure 37: Correlation between Mean Absolute Larval Fecundity and Total Length (with Standard Error Bars)

In Figure 38, 61% ($R^2 = 0.6103$) of increase in the number of larvae produced can be explained by the increase in the body weight of the berried prawn resulting in the correlation equation: $y = 2280.7x - 50815$, which differ from the perfect correlation of 1 ($\chi^2 = 0.076$, df = 1, $p<0.05$)

Comparing the relation between the number of larvae based on the total length and the wet body weight, the correlation between the number of larvae and total length is more pronounced (92%) as compared to the relation between the number of total larvae produced and body weight (61%). The difference in the correlation coefficients is significant ($\chi^2 = 0.80$, df = 1, $p<0.05$).

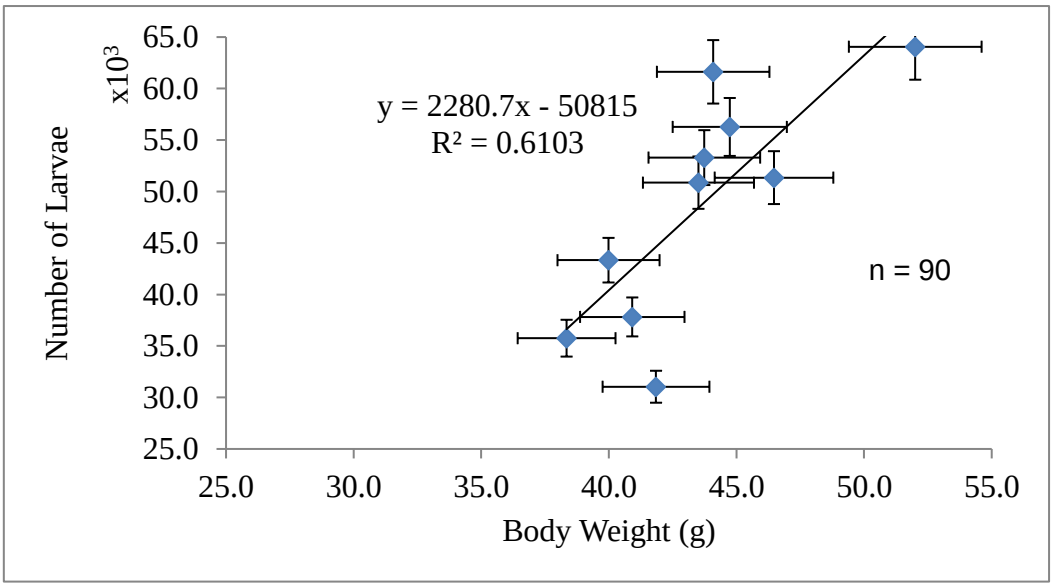


Figure 38: Correlation between Mean Absolute Larval Fecundity and Gonad Weight (with Standard Error Bars)

5.3.5 *Relation between Relative Larvae Produced, Total Length and Weight of Berried Prawns*

In Figure 39, two scenerios are presented; the relative number of larvae produced per unit length and the relative number of larvae produced per unit wet body weight in relation to the total length. The correlation between relative number of larvae per unit length ($R^2 = 0.58$)

with regression equation y = 323.39x + 863.03 and the relative number of larvae produced per unit weight (R^2 = 0.60) with regression equetion y = 103.25x – 65.27 are positively correlated with the total length of the berried pawn. The relative number of larvae produced per unit weight showed stroger correlation (R^2 = 0.60) than number of larvae produced per unit length (R^2 = 0.58); the difference is significant (X^2 = 0.99).

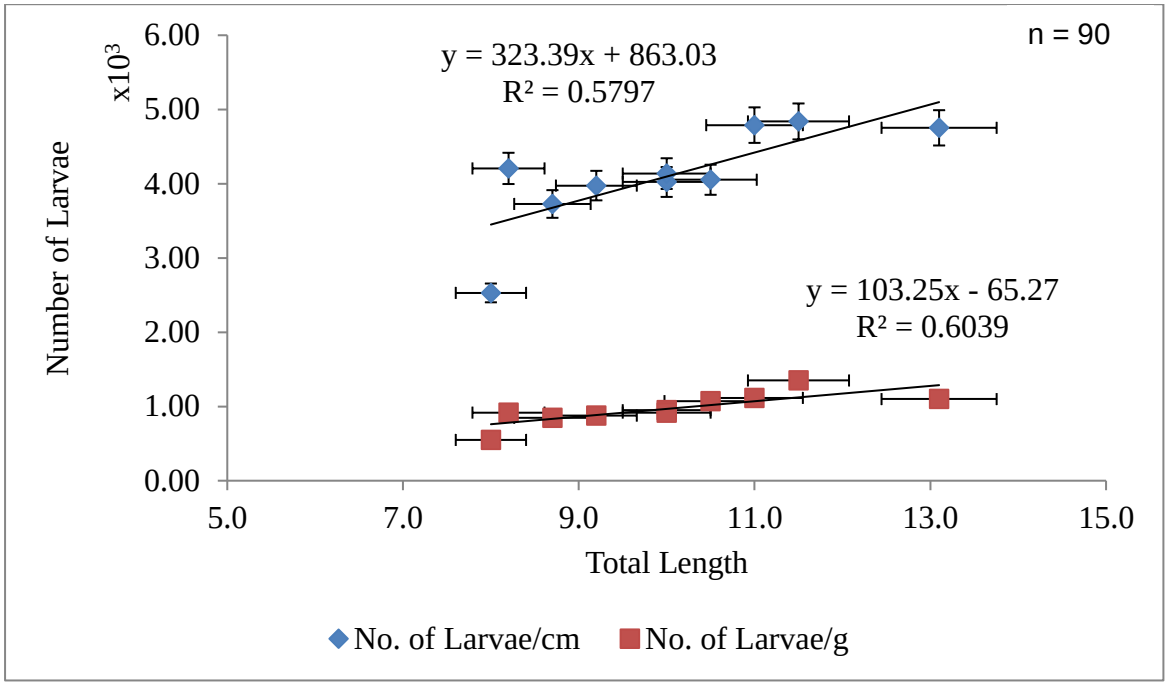


Figure 39: Relative Mean Number of Larvae Hatched and Total Length of the Berried Prawn (with Standard Error Bars)

Figure 40, is the relation between the body weight of the berried prawn and relative number of larvae produced per unit length and per body weight of the berried prawn. The correlation coefficient between the body weight and number of larvae produced per unit length, R^2 = 0.2797 indicates weak positive correlation and the correlation between body weight and number of larvae produed unit weight R^2 = 0.3684 also showed weak positive correlation, but stronger than that between relative number of larvae per unit length (R^2 = 0.2797).

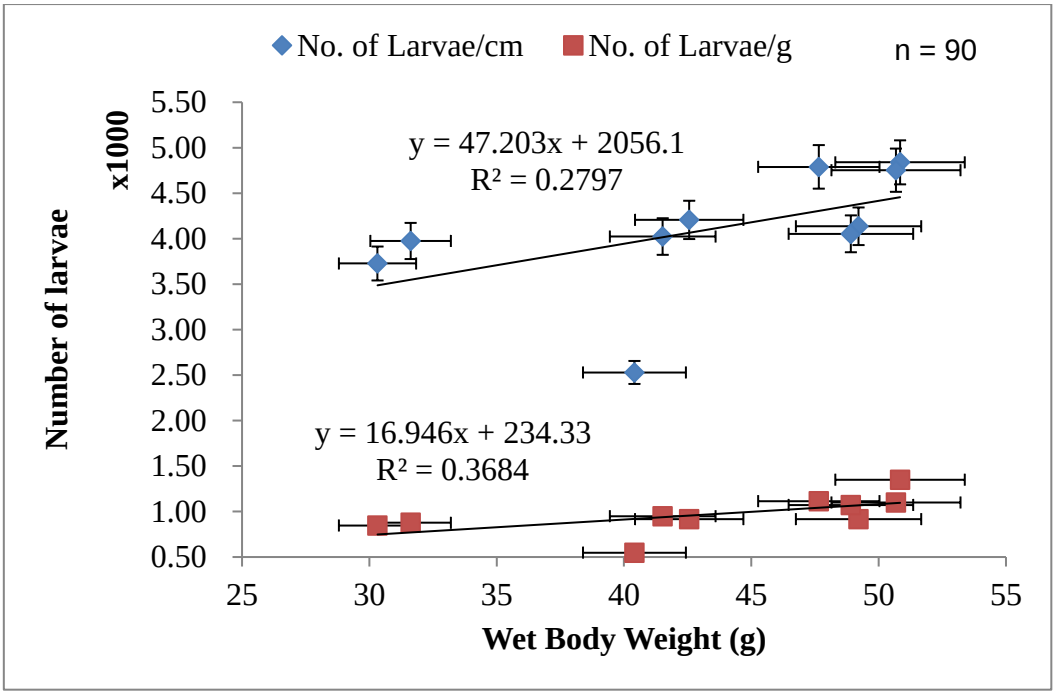


Figure 40: Relative Number of larvae hatched and wet body weight of the berried prawn (with Standard Error Bars)

5.3.6 *Maturation of Newly Hatched Larvae*

Features of the larval stages were described as suggested by Lal, *et al.* (2014; Nandlal & Pickering (2005; Choudhurry (1993) and several other authors for *M. vollenhovenii* and other members of the genus *Macrobrachium.* It was observed that during the study the larvae were highly photopositive from day 1 to about day 10 and then after became more photonegative and turn more benthic.

The newly hatched larvae of the prawns appeared as dark dots or particle (Plate 10) in the column of the culture medium. Based on their photopositive nature, the larvae are attracted to light and are aligned at the open edge of the culture medium (Plate 11). In this study eight larval developmental (Zoea) Stages (ZI to ZVII) with distinct recognizable features were observed during the 27 days of culture (Table 14).

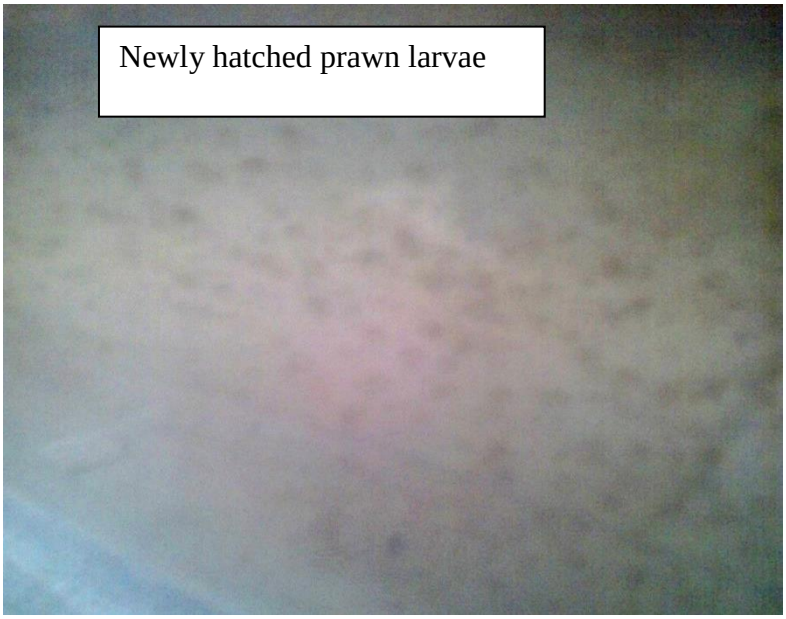


Plate 10: Appearance of newly hatched larvae of *M. vollenhovenii* (June 5, 2016)

Plate 11: Appearance of 8-day old *M. vollenhovenii* Larvae aligned at the Top Periphery of the Culture Unit.

