THE IDENTIFICATION OF SQUILLID MANTIS SHRIMP AND PRELIMINARY STUDY ON REPRODUCTION AND LARVICULTURE OF *Oratosquillina interrupta* FOUND IN PENANG WATERS

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LIST OF ABBREVIATIONS AND SYMBOLS

AFR	Absolute feeding rate
AS	Abdominal somite
CL	Carapace length
CM	Cumulative moulting frequency
cm	Centimeter
FCR	Feed conversion ratio
g	Gram
hr	Hour
IM	Intermediate
L	Liter
LT	Lateral
MD	Median
MG	Marginal
mg/L	Milligram per liter
min	Minute
mL	Milliliter
MXP	Maxilliped
n	Number of samples
pH	*Puissance d' hydrogène*
ppm	Parts per million
sp.	Species
SM	Submedian
TL	Total length
TS	Thoracic somite
°C	Degree Celsius
%	Percent
‰	Per mille

IDENTIFIKASI UDANG LIPAN SQUILLID DAN KAJIAN RINTIS PEMBIAKAN DAN LARVIKULTUR *Oratosquillina interrupta* DARI PERAIRAN PULAU PINANG

ABSTRAK

Sejak dahulu lagi, stomatopod atau udang lipan dijual murah sebagai tangkapan sampingan. Pada masa kini, udang lipan merupakan hasil ekonomi yang penting di kebanyakan negara dan ditangkap secara komersial kerana mempunyai rasa dan tekstur yang unik. Udang lipan mempunyai potensi untuk amalan akuakultur tempatan. Kajian ini akan menentukan dan menilai protokol pembiakan optima udang lipan dan memastikan peringkat perkembangan larva, khususnya *Oratosquillina interrupta*. Sebelum eksperimen pembiakan, spesies udang lipan di perairan Pulau Pinang dan makanan yang sesuai perlu dikenalpasti. Merujuk kepada petunjuk pengecaman, tiga spesies udang lipan "squillid" telah dikenalpasti, iaitu *Miyakella nepa, O. interrupta* and *Cloridopsis scorpio*. Eksperimen pemilihan makanan terdiri daripada empat jenis makanan iaitu ikan, kepah, ikan+kepah, dan diet rumusan telah dinilai. Keputusan menunjukkan bahawa kepah merupakan makanan yang diutamakan dan menunjukkan kekerapan makan tinggi serta nisbah penukaran makanan (FCR) yang rendah dan kadar kemandirian yang lebih baik. Secara amnya, kepah digunakan sebagai diet penyelenggaraan sepanjang eksperimen. *M. nepa* dan *O. interrupta* dibiak dalam dua kaedah berbeza, iaitu secara kumpulan dan pasangan. Betina yang matang dengan kelanjar simen (*cement gland*) dan perkembangan ovari peringkat III dikenalpasti dan dipasangkan dengan jantan yang bersaiz sama. Sejumlah tiga kumpulan (2 *M. nepa* dan 1 *O. interrupta*) dan 10 pasangan (6 *M. nepa* dan 4 *O. interrupta*) telah dianalisisa.

Dalam keadaan berkumpulan, betina *M. nepa* tidak bertelur tetapi 83.33% bertelur dalam keadaan berpasangan. Manakala bagi *O. interrupta*, 66.67% betina telah bertelur dalam keadaan berkumpulan dan 100% bertelur secara pasangan. Justeru, didapati kadar kemandirian *M. nepa* dan *O. interrupta* lebih tinggi dalam bentuk berpasangan. Ini menunjuk kaedah berpasangan adalah lebih sesuai untuk kadar pembiakan dan kadar kemandirian yang lebih tinggi. Seterusnya, lebih kurang 16,901 larva *O. interrupta* diternak dan diberi makan *Artemia*. Sepuluh peringkat perkembangan larva dikenalpasti berdasarkan ciri morfologi seperti karapas (*carapace*), mata (*eye*), sesungut kecil (*antennule*), sesungut besar (*antenna*), kaki berenang (*pleopod*) dan bentuk hujung ekor (*telson*). Kesimpulannya, tiga jenis spesies udang lipan telah dijumpai di perairan Pulau Pinang dan pemerhatian di makmal telah menunjukkan makanan yang digemari ialah kepah. Dua spesies berpotensi untuk membiak dalam kurungan kerana keputusan menunjuk bahawa kaedah berpasangan mempunyai pembiakan dan pengeluaran larva yang lebih berjaya. Penemuan preliminari ini merupakan asas terhadap kemajuan industri akuakultur udang lipan pada masa hadapan.

THE IDENTIFICATION OF SQUILLID MANTIS SHRIMP AND PRELIMINARY STUDY ON REPRODUCTION AND LARVICULTURE OF *Oratosquillina interrupta* FOUND IN PENANG WATERS

ABSTRACT

Stomatopods or mantis shrimps were historically sold as cheap by-catches. Presently, with increased economic importance in various countries and it is commercially captured for its unique taste and texture. Mantis shrimp has a potential for local aquaculture practices. This study focuses on assessing optimal breeding protocol and determination of larval developmental stages, particularly for the mantis shrimp *Oratosquillina interrupta*. Before the breeding experiment, it is necessary to identify the species of mantis shrimp found in Penang waters and their feeding preferences in captivity. Following taxonomic keys, three squillid mantis shrimps species, namely *Miyakella nepa*, *Oratosquillina interrupta* and *Cloridopsis scorpio* were identified. A feeding preference experiment of four diets, namely fish-only, clams-only, a fish-clam mix diet and formulated diet were evaluated. Results showed that clams were preferred with high feeding frequency, low feed conversion ratio (FCR), high survival rate and better growth performances. Generally, clams were used as maintenance diet throughout the experiments. *M. nepa* and *O. interrupta* were bred in two different methods, namely group and single pairings. Mature females with stage III cement gland and ovarian development were identified and paired with similar size males. A total of three groups (2 *M. nepa* and 1 *O. interrupta*) and 10 pairings (6 *M. nepa* and 4 *O. interrupta*) were analyzed. Female *M. nepa* did not spawn in a group setting while as pairs, 83.33% of the females spawned. For *O. interrupta*, 66.67% of

female spawned in groups while 100% spawned as pairs. Moreover, survival rates of *M. nepa* and *O. interrupta* were higher in the pairing method. These results showed that the pairing method was more favourable with higher spawning and survival rate. Subsequently, approximately 16,901 larvae of *O. interrupta* were cultured and fed with enriched *Artemia*. Larval development stages were achieved from stage I to stage X (1 to 10) following observations and identified morphological features such as carapace, eyes, antennule, antenna, pleopod, thoracopods and telson. In conclusion, the three types of mantis shrimp species found in Penang waters are *Miyakella nepa*, *Oratosquillina interrupta* and *Cloridopsis scorpio*, and laboratory observations indicated their preference for a clam diet. Potentially, two species can be bred in captivity as results showed that single pairing method had a more successful breeding and larvae production outcome. These preliminary findings are a foundation towards the development of the future mantis shrimp aquaculture industry.

