

EFFECT OF DIFFERENT LIGHTING CONDITIONS ON FEEDING ACTIVITY AND EYE ADAPTATION OF POST LARVAE *PENAEUS VANNAMEI*

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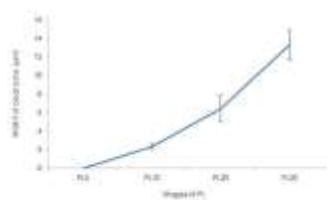
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Graphical abstract



Abstract

This study was conducted to investigate the effect of light and dark conditions on feeding activity and eye adaptations of post larvae (PL5, PL10, PL20 and PL30) *Penaeus vannamei* fed with frozen *Artemia*. Shrimp PL were placed individually in beakers and after acclimatization under the light or dark condition, PL were left to ingest known number of *Artemia* for 30 minutes. Thereafter, each PL was subsequently anesthetized by putting an ice cube into the beakers followed by adding few drops of paraformaldehyde. The results showed that the PL5 ingested significantly more frozen *Artemia* under light condition compared to dark condition ($P<0.05$). The eye structures of PL5 comprises of crystalline cone, rhabdom and fasciculated zone. However, it was incomplete due to the lack of clear zone and no migration of the screening pigment granules was observed under light and dark conditions. On the contrary, the number of frozen *Artemia* ingested by the latter stages PL10, PL20 and PL30 showed no significant differences ($P>0.05$) under both light conditions and these PL have complete eye structures with define clear zone. The width of clear zone was found to increase proportionally with the growth of the PL. Besides that, the screening pigment granules were able to migrate under light and dark conditions. The ability of the PL10, PL20 and PL30 to ingest almost similar numbers of *Artemia* under light and dark conditions suggests that different lighting conditions did not affect the feeding activity of the PL and other sensory organs may play roles in detecting food, while PL5 need light to improve their feeding activity. Based on these results, we suggest that in aquaculture practice, during the rearing of early stage of PL (<PL5), a brighter environment or light should be provided to enhance larval feeding activities. Whereas, later stages of PL (\geq PL10) can be cultured under any light condition.

Keywords: Light and dark conditions, feeding activity, eye structure, *Penaeus vannamei*, post larvae

Abstrak

Kajian ini dijalankan untuk mengkaji kesan keadaan terang dan gelap terhadap aktiviti pemakanan dan adaptasi mata udang *Penaeus vannamei* di peringkat pasca larva (PL5, PL10, PL20 dan PL30) dengan menggunakan *Artemia* yang telah dibekukan. Udang diletakkan secara individu di dalam bikar dan sebaik sahaja udang beradaptasi di dalam keadaan terang atau gelap, udang dibiarkan selama 30 minit untuk memakan *Artemia* yang telah disediakan. Setelah itu, udang dilemahkan dengan meletakkan kiub ais dan beberapa titis paraformaldehid ke dalam bikar tersebut. Hasil kajian mendapati bahawa PL5 menunjukkan kecenderungan yang tinggi memakan *Artemia* yang telah dibekukan di dalam keadaan terang berbanding gelap ($P<0.05$). Manakala pemerhatian histologi mendapati struktur optik PL5 merangkumi kon berhablur (crystalline cone), rhabdom dan zon fasikulat (fasciculated zone). Tetapi struktur optik ini adalah kurang lengkap kerana ketiadaan zon terang (clear zone). Oleh itu, kedudukan penapis pigmen granul (screening pigment granules) tidak berubah di dalam keadaan gelap atau terang. Sebaliknya, PL10, PL20 dan PL30 dapat memakan *Artemia* pada kadar yang sama di dalam keadaan terang dan gelap ($P>0.05$). Manakala, struktur optik udang pada peringkat ini adalah sama seperti udang diperingkat PL5 tetapi dengan penambahan zon terang. Kelebaran zon terang didapati meningkat sejajar dengan tumbesaran udang. Selain itu, kedudukan penapis