Table 14 A: Features of Larval Stages of *Macrobrachium vollenhovenii* Larvae after Lal *et al*. (2014)

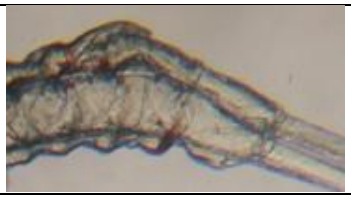
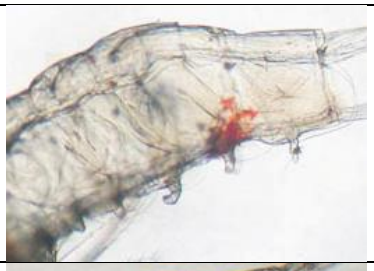
Age (days)	Larval stage	Observed Features	Description of Features of Larval Stages of prawn larvae
3	ZI		Sessile eyes; telson and uropod fused, buds for the first two pairs of walking legs (pereiopods) present.
7	ZII		Visible Stalk eyes, first two pereiopod emerged, pereiopods 3 & 5 buds appear, rudimentary uropod present.
10	ZIII		Telson narrows, one uropods developed, first dorsal tooth visible on the rostrum, forth pereiopod buds and others (1^{st}, 2^{nd} 3^{rd} & 5^{th} better developed.
13	ZIV		2 visible dorsal teeth, both uropods developed, the 5^{th} pereiopod uniramous and becomes the largest.
17	ZV		Narrow and elongated Telson with a spine, uropods further elongated, pereiopods well developed.
20	ZVI		Pleopod buds present.

Table 14 B: Features of Larval Stages of *Macrobrachium vollenhovenii* Larvae after Lal *et al*. (2014)

24	ZVII		Pleopods biramous
27	ZVIII		Pleopods with setae

5.3.7 *Larval Development*

Table 15 is the summary result of analysis of the larvae per treatment to determine growth in total length at various developmental stages (Appendix G). From the table, ZVIII was first observed on the 23rd day of growth. The transition from one stage to other took 3 to 4 days on average. The Freshwater treatment ended up with the first Zoea stage (Z1) on the third day. The freshwater treatment was therefore discontinued from the third day and therefore discarded. The other three treatments continued to record substantial number of larvae up to the 27th day of culture. The total length of the larvae in the first Zoea stage (ZI) ranged from 1.18 to 1.56 mm in 3 days of growth to get to the second Zoea stage (ZII) recording total length range from 1.72 to 1.78 mm. The second Zoea stage (ZII) to the third Zoea stage (ZIII) took 4 days recording total length range from 2.81 to1.88 mm. From ZIII to ZIV took another 4 days with total length range from 1.90 to 2.01 mm; from ZIV to ZV took further 3 days ending in length range from 2.04 to 2.10 mm. From ZV to ZVI took further 3 days with total length range from 2.14 to 2.21 mm; from ZVI to ZVII took 4 days ending in total length range

from 2.26 to 2.32 mm; from ZVII to ZVIII took another 4 days ending in total length range from 2.38 to 2.56 mm. A Single Factor ANOVA analysis shows no significant differences in the growth gains for all the four treatments. $F(2, 18) = 0.71$; F critical = 3.55; $p<0.05$.

Table 15: Mean Number of Larvae emerging at various Development Stages in 50 Sample

Age (Day)	Stage	TL (mm)	Medium			
			ASS	FW	RW	SW
			Number out of 50 sample			
1- 3	ZI	1.18 - 1.56	20±4	5±3	19±1.2	23±0.2
3 - 7	ZII	1.72 - 1.78	21±8	0	19±0.5	23±0.8
7 - 10	ZIII	1.81 -1.88	23±5	0	19±0.8	25±1.2
10 - 13	ZIV	1.90 – 2.01	28±3	0	19±06	28±1.5
13 - 17	ZV	2.04 -2.10	31±3	0	20±2.1	38±1.4
17 -20	ZVI	2.14 - 2.21	34±6	0	20±0.3	36±0.6
20 - 23	ZVII	2.26 - 2.32	28±2	0	21±0.6	35±1.1
23 - 27	ZVIII	2.38 - 2.56	31±3	0	20±1.1	38±3.1

The Larval Stage Index (LSI) at various develomental stages in the culture treatments is described in Figuure 41. The higher the index the better the treatment in supporting the growth of the larvae. In the end Seawater treatment (SW) was the highest in support of the growth of the larvvae followed by the Artificial Sea Salt (ASS) treatment with the Rock Salt (RS) treatment being the least performer. The Artificial Sea Salt medium performed slightly better than the Seawater medium from stage ZIII to ZV and merged with the Seawater treatment at stage ZVI; it dropped to 2 at stage ZVIII. The Seawater medium performed better than the Artificial Salt medium from stage ZVII to stage ZVIII. At ZI stage, all the three culture

treatmemts started at Index score of 1, the Artificial Salt and Seawater treatments scored above 2.5 from ZIV to ZVI.

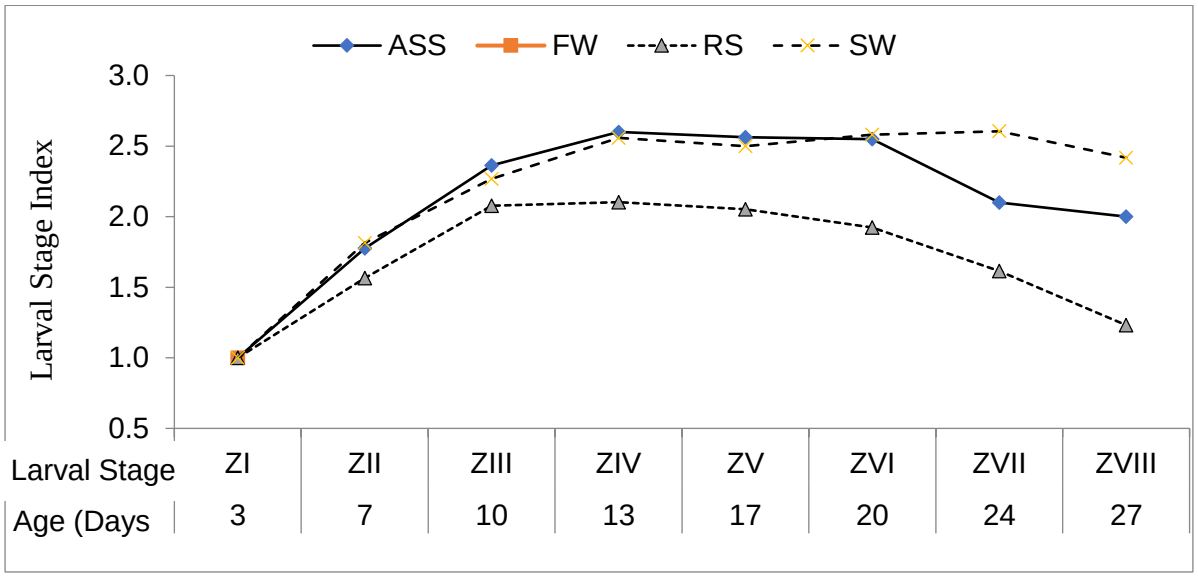


Figure 20: A graph showing the Larval Stage Index (LSI)

5.3.8 *Growth Performance of the Prawn Larvae under different Culture Conditions*

Figure 42 shows the growth of the larvae under Artificial Sea Salt (ASS), Rock Salt (RS) and Seawater treatment (SW) media. While the larvae in Artificial Sea Salt and Seawater treatment media attained the length of 2 mm at ZIV stage in 13 days of growth, the larvae in Rock Salt treatment attained the 2 mm total length at ZV in 17 days. The Seawater treatment had largest larvae throughout the culture period followed by Artificial Sea Salt with the Rock Salt producing smallest size larvae.

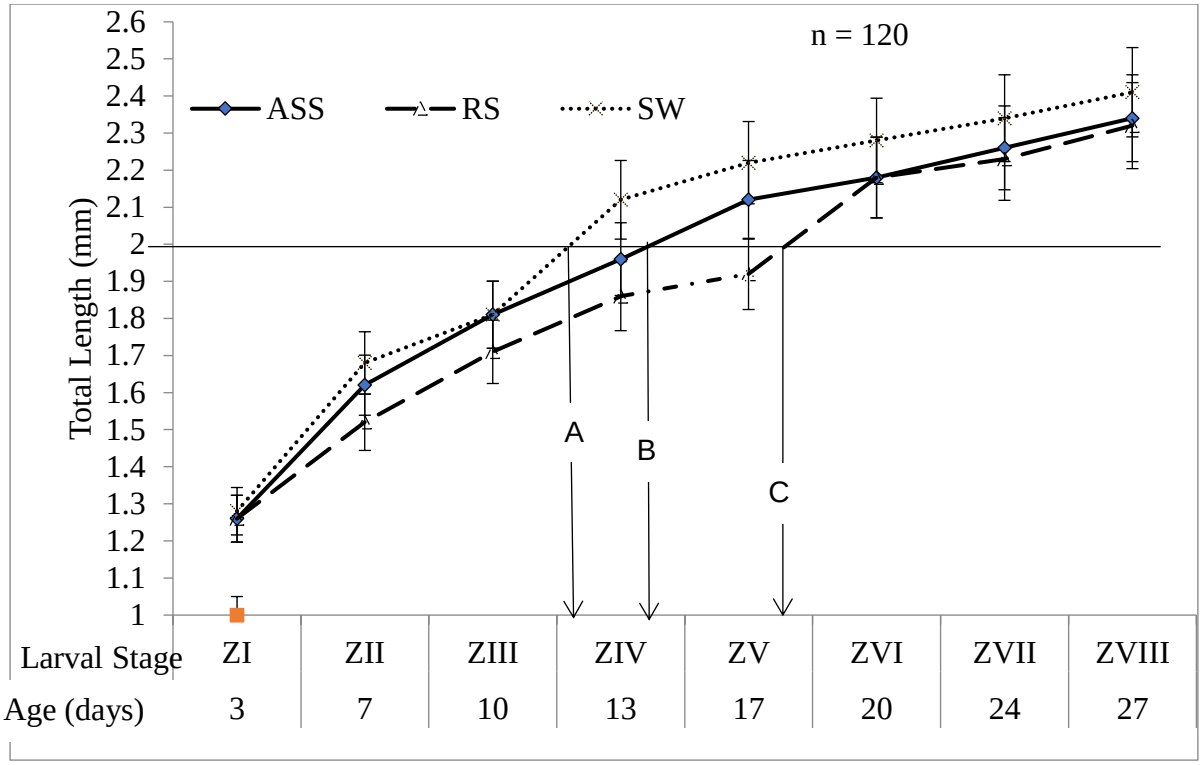


Figure 21: Mean Larval Growth at Various Stages in the Culture Treatments (with Standard Error Bars)

The result of mean daily growth rate of 120 randomly selected larvae from each treatment is displayed in (Figure 43) using equation of straight line: y = mx + a; where the "m" is the slope and rate of performance of "x". The "m" is therefore treated as growth rate of the prawn larvae in this study. The value "m" from the graph (rate of growth) in all the three treatments is approximately 0.04 mm/day. The larval growth performance in the Rock salt treatment however appeared to be the lowest (0.035 mm/day) and the Sea Water Treatment appeared to register the best growth rate of 0.038 mm/day followed by the Artificial Salt Treatment of 0.037. The One Factor Analysis of Variance indicated no statistical differences in the growth rates, $F(2, 21) = 0.43$; F critical = 3.47; $p<0.05$ among three treatments in the 27 days of growth.

