THE ROLE OF SENSORY ORGANS ON THE FEEDING ACTIVITY OF *Penaeus vannamei* UNDER LIGHT AND DARK CONDITIONS

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ABSTRACT

Penaeus vannamei is one of the important shrimp species in aquaculture. The use of light during the *P. vannamei* post larval rearing is not well documented. Thus different hatcheries applying different lighting conditions during the post larval rearing. This study was conducted to examine the effects of light or dark condition on the feeding activity and the effect of extended light or dark condition on the growth and survival of *P. vannamei*. To achieve these objectives, ingestion experiments were conducted using different sizes of shrimps; 0.5cm total length, TL (PL5), 1.0cm TL (PL10), 1.5cm TL (PL20) and 2.0cm TL (PL30) by providing them live or frozen *Artemia* under light or dark condition. After the ingestion experiment, the shrimps were preserved in Bouin's solution for histological observation of the eye and setae. Besides that, shrimps (1.0cm TL~PL10) were subjected to different lighting conditions 24 hours light (24L), 24 hours dark (24D) and a natural diurnal cycle (12LD) to examine the effect of extended lighting conditions on the growth performance for three weeks. The ingestion experiments revealed that the 0.5cm TL shrimp showed high preference in ingesting frozen *Artemia* under light condition compared to dark condition. However, the ingestion of live *Artemia* showed no significant difference ($P>0.05$) under both lighting conditions. Histological observation showed that the optical properties of the 0.5cm TL shrimp comprises of crystalline cone, rhabdom and fasciculated zone. However, it was incomplete due to the lack of clear zone which indicates that the shrimps are unable to adapt to dark conditions. On the contrary, the ingestion rate of live and frozen *Artemia* under both lighting conditions by bigger sizes of shrimps (1.0, 1.5 and 2.0cm TL) showed no significant differences ($P>0.05$). Histological observation showed that shrimps from the size of 1.0cm TL (PL10) onwards, have a complete eye structure with the appearance of clear zone which indicates the ability of the shrimp to adapt in dark condition. The width of clear zone increased proportionally with the growth of the shrimps. Through setae observation, it was found that setae densities were different at each appendage of shrimp. The highest density of setae was recorded at the antennules while the lowest density at the antenna and non-chelae pereiopod. The setae density of the appendage did not increase with the shrimp size (0.5cm to 2.0cm TL) except at the maxilliped where increased of density was observed from 0.5cm TL to 1.5cm TL shrimp. Result of the rearing experiment, found that the shrimps were not affected by the extended period of dark or light condition as the growth, feed utilization and survival rate were not differed significantly ($P>0.05$) among the treatments. Overall, this study found that 0.5cm TL shrimp~PL5 depend more on the visual receptor in searching food. Providing the shrimps with frozen *Artemia* under dark condition minimized the function of the visual and mechanoreceptor. However, the ability of the shrimps (PL5-PL30) to detect food under these conditions indicating that other sensory organs are playing role in detecting food. In practical, this study would suggest that brighter condition to be used in the rearing of 0.5cm TL shrimp and from 1.0cm TL onwards (>PL10) any lighting regimes can be used.

