Use of enriched live prey in promoting growth and maturation of Tiger Shrimp (*Penaeus monodon*)

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Abstract

This study was undertaken to determine the effect of nutritional management of broodstock of *Penaeus monodon* on growth and maturation. Test specimens were obtained from a grow-out pond before attainment of maturity and were reared in hatchery tanks. Four types of dietary treatments (M1–M4) were given to separate batches that were run in duplicate. Feeding trials continued for five months. A diet with live bloodworm, bioencapsulated to contain tricalcic phosphate as its major component, was found to be the most efficient. Specimens of this particular batch assimilated food more efficiently, grew at a faster rate and attained maturity earlier than other groups. Bloodworm provided the lipid fractions for which there is no *de novo* synthesis in shrimp. The enrichment product acted by promoting somatic growth and increasing transfer of biochemical constituents needed by the ovary for development.

Introduction

Penaeus monodon is the most widely used species of penaeid shrimp in aquaculture (Fig. 1). Supply of high quality broodstock is often a major constraint in the production of good quality seed. Most hatcheries depend on wild stocks, whose supply and quality are variable. Inducing maturation and spawning in captivity provides a more dependable means of seed production. The results are promising if the specimens are nourished properly and provided with enriched live prey. A survey of literature reveals that a sustained rearing of shrimp for production of seed in tropical countries is rare. The easier method of holding wild-caught

Fig. 1. Tiger prawn (*Penaeus monodon*) broodstock.

shrimp in captivity for spawning is widely practiced. An attempt was made in this study to collect pond-reared *P. monodon* and grow them in hatchery tanks to maturity. The results outlined in this paper are based on experiments that involved four feeding regimes, with two consisting of bioencapsulated diets. The performance of all the four batches of feeding treatments in terms of growth and maturity is presented.

Materials and Methods

The *P. monodon* used in this experiment were procured from grow-out ponds and sorted according to size. The average initial weights of the females and males were 34.2±8.6 g and 32.6±5.2 g, respectively. After acclimation to hatchery conditions for a week, the specimens were individually weighed and stocked in black fiberglass tanks of 3.8 t capacity. The specimens were divided into four groups and stocked in eight maturation tanks at a density of six specimens/m^2 with a 1:1 male to female ratio as suggested by Bray and Lawrence (1992). The specimens were exposed to a natural photoperiod which was almost 12 h light and 12 h

dark. Water depth of approximately 1 m was maintained throughout the experiment. Water was renewed daily and aerated. The physico-chemical parameters of water routinely monitored on a daily basis included temperature, salinity, pH and dissolved oxygen. The average values of water temperature, salinity, pH and dissolved oxygen were 28.1±1.5 °C, 29.8±1.7 ppt, 7.33±0.25 and 6.43±0.88 mg/L, respectively. These values are within the ranges suitable for *P. monodon* in a hatchery (Rosly 1990; Sabah Fisheries Department 1991).

Test specimens were offered four different dietary treatments (Table 1) with two replicates each. The experiment was continued for five months. The feeding regime consisted of four feedings/day at the rate of 10 per cent body weight/day (wet weight basis) at fixed intervals (0800 h, 1300 h, 1800 h and 2300 h). At these times, feeding represented 30, 40, 20 and 10 per cent of total food supplied in a 24 h period, in that order. During feeding, the water renewal process was suspended for one hour to avoid loss of feed through the effluent water.
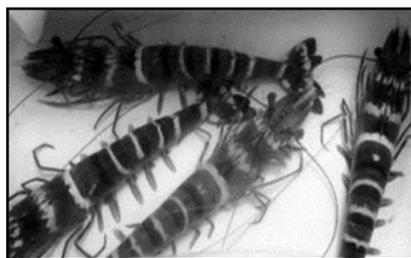
Table 1. Composition of the test diets.