pigmen granul menunjukkan perubahan di dalam keadaan terang mahupun gelap. Kebolehan PL10, PL20 dan PL30 untuk memakan *Artemia* pada kadar yang sama menunjukkan bahawa aktiviti pemakanan tidak dipengaruhi oleh keadaan terang atau gelap dan terdapat organ deria lain yang mungkin digunakan pada tempoh tersebut. Manakala, PL5 memerlukan cahaya untuk meningkatkan aktiviti pemakanan. Kajian ini mencadangkan pengkulturan udang pada peringkat awal pasca larva (<PL5) perlu dijalankan di dalam keadaan terang. Manakala udang yang lebih besar (\geq PL10) boleh dikultur di dalam keadaan terang mahupun gelap.

Kata kunci: Keadaan terang dan gelap, aktiviti pemakanan, struktur mata, *Penaeus vannamei*, pasca larva

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1.0 INTRODUCTION

The Pacific white shrimp, *Penaeus vannamei* is a native species to the Eastern Pacific coast of Latin America stretching from the south of Peru to the north of Mexico [1]. In 1996, this species was introduced commercially into China and Taiwan while other coastal Asian countries started to culture this species in early 2000 [1]. Since then, *P. vannamei* became one of the most cultured species worldwide [2].

The uses of light are 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 [3]. In some other countries, the hatcheries are equipped with transparent roofs to allow natural penetration of sun light to the rearing tanks [4]. Whereas, other hatcheries provide a 24 hours light condition in the rearing tanks [5]. However, the used of light in most hatcheries was for human convenience [5].

Studies have been conducted to investigate the effects of light on the growth and survival of the shrimps at different life stages [6, 7, 8]. It was found that light can influence the growth [7], survival [8], behaviour [9], physiology [10] and molting [11] of some penaeid species such as the banana shrimp, *Penaeus merguensis* [7], Chinese shrimp, *Fenneropenaeus chinensis* [12] and whiteleg shrimp, *Penaeus vannamei* [8]. 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* [13]. 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 [14]. While other crustacean species such as *Lucifer faxoni* can feed well under both light and dark conditions [15]. 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 consists of optical structures called the cornea and crystalline cone. The ommatidia are stacked on top of rhabdoms which are capable in sensing light [16, 17]. Lay underneath the rhabdom, there are screening pigment granules that can control light that enter the eye [18]. It has been reported that many crustaceans have the ability to change the optical properties of their eyes under light and dark adaptation [19]. The changes of the optical properties under light and dark condition were reported to be controlled by diel biological rhythms, and also influenced by hormones and/or ambient light intensities [20]. According to Meyer-Rochow [19], any component of the crustacean's compound eyes can be affected by dark or light adaptation. However, the most obvious changes are the position of the screening pigment granules and the position, size and shape of the rhabdom [19]. Although the adaptations of the compound eye under light and dark conditions are well studied on the juvenile and sub-adult of *P. vannamei* [21], there is a lack of information on the eye structures and adaptation of the post larvae. This study was conducted to examine the effect of light and dark conditions on the feeding activity and the eye adaptation of post larval stages of *P. vannamei* which can contribute to improve the rearing techniques in the hatchery.

2.0 EXPERIMENTAL

2.1 Feeding Activity Experiment

The PL (PL3) were obtained from a private hatchery and acclimatized to experimental condition in shrimp hatchery of Borneo Marine Research Institute,