CHAPTER 1
INTRODUCTION

1.1 Background

Mantis shrimps are marine crustaceans belonging to the Order Stomatopoda. Neither a praying mantis nor a shrimp, their common name was given due to their shrimp-like appearance and having appendages that resemble the praying mantis (Ahyong, 2001). Generally, mantis shrimps can be categorized into two major groups, the 'spearers' and the 'smasher', with different ecological niches or roles played in the environment (Ahyong, 2001; Manning, 1998). Spearers have claws that are elongated, slender, noticeably toothed and are not inflated basally while the smashers have more broadened and are inflated basally claws forming calcified clubs. These distinct morphologies allow spearers to prey on soft-bodied prey such as fish, squid and polychaetes; while the smashers hunt hard-shelled prey such as crabs, snails and clams. Habitat occupied by spearers and smashers also differs. Smashers lives in hard substrates or pre-existing cavities whereas spearers live in self-excavated burrows made of sand and mud (Hernáez et al., 2011). Due to the different burrow preference, smashers can be found in shallower waters and are generally more aggressive as burrows are much limited while most spearers live at deeper waters where burrows are abundant.

Mantis shrimps were usually by-catches from shrimp trawlers, collectively sold cheaply for pellet production as fish meal and poultry feed or for fertilizer production (Baigtu & Echem, 2018). Many countries have commercially fished and learned to enjoy mantis shrimp meat as a delicacy due to its unique taste and texture. These includes *Squilla mantis* in the Mediterranean countries; *Oratosquilla oratoria* in Japan

and China; *Miyakella nepa* in India and Malaysia; *Oratosquillina stephensoni* in Australia; *Harpiosquilla raphidea* in Thailand and *H. harpax* in Malaysia (Ahyong, 2001; Hernáez, et al., 2011; Arshad, et al., 2015). Price of mantis shrimp is highly dependent on the consumers' demand, size and season, which affects the daily catches of fishermen. For example, large mantis shrimp were sold at RM250/kg to Hong Kong consumers (Lo, 2020), while at local restaurants smaller mantis shrimps were sold at about RM33/kg and larger ones can fetch up to RM200/kg. In Malaysia, mantis shrimps were first recorded as valuable fisheries product in 2008 with 539 tonnes of landings recorded (Department of Fisheries Malaysia, 2021). Fisheries landing of mantis shrimps have grown immensely to 10,028 tonnes per year in 2012, however, supplies have decreased to 3,219 tonnes per year in 2020 (Department of Fisheries Malaysia, 2021). This showed that there was a growing demand for mantis shrimp meat and wild stock supplies may have dwindled as years goes by. The growing human population and the decreasing wild stock supplies suggests that aquaculture practices of mantis shrimp should be established as an alternative for future food security.

Mantis shrimp aquaculture involves three components, the feeding of larvae and adults, breeding of adult mantis shrimp/broodstocks and the grow-out culture of postlarvae/juveniles (Xing, 2015). In Malaysia, mantis shrimp supplies still relied heavily on landings from fishing industry but recent news showed that a local farmer has been exporting mantis shrimps to Hong Kong consumers (Lo, 2020). However, because of the COVID-19 pandemic, mantis shrimps were sold at a cheaper price to local restaurants as mantis shrimps can only be kept for up to three days (Lo, 2020). While local farmer culture mantis shrimps in concrete tanks, China culture mantis shrimps in earthen ponds where broodstocks were successfully bred and larvae were

grown to a marketable size of 11cm (Fu, et al., 2013; Xing, 2015). Information related to the three components, namely feeding, breeding and culturing mantis shrimps will be crucial for the country to establish mantis shrimp aquaculture (Xing, 2015).

1.2 Problem statement

There's currently no practice and protocol for mantis shrimp aquaculture in Malaysia. Limited literature on mantis shrimp aquaculture is available in foreign language from countries such as Japan, Thailand and China, suggesting potential aquaculture practices for these organisms (Wang & An, 2004; He, et al., 2005; Fu, et al., 2013; Luo, 2016). Moreover, their aquaculture approach is outdoor and pond-based where information was directed for mass scale production. Before breeding mantis shrimps in captivity, it is necessary to answer what species are available, particularly in Penang waters and determine the feeding preference of mantis shrimps. Once determined, breeding protocols can be evaluated and success of breeding mantis shrimp in captivity can be assessed. Subsequently, the different developmental stages of larvae are included in this study. From the above constraints, two main objectives are formed for this study, potentially kick-starting development of mantis shrimp aquaculture activities in Malaysia.

1.3 Objectives

(1) To determine and assess optimal breeding protocol of the squillid mantis shrimps.

(2) To determine larval developmental stages of *Oratosquillina interrupta*.