Dietary treatment	Feed component
M1	Squid (40 %)+ mussel (30 %) + trash fish (30 %)
M2	Squid (30 %)+ mussel (25 %) + trash fish (25 %) + enriched bloodworm (20 %)
M3	Squid (30 %)+ mussel (25 %) + trash fish (25 %) + Madmac (20 %)
M4	Squid (30 %)+ mussel (25 %) + trash fish (25 %) + enriched artemia (20 %)

The fresh feeds were rinsed, drained and manually sliced into 1 to 1.5 cm pieces. The appropriate amounts for each tank were weighed and labeled before putting them into the freezer. The feeds were partially thawed and rinsed before they were offered to *P. monodon* (Robertson et al.1993).

Artemia cysts were incubated in an artemia tank for 14 to 16 h. Newly hatched artemia were harvested, rinsed with clean seawater and transferred into a bucket. Live bloodworms and newly hatched artemia were enriched by the bioencapsulation technique. They were exposed to a suitable volume of filtered seawater containing tricalcic phosphate to a concentration of 1 per cent for one hour.

When the stocked *P. monodon* attained a body weight of some 60 g, unilateral eyestalk ablation was performed. One of the eyestalks was ligatured at the base by a surgical thread and just anterior to this portion the stock was cut off with scissors. After eyestalk ablation, the condition of the ovary was monitored once every two days between 1800 h and 1900 h using an underwater dive torch light. Gravid spawners were identified by the dark outline of the abdominal portion (Chwang et al. 1986). Each gravid spawner was transferred to a 250-liter tank for spawning, following the recommendation of Millamena et al. (1986). Gentle aeration was maintained throughout.

The specimens in each treatment group were individually weighed every month to estimate the food conversion ratio (FCR), food conversion efficiency (FCE %), body specific growth (BSG %), specific growth rate (SGR per day) and daily growth rate (mg/day/individual). These parameters were calculated as described by Mustafa and Ridzwan (2000).

Results

Data pertaining to growth and reproductive performance of *P. monodon* given different types of diets are summarized in Table 2. The body weight increased in all the treatment groups, but differences were evident between the groups.

Table 2. The FCR, FCE, BSG, SGR and daily growth of *P. monodon* fed four different diets.

Parameters	Diet			
	M1	M2	M3	M4
Average initial weight (g)	38.88 ± 1.68	37.12 ± 3.09	37.98 ± 3.74	38.03 ± 2.26
Average final weight (g)	60.54 ± 4.72[a]	71.81 ± 10.63[b]	68.39 ± 6.26[b]	58.84 ± 4.54[a]
FCR	5.24[a]	3.55[b]	3.78[b]	5.72[a]
FCE (%)	3.81[a]	5.65[b]	5.45[b]	3.48[a]
BSG (%)	55.70[a]	93.50[b]	80.10[b]	54.50[a]
SGR (%)	0.30[a]	0.44[b]	0.39[b]	0.29[a]
Daily growth rate (mg/day/individual)	144.40[a]	231.30[b]	202.70[b]	133.60[a]
Maturation rate	1/24	6/24	3/24	2/24

Values in the same row that are denoted by different superscripts are significantly different (P<0.05).

Growth and FCR were influenced by dietary composition and ration size, while FCR was affected by the quality of feed. Not all the food is converted to body mass. A certain proportion is eliminated from the body in the form of metabolic waste. Generally, in aquaculture, efforts are made to establish a more efficient feeding system that accelerates growth without increasing the FCR. Any rise in FCR beyond an established 'normal' profile is a matter of concern and calls for in-depth analysis of the whole feeding regime as well as the culture system. Feeding in excess of appetite wastes feed, fouls the culture medium, increases FCR and raises the production cost. Underfeeding reduces growth and also increases the FCR for a different reason. Examination of these relationships is important in developing feeding and growth models and in the interpretation of production data. When growth profiles are established using data from underfed specimens, or feeding models are developed on the basis of the data pertaining to FCR values from either underfed or overfed batches of shrimp, the information derived will be faulty (Talbot 2002).