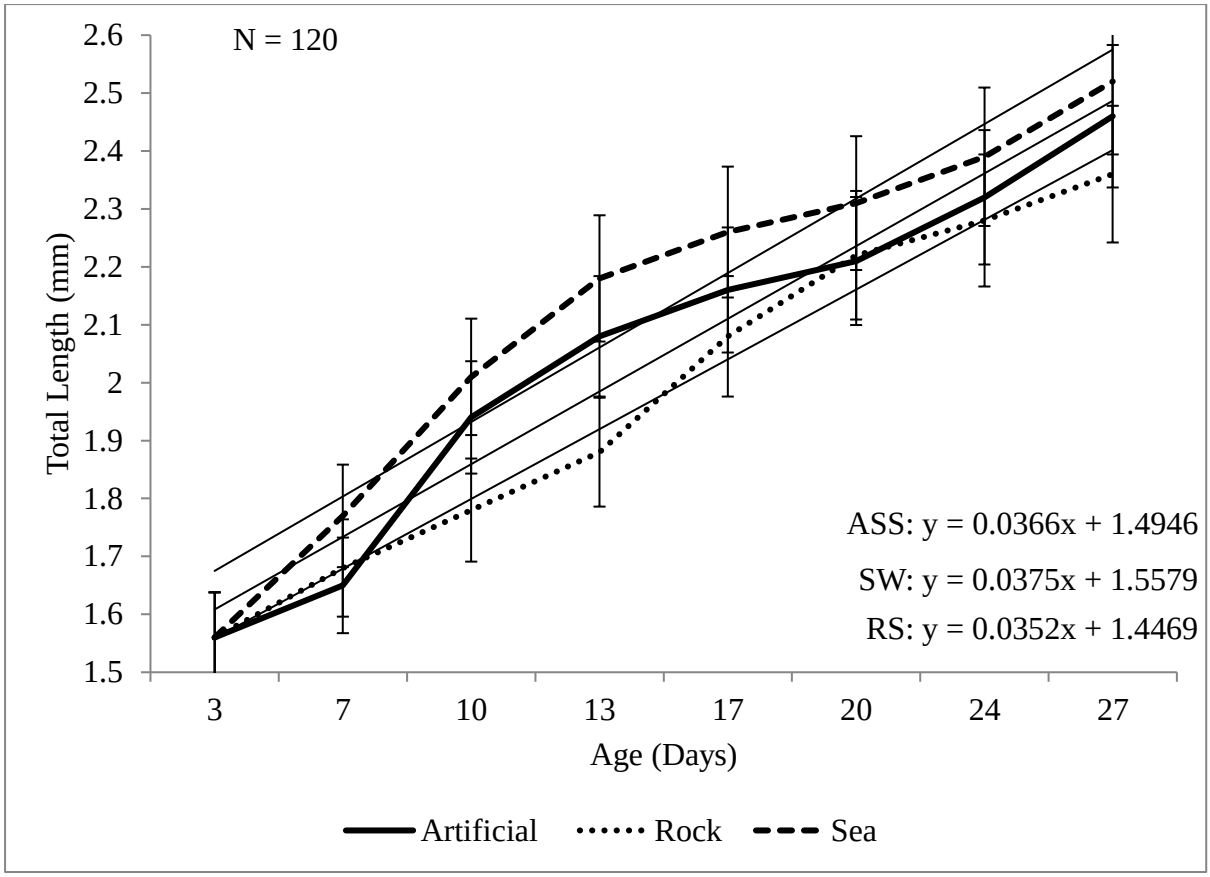


Figure 223: Mean Growth Rate of Larvae in the Four Treatment Media (with Standard Error Bars)

5.3.9: *Survival Rate of M. vollenhovenii Larvae Under Varying Stocking Densities*

The best performing culture medium, Seawater, was used to assess the effect of the varying stocking densities (Figure 44). On day 2, stocking density of 50 larvae/L was about 89%; survival then decreased in stocking densities of 100 larvae/L and 150 larvae/L and then picked up at 200 larvae/L. The best performed stocking density was the 50 larvae/L on day 2 followed by 200 larvae/L stocking density. On day 3, 100 larvae/L stocking density was the best performer followed by the 50 larve/L. In both day 2 and 3, the 150 larvae/L appeared to be the poor performer.

The result was subjected to Forecasting –Lsin Regression analysis. The number of days was compared against number of larvae per litre, the $R^2 = 0.0371$ with standard error ±60.60.86.

The F-Statistic (0.2502) < F-Critical (3.6823) indicates no significance difference at 95% confidence level. The Durbin-Watson Statistic gives 2.81, indicating no correction among the treatments and survival rate.

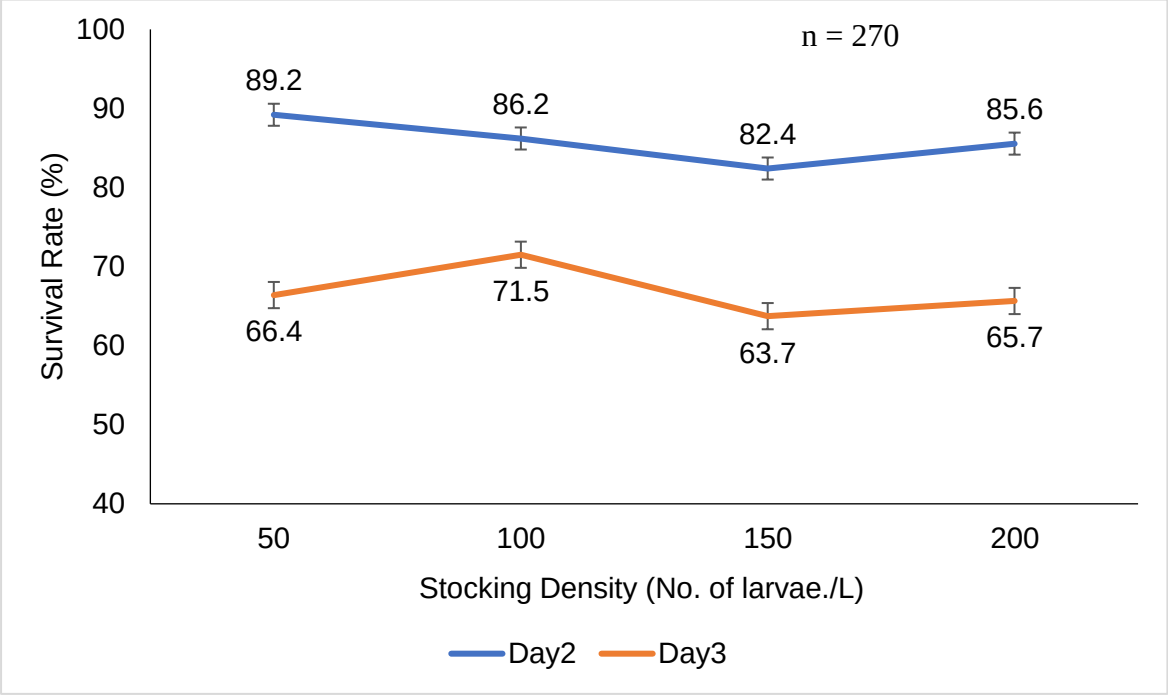


Figure 23: Survival Rate of *M. vollenhovenii* Larvae at Varying Stocking Densities from Day I – Day 3 in Seawater (with Standard Error Bars)

Figure 45 compares the survival of the larvae under the 50, 100, 150 and 200 larvae/L for day 5, 10, 15, 20 and 25. On day 5, the survival in all the stocking densities ranged between 50 – 70% with 100 larvae/L leading with 70% and 200 larvae/L trailing with 50%. The highest survival rate (38%) on day 25 was observed in the 50 larvae/L stocking density and the lowest survival rate (19%) was observed in 200 larvae/L.

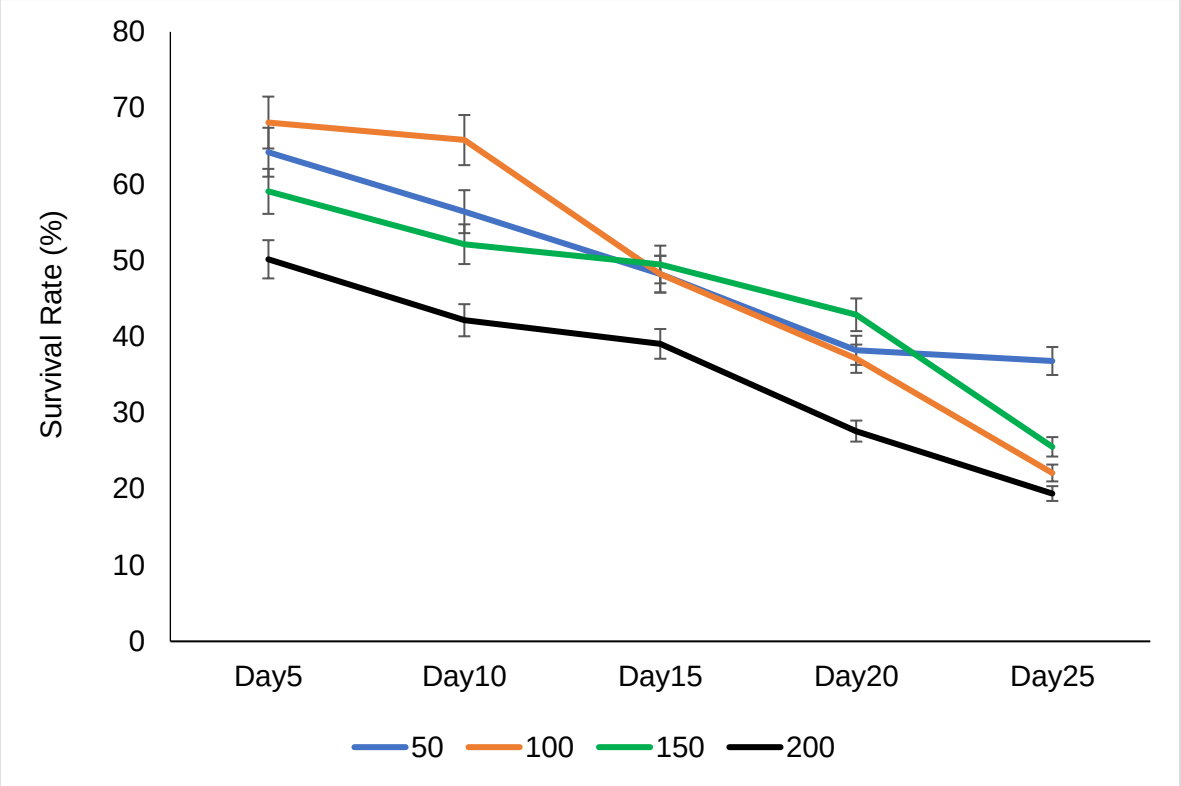


Figure 45: Larval Survival Rate at Varying Stocking Densities from Day 5 to 25 in Seawater Treatment (with Standard Error Bars)

In the culture media, survival rate was about 25% in Seawater treatment, 20.5 in the Artificial Sea Salt treatment and about 12% in the Rock Salt treatment (Figure 46). Seawater recorded the highest survival throughout the culture period with Rock Salt treatment recorded the lowest survival rate.