CHAPTER 2
LITERATURE REVIEW

2.1 Taxonomy and geographical distribution

Among the six classes of Crustacea, the class Malacostraca is the largest with about a total of 28,000 species and is the most diverse morphologically and ecologically (Richter & Scholtz, 2001). Class Malacostraca can be further categorized into three subclasses, namely the Eumalacostraca, Hoplocarida and Phyllocarida. The order Stomatopoda or commonly known as mantis shrimp belongs to the subclass Hoplocarida. In Stomatopoda, there are seven superfamilies and 17 families (Ahyong, 2001), with at least 480 species in more than 100 genera recorded worldwide (Ahyong, 2004; Ahyong, 2012; Van Der Wal, et al., 2017). The seven superfamilies are Bathysquilloidea, Erythrosquilloidea, Eurysquilloidea, Gonodactyloidea, Lysiosquilloidea, Parasquilloidea and Squilloidea. Squilloidea which has only a single family Squillidae is the most abundant and speciose among other superfamilies. There are currently 49 genera recognized which consists of more than 185 species (Van Der Wal, et al., 2017). In summary, higher classification of squillid mantis shrimps are as follow:

Subphylum: Crustacea

Class Malacostraca

Subclass: Hoplocarida

Order: Stomatopoda

Superfamily: Squilloidea

Family: Squillidae

Of the 49 genera, 39 are found in the Indo-West Pacific region, which include the Indian and Pacific Ocean (Schram, et al., 2013). The land of Malaysia, namely Peninsular Malaysia and East Malaysia is separated by the South China Sea (SCS), located in western Pacific Ocean separates. A checklist of mantis shrimps species found in the SCS was provided by Moosa (2000). A total of 61 species belonging to 32 genera of Squilloidea were recorded. Penang island is located in western Malaysia, within the Straits of Malacca and is connected to the eastern part of the Indian Ocean. To date, four species of mantis shrimp are found in Straits of Malacca (Perak) where *M. nepa*, *Oratosquillina perpensa, H. raphidea* and *Cloridopsis scorpio* have been reported (Ng, 2013).

2.2 Anatomy

Body of malacostracan is typically divided into three parts, the head, thorax and abdomen. For mantis shrimps, they possess a cephalothorax, thorax and pleon, though cross-sectionally body shapes vary slightly among superfamilies (Schram, et al., 2013). The general morphologies, taxonomic terms and abbreviations used for species identification can be referred in Figure 2.1 which was modified from Ahyong (2001) and Ahyong (2012). Detailed morphologies were described by Schram, et al. (2013), but in general, the cephalothorax refers to the head (termed cephalon) which includes a pair of highly complex eyes, five cephalic somites which bears the antennules, antennae and three mouth-parts (mandibles, maxillules and maxillae), as well as part of the thorax in which five thoracic somites (thoracomeres) bears a maxilliped each. Though the first four thoracomeres are fused together with the head and are covered by a carapace, the 5^{th} thoracomere was not fused and with the remaining 6^{th}-8^{th} thoracomeres, forms the thorax. The 6^{th}-8^{th} thoracomeres bears a pair of walking legs

(termed pereiopods) each. The pleon refers to the abdomen which includes six abdominal somites (AS) and a tail fan (telson). Each AS have pleomeres where AS1-5 bears a pair of swimming legs or pleopods while AS6 have lateral uropodal articulation.

The eyes are one of the most unique features of a mantis shrimp by having the most photoreceptor types in the world, which respond to not only visible light but also ultraviolet and polarized light (Marshall, et al., 2007). In contrast, most humans have only three types of photoreceptors which visualized red, green and blue (Kolb, 2005). Birds, reptiles and some freshwater fish have four photoreceptors, butterflies have five while mantis shrimps have up to 16 photoreceptors (Marshall, et al., 2007; Marshall & Arikawa, 2014). These specialized eyes have allowed scientist to understand ecological behaviour such as depth and habitat distribution of different species. Mantis shrimps live in wide range of habitat on the continental shelf or slope, reaching 1,500m depth (Ahyong, 2012; Schram, et al., 2013). Species that are common at shallower waters have more complicated eyes than species found in deep waters or murky waters (Schram, et al., 2013).

The carapace primarily provides protection and a foundation for bearing the five maxillipeds (Olesen, 2013). It also vibrates and produces low rumbling sound (20-60Hz) which may function as warning signals when defending (Patek & Caldwell, 2006). Males were observed to use the carapace as a foothold during mating as well (Dingle & Caldwell, 1972; Deecaraman & Subramoniam, 1983b). Mantis shrimps have five maxillipeds which the 1st was modified into grooming appendages, the 2nd into hunting appendages and the remaining into feeding appendages. Mantis shrimps

are able to groom almost all of its body and this evolution is due to the settlement of organisms such as microbes, algae and invertebrates which can use exoskeleton of crustaceans as substrate (Bauer, 2013). Raptorial appendages or claw is a distinctive feature in mantis shrimp, which are made up of a dactylus, propodus, carpus, merus and ishcium (Figure 2.1, B). Based on the structure of the dactylus and propodus, mantis shrimps can be either a spearer or smasher. On the merus, an indentation known as meral spot can be found either terminally or subterminally. Bright colouration, usually found in smashers have shown to play a role as threat display (Franklin, et al., 2017).

Based on anatomical characteristics, squilloids can be easily distinguished from other families and superfamilies by the distinct median carina and four or more intermediate denticles on its telson (Ahyong, 2004). Other than that, eye cornea has only two rows of hexagonal mid-band ommatidia, the propodi of 3^{rd}-4^{th} maxillipeds ovate and are not ventrally ribbed or beaded. A depressed and long body with dorsum of thorax and abdomen longitudinally carinated. Ischiomeral articulation on raptorial claws terminal and dactyl are slender, not inflated. Primary teeth of telson fixed while the submedian teeth may be movable or fixed. Uropodal protopod with one or two primary spines and exopod with terminal articulation (Ahyong, 2012).

