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Rematuration of Spent *Macrobrachium rosenbergii* Female Broodstock through Dietary Manipulation and Eyestalk Ablation

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Abstract

The single or combined effects of artificial feed and eyestalk ablation on gonadal maturation of spent *Macrobrachium rosenbergii* female brooders was studied in 48 females. Significant differences in percent weight gain, food conversion ratio, specific growth rate, gonado-somatic index, number of molts, molting interval, and ovarian development stages were observed. The highest numbers of molts and shortest molting intervals were found in eyestalk-ablated groups while higher percent weight gains were obtained in non-ablated groups. Groups fed formulated feed registered lower percent weight gains and higher FCRs than groups fed natural feed (clam and squid meat). Females in immature stages were found only in non-ablated groups while the only ovigerous females were found in the ablated group fed formulated feed. In short, unilateral eyestalk ablation together with properly formulated feed (46.1% crude protein) could induce gonad maturation of spent female *Macrobrachium rosenbergii* brooders during the non-monsoon season.

Introduction

Macrobrachium rosenbergii is one of the main candidate species for freshwater aquaculture. Popularity of this species is growing day-by-day, but inadequate availability of mature brooders has affected year-round availability of seed. The monsoon season is the peak breeding season for *M. rosenbergii* in India; breeding drops during the rest of the year due to a lack of mature females.

Maintenance of spent broodstock in captivity entails considerable expenditure. Spent females are a potential source for breeding during the non-monsoon season, but there is currently no technique for inducing maturation of

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these animals out of season. Hence, research into year-round gonad maturation and spawning of *M. rosenbergii* is of great interest.

The successful maturation and reproduction of crustaceans in captivity generally depend on nutrition, hormonal control, and manipulation of environmental factors such as temperature, photoperiod, and light intensity. Induction of maturation and spawning in prawns and shrimp by eyestalk ablation is well documented (Hillier, 1984; Browdy and Samocha, 1985; Aktas and Kumlu, 1999) but formulation of nutritionally complete diets for maturation is limited. Although extensive work has been undertaken on the use of pelleted feeds to enhance the production of *M. rosenbergii* in culture conditions, the use of cost effective quality formulated feeds to develop broodstock is yet to be accomplished. Fresh squid and clam meats have been used as traditional feed for *M. rosenbergii* brooders but reliance on these materials may not be assured at all times. Further, the availability of nutrients from these natural foods may be insufficient for proper gonad maturation. Hence, formulated feeds based on recent findings in broodstock nutrition may enhance gonad maturation. With this background, the present study examined the single and combined effects of dietary manipulation and eyestalk ablation on the rematuration process of spent *M. rosenbergii* females.

Materials and Methods

Collection of animals. Spent wild *Macrobrachium rosenbergii* female brooders were collected during the non-monsoon season (November-January) from local waters of Mumbai, India, in December 2003. They were acclimatized for 30 days in 5000-l cylindrical-conical FRP tanks provided with continuous aeration and manual water exchange every other day.

Experimental design. A 4 x 2 (feed x eyestalk ablation) factorial experiment was designed to study the effects of different feeds or eyestalk ablation and their interaction on gonad maturation in spent female *M. rosenbergii.* Forty-eight spent females (80±5 g) were distributed into eight treatments, each with three replicates of two females each, stocked in 24 cylindrical-conical FRP tanks (300-l) filled with chlorine-free well water. Each tank contained one mature male to stimulate sexual activity in the females. Water temperature was maintained at 29±2°C by thermostatic heaters. The study was conducted for 60 days.

Feed and feeding. Groups were fed either (a) natural food consisting of fresh clam and squid meat, (b) formulated diet, (c) formulated diet plus fresh clam meat, or (d) formulated diet plus fresh squid meat (Tables 1, 2). Each

Table1. Ingredients of formulated feed for *Macrobrachium rosenbergii* broodstock.