Analysis of Regression Forecasting–Lsin of number of days against number of larvae per litre (stocking density) resulted in $R^2 = 0.87$ with standard error ± 28.92. The F-Statistic (7.77) > F-Critical (3.11) indicating significance difference at 95% confidence level. The Durbin-Watson Statistic gives 1.86 shows negative Correlation between survival and progression in the days of culture.

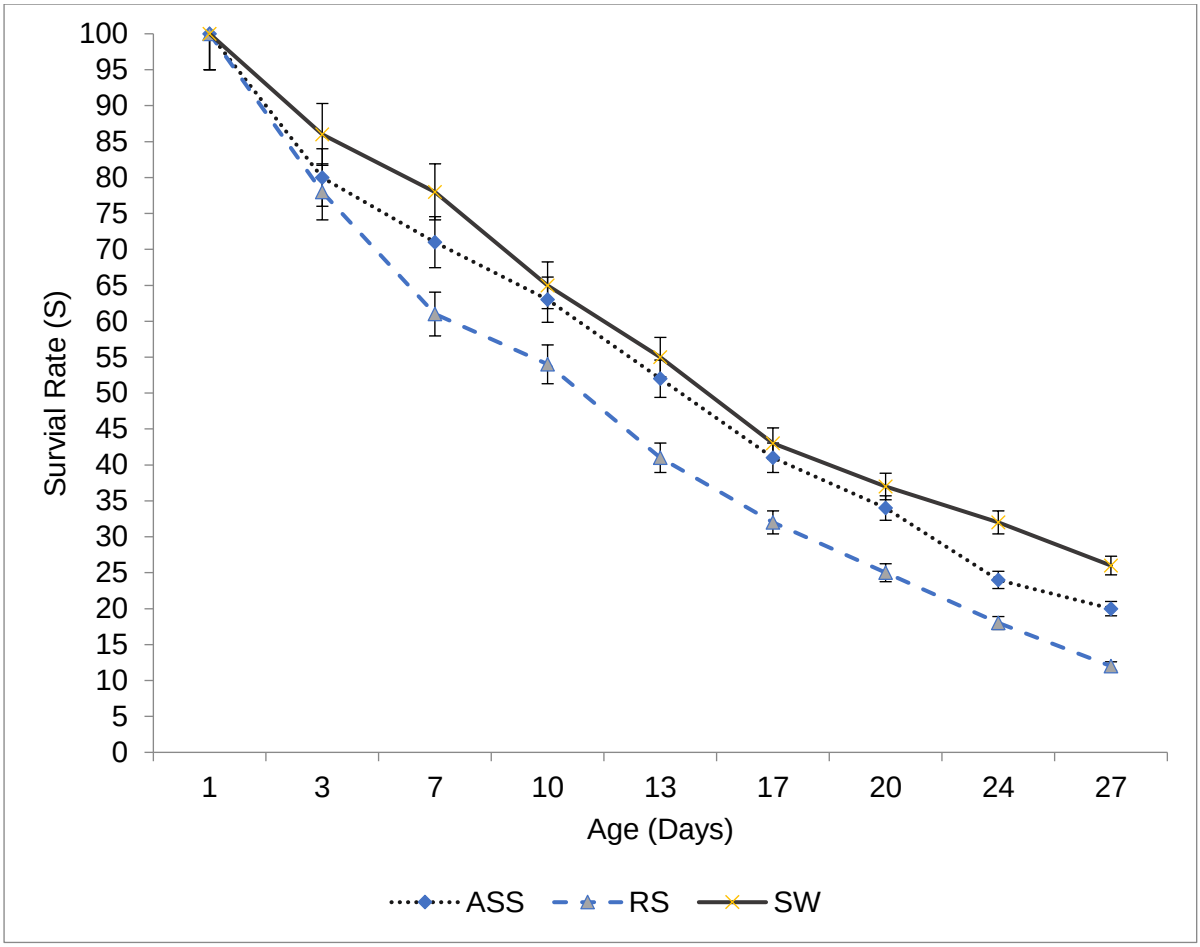


Figure 46: Survival Rate in the Culture Treatments (with Standard Error Bars)

5.4 Discussion

5.4.1 *Water Quality Parameters*

The hatching and larval development of freshwater prawns are affected by a number of factors including the culture medium. The optimal medium quality is one of the crucial conditions to ensure success in hatching of freshwater prawn eggs and larval development (Niass and Fall, 2017; Makombu *et al*., 2014; Nandlal & Pickering, 2005; Willfurhr-Nast, *et al*., 1993; Chowdhury *et al*., 1993). Analyis of the treatment media used in this study showed no significant differences ($p<0.05$) in quality parameters (Dissoved Oxygen, pH, nutrients and others measured) in the treatment media. The quality of treatment media could therefore be described as uniform and therefore any effect could be attributed to sourcec of salinity, except the Freshwater (FW) that could not even support larval growth after 48 hours of hatching.

The berried prawns of *M. vollenhonii* hatched successfully in seawater diluted to 2‰ within 48 hours as reported by severral authers for *M. rosernbergii* and prove tested by Yen & Bart (2008) who looked at hatching in 0, 6, 12, 18‰ and observed that the mean number of larvae hatched reduced with increasing salinity. From the recommendation and results from studies on *M. rosenbergii* and other *Macrobrachium* species, it is apparent that the *M. vollenhovenii* could also hatch well in both freshwater and saline water with low salinity (Niass and Fall, 2017; Makombu *et al*., 2014; Nandlal & Pickering, 2005; Willfurhr-Nast, *et al*., 1993). This recommendation was therefore supported in the current research.

5.4.2 *Hatching Performance of the Berried Prawns*

In the three samples for January, June and September, the average number of larvae varied from 21,766 to 49,963. This deviated from of Makombu *et. al*. (2014) who reported of number of larvae of *M. vollenhovenii* from 15, 000 to 26,000 in Cameroon. The observed number of larvae for the three experiments in the current study however was close to what was observed by Willführ-Nast, *et. al*. (1993) who reported of 43,430 to 44,680 larvae from berried prawns

of total lengths ranging from 10 to 10.5 cm and weight from 28.5 to 30.0 g. Comparing the currrent results for total length 8.3 to 12 cm and weight 36.2 to 51.8 g to that of Willführ-Nast, *et. al*. (1993), it appeared that size of berried prawns affects the number of larvae produced as indicated in correlation equations of $R^2 = 0.92$ and $R^2 = 0.61$ for tatal length and body weight respectively of the berried prawns. The number of larvae produced per unit length and per unit weight in this experiment also correlated positively with the total length affecting the number of number of larvae produced more than the weight {$t(2) = 93.88$, $p > 0.01$}. The number of larvae produced could therefore be a function of the size of the berried prawn.

Apart from the number of larvae producecd being influenced by the size of berried prawn, the weight of gonad is also influenced by the size and weigt of the berried female. The weight of gonads, however, appeared to be influed by total length more than body weight. Strong relation correlation of total length and gonad weight was established and this could be due to the fact that length is a structual feature that could be a function of the volume of the berried prawns. The weight of any organism could varied due to availability of food, health and physiological condition of the organism and could therefore vary depending on the conditions and environmental factors (Akinwunmi *et al*., 2014).

And again, the relative number of larvae increased from 548.67 to 1,350.15, a difference of 801.48 with increaes in body weight as against 2,529.38 to 4,839.39, a difference of 2,301.01 with increases in length. Clearly length again has more influence in number of larvae produced by the berried prawn. Though the influence of length has no statistical difference from the influence of weight on the number of larvae produced, the length correlated more positively to the relative number of larvae produced than the weight of the berried prawn. This implies that though the number of larvae produce might depend on size of the berried

prawn, number per unit length or weight could not be necessarily significant in the number of larvae produced.

5.4.3 *Growth Stages of M. vollenhovenii Larvae*

Newly hatched larvae appeared as black dots scattered in the hatching medium and later moved towards the open end of the holding facility where they aligned against the wall of the holding facility. This observation confirmed the finding that *Macrobrachium* larvae are photositive (Ratanak, 2011; Roe *et al*., 2005; Willführ-Nast *et al*., 1993) This behaviour could permit collection of the larvae from waste materials in the culure medium by siphoning the larvae from surface of the medium or removal of the waste materials from the medium by siphoning from the bottom coupled with creation of dark spot at top section of the container where siphoning could be carried out with minimal number of larvae taken along with the siphoned water.

The experiment on the prawn larval development was conducted for 27 days with the first ZVIII stage observed on the 23rd day. This can be compared to the work of Makombu *et al*. (2017) who obtained the ZVIII on the same species in 21 to 39 days using two berried prawns in Cameroon. Belsare *et al*. (2007) also reported of ZVI – ZVIII for the most commonly farmed freshwater prawn, the Giant River Prawn (*M. rosenbergii*) within 21 to 23 days while Nandlal & Pickering (2005) reported of 13 to 20 days to observe the ZVIII stage for *M. rosenbergii*. The growth and larval development of the prawn just like any other organims depend on the species, environmental conditions, quantity and quality of food. The varying growth and larval developmental stages of the *Macrobrachium* larvae under culture conditions could therefore depend on the species, water quality and type of food. Temperature plays a vital role, if it is either below or above the optimum range (28 – 32^{o}C). Below the lowest required temperature could prolong the development (Niass & Fall, 2015).