Universiti Malaysia Sabah. The PL were cultured under natural diurnal cycle and fed with *Artemia* nauplii. PL at different stages such as PL5 (0.5cm total length, TL), PL10 (1.0cm TL), PL20 (1.5cm TL) and PL30 (2.0cm TL) were used in the feeding activity experiment. The feeding experiment was conducted in a room equipped with high intensity aquarium lamps (Power-Glo lamp, 15 W, Hagen, Canada), that was also alternately used as a dark room for the dark condition experiment. For light condition, the light intensity used was 1400 ± 339.19 lux. Lux meter (Light meter Model LT300, Extech Instrument, USA) was used to measure the light intensity. Prior to the experiment, the PL were starved for 12 hours. Thirty PL of each size groups were kept individually in 500ml beakers. The PL 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 PL were left to feed for 30 minutes. The acclimatization and ingestion period used in this experiment was based on the results of preliminary experiments, where 30 minutes of acclimatization and ingestion period was enough to observe the feeding activity and the eye adaptation of the PL under light and dark conditions. The initial number of frozen *Artemia* nauplii fed to each PL5, PL10, PL20 and PL30 were 15, 30, 60 and 80 respectively. After 30 minutes feeding time, the PL were subsequently anesthetized by putting an ice cube into the beakers followed by adding few drops of 15% paraformaldehyde in order to stop the ingestion within a couple of minutes. The frozen *Artemia* nauplii left in the beaker were counted. The PL were then preserved in Bouin's solution for eye histological observation. The number of frozen *Artemia* nauplii ingested was estimated as follows [22]:

$$\text{Number of } Artemia \text{ nauplii ingested} = A_i - A_f \quad (1)$$

Where A_i is the initial number of frozen *Artemia* nauplii and A_f is the number of uneaten frozen *Artemia* nauplii.

2.2 Histology

Shrimps were soaked in Bouin's solution for 24 hours. Then, the samples were dehydrated in a series of ethanol, cleared in xylene and embedded in paraffin wax. After that, the samples were cut into $6\mu\text{m}$ thick sections using a microtome and stained with hematoxylin and eosin [21, 23, 24]. Lastly, the sectioned samples were observed under a light microscope (Eclipse 80i, Nikon, Japan).

2.3 Data Analysis

Data obtained on the numbers of *Artemia* nauplii ingested under light and dark condition were statistically analyzed with t-test (Statistical package for social sciences, SPSS ver. 21, USA).

3.0 RESULTS AND DISCUSSION

This present study was done on PL5-PL30 and study was not conducted on PL1-PL4 due to the unavailability of the PL, as the PL were obtained from a private hatchery at stage PL3. The results showed that PL5 ingested more frozen ($P < 0.05$) *Artemia* nauplii under light condition compared to dark condition (Table 1). Hence indicated that light was preferred than dark and this affected the feeding activity of the PL5. Previously, Meyer-Rochow [25] had reported that the compound eye of crustacean changed or developed from larval to adult stages. Larvae of the rock lobster, *Panulirus longipes* was found to possess the apposition type of compound eye, in which no clear zone is found. Meanwhile, the adult of this species possessed the superposition type of compound eye, where the clear zone can be observed clearly [25]. Clear zone is a region devoid of pigment or gap between the crystalline cone and photoreceptor layers [19, 26, 27]. It contains cone thread which is the elongated proximal ends of the crystalline cone [19]. Clear zone is important to improve the vision of crustacean under dim light environment [19, 21]. In this present study, histological observation showed that the eye structure of the PL5 comprises of crystalline cone, rhabdom, and fasciculated zone but lacking of clear zone (Figure 1 a-b). Due to the lack of clear zone, the eye of PL5 was unable to show clear adaptation under the dark condition. Study also found that the eye of PL1 of the grass shrimp, *Palaemonetes pugio* was unable to adapt under dark condition due to the lacked of clear zone and the crystalline cones remained circular in cross section [20]. This might help to explain the reason of the lower number of frozen *Artemia* nauplii ingested by the PL5 under the dark condition. Based on these observations and previous study, this study suggests that PL5 and most probably those PL in early stages (PL1-PL4) may need light to improve their feeding.

The numbers of frozen *Artemia* nauplii ingested by the PL10, PL20 and PL30 showed no significant difference ($P > 0.05$) under light and dark conditions (Table 1) and it may suggests that the dependency on visuals in hunting for food decreased with the growth of the PL. Likewise, this also indicates that other than the eyes, sensory organs such as setae searching under dark condition. This study is in agreement with You et al [28] reported that odours are mainly used for food detection by the juvenile of *P. vannamei* due to the ability to ingest food under dark condition. Similarly, Chong and Sasekumar [29] reported the ability of the PL banana shrimp, *Penaeus merguensis* to feed well at any time, both day and night time.