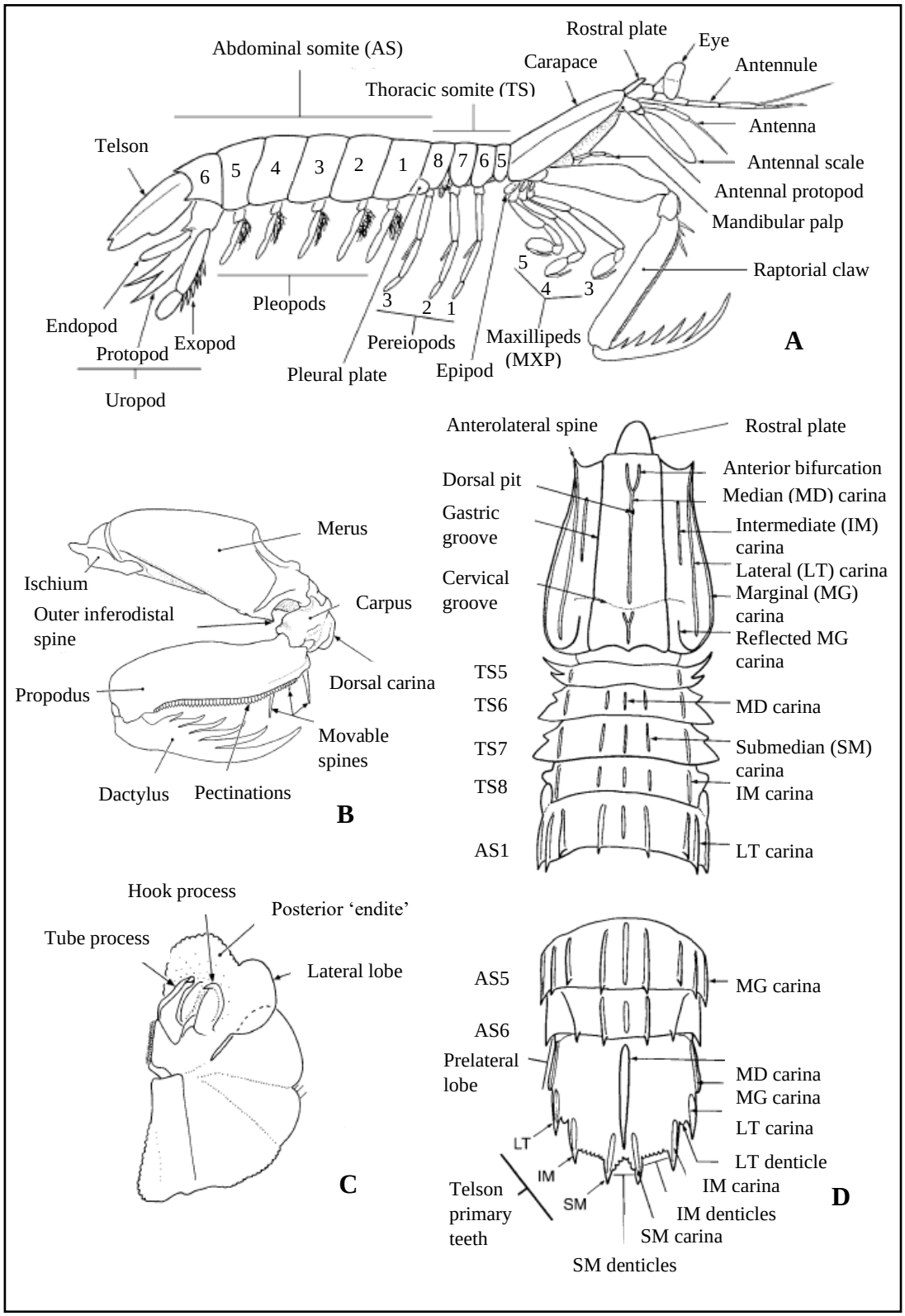

Figure 2.1: Morphology. A: general; B: right raptorial claw; C: petasma; D: dorsal carinae (Ahyong, 2001; Ahyong, 2012).

2.3 Agonistic behaviour

Agonistic behaviour in mantis shrimps involves competitive interactions exhibited between two individuals, especially in burrow defence and during courtship. Most ethological studies were carried out in relation to the ecology of two major groups, the smashers and the spearers (Schram, et al., 2013). Smashers preferred to live in corals, rocks and rubbles which are often limited in abundance, brightly coloured and lives in clear, well-lit environment. On the other hand, mud and sand habitat of spearers are widely available and due to substrate's physical nature, spearers are dull in colour and environment are often murky and turbid. These factors have led to behavioural differences among the group. For example, the *Neogonodactylus bredini* and *N. zacae* were the most aggressive in burrow defence as cavity availability is the most restricted, living in hard, often inorganic rocks (Caldwell & Dingle, 1975). Aggressiveness is followed by *Neogonodactylus oerstedii*, *Gonodactylus smithii* and *Gonodactylellus incipiens* with the latter being a generalist, living in rubble habitats ranging from atoll lagoons to exposed reef flat and occasionally in calcareous substrate near benches (Dingle, et al., 1973; Caldwell & Dingle, 1975).

Agonistic behaviour of mantis shrimps was categorized into several behavioural acts (Table 2.1) as described by Dingle (1969) and Dingle & Caldwell (1978). The act "strike" was the most aggressive behaviour (Caldwell & Dingle, 1975). For spearers, reports on agonistic interactions were much more limited most likely because aggression are less apparent. Dingle & Caldwell (1975; 1978) studied agonistic behaviour of spearer mantis shrimps found in mudflats where *Chloridopsis scorpio* and *Oratosquillina inornata* were commonly found. In intraspecific encounters where two same species indiviiduals met, the act "avoid" and

"antennular contact" were dominant, indicating lower aggression compared to smashers. Though at a lesser frequency, agonistic acts of spearers such as "lunge", "strike" and "chase" were still observed. Another spearer species *Squilla empusa* was studied by Wortham-Neal (2002a) where in addition to intraspecific interactions, inter- and intrasexual interactions were studied as well. Generally, males are more aggressive than females and aggressive acts involving physical contact are common. Moreover, interactions involving females are usually passive and non-striking.

Table 2.1: Behavioural acts displayed by mantis shrimps during an encounter with another mantis shrimp (Dingle, 1969; Dingle & Caldwell, 1978).

Acts	**Descriptions**
Meet	A 'chance' encounter with no recently preceding overt evidence of recognition.
Head-up	Cephalo-thorax is raised and eyes and antennules are directed forward.
Approach	A direct, deliberate advance toward another animal.
Meral Spread	A simultaneous outward spreading of the enlarged meri of the raptorial (2nd thoracic) appendages.
Lunge	A short rapid forward movement directed to another animal.
Strike	A blow delivered by one individual to another with the dactylus of one or both raptorial appendages.
Chase	Rapid pursuit of another individual.
Grasp	Seizing of another individual with thoracic appendages 3 to 5.
Coil	Bending over of the body so that the head is positioned directly above the telson.
Uncoil	Straightening out following a coil.
Avoid	Rapid and abrupt swimming or turning away from another animal.
Follow	An unhurried directed movement toward an animal which is moving away.
Antennular contact	Antennular palpation of the body or "fencing" with the antennules of another animal.
Does nothing	No discernible behavioural response to an act performed by another animal.