The features at the various developmental stages were similar to those observed in other *Macrobrachium* species elsewhere (Lal *et al*., 2014; Nandlal & Pickering 2005; New, 2002; Choudhurry,1993; Atkinson, 1977). The transition from one stage to another in the current study took 3 – 4 days. At ZI the total length of the larvae observed ranged from 1.2 mm to about 1.6 mm and increased to 2.4 mm to about 2.6 mm at ZVIII stage. This observation deviated from the 2.2 mm at Z1 and 3.1 – 3.2 mm at ZII stage within 12 days as recorded by Marioghae & Ayinla (1995) on the same species in Nigeria. Willführ-Nast *et al.* (1993) on the other hand observed 1.88 – 1.98 mm at ZIII and 2.88 mm at ZVII which was close to what was observed in the current study. In other species of the genus, *Macrobrachium acanthurus*, Choudhury (1970) recorded 2.25 – 2.35 mm at ZI stage and 4.4 – 4.65 mm at ZVIII stage in 22 – 26 days. Variations in larval size could therefore be a function of species involved, the culture conditions, food availability and food quality with temperature playing a vital role.

5.4.4 *Salinity Requirement for the Larval Development of the M. vollenhovenii Larvae*.

The *Macrobrachium* speices may either complete their life cycle in freshwater or they may need saline water at the early stage of growth (Marioghae & Ayinila, 1995; Miller, 1971). Those species whose larvae need saline water are most commonly reported to do well in salinity range of 12 – 15 ‰. (D'Abramo *et al.,* 2003). In the current study salinity of 12‰ was used based on the recommendations by several authors as the most tolerant salinity for the growth of *M. rosenbergii* larvae (Nandlal & Pickering, 2005; New, 2002). This assertion was further investigated by Ratanak (2011), who compared the survival of *M. rosenbergii* larvae under 9, 12 and 15‰ and came to conclude that the 12 ‰ registered the highest survival rate. Nontheless, there is an interesting report by Willführ-Nast (1993) that the optimum salinity range for *Macrobrechium vollenhovenii* larvae could be within the range of 16 – 24 ‰. In contrast to Willführ-Nast (1993), Prah (1982, 1977) reported that the *M. vollenhovenii* encountered in Ghana completed its life cycle in freshwater habitat with salinity range of

0.005 – 0.012‰. Perhabs the work of Prah (1982, 1977) could be supported by the work of Alhassan (2011; 2007). The report by Alhassan (2011; 2007) indicated the presence of *M. vollenhovenii* in commercial dimensions in the Dawhenya impoundment in Ghana which has no clear link to any saline water body. As indicated by Fielder (1970) three types of the freshwater prawns, *Macrobrachium* species are distinguished: i) those that spent their early life cycle in the marine and brackish waters producing small and numerous larvae that grow through longer larval stage ii) those that complete the life cycle in freshwater producing fewer larvae that grow through shorter larval stages iii) those that complete their life cycle with direct development as post larvae. The current species, obtained from the Volta Estuary of Ghana, should be the type that needs saline condition in the early stage of development with longer larval stages. This is evident in the result of the current work; the larvae could not survive beyond 48 hours in the freshwater medium while those in the saline medium treatments could survive up to the ZVIII stage in 27 days. In the freshwater medium, the larvae could not reach the ZII stage. Mishra and Kanaujia (2016), however reported of Gangetic prawn *Macrobrachium gangeticum* of reaching ZII before dying in a few days in freshwater.

In the current study ZVIII was reached in 23 days out the most popularly reported ZIX stages for most *Macrobrahium* species, but the larval stages varied with species and culture conditions. Niass & Fall (2015) reported of 13 larval stages of the *M. vollenhovenii* in Senegal, but most researchers who worked on the same species reported of 11 larval stages Makombu, *et al*. (2017; (Willführ-Nast (1993). The 11 larval stages are mostly reported for the *M. rosenbergii* (Ishmael & New, 2000). The variation in larval stages for one particular species of the *Macrobrachium* could be attributed to culture conditions (water temperature, feeding, other environmental and culture conditions).

5.4.5 *Growth Performance of M. vollenhovenii larvae under different Sources of Salinity*

The best culture medium was the Seawater medium followed by the Artificial Salt medium and then Rock Salt medium. The Ttest t(2.14) > p(0.72) $p<0.05$; indicated no significant differences between the performance of the Artificial Sea Salt treatment and Seawater treatments. These two media could therefore be used in producing the *M. vollenhonii* larvae. The performance of the Artificial Sea Salt, could perhabs facillitate the setting up of the prawn hatchery in areas where access to seawater poses problems or distance could not make the transportation of seawater economically feasible.

The quality of the culture media in supporing the growth of the larvae is further tested with Larval Stage Index with Seawater and Artificial Salt treaments recording high scores. The performance of Rock Salt treatment though not as good as the Seawater and Artificial Sea Salt treatments, could be used with care in the absence of Seawater and Artificial Salt.

Apart from the Seawater and Artificial Salt treaments producing various larval stages in fewer days, the size of the larvae produced were also larger than those produced in Rock Salt treatment. At ZIV in 13 days, the 2 mm total length was observed in the Seawater and Artificial Salt treatments, the same 2 mm was observed in Rock Salt treament at ZV in 17 days. The Seawater and the Artificial Salt treatments prove to be of better quality media for the larval development of *M. vollenhovenii* than the Rock Salt Treatment.

In the work of Alam, *et al*. (2003) on *M. rosenbergii*, all the larvae died in 12 days out the 25-day experiment in the Crude or Rock Salt treatment and with lower performance when 75% of the Crude Salt mixed with 25% Brine Salt (Artitifial Sea Salt). The highest survival occurred when 50% Crude Salt was mixed with 50 Brine salt. This suggests that mixing the

Rock or Crude Salt with Artificial Sea Salt could also be another way to improve the performance of the Rock Salt.

Comparing the availability and cost of Articificial Sea Salt, the use of both Artificial Sea Salt and Rock Salts offers another opportunity for over dependence on Seawater in areas where there is no sea. Soundarapandia *et al.* (1994), in India, on the other hand reported of good survival of 50.4% (50.4±0.2 PL/l) of *M. malcolmsonii* in crude salt (common or rock salt) treatment as against 47.1% (47.01 PL/l) in synthentic brackishwater treatment in India during the 46 days of culture. They compared the performance of rock salt medium to the work of Melecha (1983) in Hawai on *M. rosenbergii*, Mohanku Nair & Shahoul Hameed (1990) on Scampi species in India; New & Shingholka (1985) on *M. rosenbergii* in Thailand also gave favourable result on the use of the rock salt. These are clear indication that rock salt if carefully used could compare favourably with seawater and artificial sea salt.

Despite the poor performance of the Rock Salt treatment in this work, the growth rate of larvae in all the three saline treatmets was about 0.04 mm/day: Rock Salt treatment: 0.035 mm/day; Artificial Sea Salt: 0.037 mm/day and Seawater: 0.038 mm/day. The Rock Salt might not support good survival rate for long time, but has no effect on the growth rate and can therefore be used with precautionary measures.

5.4.6 Growth Performance under varying Stocking Density

The stocking density of farmed aquatic organisms should commensurate with the carrying capacity of the culture medium for good and healthy growth deviod of deterioration or breakdown of the medium, unhealthy competition and cannibalism as in the case of the *Macrobrachium* and other canivorous speceis. In the current study 50, 100, 150 and 200 larvae per litre were scrutinized. All four stocking densities performed well in first three days after hatching (larvae/L on day 3: 50-66.4%, 100-71.3%, 150-63.7%, 200-65.7%), this

implies that upto the third day after hatchng, larval stocking density could be as high at 200 larvae/L without heavy mortlities. The negative impact of high stocking density started to show up from day 5, reducing the number larvae per litre of water appeared to be necessary on day 3 to avoid high mortalities. As indicated by The Nhan *et al*. (2010) stocking density could be enhanced with appropiate water conditions and feeding.

The best stocking density in this study at end of the 27 days of work was 50 larvae/L, this was supported by the work of Negrini *et al*. (2017) and Yamasaki-Granados *et al*. (2013) in Brazil and Mexico respectively; they also discovered the best stocking density at 50 larvae/L for *M. rosenbergii* and *M. americanum* respectively in their respective locations. Wilder *et al*. (2004) recommended stocking density from 60-120 larvae/L from hatching to post larval stage in both static "green water" and recirculating systems for *M. rosenbergii*.

The Nhan *et al*. (2010) observed that survival and growth were better at stocking density of 100 and 200 larvae/l with increased amount of feed and frequency of feeding. Too low stocking densities could also be disastrous as reported by Yamasaki-Granados *et al.* (2013), low survival (70.3%) occurred at stocking density at 10 larvae/l and increased 90.6% as the stocking density increased to 50 larvae/l. To keep stocking densities higher than 50 larvae /l, more feed and more frequency of feeding should be ensured for good survival. This not withstanding thinning, increasing the water volume or the size of the holding facilities will be required with age of the larvae as they grow bigger and fast growerrs (jumpers) turn to cannibalise on the smaller and weaker ones.

5.4.7: Survival Rate of M. vollenhovenii larvae under Varying Stocing Densities

The survival rate at the end of 27 days of work varied from 19.4% (200 larvae/L), 22.1% (150 larvae/L), 25.5% (100 lavae/L) and 36.8 (50 larvae/L). The highest survival rate was therefore observed in the 50 larvae/L stocking density. The survival rate appeared to be low, but

comparable to the work of Davassi, (2011) who obtained survival rate from 13 – 40% at stocking density of 30 larvae/L in accessing protein levels on the growth of *M. rosenbergii* larvae. Lal (2014) reported of 0.08% at end of his work in Fiji and compared this result to <0.1% survival in the wild. Naturally, the larviculture of the crustaceans according Lal (2014) is faced with low survival rate until the process of culture is refined and perfected. The survival rate in the current work though appeared low had laid solid foundation for future work. According to Ling (1961), the initial larviculture of *M. rosenbergii* recorded survival rate from 16 – 17%, but today 60 – >80% are being reported as culture techniques improved. Even then Wilder *et al*. (2004) obtained 27.4% – 52.5% and 27.7% – 41.7% in comparing the performance of *M. rosenbergii* larvae in re-circulating and green water systems repectively. Low stocking density is thefore the norm in any new larviculture system. Despite perfection in the larviculture techniques, low survival could still be recorded at any time in the larval development process.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1: **Conclusions**

6.1.1: *Occurrence and Distribution of Prawns and Shrimps in the Volta Estuary*

- Three families namely: Atyidae, Palaemonidae and Penaedae with single representative genus of *Atya, Microbrachium* and *Penaeus* respectively were encountered in the survey.
- Two freshwater prawn species (*Macrobrachium macrobracion, Macrobrachium vollenhovenii)* and one crayfish (*Atya gabonensis*) and unidentified shrimp species (*Panaeus spp.)* were encountered during the study in the Volta Estuary and its environs.
- The most dominant freshwater crustacean species encountered in the Volta estuary was the freshwater prawns *Macrobrachium* species (77.38%) followed by the marine *Penaeus* species (22.58%) and Atya *gabonesis* (0.03%).
- Relatively higher proportion (82%) of *Penaeus* were found in areas close to the mouth of the estuary with comparatively higher salinity (23‰ on average) while the *Macrobrachium* species occurred in both the freshwater (0‰ salinity) and brackish estuarine waters (25‰ salinity) with higher proportion (96%) in the freshwater zones in the estuary.
- The *Atya* species were not observed in Zone A (23‰ salinity on average), but were found in Zone B (average salinity of 19‰) and mostly in the upper reaches of the river such as Torgome, in the more northern riverine part of the Volta River, where they were the dominant species in the sample (86%) compared to *Macrobrachium* species (14%).

