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Effects of Eyestalk Ablation on Feeding, Gonad Development, and Energy Conversion of *Penaeus monodon* Broodstock

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ABSTRACT

The present study aimed to evaluate the effects of eyestalk ablation on feeding, gonad development, and energy conversion of *Penaeus monodon* broodstock. An experiment of 30 days was carried out on broodstock (average body length 28.4 ± 0.95 cm, average body weight 96.7±6.48 g) with (treatment) or without eyestalk ablation (control), cultivated under a water temperature of 27.0±1.5 °C, salinity of 30–32, pH of 8.0–8.3, and light intensity of 1000 lx. Results showed that food consumption and feeding intensity of the eyestalk-ablated broodstock were higher than those without eyestalk ablation. The night-time feeding intensity was 1.46-fold and 1.31-fold higher than the daytime feeding intensity in the treatment and control groups, respectively ($P<0.01$). Food consumption in the treatment group increased continuously during sexual maturation acceleration, whereas that of the control group fluctuated. At the end of the experiment, the gonadosomatic index (GSI) increased by 607.14% and 100.00% in the treatment and control groups, respectively ($P<0.01$). The gonadal wet and dry mass ratio (G_d/m) was higher in the treatment group than those in the control group, but the difference was not significant ($P>0.05$). Furthermore, the body energy values (E_b) slightly decreased in both groups. The increase in the gonad energy value (E_g) was significantly higher in the treatment than control group ($P<0.01$). Thus, the broodstock showed negative growth in their breeding season from the perspective of body energy value. During the experimental period, food conversion efficiency (FCE) and energy conversion efficiency (ECE) values of the treatment group were significantly higher than those of the control, with FCE values of 11.06% and 1.89%, respectively, and ECE values of 15.45% and 2.64%, respectively. These results indicated that eyestalk ablation promoted food consumption, feeding intensity, gonad development, and energy conversion efficiency of *P. monodon* broodstock. During gonad development, food was the chief, though not the only, source of energy, with tissues and organs also important energy suppliers. The results of the present study will hopefully improve our understanding on the characteristics of feeding, gonad development, and energy conversion of *P. monodon* broodstock during their breeding season, and provide a theoretical foundation for the scientific cultivation of *P. monodon* broodstock.

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Authors' Contribution
SGJ, SJ and FLZ designed the study. QBY and JHH took part in the execution of study. LSY, QBY and SJ implemented the study and involved in sampling as well as testing. FLZ, SJ and SGJ drafted the manuscript.

Key words
Eyestalk ablation, *Penaeus monodon*, Broodstock, Feeding, Gonad development, Energy conversion.

INTRODUCTION

In crustaceans like shrimp and crabs, gonad development and molting are regulated by the gonad-inhibiting hormone (GIH) and molt-inhibiting hormone (MIH) stored and released by the X organ-sinus gland complex, which is a part of the neurosecretory system located in the eyestalk (Pervaiz *et al.*, 2011). Unilateral or bilateral eyestalk ablation can accelerate molting and promote gonad development and maturation (Snyder and Chang, 1986; Kang *et al.*, 2000). At present, eyestalk ablation is a typical method for development and maturation acceleration in the breeding of economically important crustaceans (Zhu *et al.*, 2011). Eyestalk ablation also affects the feeding, growth, energy conversion, and osmoregulation of freshwater shrimp and crabs; however, such research has mainly focused on the larval stage of the organisms studied (Maynard and Sallee, 1970; Koshio *et al.*, 1992; Bernardita, 1994; Venkitraman *et al.*, 2004; Kumari and Pandian, 1987; Juinio and Ruinata, 1996; Arcos *et al.*, 2003; Chen and Chia, 1995; McNamara *et al.*, 1990). *Penaeus monodon* is an important cultured shrimp species in southern China.

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With the expansion of cultivation, increasing attention has been paid to the gonadal development and spawning of *P. monodon* broodstock (Huang *et al*., 2006; Wang *et al*., 2017). In the present study, the characteristics of *P. monodon* broodstock during gonad development with or without eyestalk ablation were investigated, and the effects of eyestalk ablation on the feeding, gonad development, and energy conversion of *P. monodon* broodstock were analyzed to provide a theoretical foundation for the scientific management of *P. monodon* broodstock during the breeding season.

MATERIALS AND METHODS

Experimental materials

Two hundred female broodstock ('Nanhaiyihao' variety, average body length 28.4 ± 0.95 cm, average body weight 96.7±6.48 g) were obtained from our laboratory's breeding conservation pond. The broodstock were collected by shrimp traps, surface sterilized in formaldehyde solution (15 ppm) for 60 s, and temporarily maintained in a cement pond (5m×5m×2m). The broodstock were fed with fresh *Ommastrephes bartramii* purchased from a local seafood market. The squid had their internal organs and heads removed, before being washed with water and cut into small pieces by a meat grinder.