Ingredient	*%*
Fish meal	50.0
Prawn head meal	20.0
Acetes meal	8.0
Wheat flour	10.5
Carboxymethyl cellulose	1.0
Oil	6.0
Vitamin-mineral mix*	2.0
Cholesterol	0.5
Soy lecithin	2.0
Supplements	
Vitamin A	500 mg/kg
Vitamin C	130 mg/kg
Vitamin E	250 mg/kg
β-carotene	40,000 mg/kg

* Emix Plus, 0.5 kg contains: 5.5 million IU vitamin A, 1.1 million IU vitamin D3, 22,000 mg vitamin B, 750 mg vitamin E, 1.0 mg vitamin K, 1000 mg vitamin B6, 6 mcg vitamin B12, 25,000 mg calcium pantothenate, 10 mg nicotinamide, 150 g choline chloride, 27,000 mg Mn, 1000 mg I, 7500 mg Fe, 5000 mg Zn, 2000 mg Cu, 450 mg Co, 500 g Ca, 300 g P, 10 g L-lysine, 10 g DL-methionine, 50 ppm selenium, 250 ppm Satwari, 120 million units lactobacillus, 30,000 million units yeast culture.

Table 2. Proximate composition (%) of four dietary treatments for *Macrobrachium rosenbergii* broodstock.

	Dietary treatment			
	Formulated feed	*Clam*	*Squid*	*Clam and squid*
Crude protein	46.10	67.0	80.3	74.5
Fat	10.70	4.10	8.13	6.36
Crude fiber	4.20	4.00	0.69	2.70
Nitrogen free extract	23.3	13.8	5.03	8.81

type of feed was fed to eyestalk-ablated groups and non-ablated groups. The formulated diet was prepared as follows. Ingredients were weighed and manually mixed with water to prepare the dough. The dough was cooked in a pressure cooker for 1 h. Vitamin and mineral mixtures were mixed after cooling. The dough was pressed through a hand pelletizer with a diameter of 2 mm. The pellets were air dried and kept in an oven at 60°C until completely dried. Feed was given twice daily at 09:00 and 16:00 at 3% of the body weight of the *M. rosenbergii*.

Maturation study. Female maturation stages were identified according to Chang and Shih (1995). Ovarian development was classified into five stages based on the size and color of the ovary. White ovaries indicated previtellogenesis (stage 1), small yellow masses of ovarian tissue near the epigastric tooth indicated previtellogenic and vitellogenic oocytes (stage 2), orange ovaries indicated vitellogenesis (stage 3), dark orange ovaries indicated advanced vitellogenesis (stage 4), and reddish ovaries extending from behind the eyes to the first abdominal segment indicated the ovigerous stage (stage 5). Molts and molting intervals of all animals in all tanks were followed daily.

Growth parameters. Food conversion ration (FCR), specific growth rate (SGR), weight gain (%), and gonado-somatic index (GSI) were calculated as follows: FCR = dry wt of feed given/wet wt of body gain, SGR = 100(Log_e final wt - Log_e initial wt)/no. days, percent wt gain = 100(final wt - initial wt)/initial wt, and GSI = 100(wt of gonad/wt of prawn).

Water quality. Water samples were collected from each tank every week. Temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, ammonia, nitrite, and phosphate were analyzed according to standard methods (APHA, 1998).

Statistical analysis. Mean values of treatment groups were analyzed by two-way ANOVA. Duncan multiple range tests were used to compare means. Differences among treatments were tested for significance at a 5% level. SPSS (version 14) was used to analyze data.

Results

Gonad maturation. The GSI of females was significantly affected by eyestalk ablation, feed, and the interaction of the two (Table 3). Whether ablated or not, groups fed formulated feed had higher GSI values than groups fed natural feed (squid and clam meat). Supplementation of formulated feed with clam meat produced a higher GSI than supplementation with squid meat.

The number of molts was significantly affected by eyestalk ablation but not by feed or the interaction of the two. Ablated groups had a greater number of molts than non-ablated groups. In contrast, the molting interval was affected by ablation, feed, and the interaction of the two with the lowest molting intervals in ablated groups, highest in groups fed natural feed (clam and squid), and no significant difference among groups fed artificial feed, whether supplemented or not.

Table 3. Gonad maturity parameters of eyestalk ablated and non-ablated female *Macrobrachium rosenbergii* broodstock fed different diets.