2.4 Food and feeding habits

Mantis shrimps are benthic predators which feed on a wide variety of prey/food items, ranging from fish, crab, clam, shrimp, polychaete worm and squid (Ahyong, 2004). Gut analyses were mostly used where recognizable fragments of prey such as fish scale and bone, crustacean cuticle, operculum and shell of mollusc, squid tentacle and annelid setae are identified (Dingle & Caldwell, 1975; Sreelatha & John, 1996; Ng, 2013; Zamri, 2015). Though both spearers and smashers share the same food resources (deVries, 2017), morphological structures of raptorial appendages affect hunting abilities, leading to different diet composition of mantis shrimps. Smashers with hammer-like claws are stronger hard-shell predators than spearers while spearers with sharp tooth are stronger soft-bodied predators (deVries, 2017).

However, smasher mantis shrimps like *Neogonodactylus bredini* may have a generalized diet where soft-bodied prey were found consumed as much as hard-shelled prey (deVries, et al., 2016). Similarly, spearers such as *Miyakella nepa* also have a generalized diet where both types of prey were consumed (Ng, 2013). Other spearers such as *Harpiosquilla* sp. have strong preference for fish (Dingle & Caldwell, 1978; Ng, 2013; Rajendra & Yedukondala, 2015) as sharp spines on its claws prevents the fast-moving fish from escaping and with a large pair of eyes, *Harpiosquilla* sp. are specialized fish hunter (Dingle & Caldwell, 1978).

Mantis shrimps are also known as stomatopods predators as remains of mantis shrimps were found in guts of *Cloridopsis scorpio* (Dingle & Caldwell, 1975; Dingle & Caldwell, 1978) and *Oratosquilla oratoria* (Bo, et al., 2020). Dingle & Caldwell (1978) observed that *C. scorpio* have heavier raptorial appendages and invests heavily

on burrow construction, therefore this species are more agonistic and will compete aggressively for both food and shelter. On the other hand, *O. oratoria* may have turned to cannibalism as an alternate feeding strategy due to the lack of food availability in the habitat (Bo, et al., 2020). Such behavourial change was because of the chain reaction from rapid urban development, habitat loss, decreased food availability and increased cannibalism (Bo, et al., 2020).

2.5 Reproduction biology and behaviour

Reproduction of mantis shrimp were studied in various field, namely morphologically (Wortham-Neal, 2002b, Subramoniam, 2017), histologically involving oogenesis and spermatogenesis (El-Sherif, et al., 2012; Subramoniam, 2017), histochemically involving sex pheromone and neurosecretory mechanisms (Deecaraman & Subramoniam, 1982; Deecaraman & Subramoniam, 1983a, Subramoniam, 2017), as well as behaviourally involving mating system and parental roles in caring of offspring (Dingle & Caldwell, 1972; Hatziolos & Caldwell, 1983; Hamano & Maatsuura, 1984; Hamano, 1988; Caldwell, 1991; Christy & Salmon, 1991). Mantis shrimps are dioecious where individuals can either produce male or female gametes. Males have a pair of penes while females have a genital region, comprising a genital slit for copulation and a medial cement-gland pore for cement gland secretion. Cement gland secretion are female accessory sex organ which facilitates holding individual eggs together, forming an egg mass which the female will then hold and care for it. However, unlike shrimps, prawns or crabs which commonly nurse their eggs on their pleopods, mantis shrimps nurse their eggs within its maxillipeds, without any attachment to its body. Besides cement gland, females also have a storage organ known as the seminal receptacles which help store viable sperms during copulation

(Wortham-Neal, 2002b). The storage durations vary between species from only a few weeks in *N. bredini* and up to months in *Oratosquilla oratoria* (Christy & Salmon, 1991).

Mantis shrimps displayed courtship before copulation where studies were mainly on species from Gonodactyloidea, Lysiosquilloidea and Squillidae. Mating patterns in mantis shrimps vary in terms of searching behaviour for mates, courtship, mating location, number of mates required per brood, availability of sperm storage, bonding between pairs and the role of male in breeding (Caldwell, 1991). From Dingle & Caldwell (1972), both male and female smasher *N. bredini* search for mates though it is more common for males to search for females. After mating, males remained in the burrow and repeatedly mate with the female until the female spawns. Males leaves after the female has spawned and aggression were observed in females if the male returned. Another smasher *Haptosquilla trispinosa* performed courtship for only 30min and mating occurs for only a few seconds at the entrance with the male leaving immediately after copulation (Caldwell, 1991; Christy & Salmon, 1991). In contrast to *N. bredini* where males are usually as large as or slightly larger than the females, *H. trispinosa* are more willing to mate with males of smaller size than her. Mating of smaller males with larger females were also observed in spearer *Pseudosquilla ciliata*, however, courtship was performed by the female and males were forced to mate (Caldwell, 1991). In spearer *Lysiosquilla* sp., mantis shrimps formed lifelong pairing where individuals obtained from the wild are always in single, male-female pairs (Caldwell, 1991). Males usually only leaves when the females die or are removed from its burrow. In reverse, females do not leave but may recruit new partner (Caldwell, 1991). Mating of both *O. oratoria* and *Miyakella holoshista* were promiscuous where

females mate repeatedly with any male, courtship is brief and males will leave after mating (Deecaraman & Subramoniam, 1983b; Hamano, 1988). *O. oratoria* was capable of storing viable sperms for up to a few months (Hamano, 1988). Deecaraman & Subramoniam (1983b) found that proteins from male accessory gland secretions were incorporated into the female's ovary and multiple mating may be required to accumulate these proteins.

2.6 Larval anatomy and life cycle

Mantis shrimp larvae are free-living and have unique metamorphic stages, with a large raptorial claw and can grow up to 50mm (Ahyong, et al., 2014). Mantis shrimp larvae undergo four growth stages, an early-larval stage, late-larval stage, post-larval stage and juvenile stage. Depending on superfamilies, early-larval stage may be either an antizoea or pseudozoea which will then develop into the late-larval stage known as erichthus or alima (Ahyong, et al., 2014). Morphological structures and terms in Figure 2.2, obtained from Hamano & Maatsuura (1987) were used for identification. Squilloidae larvae hatched as pseudozoea and develops into alima type. Gonodactyloidea, Parasquilloidea and Eurysquilloidea larvae hatched as pseudozoea but develops into erichthus type while only Lysiosquilloidea hatched as antizoea and develops into erichthus type. These larval types and its morphological features were first introduced and described by Giesbrecht (1910, cited in Pyne, 1972). Later, some authors categorized larval into propelagic, pelagic and post-larval stage following ecological and functional role (Pyne, 1972; Hamano & Maatsuura, 1984). Propelagic stage is usually difficult to study as larvae are benthic and remained inside the burrow with the female. Hence, larval studies were mostly derived from planktonic materials

and only pelagic stages were identifiable (Gurney, 1946; Alikunhi, 1952; Townsley, 1953; Michel & Manning, 1972; Feller, et al., 2013).