In this experiment, the highest weight gain (231 mg/day) was recorded in the treatment with the diet that was supplemented with bioencapsulated bloodworm (M2). These specimens also showed a higher value of BSG (93.5 per cent) and SGR (0.44 per cent) and a lower value of FCR (3.55), indicating better assimilation of food into body tissues and faster growth in biomass as compared with other dietary treatments. The performance of dietary treatment M3 in terms of growth was next to M2. Test specimens maintained on M3 diet grew at a rate of 202.7 mg/day. No significant difference in growth was seen between M2 and M3 treatments (P<0.05). *P. monodon* fed the other two diets showed slower growth rate: 144.4 mg/day (M1) and 133.6 mg/day (M4). Treatment M2 also gave better results in reproductive efficiency in addition to supporting the highest growth out

of all the test groups. In this batch the first sign of maturity was observed four weeks after the eyestalk ablation and the number of individuals with mature gonads was also the highest.

For managing growth and optimizing FCR, an analysis of good growth with low FCR or low growth with high FCR is required. The real cause of slow growth and high FCR can be determined by growth-ration analysis. Increase in SGR with ration size, and decline in FCR in a rearing system when specimens are given a particular dietary treatment requires attention for increasing the nutritional status by manipulation of quality or quantity of the diet.

Discussion

Nutrition is a major factor in growth (Fenucci et al. 1980, 1981; Piedad-Pascual 1986; Bautista and Subosa 1997; Deering et al. 1997; Sudaryono et al. 1999; Davis and Arnold 2000) and reproductive performance of penaeid shrimps in captivity (Harisson 1990; Bray and Lawrence 1992; Naessens et al. 1997). However, comprehensive information on nutrient requirements of *P. monodon* is limited and is urgently needed for a successful management of broodstock in hatcheries. Products such as squid, mussel, bloodworm, clam and trash fish in raw form have demonstrated their role in nourishing the captive stock of *P. monodon,* but there is no explicit indication of their direct or specific influence on reproduction. Generally, well-nourished shrimps are expected to be more fertile. It is unknown if any of these or other natural food items selectively act on the reproductive system to stimulate the cycle of maturation and increase the fertility. A diet that contains substances which can target the gonads is, therefore, of great importance in broodstock management. It can reduce the duration of broodstock rearing and cost of hatchery operations. Bioencapsulation offers a means of developing such a diet. Results of this study suggest that this approach can be employed to develop

a diet for manipulating the fertility of shrimp in hatcheries for high quality seed production.

Noticeable differences in the FCR in *P. monodon* fed different diets indicated the essential differences in the efficacy of assimilation of feed components and accomplishing growth of the animal. In fish culture, an FCR value of up to 2.0 is considered suitable for commercial feeding. In the case of *P. monodon*, such a generalized scale has not been established, but FCR values ranging from 1.58 to 1.71 (wet weight) and 5.49 to 6.62 (dry weight) have been reported (Higano and Pichitkul 2000) from commercially viable shrimp farms. Sarac et al. (1993) observed that FCR values varied with the stage of growth: 8.18 to 12.25 in larger *P. monodon* (32 g) and 1.69 to 2.98 in smaller animals (1.5 g) given the same diet. In adult *P. monodon,* intake of feed was consistently high but the FCE was low as compared to their juvenile stage. Evidently, small animals show an accelerating growth pattern with time, while the large-sized ones grow at a slower rate on similar diets. Similar results were obtained for other species of shrimp. Choe (1971) found that growth declined in *P. japonicus* with size and age. Wyban et al. (1995) reported that growth and feeding rate were directly related to temperature but varied inversely with size in *P. vannamei.* Obviously, at the younger stage shrimps assimilate nutrients more efficiently than the older individuals and this accounts for their lower FCR. In the light of these findings, the determination of economic feasibility of feeding requires a consideration of a multitude of factors, including FCR, stage of growth and total cost of compound rations, among others. In addition to the type of food, the physiological efficiency and utilization of diets also influence the FCR and FCE in *P. monodon.* Piedad-Pascual (1986) noticed that the FCR values varied from 7.46 to 13.72 in an experiment that involved supplementing lecithin and lipid in the diet offered to *P. monodon* juveniles. Buchanan et al. (1997) obtained FCR values in the range of 1.98 to 3.06 for juveniles of this

species given canola meal mixed with enzymes. Significantly lower FCR values (2.3 to 2.9) were observed in *P. monodon* when fed with different types of legume meals (Sudaryono et al. 1999).