ABSTRAK

PERANAN ORGAN DERIA TERHADAP AKTIVITI PEMAKANAN *Penaeus vannamei* DI DALAM KEADAAN TERANG DAN GELAP

Penaeus vannamei ialah spesies udang yang popular dalam bidang akuakultur. Walau bagaimanapun, tidak ada dokumentasi lengkap tentang penggunaan cahaya dalam pengkulturan larva ini. Oleh itu penggunaan cahaya untuk untuk setiap hatceri adalah berlainan. Kajian ini dijalankan bagi mengkaji kesan keadaan terang atau gelap terhadap aktiviti pemakanan dan kesan pencahayaan yang panjang terhadap tumbesaran dan kemandirian udang. Bagi mencapai objektif ini, eksperimen pemakanan dijalankan menggunakan udang yang berlainan saiz iaitu 0.5cm (PL5), 1.0cm (PL10), 1.5cm (PL20) dan 2.0cm (PL30) dan udang tersebut diberi makan *Artemia* hidup atau beku di dalam keadaan terang atau gelap. Setelah itu, udang diawet di dalam larutan Bouin's bagi tujuan pemerhatian histologi terhadap mata dan seta. Selain itu, bagi mengkaji kesan pencahayaan yang panjang terhadap tumbesaran udang, udang bersaiz 1.0cm telah dikultur dalam keadaan pencahayaan yang berbeza iaitu 24 jam bercahaya (24L), 24 jam gelap (24D) dan di dalam keadaan ritma diurnal (12LD) selama tiga minggu. Hasil eksperimen pemakanan mendapati bahawa udang bersaiz 0.5cm, menunjukkan kecenderungan yang tinggi memakan *Artemia* beku di dalam keadaan terang berbanding gelap ($P<0.05$). Tetapi, kadar pemakanan *Artemia* hidup di dalam keadaan terang dan gelap tidak menunjukkan perbezaan bererti ($P>0.05$). Pemerhatian histologi mendapati bahawa struktur optik udang yang bersaiz 0.5cm merangkumi kon berhablur, rabdom dan zon fasikulat. Tetapi struktur optik ini adalah kurang lengkap kerana ketiadaan zon terang yang menunjukkan udang pada peringkat ini tidak dapat beradaptasi di dalam kedaan gelap. Hal ini berbeza dengan udang bersaiz lebih besar (1.0, 1.5 dan 2.0cm) kerana kadar pemakanan *Artemia* hidup dan beku tidak menunjukkan perbezaan bererti ($P>0.05$). Pemerhatian histologi mendapati udang bersaiz besar mempunyai struktur optik yang lengkap dengan kehadiran zon terang. Ini menunjukkan udang berkebolehan beradaptasi di dalam keadaan gelap. Kelebaran zon terang didapati meningkat sejajar dengan tumbesaran udang. Pemerhatian terhadap seta mendapati kepadatan seta adalah berbeza di setiap anggota badan udang. Kepadatan seta yang tinggi direkodkan di antenul manakala seta yang terdapat di sensungut antena dan pereiopod yang tidak bersepit mencatatkan kepadatan yang terendah. Walaupun udang membesar, kepadatan seta pada setiap anggota badan udang adalah sama kecuali di bahagian maksiliped di mana, kepadatan seta meningkat ketika udang membesar dari saiz 0.5cm kepada 1.5cm. Hasil kajian pengkulturan udang mendapati kesan pencahayaan yang panjang tidak mempengaruhi udang kerana kadar tumbesaran dan kemandirian tidak menunjukkan perbezaan bererti ($P>0.05$). Secara keseluruhannya, kajian ini mendapati udang bersaiz 0.5cm~PL5 lebih bergantung kepada mata dalam pencarian makanan. Pembekalan *Artemia* beku di dalam keadaan gelap telah meminimumkan fungsi deria penglihatan dan mekanoreseptor udang. Tetapi, udang (PL5-PL30) masih mampu mengesan makanan di dalam kedaan gelap, menunjukkan bahawa terdapat organ deria lain yang memainkan peranan dalam pengesanan makanan. Secara praktikal kajian ini mencadangkan agar keadaan pengkulturan udang bersaiz 0.5cm (<PL5) dijalankan di dalam keadaan terang dan udang bersaiz 1.0cm dan seterusnya (>PL10) boleh dikultur di dalam kedaan terang mahupun gelap.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
PL	Post larvae
SEAFDEC	Southeast Asian Fisheries Development Center
SGR	Specific growth rate
SPSS	Statistical Package for Social Sciences
TL	Total length
12LD	12 hours light and dark condition
24D	24 hours dark condition
24L	24 hours light condition

LIST OF SYMBOLS

%	Percentage
^oC	Degree Celcius
cm	centimeter
g	gram
L	liter
M	meter
mg	milligram
ml	milliliter
mm	millimeter
ppt	parts per thousand
µm	micrometer

CHAPTER 1

GENERAL INTRODUCTION

1.1 Whiteleg Shrimp, *Penaeus vannamei*

Penaeus vannamei (Boone, 1931) is commonly known as whiteleg shrimp and locally as *udang putih*. *P. vannmaei* is a native species to the Eastern Pacific coast from the Gulf of California, Mexico to Tumbes and to North of Peru (FAO, 2004; Wakida-Kusunoki, 2011).