In the present study, the histological observation found that the eye structures of the PL10, PL20 and PL30 were similar with that of PL5 except for the lack of a defined clear zone (Figure 1 c-h). Besides that, it

was also found that the width of clear zone increased proportionally with the growth of the PL (Figure 2). The width of the clear zone in the eye at different developmental stages has not been well documented on *P. vannamei* and the present study might be the first finding.

Due to the presence of clear zone, the eye of PL10, PL20 and PL30 were able to adapt to any lighting conditions. This is based on the position of the screening pigment granules (Figure 1 c-h). In response to light condition, the screening pigment granules moved upward insulating the rhabdom while under dark condition, the screening pigment granules migrated away from the edge of the rhabdom. The pigment migrations are the important mechanism of light and dark adaptation in many

compound eyes [18, 20, 27]. The reason of these adjustments under the dark condition is to allow more light to enter the eye so that the photon capture rate by the molecules of the photopigment can be improved [30]. While for the light adapted eye, these adjustments occurred to reduce the light flux at the retina of the compound eye [18, 27]. The migration of the screening pigment granules were also reported to occur on the eyes of other crustacean species that were subjected to light and dark adaptation such as the giant freshwater prawn, *Macrobrachium rosenbergii* [31], spiny lobster, *Jasus edwardsii* [19], elongated crab, *Petrolisthes elongates* [30], freshwater prawn, *Macrobrachium heterochirus* [19] and the juvenile and sub-adult of *P. vannamei* [21].

Table 1 The number of frozen *Artemia* nauplii ingested by the different stages of *P. vannamei* post larvae under light and dark conditions.

PL stages	The number of frozen <i>Artemia</i> nauplii ingested by different stages of PL	
	Light	Dark
5	9.3 ± 2.26 ^b	5.9 ± 1.95 ^a
10	26.43 ± 4.06 ^b	26.93 ± 2.70 ^b
20	40.40 ± 15.27 ^b	42.77 ± 11.93 ^b
30	67.94 ± 11.14 ^b	59.40 ± 12.05 ^b