Acclimatization of broodstock

The seawater (27.0±1.5 °C, salinity from 30 to 32 psu and pH from 8.0 to 8.3) which the broodstock temporarily cultivated was extracted from the adjacent sea area was sieved through a sand filter, and sterilized by ultraviolet light. Nitrate(NO_3-N) was too low to detect. Nitrite (NO_2-N) and ammonia nitrogen (NH_4-N) ranged from 0.52 to 0.73 μmol·L^{-1} and 5.40 to 5.73 μmol·L^{-1} respectively. Half of the water was changed every day.

Experimental design

The experimental period was 30 d, from May 15 to June 14, 2017. After 7 d of acclimatization cultivation, 99 broodstock with uniform size and undeveloped gonads were selected and weighed by an electronic balance to an accuracy of 0.01 g. Ninety were randomly selected and equally distributed into 18 glass fiber reinforced black tanks (500 L, containing 400 L of water) for maturation acceleration. Three parallel groups were established in the control (without eyestalk ablation) and treatment (with eyestalk ablation) groups, respectively, with three tanks in each group and five shrimp in each tank. The left eyestalks of the treatment groups were ablated using burning tweezers (Zhou *et al*., 2017). The remaining nine shrimp were randomly divided into three groups for analysis of initial body weight, wet and dry weight of gonads, and energy values.

Collection and pretreatment of samples

Food samples were collected in triplicate for energy detection. The broodstock were fed four times every day (at 7:00, 12:00, 17:00, and 22:00, respectively). Two hours after feeding, the residual feed was collected by siphoning, and food consumption of *P. monodon* was calculated. Waste material was also removed by siphoning. At the end of the experiment, the shrimp were dissected on ice, with the body and wet gonads weighed and then dried to a constant weight at 65 °C in an oven. The body and gonad dry weights were recorded. The body (gonad removed, (E_b), gonad (Eg), and diet (E_d) energy values were determined using an oxygen bomb calorimeter.

Calculations

Gonad weight increase (G_g), body weight increase (G_b), food consumption (C), feeding intensity (I_f), gonadosomatic index (GSI), gonadal wet and dry mass ratio ($G_{d/m}$), food conversion efficiency (FCE, %), and food energy conversion efficiency (ECE, %) were calculated as follows:

Gonad weight increase (G_g) = $m_{gt} – m_{g0}$

Body weight increase (G_b) = $m_{bt} – m_{b0}$

Food consumption (C) =$D_p – D_r$

Feeding intensity (I_f) = C / m_{bt}

Gonadosomatic index (GSI) = m_{gt} / m_{bt}

Gonadal wet and dry mass ratio ($G_{d/m}$) = m_{gd} / m_{gt}

Food conversion efficiency (FCE, %) = $100×(m_{gt}- m_{g0})/C$

Food energy conversion efficiency (ECE,%)=$100×E_g /E_d$

Where, G_g and G_b are the weight gains of the gonad and shrimp body, respectively; m_{g0} and m_{b0} are the initial wet weights of the gonad and shrimp body, respectively; m_{gt} and m_{bt} are the wet weights of the gonad and shrimp body at the end of the experiment, respectively; m_{gd} is the dry weight of the gonad at the end of the experiment; C is the food consumption; D_p is the feeding amount; D_r is the amount of residue diet; E_g is the energy of the gonad; and E_d is the energy value of the diets (Huang *et al*., 2005; Xie, 2002).

Statistics

One-way analysis of variance (ANOVA) was adopted for data analyses, with *P* values of < 0.05 and < 0.01 considered to be statistically significant and extremely significant, respectively.

RESULTS

Daily food consumption and feeding intensity

The daily food consumption and feeding intensity of the treatment group were 6.58±1.37 g/ind and 5.68%, respectively, which were significantly higher than those of the control group (4.08±0.61 g/ind and 4.41%, respectively). The food consumption and feeding intensity showed significant diurnal variation in both the control and treatment groups ($P < 0.01$). Night-time food consumption was significantly higher than that of daytime, and was 1.46-fold and 1.31-fold higher in the control and treatment group, respectively. Food consumption of the treatment group increased continuously, whereas that of the control group fluctuated during the whole maturation acceleration period. Thus, eyestalk ablation significantly promoted food consumption and feeding intensity of *P. monodon* broodstock.