The main reason for the importation of *P. vannamei* to the Asian country is due to the disease outbreak of tiger prawn, *Penaeus monodon* aquaculture that led to low production and huge economic losses. In Malaysia, *P. monodon* had been a major cultured species. However, disease outbreak (Senanan *et al.,* 2007) such as yellow head virus and white spot viruses (Briggs *et al.,* 2004; Wyban, 2007) and poor growth performance (Briggs *et al.,* 2004; Senanan *et al.,* 2007) had lowered the production and price for this species (Senanan *et al.,* 2007). This pressured the aquaculturist in Malaysia to culture other species such as *P. vannamei* as an alternative species. The same problems were also reported in Thailand, Indonesia, Philippines, Myanmar and some other countries (SEAFDEC, 2005). In Malaysia, the Department of Fisheries rejected a proposal by foreign investors in 2000 to introduce *P. vannamei* as substitute to *P. monodon* (FAO, 2004), due to their concern on the ability of *P. vannamei* to carry taura syndrome virus (FAO, 2004). Despite the diseases concern, *P. vannamei* has been produced worldwide since 2000 (Figure 1.1). The global production of *P. vannamei* started globally in 1980. It is reported that China is the main producer followed by Thailand, Indonesia and Vietnam in 2004 (FAO, 2015a).

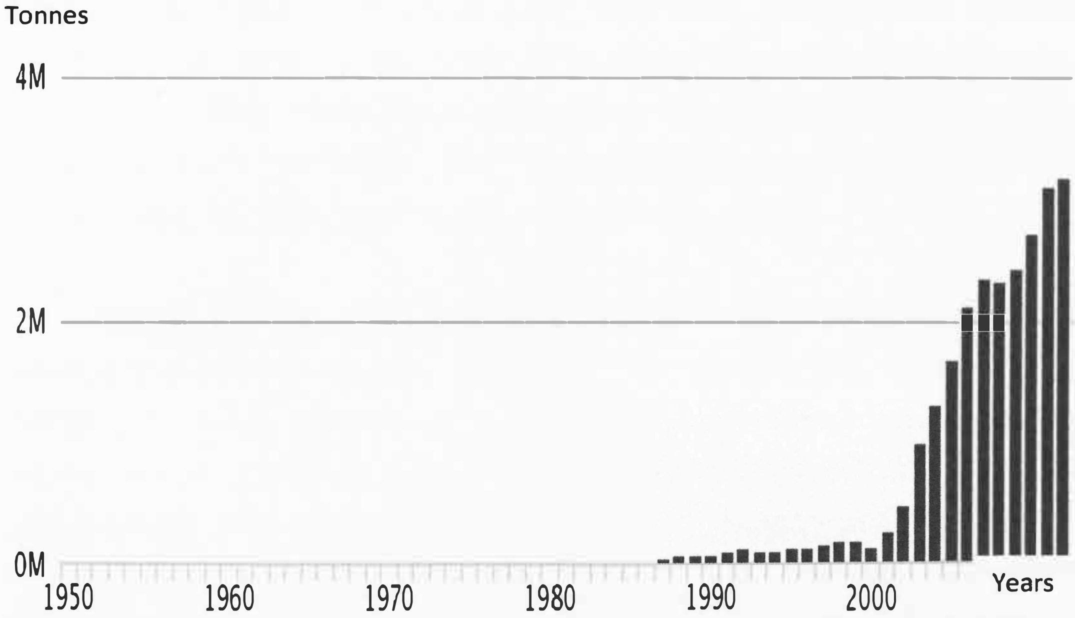

Figure 1.1 : Global aquaculture production of *P. vannamei*.
Source : Food and Agriculture Organization (2015a)

As the aquaculture interest for *P. vannamei* increases, research on the reproduction, behavior and nutrition has been extensively conducted. Meanwhile, there are many factors have been documented to influence the growth and survival of shrimp such as the stocking density, water temperature, salinity, dissolve oxygen and pH (Moullac and Haffner, 2000). However, the influence of light received little attention and still remains controversial especially in shrimp culture. As a result, different hatcheries introduced or exposed their cultured shrimp with different light condition without considering or understanding the ability of the cultured shrimp to feed under those conditions.