Treatment	*GSI*	*No. of molts*	*Molt interval (days)*
Non-ablated	5.03±0.10^{b}	2.00±0.12^{b}	26.08±0.47^{a}
Ablated	7.90±0.10^{a}	3.42±0.12^{a}	17.75±0.47^{b}
Clam + squid	5.40±0.14^{d}	2.68±0.17	26.17±0.67^{a}
Formulated	7.34±0.14^{a}	3.00±0.17	19.50±0.67^{b}
Formulated + clam	6.80±0.14^{b}	2.50±0.17	21.50±0.67^{b}
Formulated + squid	6.30±0.14^{c}	2.68±0.17	20.50±0.67^{b}
Non-ablated/clam + squid	3.39±0.19^{g}	2.33±0.24	33.33±0.94^{a}
Non-ablated/formulated	6.30±0.19^{d}	2.00±0.24	23.00±0.94^{b}
Non-ablated/formulated + clam	5.54±0.19^{e}	1.68±0.24	24.00±0.94^{b}
Non-ablated/formulated + squid	4.88±0.19^{f}	2.00±0.24	24.00±0.94^{b}
Ablated/clam + squid	7.42±0.19^{c}	3.00±0.24	19.00±0.94^{c}
Ablated/formulated	8.38±0.19^{a}	4.00±0.24	16.00±0.94^{d}
Ablated/formulated + clam	8.05±0.19^{ab}	3.33±0.24	19.00±0.94^{c}
Ablated/formulated + squid	7.73±0.19^{bc}	3.33±0.24	17.00±0.94^{cd}

Means in a column bearing different superscripts vary significantly (p<0.05).

Immature stages were found only in non-ablated groups while ovigerous females were found only in the ablated group fed formulated feed (Table 4).

Growth parameters. The percent weight gain and SGR were significantly affected by eyestalk ablation and feed with higher values in non-ablated groups than ablated groups and lower values in groups fed formulated feed (Table 5). FCR was lower in non-ablated groups and higher in groups fed only formulated feed.

Water quality parameters. Mean water temperature ranged 27.7-31.7°C, mean dissolved oxygen 7.39-7.66 mg/l, and average total alkalinity 67.6-71.6 mg/l. Mean ammonia (NH_3) ranged 0.08-0.13 mg/l while nitrate-N ranged 0.01-0.09 mg/l. Nitrite-N and PO_4 were within the optimum ranges of 0.001-0.005 mg/l and 0.08-0.1 mg/l, respectively.

Discussion

GSI was highest in the ablated groups fed formulated feed and lowest in the control groups (non-ablated fed clam and squid), suggesting that maturation of *M. rosenbergii* can be manipulated through diet and eyestalk ablation. The GSI of eyestalk-ablated groups was consistently higher than un-ablated groups in all feed treatments, indicating the significant role of eyestalk ablation on gonad maturation, as reported by Wilder et al. (1991). The GSI of groups fed formulated fed was consistently higher than groups fed clam and squid, whether ablated or not, indicating the positive role of formulated feed alone or in combination with natural feed, on gonad maturation. While both eyestalk ablation and formulated feed significantly affected the GSI, the synergistic interaction of both induced even better maturation. A negative correlation was found

Table 4. Number of females in each maturation stage* in each treatment.

Treatment	*Immature*	*Stage 1*	*Stage 2*	*Stage 3*	*Stage 4*	*Stage 5*
Non-ablated/clam + squid	1	5	0	0	0	0
Non-ablated/formulated	0	0	1	0	5	0
Non-ablated/formulated + clam	1	4	1	0	0	0
Non-ablated/formulated + squid	2	4	0	0	0	0
Ablated/clam + squid	0	0	6	0	0	0
Ablated/formulated	0	0	0	0	3	3
Ablated/formulated + clam	0	0	1	3	2	0
Ablated/formulated + squid	0	0	2	4	0	0

* Stage 1 = previtellogenesis; stage 2 = previtellogenic and vitellogenic oocytes; stage 3 = vitellogenesis; stage 4 = advanced vitellogenesis; stage 5 = ovigerous (Chang and Shih, 1995).