GSI and gonadal wet and dry mass ratio

As shown in Table I, the GSI in the treatment group increased from 0.014±0.003 to 0.099±0.026 by the end of the experiment, an increase of 607.14%; whereas, that in the control increased to 0.028±0.013, an increase of 100.00%. Thus, the treatment group showed a highly significant 6.07-fold increase compared with the control group (P<0.01). In addition, the gonadal wet and dry mass ratios increased in both groups (P<0.05) by the end of the experiment, with the treatment group higher than that of the control, although the difference was not significant (P>0.05). Body weight analysis showed that the initial body weights of the control and treatment groups were 97.3±2.19 g and 96.4±2.55 g, respectively, and the final body weights were 103.3±2.09 g and 99.8±1.97 g, respectively. However, the increases were not significant (P>0.05). Considering that the *P. monodon* broodstock were in a period of growth stagnancy during the breeding season, the body weight increase might be due to the increases in gonad and hepatopancreas weight. The eyestalk ablation further promoted an increase in gonad weight. During the experimental period, the food consumption of the treatment group was significantly higher than that of the control group (P<0.01).

Feeding and energy conversion

Relationship between feeding, growth, and gonad development

The initial E_b value was 20.486±1.628 kJ/kg, which decreased slightly in both the treatment and control groups by the end of the experiment. The decrease in the treatment group was more significant than that of the control (P<0.05). From the perspective of energy, the *P. monodon* broodstock showed negative growth during the breeding season. The initial E_g value was 17.931±0.448 kJ/kg, which increased significantly in both the treatment and control groups by the end of the experiment (P<0.05). The increase in the treatment group was more significant than that of the control group (P<0.01) (Table II). The energy gains of the shrimp body were negative in both the treatment and control groups. Thus, except for food, which plays a key role in gonad development, stored energy in the body contributed to gonad development to a certain degree, resulting in the continuous increase in GSI and Gd/m. In addition, the spawning of individual shrimp led to a transfer of gonad energy to the larvae, which resulted in a decrease in energy and body weight, and thus temporary negative growth. Afterwards, with the considerable food consumption, gonad energy accumulated again. Thus, the body weights of the spawning shrimp showed alternating positive and negative growth.

Table I.- Feeding, growth, and gonad development of *P. monodon* broodstock.

Experimental state	Group	Body weight (g)	GSI	Gd/m	I_f
Start	Control	96.4 ± 2.55	0.014 ± 0.003^a	0.248 ± 0.043^a	
	Treatment	97.3 ± 2.19			
End	Control	99.8 ± 1.97	0.028 ± 0.013^b	0.296 ± 0.023^b	4.41^a
	Treatment	103.3 ± 2.09	0.099 ± 0.026^c	0.314 ± 0.054^b	5.68^b

All values are presented as means±SD. Different superscript letters indicate significant differences among sampling times within each treatment.

Table II.- Feeding and energy conversion of *P. monodon* broodstock.

Experimental state	Group	Body energy value (kJ·kg^{-1})	Gonad energy value (kJ·kg^{-1})	FCE (%)	ECE (%)
Start	Control	20.486 ± 1.628^a	17.931 ± 0.448^a		
	Treatment				
End	Control	19.046 ± 1.742^a	19.254 ± 0.848^b	1.89	2.64
	Treatment	18.841 ± 1.025^b	21.451 ± 2.408^c	11.06	15.45

All values are presented as means±SD. Different superscript letters indicate significant differences among sampling times within each treatment.

Relationship between food consumption and energy conversion

Based on gonad weight increase and gonad energy, the FCE and ECE values of the treatment and control broodstock groups were 1.89% and 11.06%, respectively, and 2.64% and 15.45%, respectively (Table II). The treatment group showed higher food utilization efficiency than that of the control, and transformed food into energy for gonad development more effectively. However, most of the energy was still consumed for the maintenance of body metabolism (respiration and excretion). In addition, the shrimp in the control group were frequently active, whereas those in the treatment group were relatively stationary for much of the time. This behavior difference likely led to the feeding and energy consumption differences observed between the two groups.

DISCUSSION

Effects of eyestalk ablation on feeding

Aquatic animal feeding is not only affected by the external environment, but also by innate day and night feeding rhythms (Zhou *et al*., 2000). In the present study, both the control and treatment groups showed higher night-time food consumption and feeding intensity than during the daytime. The food consumption and feeding intensity of the eyestalk-ablated *P. monodon* broodstock were higher than those without eyestalk ablation. In the whole experimental period, the daily food consumption of the control fluctuated with a general increasing trend, whereas that of the treatment group increased continuously, indicating that eyestalk ablation affected the feeding state of the shrimp, mainly by the increase in average daily food consumption and feeding intensity. The eyestalk-ablated shrimp also increased their nutrition by increasing food consumption and feeding intensity. Several studies have indicated that food consumption and feeding intensity are related to temperature, salinity, light, and food species (Xie, 2002; Chen *et al*., 2001; Li *et al*., 2005a, b; Yu *et al*., 2007; Wang *et al*., 2003). The low temperature during the experiments, as well as the diurnal temperature changes, may have reduced food consumption and feeding intensity of the shrimp to a certain degree. Thus, in practical shrimp production, both innate feeding habits and environmental effects on feeding should be considered, with the local environment appropriately regulated to guarantee rapid body growth and gonad development.