1.2 Significant of Study and Problem Statements

The use of light is noticeably different in different hatcheries. In Taiwan, the larvae are cultured in a total dark condition until they reached the post larval stages or the rearing tanks are shaded during the light sensitive zoeal stages (Liao, 1992). In some other countries, the hatcheries are equipped with transparent roofs to allow natural penetration of sunlight to the rearing tanks (FAO, 2002). Whereas, other hatcheries provide a 24 hours light condition in the rearing tanks (Smith *et al.*, 1992). Although, it was commonly known that continuous lighting was intended to enhance the growth of the algae or to promote the growth of the shrimp, the used of lighting in the larval rearing is generally for human convenience (Smith *et al.*, 1992).

The ability of several species of crustacean to feed under light and dark condition had been investigated thoroughly. The northern krill, *Meganyctiphanes norvegica* was found actively hunt and consumed more *Calanus* spp. and *Metridia longa* at low, realistic light intensities than in total dark condition. The provided foods were ingested three times higher in the low light intensities than under total dark condition by the *M. norvegica* (Togersen, 2001). The spiny water flea, *Bythotrephes longimanus* was also reported to feed relatively more *Daphnia* under the light condition, and no *Daphnia* was ingested under low light intensities (Pangle and Peacor, 2009). While other crustacean species such as *Lucifer faxoni* can feed well under both light and dark conditions (Vega-Perez *et al.,* 1996). However, the ability of the penaeid shrimp especially the *P. vannamei* to feed under different lighting conditions is not well documented.

The ability of crustacean to search for food under the light condition is usually assisted by the visual receptor. In crustacean, the compound eyes are composed of repetitively identical visual units called ommatidia and each of the ommatidium consist of optical structures called the cornea and crystalline cone. The ommatidium that are stacked on top of rhabdoms are capable of sensing light (Schoenemann, 2013; Cronin and Jinks, 2001). Lay underneath the rhabdom, there are screening pigment granules that control light that enter the eye (Yahaya *et al.*, 2012). It has been reported that many crustaceans have the ability to change the optical properties of their eyes under light and dark adaptation (Meyer-Rochow, 2001). The adaptations of the compound eye under light and dark conditions are well studied on the juvenile and sub-adult of *P. vannamei* (Matsuda and Wilder, 2014) but not on the early stage of post larvae.

Other sensory organs such as setae are also responsible in helping crustacean in searching food (Wroblewska *et al.,* 2002; Corotto and O'Brien, 2002; Steullet *et al.,* 2001; Derby *et al.,* 2001). Setae can be found from all body parts of shrimp such as antennules, antenna, maxilliped, pereiopods, pleopods and uropod (Chan *et al.,* 1988). A lot of studies have been conducted to investigate the role of setae in searching food. However, study on the distribution of setae at different appendages of shrimp is not well documented.

This research was undertaken to determine the feeding activity of *P. vannamei* post larvae under light and dark conditions. Besides that, this research examined the role of the eyes and setae distribution at different sizes of post larvae and juveniles. Results of this research can help to improve the rearing technique of *P. vannamei* post larvae and juveniles in the hatchery and provide information on the importance of eyes and setae during the feeding activity of this species.

1.3 Objectives

In order to understand the feeding activity of *P. vannamei* under light and dark condition, this study was divided into three objectives. These objectives are:

1. To investigate the feeding activity and eye adaptation of *P. vannamei* with live or frozen *Artemia* nauplii under light or dark condition.
2. To investigate the setae distribution at different sizes of post larvae.
3. To investigate the effect of extended light or dark condition on growth and survival of *P. vannamei.*

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of *Penaeus vannamei*

P. vannamei is a marine crustacean and it is classified scientifically as shown in Figure 2.1. This species has same anatomy and life cycle of others Penaeidae shrimp (Galitzine *et al.,* 2009). Normally, the adult has translucent and bluish-green colour. This colour is due to the pigmentation of chromatophores which are the molecules that developed to collect and reflect light. This species can be distinguished by a rostrum armed with 1-3 ventral teeth and 8-9 dorsal teeth and the rostrum slightly curves down (Wakida-Kusunoki, 2011; Bailey-Brock and Moss, 1992).

