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Feeding Activity and Growth Performance of Shrimp Post Larvae *Litopenaeus vannamei* Under Light and Dark Condition

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Abstract

The present study was conducted to elucidate the effects of light and dark condition on the feeding activity of shrimp, *Litopenaeus vannamei*. Examination on the ingestion rate of shrimps at different sizes (0.5, 1.0 and 1.5 cm total length, TL) under light and dark condition was conducted using newly hatched frozen *Artemia* nauplii. For each condition, shrimp were let to ingest known number of *Artemia* nauplii for 30 minutes, thereafter the remaining *Artemia* nauplii was counted. For the observation of eye structures under light and dark conditions, 1.0 cm TL shrimps were preserved in Bouin's solution for histological observation. Another feeding trial was conducted to examine the growth performance and survival of shrimps (initial size 1.0± cm, TL) under different photoperiod regimes (24 hours dark: 24D, 24 hours light: 24L and 12 hours light and dark: 12LD) for three weeks. Results showed that, the 0.5cm TL shrimp significantly ingested more *Artemia* nauplii under light condition compared to dark condition (P = 0.000). The 1.0 and 1.5 cm TL shrimps consumed *Artemia* naulplii equally under both conditions. The shrimp attained a complete eye structure which can be differentiated into crystalline cone, clear zone, rhabdom and fasciculated zone at 1.0cm TL. This study also showed that growth (P = 0.557) and survival (P = 0.686) of shrimps did not vary significantly among different photoperiod. This study suggests that the feeding activity of the smallest shrimp (0.5 cm TL) is affected by light condition. However, feeding activity, growth and survival of bigger sized shrimp (> 1.0 cm TL) were not affected by light and photoperiod regimes.

Keywords: dark condition, ingestion, growth performance, light condition, *Litopenaeus vannamei*, photoperiod

1. Introduction

The Pacific white shrimp, *Litopenaeus vannamei* is originated from the Western Pacific coast of Latin America stretching from the south of Peru to the north of Mexico (Briggs, Smith, Subasinghe, & Philip, 2004). This species was introduced commercially into China and Taiwan in 1996 and other coastal Asian countries started to culture this species in early 2000 (Briggs et al., 2004). Since then, *L. vannamei* became one of the important penaeid species farmed worldwide (Balakrishnan et al., 2011).

Light is considered to be one of the most important factors in directing locomotor rhythm in crustacean (Hughes, 1968). In marine fish, feeding activity is mostly assisted by visual receptor in light environment. While other sensory organs such as lateral line play role in detecting prey under dim or dark condition. Several studies on ingestion experiments of crustacean species are performed to investigate the optimum food density (Yufera, Rodriguez & Lubian, 1984; Chu & Shing, 1986; Zhang, Lin & Creswell, 1998) and feeding schedule (Vega-Perez, Ara, Liang & Pedreira, 1996; Barros & Valenti., 2003; Martin et al., 2006). However, limited information available on the feeding activity of *L. vannamei* under light and dark condition and the sensory organ/s that are responsible in food searching under those conditions. Shrimp has compound eyes which are the general characteristic of eutrhopods (Schoenemann, 2013). The compound eyes are believed to have a complicated structure (Cronin & Jinks, 2001) which composes of repetitively identical visual units called ommatidia. Each of the ommatidium composed of optical structures of cornea, lens and crystalline cone. The ommatidium is stacked on top of rhabdoms (Schoenemann, 2013; Cronin, 1986; Cronin & Jinks, 2001). Rhabdom is a light-guiding structure (Land & Fernald, 1992) which is formed by 7-8 retinular cells (Schoenemann, 2013; Cronin, 1986; Cronin & Jinks, 2001; Martin, Crandall, & Felder, 2009). In some species of crustacean such as the rock lobster *Jasus edwardsii*, the size and shape of the rhabdom are affected by dark or light adaptation (Meyer-Rochow, 2001). While some species such as *Penaeus monodon*, the size and shape of the rhabdom did not change as an effect to light or dark adaptation. However, the position of the screening pigment granules were changed, as in light condition the screening pigment granules insulating the rhabdom while under dark condition it is migrated away from the edge of the rhabdom (Matsuda & Wilder, 2010).