Table 5. Growth parameters of eyestalk ablated and non-ablated female *Macrobrachium rosenbergii* broodstock fed different diets.

Treatment	*Wt gain (%)*	*SGR (%/day)*	*FCR*
Non-ablated	17.85±0.74^{a}	1.78±0.02^{a}	3.40±0.21^{b}
Ablated	14.04±0.74^{b}	1.67±0.02^{b}	4.60±0.21^{a}
Clam + squid	17.73±1.05^{a}	1.79±0.03^{a}	3.39±0.30
Formulated	12.34±1.05^{b}	1.59±0.03^{b}	5.33±0.30
Formulated + clam	16.79±1.05^{a}	1.80±0.03^{a}	3.65±0.30
Formulated + squid	16.92±1.05^{a}	1.73±0.03^{a}	3.61±0.30
Non-ablated/clam + squid	19.41±1.48	1.84±0.05	2.97±0.42^{b}
Non-ablated/formulated	15.61±1.48	1.70±0.05	3.87±0.42^{b}
Non-ablated/formulated + clam	18.74±1.48	1.83±0.05	3.25±0.42^{b}
Non-ablated/formulated + squid	17.63±1.48	1.74±0.05	3.50±0.42^{b}
Ablated/clam + squid	16.05±1.48	1.74±0.05	3.81±0.42^{b}
Ablated/formulated	9.08±1.48	1.48±0.05	6.79±0.42^{a}
Ablated/formulated + clam	14.84±1.48	1.76±0.05	4.05±0.42^{b}
Ablated/formulated + squid	16.20±1.48	1.72±0.05	3.71±0.42^{b}

Means in a column with different superscripts vary significantly (p<0.05).

between molting interval and GSI, y = -3.0388x + 41.551, r^2 = 0.9196 (Fig. 1).

The lowest percent weight gain was obtained in the eyestalk ablated group fed only formulated feed, suggesting that the interaction of the two induced maturation at the expense of growth. This concurs with findings of Browdy and Samocha (1985) who reported that the increased energy demand for gonad development after eyestalk ablation

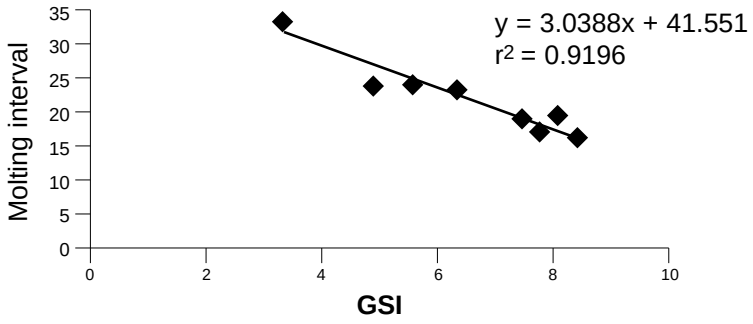

Fig. 1. Relationship between gonado-somatic index (GSI) and molting interval.

is reflected by a lower growth rate. Higher growth rates were observed in the non-ablated groups fed clam and squid, perhaps due to the combined effect of amino acids in fresh clam and squid that is similar to that of prawn meat (Deshimaru and Shigeno, 1972).

Venkataramani et al. (2002) observed that feed incorporated with animal protein had a positive effect on broodstock development of *M. rosenbergii*. Millikin et al. (1980) reported that a dietary protein level of 47-54% is best for broodstock development and that 40% is best for growth. In addition to animal protein, our formulated feed contained cholesterol, lecithin, β-carotene, minerals, and vitamins that are associated with reproduction. Cholesterol, for example, plays an important role in maturation and reproduction in prawns (Cahu et al., 1994). In accordance, groups fed formulated feed had a higher FCR and greater fecundity than somatic growth. Only the ablated groups fed formulated feed reached the most advanced stage of development, the ovigerous stage, suggesting that maturation can be induced by eyestalk ablation together with an adequate formulated feed.

In conclusion, a properly formulated feed in combination with unilateral eyestalk ablation could induce rematuration of spent *M. rosenbergii* females during the non-monsoon season, leading to more efficient use of broodstock.

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