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Family: Penaeidae
Genus: *Penaeus*
Species: *Penaeus vannamei*

Figure 2.1 : The taxanomic hierarchy of *P. vannamei.*
Source : Integrated taxanomic information system (ITIS)

2.1.1 External Anatomy of *Penaeus vannamei*

The body of a shrimp can be divided into two main divisions which are the cephalothorax and the abdomen (Figure 2.2). Cephalothorax contains the antennules, antennae, compound eyes, maxillipeds and pereiopods. While the abdomen of a shrimp includes pleopods, uropods and telson and the abdomen are segmented into five segments (Stamhuis and Videler, 1998).

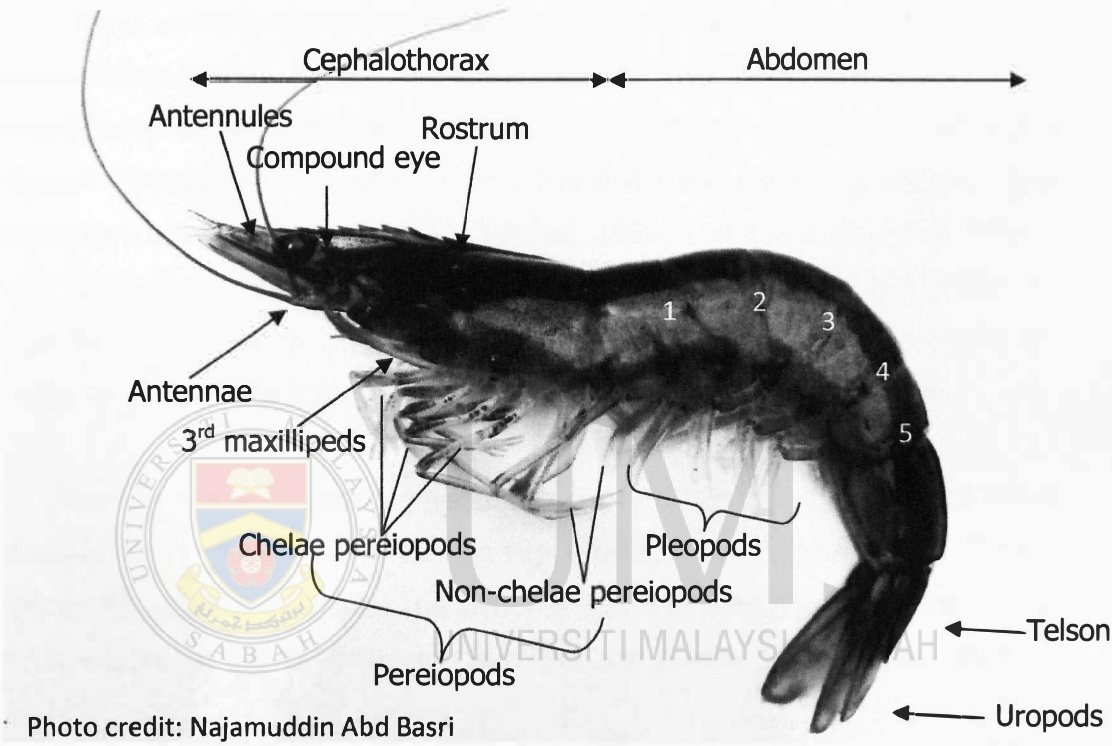

Figure 2.2 : External anatomy of *P. vannamei.*
Source : FAO (1999)

There are two pairs of antennae affixed to the head of a shrimp and both of them perform sensory function. The first pair of antenna known as antennules and it is located just ventral to the compound eye (Daniel *et al.,* 2008; Johnson and Atema, 2005; Schmidt and Derby, 2005). The antennules have two flagellums which are the lateral flagellum and medial flagellum (Horner *et al.*, 2008a, 2008b; Johnson and Atema, 2005; Schmidt and Derby, 2005; Steullet *et al.,* 2001). While the second pair of antennae are much longer than the antennules and sometimes the length of the antenna is three times that of the body length (Denton and Gray, 1985).