In shrimp farming, abiotic factors such as light and photoperiod are believed to play important roles in promising successful grow-out period. Light is able to influence the behaviour (Sulkin, 1984; Minagawa & Murano, 1993), physiology (Ravi & Manisseri, 2012), cannibalism (Hecht & Pienaar, 1993) and molting (Vijayan & Diwan, 1995) of decapods larvae. The effects of photoperiod on larval survival and growth have been studied in several species of crustacean and the results varied with species. Study on the larvae of marine blue swimmer crab, *Portunus pelagicus* at zoea until megalopa stage showed no significant difference on survival under different photoperiod regimes (6L:18D, 12L:12D, 18L:6D) (Ravi & Manisseri, 2012). Gardner and Meguire (1998) also reported the insignificant difference of survival rate on the zoea of the Australian giant crab, *Pseudocarcinus gigas* reared under different photoperiod regimes (0L:24D, 6L:18D, 12L:12D, 18L:6D, 24L:0D). The early juvenile stage, 26-27 mm total length (TL) of *Penaeus indicus* also revealed no significant difference on growth, cultured under different photoperiod regimes (24L:0D, 12L:12D, 0L:24D) (Vijayan & Diwan, 1995). Despite the insignificant results, Hoang, Barchiesis, Lee, Keenan and Marsden (2003) stated that photoperiod had significant effect on the growth of banana prawn, *Penaeus merguinses* juveniles (3.13-3.77 g, initial weight) but not on the survival rate.

There is lack of information on the effects of light condition on the feeding activity of *L. vannamei*. Furthermore, the varied results of different photoperiod on the growth and survival of shrimp have led to this present study. This study was conducted to examine the influence of light and dark condition on the feeding activity and the different photoperiod regimes on growth and survival of *L. vannamei*.

2. Materials and Methods

2.1 Experiment on Ingestion of Post Larvae Under Light and Dark Condition

The shrimp (PL3) were obtained from a private hatchery and acclimatized to experimental conditions in shrimp hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. The shrimps were fed with *Artemia* nauplii until they reached 0.5 cm of TL (PL 5).Then, shrimp with the size of 0.5, 1.0 and 1.5 cm TL were used in the ingestion experiment, fed with frozen *Artemia* nauplii under light and dark conditions. The ingestion experiments under light and dark condition were alternately conducted in a dark room which equipped with fluorescent lamps (Power-Glo lamp, Hagen, Canada). For light condition the light intensity used was 1400 ± 339.19 lx (measured by Light meter Model LT300, Extech Instrument, USA) while for dark condition the lights were switched off. The light intensity used in this experiment was slightly higher with the next experiment without much difference. In order to maintain the temperature, air conditioner and a mechanical fan was switched on during the ingestion experiment under light condition to ventilate the room. While the air conditioner and mechanical fan was switched off under dark condition. Prior to the experiment, the shrimp were starved for 12 hours before the feeding experiments. Thirty shrimp of each size were kept individually in 500 ml beakers. The shrimps were then acclimatized to light or dark condition for 30 minutes. Thereafter, known numbers of frozen *Artemia* nauplii were placed into the beaker and the shrimps were left to feed for 30 minutes. The shrimp were subsequently anesthetized by putting an ice cube into the beakers followed by adding few drops of 15% paraformaldehyde into the beakers in order to stop the ingestion within a couple of minutes and left over *Artemia* nauplii were counted. This trial was repeated for different sized shrimp following the condition as stated in Table 1. Ingestion rate of *Artemia* nauplii was estimated as follows:

$$\text{Ingestion rate (\%)} = [(A_i - A_f) / A_i] \times 100 \quad (1)$$

Where A_i is the initial number of given *Artemia* nauplii and A_f is the number of uneaten *Artemia* nauplii. For 1.0 cm TL shrimp, the shrimps were preserved in Bouin's solution for observation on the eye structure after adaptations under light and dark conditions. The samples were dehydrated with an ascending series of ethanol and cleared with xylene. After that, the samples were embedded in paraffin, cut into 6μm thick sections and lastly, stained with hematoxylin and eosin (Yahaya et al., 2011; Matsuda & Wilder, 2010; Mukai, 2006) before observation under light microscope (Eclipse 80_i, Nikon, Japan).