- By size, larger size (9.8±1.1 cm Total length) of the *Penaeus* were observed in higher salinity area (Zone A) as compared to 8.7±1.6 cm Total Length in Zone B. On the contrary, larger size (12.7±1.7 cm Total Length) of *Macrobrachium* species were found in the freshwater areas compared to 10.6±2.0 cm Total Length in the more saline areas. There was migration therefore of the *Penaeus* species from the low salinity zone to high salinity zone as the mature with increase in size. This pattern is reversed in the *Macrobrachium* and *Atya* species that migrate to low salinity zones as they mature and increase in size.
- Both the *Macrobrachium* and the *Penaeus* species occurred throughout the year, but *Macrobrachium* occurred more in the rainy season as compared with *Penaeus* species that appeared more in the dry months of the years
- Between the two species of the *Macrobrachium*, the *M. vollenhovenii* was more dominant species (75%) compared to *M. macrobrachium* (25%) and by size.

6.1.2: *Aspects of Reproductive Biological Study of M. vollenhovenii*

- The females were significantly ($p<0.05$) more than the males in all the monthly samples resulting in the overall male to female ratio of 1:1.3.
- Total berried prawns encountered constituted 46% and non-berried female prawns 54% resulting in significant ($p>0.05$) berried to non-berried prawn ratio of 1:1.7.
- The berried prawns were available throughout the year with comparatively more in the rainy months than in the dry months.
- Gonad weight and fecundity were directly proportional to total length and body weight of the berried female with total length increases playing more role in the increase of gonad weight and fecundity than increases in body weight.
- The size of the egg was not proportional to size and weight of the berried female.
- Increased in gonad weight was a function of number of eggs rather than size of egg.

6.1.3 *Hatching Performance of Berried Macrobracium vollenhovenii female.*

- The berried *M. vollenhovenii* could hatch in water with salinity of 2‰ or lower, but the newly hatched larvae could not survive in freshwater beyond 48 hours.
- The number of larvae produced is directly proportional to the size of the berried prawns and gonad weight.
- The newly hatched prawns were photopositive up to 10th day and then after they turned to be photonegative.

6.1.4: *Development of the M. vollenhovenii Larvae*

- The larvae reached stage the 8th stage (ZVIII) within 23 days of culture. During this period the larvae increased from size range of 1.2 – 1.6 mm total length to a maximum size range of 2.3 - 2.6 mm at ZVIII mm total length.
- Larval growth was successful with salinity of 12‰.
- Artificial Sea Salt, Rock Salt and Seawater medium could support larval development after 48 hours, the Freshwater treatment medium could not support larval growth beyond 48 hours.
- Seawater was the best larval culture medium followed by Artificial Sea Salt and then Rock Salt medium. The performance of Seawater was however not scientifically different ($p<0.050$ from that of Artificial Salt media.
- Growth rate in all the three treatments was 0.04 mm/day, the Rock Salt medium though could not support high survival, it had no negative effect on growth rate.
- Survival rate was found to be significantly ($p<$) indirectly proportional to increasing stocking density.
- The best stocking density at end of 27 day the study was found to be 50 larvae/litre.

- The highest survival rate of 36.8% was observed in 50 larvae/L treatment at the end of 27 days of work and the lowest was 19.4% in the 200 larvae/L in the Seawater treatment.

6.2: Recommendations

- Work could be carried out to sample the prawns in other estuaries such as Pra River, Ankora and other water bodies in the country to determine the ecological, seasonal distribution, availability, health and diversity of the freshwater prawns to serve as alternative source of brood prawns for culture.
- Long and sustained sampling in the Volta Estuary and its environs would provide more information on the population dynamics of the prawns in the area.
- Studies on brood stock development of the prawns should be undertaken to provide extra information for sustained development of prawn culture in Ghana.
- Research into breeding and culture potential of *M. macrobrachiom* could be carried out to enhance their culture in the country and widen the species base for culture.
- High stocking densities of the prawn could be desirable at initial larval development, but the need to thin down the number, increase the volume of water or size of holding facility from 72 hours after hatching would ensure low mortalities and good growth.

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APPENDICES

APPENDIX A: PRAWNS AND SHRIMPS

General View

The prawns or shrimps occur in fresh, brackish and marine waters across the temperate and tropical climates with greater number found in shallow and moderately deep waters. Although some are pelagic, the majority are benthic living on rocky, muddy, peat, sandy, fragmented or mixed substrates. Except a few, the sexes are separate (Powell, 1982).

In commercial farming and fisheries, the terms "shrimp" and "prawn" are often used interchangeably. However, recent aquaculture literature increasingly uses the term "prawn" only for the freshwater forms of the Palaemonids and "shrimp" for the marine penaeids (Pillay 1993).

Distinguishing Features of Prawns and Shrimps ((Valencia, *et at*., 2007; New, 2002).

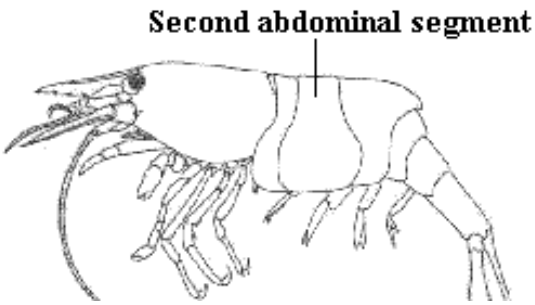


Figure 1: Prawn

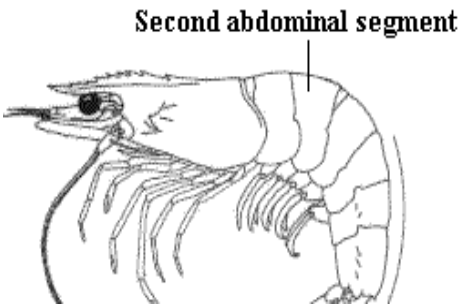


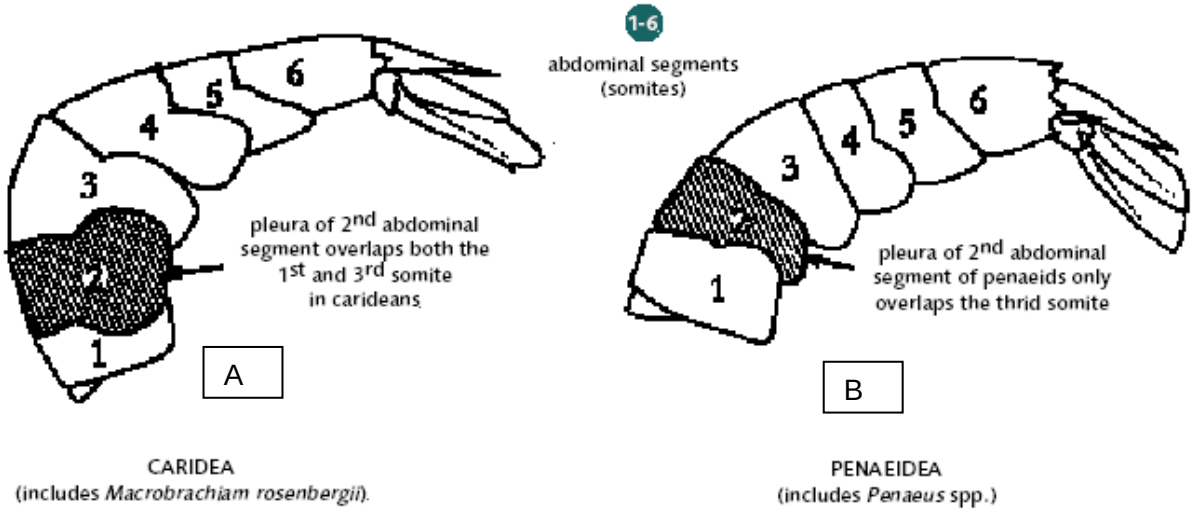
Figure 2: Shrimp

The freshwater prawns can be distinguished from the penaeid shrimps using the second pleura on the abdomen

Caridea prawns (*Macrobranchium sp*.) - pleura of second abdominal segment overlaps both the 1st and 3rd somites/segemants (Figures. 1 and 3 A); only the first two pairs of legs are chelated (Figure 4). The abdomen shows a pronounced *caridean bend*.

Penaeidea shrimps (*Penaeus* sp) - the pleura of second abdominal segment only overlaps the 3rd somite, and is overlapped by the first. The shrimps therefore have sequential overlapping body segments (presiding segment overlapping

the subsequent segment) Figure 2 and 3 B; chelate (claw like) on the first three pairs of legs (Figure 5). The abdominal segments are even-sized.



Source: Wickins (1976)

Finger 3: The prawn and shrimp

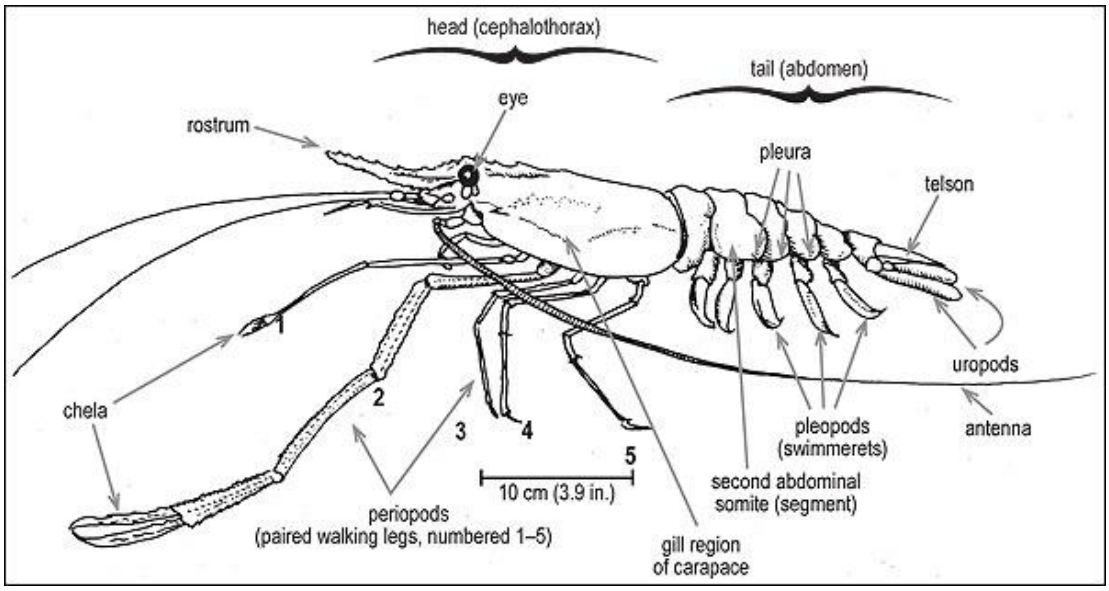
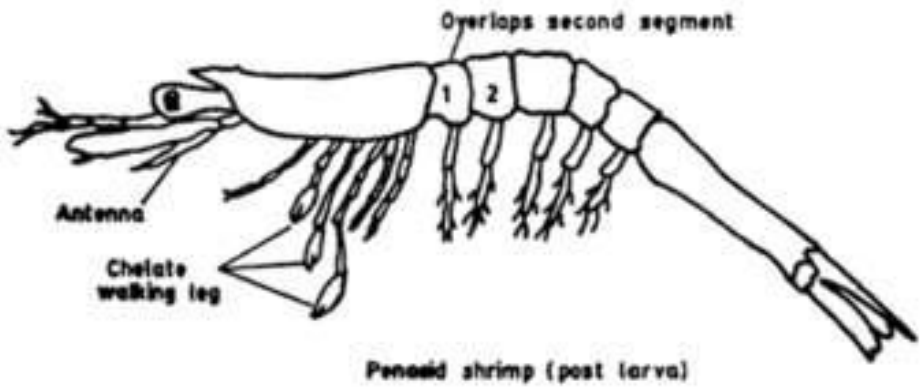


Figure 4: External anatomy of Freshwater Prawn (from Hicks, & Pierce II, 2011).