Maxillipeds are the first three pairs of appendages that can be found around the cephalothorax. Structurally, the third pair of maxillipeds closely similar to the pereiopod (Young, 1959). The function of the third maxilliped is to grasp large food particles and hold the food next to the pereiopod with chelae for further size reduction and move the food to the mouthpart for swallowing (Garm, 2004a; Wroblewska *et al.,* 2002; Hunt *et al.,* 1992; Derby and Atema, 1982; Young, 1959).

There are five pairs of pereiopods which also known as walking legs. In Penaeid species, the pereiopods are relatively long and slender and have chemosensory setae that responsible in discriminating edible and non-edible materials (Hindley and Alexander, 1978). The first three pairs of pereiopods bear small chelae with sharp cutting edges (Young, 1959) and it is a diagnostic feature of the family penaeidae (Hindley and Alexander, 1978). According to Hindley and Alexander (1978), shrimp used one or more chelae to transfer food particles to the mouthpart. While the last two pairs of pereiopods are the non-chelae limb.

Each of the abdominal segments of a shrimp bears a pair of pleopods which also called as swimmerets or swimming legs (Stamhuis and Videler, 1998). Totally, there are five pairs of pleopods. The pleopods help the shrimp to propel themselves forward rapidly for a great distances (Stamhuis and Videler, 1998; Young, 1959).

According to Young (1959), the tail fan of a shrimp is made up of the telson and uropods, which are projecting from the posterior end of the 6^{th} abdominal segment. Usually, the telson bears the anus and normally considered as an unsegmented body part. The main function of the tail fan (uropods and telson) is to draw the shrimp backwards through the water (Stamhuis and Videler, 1998; Young, 1959).

2.1.2 Life Cycle of *P. vannamei*

P. vannamei is known as euryhaline and eurythermal species. This is based on the life cycle of this species which comprise several stages and in each of the stages the shrimp inhabit variety of habitats (Bailey-Brock and Moss, 1992) as shown in Figure 2.3. Once the *P. vannamei* reach adult stage, it will mature, mate and spawn their eggs in coastal tropical water at depths of about 10 – 80m (Wyban *et al.,* 1995). After hatching, the larvae will undergo larval development for about two to three weeks followed by inshore migration by the post larvae when the larvae size are about 6 – 14mm. Normally, there are 11 larval stages including 6 stages of nauplii, 3 stages of protozoea and lastly 3 stages of mysis. Then the post larvae and juveniles will adapt to a shallow nursery environment in mangrove estuaries with a brackish waters (Fast, 1992). After that, the shrimp will leave the estuaries and migrate to offshore once the shrimp reach a larger size which is around 100 – 200mm of total length (TL) and a body weight of 8 – 10g (FAO, 2015a). Besides that, penaeids shrimp passed through three stages in their life cycle (FAO, 2015a). The first stage is larvae whereby the larvae are adapting to oceanic salinities and surface temperature. As they grow up to juvenile stage, they will adapt to estuarine salinities and coastal temperature and lastly the adult will tolerate to oceanic salinities and bottom temperature (Holthuis, 1980).