Table 1. Number of *Artemia* nauplii given to different sized shrimp

Total length (cm)	Number of *Artemia* nauplii
0.5	15
1.0	30
1.5	60

2.2 Experiment on Growth Performance of Post Larvae Under Different Photoperiod Regimes

The shrimp size of 1.0 cm TL with the initial body weight 15.00 ± 3.00 mg was used for feeding trial under different photoperiod regimes. Groups of 30 individual of shrimp (6 individuals L^{-1}) were introduced into 7 L plastic aquaria where all sides of the walls including the bottom were wrapped with black plastic sheets and filled with 5 L of seawater. The shrimp were reared under three photoperiod regimes which were continuously dark conditions (24 hours dark, 24D), continuously light conditions (24 hours light, 24L) and natural diurnal cycle (12 hours light and 12 hours dark, 12LD). Each treatment was done in five replicates. For the 12LD and 24L, the shrimps were exposed to 1368 ± 235 lx of light intensity. Meanwhile, under continuous dark conditions no light was provided. The shrimps were fed with extruded commercial pellet (starter crumble number 3, 42% protein, 6% lipid, Cargill, Malaysia) twice daily in the morning (0900) and evening (1600) at a feeding rate of 15 % of total biomass. Every morning, 20% of the water was changed and the water temperature during the experiments was recorded in the range of 28.0-29.0 ^{o}C. For continuous dark condition, the aquaria were exposed to light for about 10 minutes in order to change the water and to feed the shrimp. The experiment was conducted for three weeks and routine body weight measurement was conducted once a week to estimate the growth performance. The weight of the shrimps was weighed individually to the nearest 0.0001 g using the analytical balance (model PA214C, OHAUS Pioneer, USA). At the end of the rearing trial, numbers of shrimp survived were recorded. Weight gain, food conversion ratio, specific growth rate and survival rate were determined as follows:

$$\text{Weight gain (\%)} = [(W_f - W_i) / W_i] \times 100 \quad (2)$$

$$\text{Food conversion ratio, FCR} = \text{total dry feed intake (g)} / \text{weight gain (g)} \quad (3)$$

$$\text{Specific growth rate, SGR (\%/day)} = [(\ln W_f - \ln W_i) / \text{day}] \times 100 \quad (4)$$

$$\text{Survival rates (\%)} = (\text{final number of shrimp}/\text{initial number of shrimp}) \times 100 \quad (5)$$

Where the W_i is the initial weight (mg) and W_f is the final weight (mg) of the shrimps (Ju, Deng, & Dominy, 2012; Kim, Rahimnejad, Kim, & Lee, 2013).

2.3 Statistical Analysis

Data obtained on the ingestion rates of frozen *Artemia* nauplii, were statistically analyzed with t-test (Statistical package of social sciences, SPSS ver. 21, USA). Growth, survivals and feed utilization of shrimp were analyzed with One-way analysis of variance.

3. Results

3.1 Ingestion Experiments

The frozen *Artemia* nauplii ingested by the *L. vannamei* at different sizes under light and dark condition is presented in Table 2. Smallest sized shrimp (0.5 cm TL) significantly ingested more frozen *Artemia* nauplii under light condition compared to dark condition (P = 0.000). However, the ingestion rate of frozen *Artemia* nauplii did not differed significantly under light and dark condition by 1.0 cm TL (P = 0.576) and 1.5 cm TL (P = 0.506) of shrimp.

Table 2. Ingestion rates of frozen *Artemia* nauplii by *L. vannamei* under light and dark condition

Total length (cm)	Ingestion rates (%) of frozen *Artemia* nauplii	
	Light	Dark
0.5	62.00±15.08^a	39.33±13.03^b
1.0	88.11±13.52^a	89.78±9.01^a
1.5	67.33±25.45^a	71.28±19.89^a

*Values expressed mean ± standard deviation, and values with different letters within the same row indicate significant value (P < 0.05).