APPENDIX B: THE KEY FEATURES OF THE CRUSTACEANS ENCOUNTERED DURING THE STUDY

Family Palaemonidae

***Machrabrachium macrobrachion* (Herklot 1851).**

1. Rostrums are long, reaching and usually passing the end of antennal scale, slender depth (excluding spines) about 15% of length; rostral tip long, tapered, usually upturned and bearing 1-2 apical teeth. (Rutherford 1971) (Plate 2)

2. Second chelipeds with carpus slightly longer than palm, palm much longer than fingers which are straight and covered with fur-like dense layer of short soft hairs; chela, carpus and merus uniformly dark coloured with row of visible spines along inner margin; ischium pale-coloured. (Powell 1982) Plate 3.

3. Body dark, with dorsal parts of last 3-4 abdominal somites light coloured; side of carapace with a dark line running from below eye towards base of 2nd cheliped. Body transparent or lightly pigmented; side of carapace with 3 vertical/oblique lines (sometimes very faint) converging towards base of 3rd - 4th legs. (Powell, 1982)


Plate 1: *M. macrobrachion*


Plante 2: Head region of *M. macrobrachion*


Plate 3: 2nd Pereiopod of *M. macrobrachion*

Macrobrachium vollenhovenii (Herklot 1957) African River Prawn (Plate 5)

1. Number of dorsal teeth is 11-14 on rostrum. (Plate 6)
2. No apical teeth; rostrum is equal (more usually in adults) or shorter than antennal scale; dorsal edge convex over eye; tip lacking prolonged toothless portion. (Plate 7)
3. Second chelipeds with carpus shorter than palm, movable finger with a single large tooth at midlength of finger (in large adults). (Plate 8)
4. Colour is generally pale without speckling or mottling; thin dark longitudinal lines on carapace and transverse ones on abdomen; a thin unbroken line along ventral margin of carapace.
5. Third maxillipeds are bright yellow (white in small juveniles).
6. Fingers of 2nd cheliped are dark blue, with yellow patch at articulation with palm; tips of fingers white in juveniles. (Rutherford, 1971 and Powel,l 1982)

Plate 5: *M. vollenhovenii*

Plate 6: Dorsal teeth of *M. vollenhovenii*

Plate 7: Antennular scale of *M.vollenhovenii*

Plate 8: 2nd Pereiopod of *M.vollenhovenii*

Family Atyidae

Atya gabonensis (Giebel 1875) Gabon Shrimp (Plate 4)

Rostrum is short, lacking dorsal teeth and flanked by a pair of lateral teeth. (Powell, 1982)

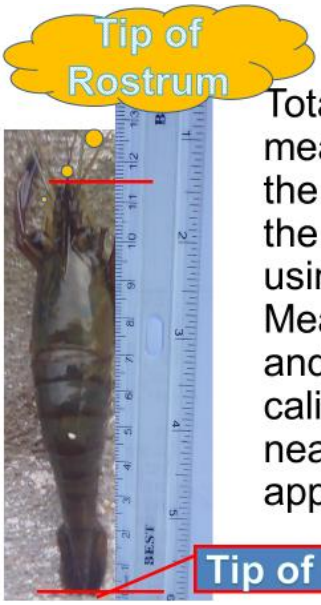
1. First and 2nd walking legs (chelipeds) are reduced and specialized, appearing as mouthparts, their modified chelae bearing brushes of setae as long as the chelae. (Powel, 1982)

2. Third to 5th walking legs are stout, ambulatory, the third much larger than the 4th or 5th. Plate 4,

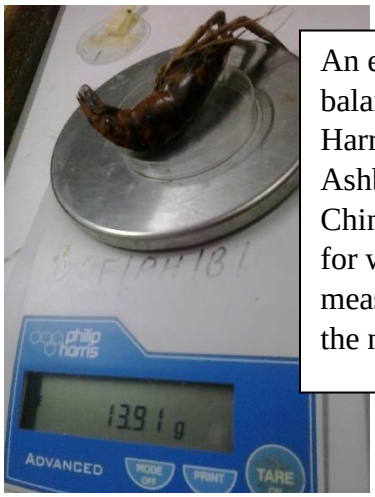
3. Colour is uniformly dark grayish and no mid-dorsal stripe.

Plate 4: *A. gabonensis*

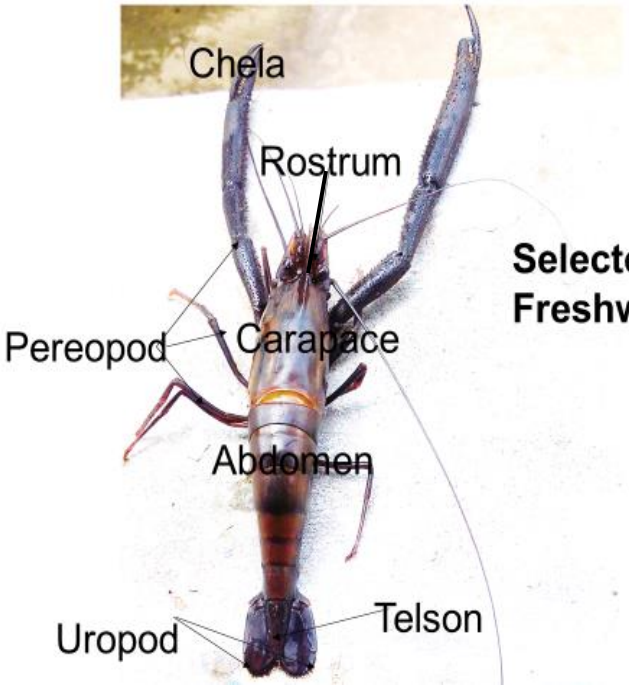
Appendix C: Measurement of Total Length and Weight



Total length was measured from the tip of telson to the tip of rostrum using Fish Measuring Board and a Vernier calipers to nearest cm when appropriate.



An electronic balance (Philip Harris No A20002, Ashby Park, China) was used for weight measurement to the nearest 0.1 g.



Selected labelled Parts of Freshwater Prawn

APPENDIX D: FEATURES OF MALE AND FEMALE *M. vollenhovenii*

Space between 5th Walking legs: Narrow or closed in Males and Wide or spaced out in Females

Male *M. vollenhovenii*

Female *M. vollenhovenii*

Wide space between 4th and 5th pairs of walking legs

***Macrobrachium vollenhovenii*, Herklots 1857**

Sex was determined by visual inspection of the base of the fifth pair of periopods described by Kingdom and Erondu (2013)

APPENDIX E: HATCHERY SET UP AT DEPARTMENT OF MARINE AND FISHERIES SCIENCES, UNIVERSITY OF GHANA

Hatching of the African River Prawns *M. vollenhovenii* (Herklots, 1857)
In a small hatchery set up

Plate A: Hatchery shed under construction at the Department of Marine and Fisheries Sc., UG

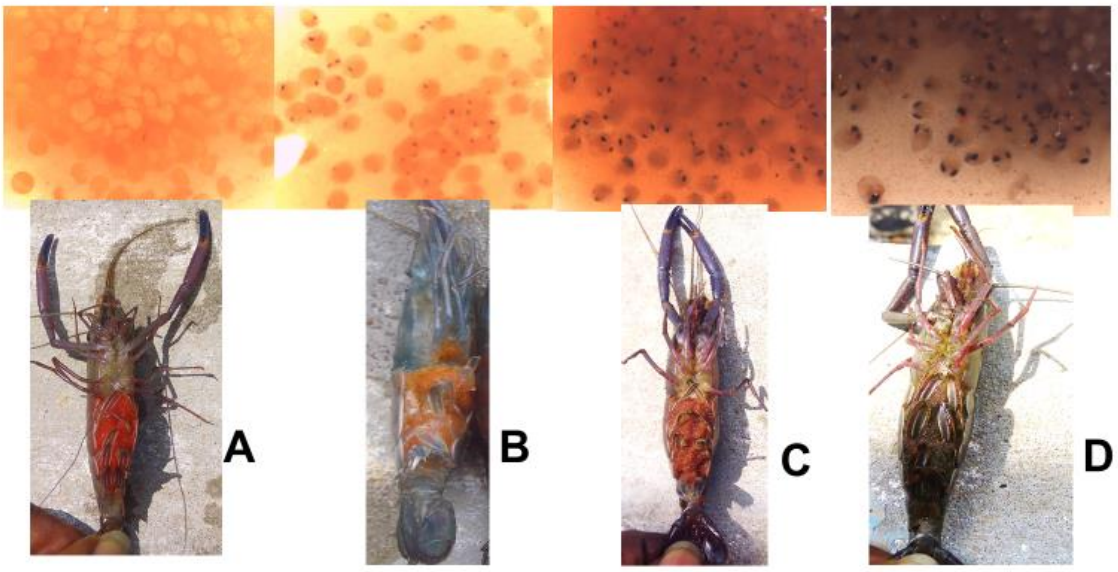
Plate B: Hatchery Shed with some fittings at the Department of Marine and Fisheries Sc.