Duration and the number of larval developmental stages varies among species and is affected by the environmental factor, water temperature (Hamano & Matsuura, 1987; Morgan & Goy, 1987). *Neogonodactylus oerstedii* and *N. bredini* undergoes three propelagic and four pelagic stages in 35-40 days when cultured at 25°C with salinity of 36‰ (Provenzano & Manning, 1978; Morgan & Goy, 1987). Morgan & Goy (1987) also noted that higher water temperature (30°C) may reduce the duration of larval stages (30 days). Ngoc, et al. (2018) studied *Harpiosquilla harpax* which undergo eight larval stages in about 42 days with culturing condition of salinity 27.5-29.0‰ and water temperature of 27.5-28.5°C. *Oratosquilla oratoria* takes 36-59 days and developed through 11 stages at 21-28°C (Hamano & Matsuura, 1987). The temperate species (13.5°C in total darkness), *Pterygosquilla schizodontia* undergo 11 developmental stages as well, however, the duration of its larval life was almost 9 months (Pyne, 1972). Another temperate species, *Heterosquilla tricarinata* cultured at 15°C has a longer larval life of 60-70 days even though it had only three developmental stages as compared to *O. oratoria* (Greenwood & Williams, 1984).

The major challenge in studying mantis shrimp larvae was determining the larval feeding habits. Many scientists have attempted to feed pelagic larvae after yolk was depleted, however results were deemed unsuccessful (Townsley, 1953; Manning & Provenzano, 1963; Pyne, 1972). Food items tested include echinoderm eggs, eggs and larvae of *Polystoma* worms, zoeae of decapods, copepods, pieces of mussels and powdered egg yolk (Manning & Provenzano, 1963; Pyne, 1972). Pyne (1972)

mentioned that live food are more acceptable than powdered egg yolk as water quality deteriorates very rapidly. Later, Provenzano & Manning (1978), Williams, et al. (1985), Morgan & Goy (1987) and Hamano & Matsuura (1987) was able to complete larval developmental stages of various mantis shrimp species by feeding *Artemia*. The amount of food given was the reason for the previous failed attempts (Provenzano & Manning, 1978). However, stocking density of food given was not recorded and other than *Artemia*, feeding habits and diets of larval mantis shrimps is still practically unknown.

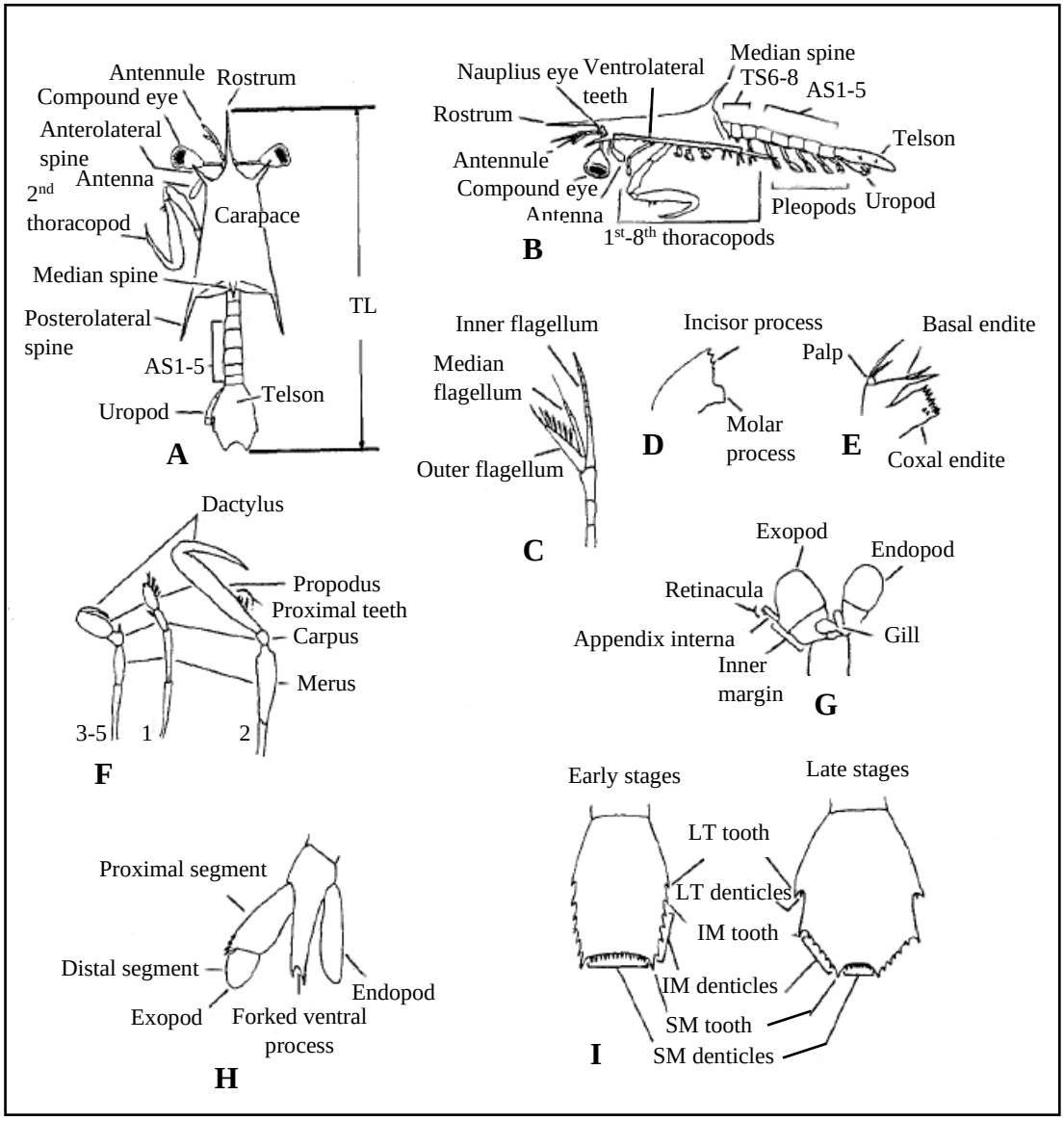

Figure 2.2: Morphology (Hamano & Matsuura, 1987). A: dorsal view; B: lateral view; C: antennule; D: mandible; E: first maxilla; F: first to fifth thoracopods (maxillipeds); G: pleopod; H: uropod; I: early and late stage telson.