Histological observations on the eye of 1.0 cm TL shrimp under light and dark conditions are shown in Figure 1. It is found that there are four optical properties observed which are crystalline cone, clear zone, rhabdom and fasciculated zone. It showed that, the eyes were able to adapt to light and dark condition. This is based on the position of the screening pigment granules in the rhabdom. Under light condition, screening pigment granules moved upward insulating the rhabdom while the screening pigment granules migrated away from the edge of the rhabdom under dark condition.

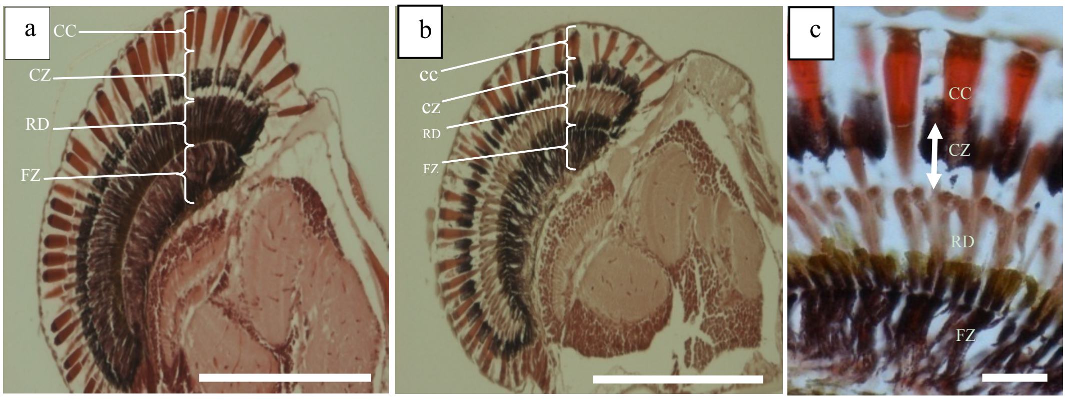

Figure 1. Histological observation on the eye of 1.0 cm TL shrimp, a) kept under light condition. Scale bar 100 µm. b) kept under dark condition. Scale bar 100 µm. c) a portion of eye of the shrimp, arrow shows the area of clear zone. Scale bar 20 µm. CC, crystalline cone; CZ, clear zone; RD, rhabdom; FZ, fasciculated zone

3.2 Post Larval Rearing Under Different Photoperiod

The average body weight of the shrimp after three weeks of rearing period showed no significant different among the treatment groups (P = 0.557) (Table 3). The body weight gain of shrimp under 24L was 810.64% followed by 24D, 12LD with the value of 799.71% and 778.12% respectively. Results of SGR (P = 0.495) and FCR (P = 0.890) also showed similar trend as weight gain without significant difference. Survival rate of shrimp also showed no significant different among the groups (P = 0.686).

Table 3. Growth performance and feed utilization of *L. vannamei*

Photoperiod	Initial weight (mg/individual)	Final weight (mg/individual)	Weight gain (%)	FCR	SGR (%/day)	Survival rates (%)
24D	15±3.00	133±35.00	799.71±526.28	0.89±0.05	9.98±0.27	95.33±1.83
24L	15±3.00	138±32.00	810.64±430.54	0.87±0.05	10.04±0.22	93.33±4.71
12LD	15±3.00	131±35.00	778.12±233.25	0.90±0.05	9.87±0.12	94.67±3.80