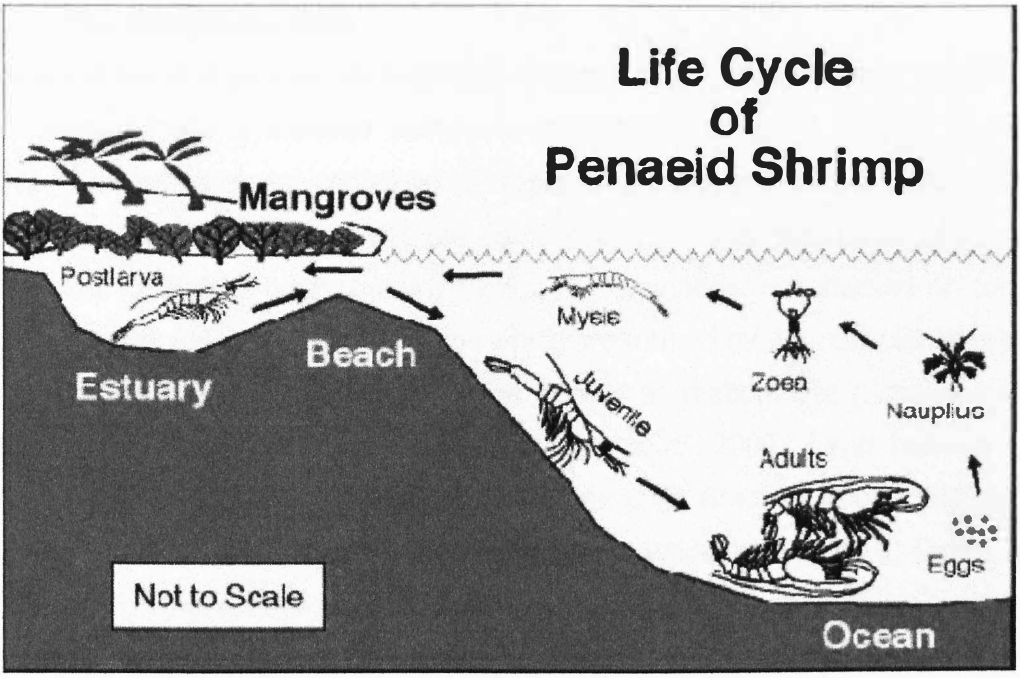

Figure 2.3 : The life cycle of Penaied species.

Source : Rosenberry (2005)

2.2 Sensory Organs

Shrimp is equipped with two major sensory organs which are the compound eye and setae. These sensory organs play different roles. Generally eye is known to provide vision and act as a visual receptor. However, the function of eyes for crustacean is differed based on the stages (Cronin and Jinks, 2001). Primarily, eye is used to focus with orientation in water column, vertical migration and avoidance of predators throughout larval stages (Cronin and Jinks, 2001). While adult vision provides navigation, prey recognition and capture, mate selection and communication (Cronin and Jinks, 2001). Meanwhile setae is a hair like projection with elongate structure (Garm, 2004b) and literally it functioned as mechanosensory, chemosensory or both (Garm *et al.,* 2003).

2.2.1 The Compound Eyes

Compound eye is a general characteristic of eutrhopods (Schoenemann, 2013) and the compound eye is believed to have a complicated structure (Cronin & Jinks, 2001). Compound eyes compose of repetitively identical visual units called ommatidia. Each of the ommatidium composed of optical structures which are cornea and crystalline cone (Cronin, 1986). The ommatidium is stacked on top of receptor layers which are the rhabdoms which are formed by 7-8 retinular cells that capable of sensing light and each cell are termed as rhabdomere (Schoenemann, 2013; Cronin, 1986; Cronin & Jinks, 2001; Martin *et al.,* 2009). Layer between the crystalline cones and rhabdoms is called the clear zone which is a region devoid of pigment and contain the cone thread (Matsuda and Wilder, 2014, 2010; Cronin and Porter, 2008; Mishra *et al.,* 2006; Meyer-Rochow, 2001; Cronin, 1986). Clear zone is an adaptation to improve vision in dim light condition and it is reported that clear zone can only be found in some malacostraca such as Euphausiaceae, Mysidaceae, and Decapoda (Matsuda and Wilder, 2014; Meyer-Rochow, 2001). Within the retinular cells and fasciculated zone lays the screening pigment granules (Matsuda and Wilder, 2014, 2010) which help to screen out stray light and to control any incident of light flux at the rhabdom (Insausti *et al.,* 2013).

Normally, the screening pigment granules will migrate or change their position as an adaptive change under light and dark condition (Matsuda and Wilder., 2014, 2010; Warrant and Locket., 2004; Meyer-Rochow., 2001; Meyer-Rochow *et al.*, 1990; Douglass and Forward, 1989; Ball *et al.,* 1986; Hariyama *et al.,* 1986). If light is present, the compound eye will adapted to the light condition by the upward migration of the screening pigment granules insulating the rhabdom to minimize the amount of light entering the receptor layers (Insausti *et al.,* 2013; Matsuda and Wilder, 2010). If there is limited amount of light the screening pigment granules will migrate away from the edge of the rhabdom to allow more rays to penetrate into the receptor layers (Matsuda and Wilder, 2010). The diagram of the ommatidium of *P. vannamei* is shown in Figure 2.4. While the functions of eye components are shown in Table 2.1.