Effects of eyestalk ablation on gonad development

GSI is widely used for the evaluation of ovarian development, and is a rapid, subjective, and measurable method (Huang *et al*., 2006). In the present study, the weight increase of *P. monodon* broodstock was mainly attributable to the increase in gonad and hepatopancreas weight during the experiment. In addition, GSI and G_d/m increased significantly, and the nutrients in the gonad continued to accumulate during the experimental period, with the eyestalk-ablated shrimp showing faster gonad development than that of the control. Following eyestalk-ablation, the X organ-sinus gland complex is destroyed, gonad development inhibition is relieved, gonad nutrient accumulation is promoted, and gonad maturation is accelerated (Wang *et al*., 1995). The water temperature in the present study was 27.0±1.5 °C. This relatively low temperature, as well as the fluctuation in diurnal temperature, might reduce food consumption and feeding intensity, and thus affect gonad development. In addition, dietary nutrient composition plays an important role in gonad development. Highly unsaturated fatty acid (HUFA), especially eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), significantly accelerate gonad maturation (Lin and Zeng, 2006; Naessens *et al*., 1997; Laufer *et al*., 1998). The *O. bartramii* food source used in the present study has much lower HUFA content than clamworms, a common food source used in breeding facilities. Thus, the results may be more significant when applying mixed feed that is more efficient in supporting food conversion efficiency and which contains HUFA or other gonad promoting factors. In addition, during the experiment, the spawning of eight eyestalk-ablated shrimp was recorded, with a body weight decrease of 1.8–4.6 g and egg number of 304,000–509,000. No significant correlation was observed between the body weight decrease and egg number. However, the food consumption of broodstock increased significantly after spawning. Thus, feeding amount should be increased after spawning, which could promote ovarian redevelopment.

Effects of eyestalk ablation on energy conversion

Many hormones synthesized and stored in glands can be secreted to the hemolymph and regulate metabolism (Rosas *et al*., 1995). To date, studies have been conducted on the regulation effects of hormones on food consumption, digestion, nutrient transformation (Rosas *et al*., 1995), lipid metabolism (Teshima *et al*., 1988), and glucose and protein levels in the hemolymph (Santos and Keller, 1993; Chen and Cheng, 1995). In the present study, the treatment group showed significantly higher FCE and ECE values, which were related to the maturation acceleration effects of eyestalk ablation. Following eyestalk ablation, the feeding rate, FCE, and ECE all increased significantly, and thus gonad development was accelerated (Ponnuchamy *et al*., 1981). Body weight also significantly affected the feeding and growth rates of the shrimp. Under the same temperature, feeding, growth, and food conversion rates have been found to decrease with an increase in body weight (Lin *et al*., 2004). The results of the present study also showed that shrimp with higher body weights had lower feeding and food conversion rates, which agrees with the results of the previous study. In addition, the feeding and food conversion rates varied greatly in different growth and development stages. Thus, to maximize breeding efficiency, the environment should be regulated rationally, and feed species and feeding method should be optimized according to the growth and development stage of the shrimp. In addition, the body energy value of the shrimp decreased (negative growth) in both the control and treatment groups. Paradoxically, body weight increased (positive growth) in both the control and treatment groups, and was basically stable during the breeding season. The body weight increase was mainly attributable to the gonad and hepatopancreas weight increases. The decrease in the body energy value might be due to energy conversion from body-stored energy to the gonad. The present study accelerated the sexual maturation of *P. monodon* in the treatment group, combining unilateral eyestalk ablation by burning tweezers and dark room cultivation. The treatment group had higher food consumption, faster gonad development, and higher food conversion efficiency than that of the control, indicating that eyestalk-ablated broodstock had higher feeding intensity and food conversion efficiency and achieved rapid nutrient accumulation and gonad development. In terms of absolute values, however, the food consumption and feeding intensity of the treatment group in the present study were not sufficiently high, and the application of comprehensive measures would be more effective than the application of a single measure in maturation acceleration. Ovarian maturation acceleration requires a combination of endocrine regulation, nutrient supply, and appropriate external environment. Lacking any one of these factors will affect broodstock gonad development to varying degrees (Huang *et al*., 2000).

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Statement of conflict of interest

The authors declare that there is no conflict of interests.

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