Plate C: Hatchery fitted with holding units for brood prawns, hatching and nursery

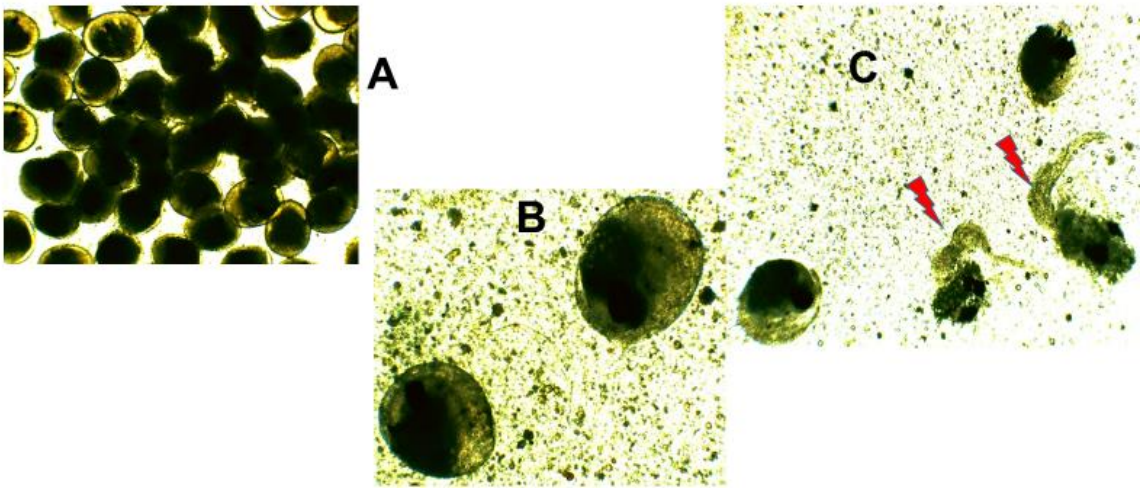
Plate D: Hatcing and Nursery Units in the Hatchery, Dept of Marine and Fisheries Sciences, UG

Plate E: Aerators and Accessories in the Hatchery, Dept of Marine and Fisheries Sciences, UG

Appendix F: Eggs of *Macrocrobrachium vollenhovenii*

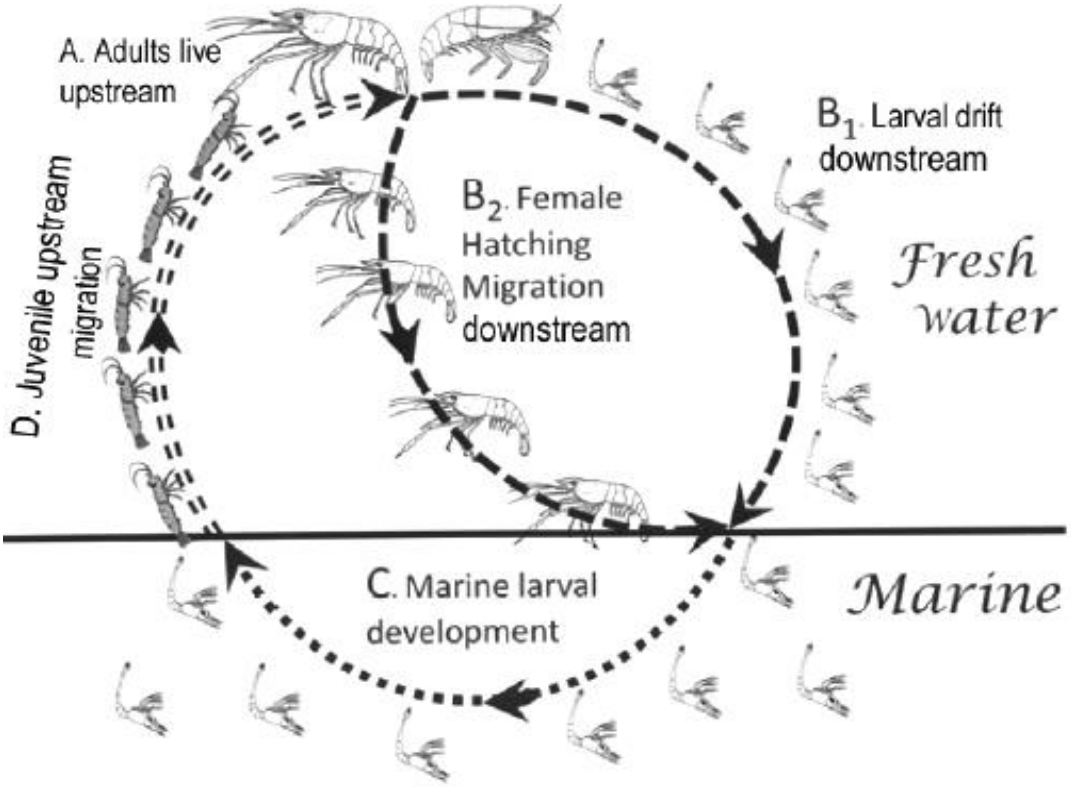


Berried *M. vollenhovenii* Females and Maturation Stages of *eggs*



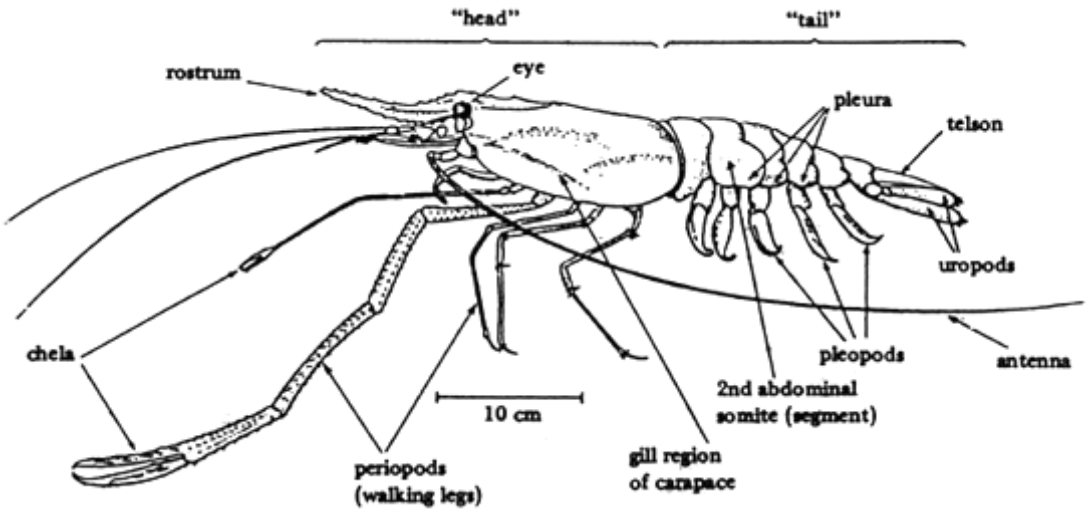
Mature Prawn Eggs

Appendix G: Life Cycle and larval Developmental Stages of *Macrobrachium* species



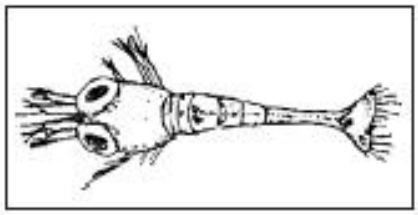
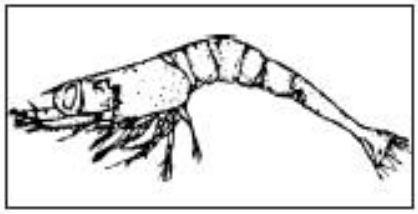
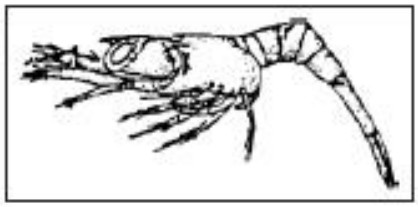
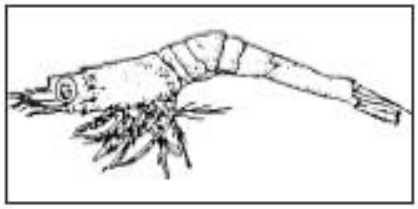
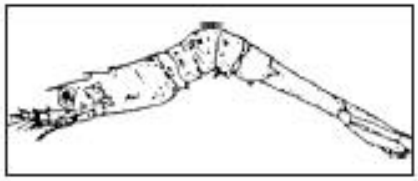
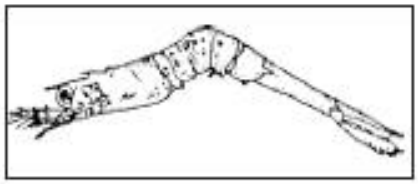
(Lal *et al*., 2014 and Bauer, 2004)

Life Cycle of Freshwater Prawns *Macrobrachium* species



Morphological Features of *Macrobrachium* species By New, 2002

Guide to Identification of the Larval Stages of *Macrpbrachium* species

Larvae Stage	Age (days)	Characteristics	Pictures
I	1	Sessile eyes 	
II	2	Stalked eyes 	
III	3-4	Uropods appear 	
IV	4-6	Two dorsal teeth on rostrum 	
V	5-8	Telson narrower and elongated 	
VI	7-10	Pleopod buds appear 	

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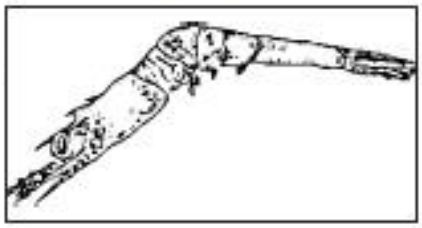
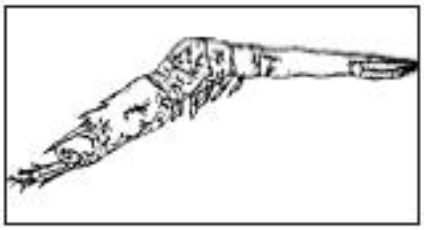
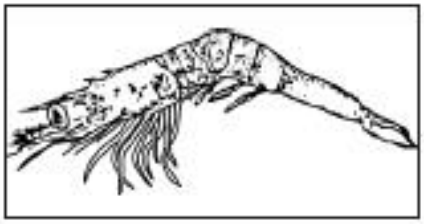
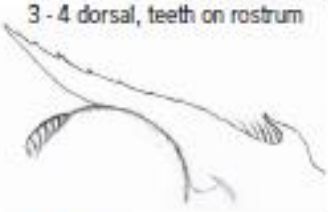
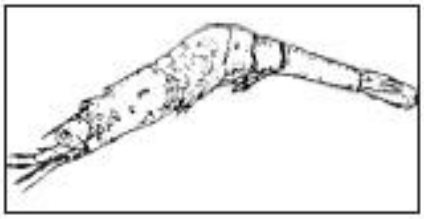
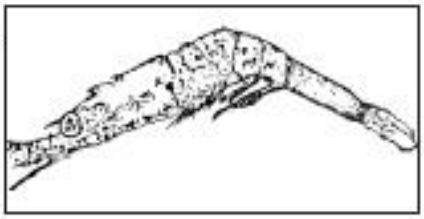
Larvae Stage	Age (days)	Characteristics	Pictures
VII	11-17	Pleopods biramous and bare 	
VIII	14-19	Pleopods with setae 	
IX	15-22	Endopods of pleopods with appendices internae 	
X	17-24	3 - 4 dorsal, teeth on rostrum 	
XI	19-26	Teeth on half of upper dorsal margin 	
Post Larvae	23-27	Now benthic, swims forwards with dorsal side uppermost. Teeth on upper and lower margin of rostrum (also behavioural changes, mainly in swimming)