2.7 Mantis shrimp aquaculture

Limited literatures were available on mantis shrimp aquaculture, namely Xing (2015) from China and Ngoc, et al. (2018) from Vietnam. Xing (2015) studied mantis shrimp aquaculture for *O. oratoria* using ecosystem-based breeding and rearing technology. Wild-caught broodstocks were cultured in concrete tanks but larvae and grow-outs of juveniles were cultured in idle ponds at salinity of 30-32‰ and temperature of 16-24℃. Ngoc, et al. (2018) studied stocking density of *H. harpax* larvae in plastic tank systems at more than 5mg/L dissolved oxygen, 7.5-8.5 pH and 28-30‰. Stocking densities of 50, 70 and 90 larvae/L were analyzed and 50 larvae/L achieved the fastest growth development and highest survival rate.

CHAPTER 3
MATERIALS AND METHODS

3.1 Animals procurement

Mantis shrimps were obtained from a trawler fisherman located at Bayan Lepas (5°18'40.5"N 100°17'36.5"E) and transported to the Centre for Marine and Coastal Studies (CEMACS), Penang. Throughout the transportation, ice-bath treatment was performed to reduce stress and upon arrival, the mantis shrimps were acclimatized into a 1-tonne quarantine tank. The following day, a 30min formalin bath treatment was performed to remove any external parasites on the mantis shrimps and was left undisturbed to recover for a day. The formalin bath was prepared at 150ppm concentration by adding 4.05mL of formaldehyde (37%) to every 10L of seawater (Mohamed, et al. 2000). No feeding was carried out throughout the acclimatization period. Any mortalities or moultings were removed and recorded.

3.2 Tank set-up and maintenance

Mantis shrimps were kept individually in tanks (23cm x 30cm x 50cm) which are compartmentalized into four columns, each for an individual. A PVC (polyvinyl chloride) pipe was provided for each mantis shrimps as artificial burrows. Filtered seawater (29-30‰), treated with Septrivet® 3.0 was stored in 1-tonne storage tanks to kill harmful microorganisms, as well as to ensure continuous supply of clean seawater for water changes. For maintenance, all tanks were connected to an outlet leading to a filter tank where filter bags, protein skimmers, bioballs and ultraviolet (UV) light were equipped as mechanical and biological filtration. Water quality were monitored daily using a multiprobe (YSI professional plus) for parameters such as temperature, dissolved oxygen and salinity. API® test kits for Ammonia (NH_3), nitrite (NO^{2-}),

nitrate (NO^{3-}), pH, calcium (Ca) and carbonate hardness (KH) levels were used before water changes. Generally, 50% water change and cleaning were carried out weekly however if NH_3 and NO^{2-} levels were higher than 0.25mg/L or NO^{3-} level was higher than 20mg/L, water changes will be carried out twice a week.

3.3 Morphology and identification of mantis shrimp

Morphology of 130 mantis shrimps were studied. Prior to observations, mantis shrimps were subjected to 5min ice-bath treatment (16-17°C). Morphology was observed following identification keys by Ahyong (2001). Photographs of specific anatomy for each species were taken and recorded. Sex was identified following references from Wortham-Neal (2002b). Mantis shrimp was then blot dried, weighed to the nearest 0.01g using an electronic balance and measured using a digital caliper measuring to nearest 0.01mm as followed (Figure 3.1):

(a) Body weight (BW)

(b) Length

(i) Carapace length (CL): medial line from the anterior margin to the posterior spine of carapace, excluding the rostrum.

(ii) Total length (TL): medial line from the anterior margin of the rostrum to the apices of the submedian teeth of the telson.