The growth rate of adult *P. monodon* maintained entirely on fresh natural food (M2) was higher than that reported for juvenile penaeid shrimp fed artificial diet (Surdayono et al. 1999). Previous studies have shown that the growth rate and FCR are greatly influenced by the type of food, life stage of the animal and the rearing environment. Sudaryono et al. (1999) noticed that juvenile *P. monodon* (4.1 g) gained an average of 83.5 mg/day when fed with soybean and lupin based diet. A higher growth rate was noted in juvenile penaeid prawns supplied with artificial diet that contained a higher concentration of protein (Dominy and Ako 1988; Sudaryono et al. 1995). Furthermore, Cruz-Suárez et al. (1992) demonstrated that the addition of 10 per cent squid meal into juvenile *P. monodon* diets consistently improved the growth rate of the shrimps that were reared in ponds and tanks. This study also indicated that FCR was lower (1.7) in specimens that were cultivated in ponds as compared to those reared in tanks (2.8).

The higher growth rate and FCE obtained from the M2 group in this investigation could be attributed to the phosphate enrichment and the bloodworm supplement. Bloodworm is known to be a natural forage organism that supports maturation in penaeid prawns due to its highly unsaturated fatty acids (HUFAs) that help in the process of gonad maturation (Middleditch et al. 1979). The better growth performance exhibited by *P. monodon* fed enriched bloodworm was probably caused by the HUFAs and certain essential nutrients needed to build body tissues. Published data have indicated the role of variable proportions of lipid and protein in growth and food conversion (Ketola 1982; Sarac et al. 1993; Merican and Shim 1996; Glencross et al. 2002), but the quantitative requirements of the fractions of these nutrients (amino

acids in the case of protein and HUFAs in the case of lipid) are not properly understood. Diets poor in essential fatty acids retard growth in tiger prawn (Sarac et al. 1993; Merican and Shim 1996). It has also been shown that not only the total quantity of lipid but also the source from where it is derived influences the growth of shrimps (Peidad-Pascual 1986; Deering et al. 1997). In fish, it is possible to reduce the protein level of the diet without growth suppression if the caloric content is maintained at a high level with lipid (Watanabe 1982), but such studies have not been reported in shrimps. It will be potentially interesting in diet formulation if information on this aspect is available.

Observations on maturation resulted in interesting information. The first spawning occurred four weeks after the eyestalk ablation in the most nourishing dietary treatment (M2). This was faster than the spawning time reported by Bray and Lawrence (1998) where unilaterally eyestalk-ablated female *P. monodon* took five weeks to spawn. Supply of HUFAs and cholesterol from bloodworm to broodstock obviously contributed to the development of gonads. Without having an intrinsic biosynthesis of these substances despite their physiological requirement, captive broodstock would obviously depend on the availability of these lipid fractions in the diet. Presence of both cholesterol and HUFAs is, therefore, a critically important factor in the reproductive processes. Incorporation of the phosphate compounds apparently facilitated transfer of cholesterol and HUFAs to the developing ovary, either by strengthening the capacity of somatic organs (especially the hepato-pancreas) to store them for subsequent transfer to ovaries, or by directly influencing the ovary to increase the uptake. An understanding of the exact mechanism requires more physiological investigations designed to elicit information on these aspects.

Conclusion

Growth and maturation of *P. monodon* are influenced by nutrition. Live prey in the form of bloodworm is quite effective in nourishing *P. monodon* broodstock. Bioencapsulation of bloodworms with tricalcic phosphate strengthens the capacity of somatic tissues to support development of the ovary. The enrichment product may also act on the ovary to facilitate the uptake of required chemical substances such as cholesterol and highly unsaturated fatty acids required for the development of ovaries and formation of high quality eggs. An understanding of the exact mechanism of action of the bioencapsulated phosphorylating compound requires further investigation.

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