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

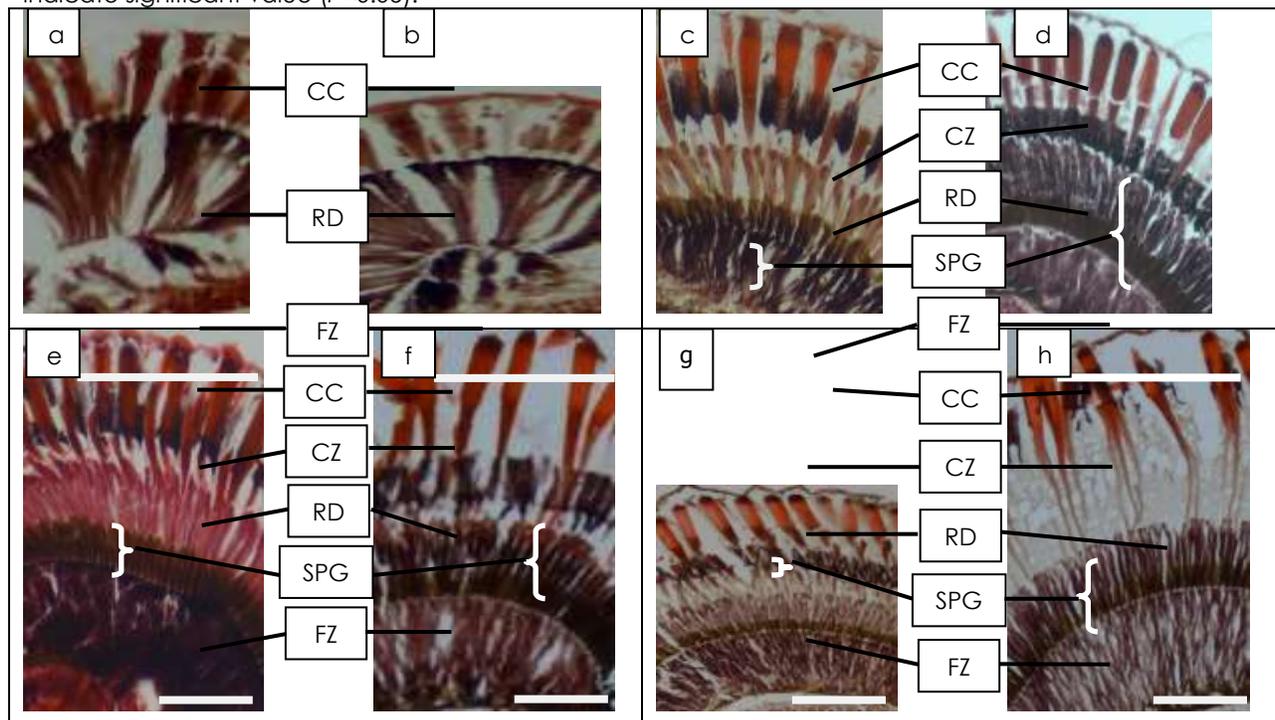


Figure 1 Eye sections of *P. vannamei*. a) Dark adapted of PL5. b) Light adapted of PL5. c) Dark adapted of PL10. d) Light adapted of PL10. e) Dark adapted of PL20. f) Light adapted of PL20. g) Dark adapted of PL30. h) Light adapted of PL30. CC: crystalline cone. CZ: clear zone. RD: rhabdom. SPG: screening pigment granules. FZ: fasciculated zone. Scale bar 100µm.

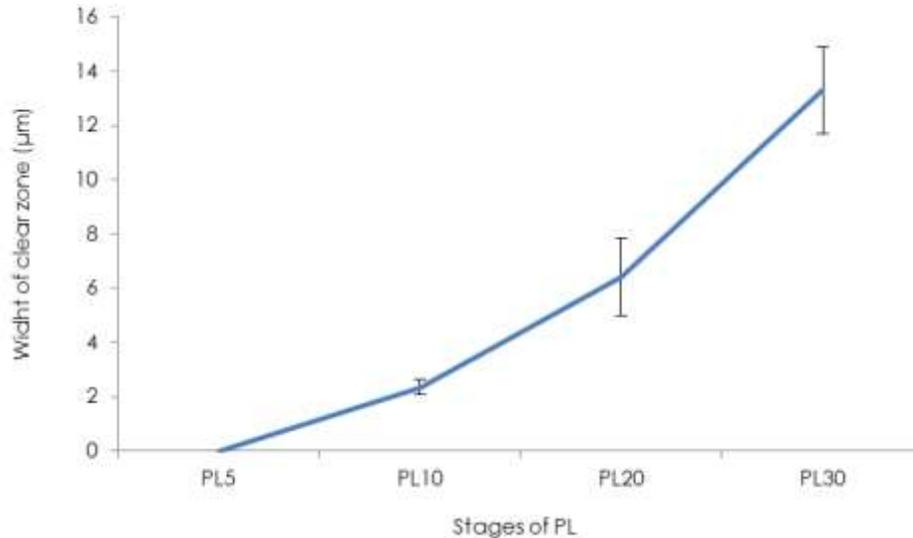


Figure 2 The width of the clear zone of the *P. vannamei* post larvae at different stages under light condition (n=12).

4.0 CONCLUSION

Overall, this present study showed that the different lighting conditions affected the feeding activity of the PL5. These early stage PL feed better under light condition, suggesting the need for light to assist them in the feeding activity because the eyes of PL5 were unable to adapt to dark condition due to the lack of clear zone. However, as the PL grow (\geq PL10), complete eye structures are developed, making them well adapted to both light and dark conditions, and thus, the feeding activity was not influenced by the different lighting conditions.

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