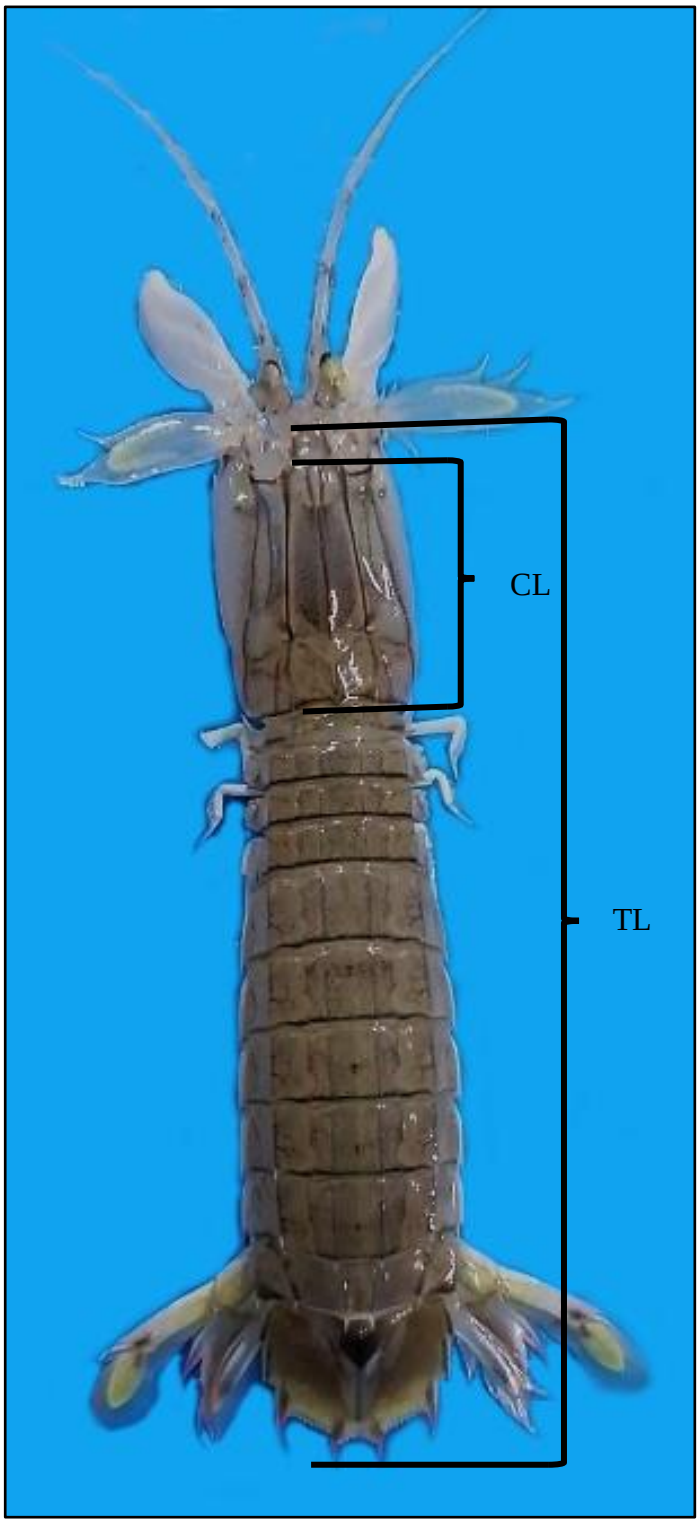

Figure 3.1: Dorsal view showing carapace length (CL) and total length (TL) of mantis shrimp.

3.4 Feeding preferences of mantis shrimp in captivity

3.4.1 Feed preparation

Two types of feed were utilized in this study, namely fresh and formulated feed. Fish (*Rastrelliger* sp.) and clams (*Meretrix* sp.) were bought from the local market. Fish were eviscerated and beheaded while the clams were shucked and drained. After processing, about 200g of raw muscle was weighed and oven-dried at 60°C for 24hrs. Dried fish and clam were then blended, ground into powder, and kept frozen in 50mL Eppendorf tube. Remaining fresh fish and clams were kept frozen as well until feeding trial starts.

Pelleted feed was formulated to constitute 45% protein and 10% lipid levels with ingredients listed in Table 3.1. Proximate analysis was performed for dry ingredients used in the formulation prior to pelleted feed production. Dry ingredients consisting of fish meal, casein, gelatin, corn starch, cellulose, vitamin and mineral premix (Rovimix® and Uni-Nutrich Plus®) were weighed and manually mixed until all were evenly combined. Fish oil (Seven Seas® cod liver oil) was then incorporated into the mixture until evenly combined. Approximately 200mL of distilled water was added slowly until the dough held together. Strands of pellet (diameter 0.6cm) were made using a tabletop meat mincer and were oven-dried at 60°C for 24hrs. After drying, the pellet strands were kept frozen for the feeding trials. For a final proximate analysis, 18g of pellets were ground into powder using a mortar and pestle and kept frozen in 50mL Eppendorf tube.

Table 3.1: Ingredients used in formulation of pelleted feed (g/100g dry matter).

Feed ingredients	**g/100g**
1. Fish meal	30.13
2. Casein (bovine)	11.98
3. Gelatin	12.69
4. Cod liver oil, Seven Seas®	7.97
5. Corn starch	22.98
6. Cellulose	8.75
7. [1]Rovimix®, DSM company	1.00
8. [2]Uni-Nutrich Plus®, Sanzyme Biologics (P) Ltd.	3.00

Notes:
[1] indicates each kg consists of 50 million international unit (MIU) Vitamin A; 10 MIU Vitamin D3; 130g Vitamin E, 10g vitamin B1; 25g Vitamin B2; 16g Vitamin B6; 100mg Vitamin B12; 10g Vitamin K3; 500mg Biotin; 56g Pantothenic Acid; 8g Folic Acid; 200g Niacin; 20g Anticaking Agent and 0.2g Antioxidant;
[2] indicates each kg consists of 5 MIU Vitamin A; 625,000 IU Vitamin D3; 800mg Vitamin E; 2,000mg Vitamin B2; 400mg Vitamin B6; 0.1mg Vitamin B12; 4,000mg Calcium Pantothenate; 10,000mg Choline Chloride; 2,000mg Copper; 3,500mg Manganese; 7,500mg Iron; 15,000mg Zinc; 1,000mg Iodine; 27.25% Calcium; 7.45% Phosphorus; 10mg Selenium; 40,000mg Magnesium and 100 x 109 probiotics.

3.4.2 Proximate analysis

Proximate analysis includes moisture, crude lipid, crude fibre, ash and crude protein content determination. Dried fish and clams were subjected to proximate analysis. For formulated feed, an initial proximate analysis was carried out on each feed ingredient and data was used to tabulate and formulate feed. Completed feed were then subjected to another proximate analysis. In the following procedures, samples referred to dried fish, clam, feed ingredients or formulated feed. All samples were tested in triplicates.

(a) Moisture content determination

Moisture content was determined by using an Infrared Moisture Determination Balance (AND 4712), following Nielsen (2017). Samples were weighed (5g) and moisture content was determined. Dried samples were stored in 50mL Eppendorf tube

and were kept in a desiccator. These samples were subsequently used for lipid, fibre, ash and protein content determination.

Calculation:

$$\% \text{ Dry matter} = \frac{\text{weight of dried sample}}{\text{weight of initial sample}} \times 100\%$$

% Moisture = 100 - % dry matter

(b) Crude lipid content determination

Crude lipid was determined using extraction method Bligh & Dyer, 1959. Dry samples were weighed (2g) into a test tube and chloroform:methanol (2:1 ratio) solution was added. The sample was homogenized for 2min and the homogenate obtained was then filtered in a Buchner funnel with a filter paper (Whatman No.1). Fat-free residue left on the filter paper were set aside for drying before storing it in a falcon tube and desiccator for further crude fibre analysis, if necessary. The filtrate was poured into a separating funnel and 20mL distilled water was added. The separating funnel was capped, shaken vigorously and left to stand overnight for a 2-layer separation, the methanol-distilled water (top layer) and chloroform-lipid layer (bottom). The next day, empty beakers were dried in an oven for an hour (80°C) and weight was recorded. Chloroform-lipid layer was collected into the empty beakers and placed in the oven at 80°C for at least 4hrs to evaporate the chloroform. After drying, weight of the beaker and dried sample was recorded. Crude lipid (%) was then calculated as followed.

Calculation:

$$\% \text{ Lipid} = \frac{(a) - (b)}{\text{sample weight (g)}} \times 100$$

whereby a – weight of beaker + dried sample

b – weight of beaker