4. Discussion

This present study showed that different lighting condition (light and dark) affects the feeding activity of the smallest shrimp (0.5 cm TL) based on the ingestion rate of frozen *Artemia* nauplii. The smallest shrimp ingested significantly more frozen *Artemia* nauplii under light condition compared to dark condition. The ingestion rates of frozen *Artemia* naupliiunder light condition was 1.58 times higher than under dark condition, suggest that vision is important in the feeding activity of the shrimp in the light phase. However, dependency on eyes in hunting food decreased with the shrimp growth. The insignificant results of the ingested frozen *Artemia* nauplii by 1.0 and 1.5 cm TL of shrimp under light and dark condition suggest that the feeding activity of the shrimp was not affected by the lighting condition. This indicates that other sensory organs than eyes are also responsible in food searching especially under dark condition. Setae are part of sensory system of shrimp which either function as mechanosensory, chemosensory or both (Garm, Hallberg, & Hoeg, 2003). These setae can be found around the appendages of the shrimp such as uropods, pleopods and antennules scales (Chan, Rankin, & Keeley, 1988). In this study, frozen *Artemia* was provided, and hence no vibration was produced in water column during the feeding. However, the ability of shrimps to detect and consume frozen *Artemia* nauplii particularly under dark condition may be due to the functionality of the chemosensory of the setae. This study is in agreement with You et al. (2006) reported that odours are mainly used for food detection by the juvenile of *L. vannamei* due to the ability to ingest food under dark condition. Kumlu (1999) also stated that decapods larvae were found to use other sensory organs than eyes in hunting for prey and stated that dark condition did not affect the growth of the larvae under longer dark hour.

Although the shrimp used other sensory organ in detecting food, the eyes were remarkably found to be well adapted to light and dark condition at 1.0 cm TL of shrimp. Through the histological observation, it is found that clear zone is present in the eye of 1.0 cm TL of shrimp. Clear zone is a region devoid of pigment or a gap between the dioptric apparatus (cornea and crystalline cone) and the rhabdom (Cronin & Porter, 2008;

Meyer-Rochow, 2001). The function of clear zone is to improve crustacean vision in dim light environment (Matsuda & Wilder, 2010; Meyer-Rochow, 2001). Thus, the eyes of the shrimp were able to adapt well especially under dark condition. This is based on the rhabdom part of the eye which is formed by 7-8 retinular cells that is capable of sensing light (Schoenemann, 2013; Cronin, 1986; Cronin & Jinks, 2001; Martin et al., 2009). Under light condition, screening pigment granules moved upward insulating the rhabdom while the screening pigment granules migrated away from the edge of the rhabdom under dark condition. Juvenile and sub-adult of *L. vannamei* were also having the ability to change their optical properties of the eyes by light or dark adaptation through the position of the screening pigment granules (Matsuda & Wilder, 2010). Since the eyes are able to adapt under light or dark condition, eyes might play a role in food searching under light condition for the 1.0 and 1.5 cm TL of shrimp. Other sensory organs which are setae may also function for food hunting under light and dark condition.

This present study also showed that the growth, survival and feed utilization of *L. vannamei* were not significantly affected by the photoperiod regimes. This might be due to the ability of the shrimp to detect and consume food under light and dark condition which has been showed through the ingestion experiment. Some researchers found that the ability to consume feed in any lighting regime might not affect the survival of decapods larvae (Gardner & Maguire, 1998). Other studies also revealed the insignificant result of different photoperiod regimes on the survival and growth of crustacean species. Ravi and Manisseri (2012) revealed that the survival rate of blue swimmer crab, *Portunus pelagicus* larvae was not affected by different photoperiod regime (6L:18D, 12L:12D, 18L:6D). Survival rate of the zoea of Australian giant crab, *Pseudocarcinus gigas* were also reported to have no significant effect after being cultured under different photoperiod regimes (0L:24D, 6L:18D, 12L:12D, 18L:6D, 24L:0D) (Gardner & Maguire, 1998). Matsuda, Abe and Tanaka (2012) also revealed the insignificant result of different photoperiod (10L:14D, 12L:12D, 14L:10D) on the growth of phyllosoma larvae of the Japanese spiny lobster, *Panulirus japonicus*.

Overall, this present study showed that the light conditions affected the feeding activity of the smallest shrimp (0.5 cm TL), where they depend more on eyes to search for food. As the shrimp grow (up to 1.0 and 1.5 cm TL), light or dark conditions did not influence the feeding activity as well as the growth performance, survival and feed utilization. The eyes were found to be well adapted to light and dark condition and have a complete structure at size of 1.0 cm TL.

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