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Reproductive Performance of *Macrobrachium rosenbergii* Females in Captivity

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Abstract.—The breeding frequency, fecundity, egg hatchability, larval output and viability of pond-reared, mature *Macrobrachium rosenbergii* females were individually followed up during 180 d. Sexually mature prawns were maintained under controlled laboratory conditions (28.7 C and 12-h light/d) in a 190-L freshwater recirculation system and fed a formulated diet. Ovarian development, moulting and spawning events were checked daily. At least six consecutive moults were recorded for each of the 18 females. The duration of the intermoult period averaged 27.5 d and was not affected by ovarian development or spawning, but intermoult periods followed by spawning had significantly lower growth rates. From a total of 126 moulting events recorded, egg laying successfully followed 76 (60.3%) of them. The number of eggs per spawn (NES) varied from 26,587 to 74,775 for females weighing 20.0 to 55.8 g. The relationship between NES and female size (W; in g) was found to be NES = 484 + 1454W (r^2 = 0.74). Results suggest that the number of viable larvae produced per egg clutch may be increased by *in vitro* incubation, as the losses of eggs, which usually occur under *in vivo* incubation, are prevented. The present study illustrates that under adequate and stable rearing conditions, *M. rosenbergii* females are able to spawn up to five times during 180 d, in comparison to three to five times per year as reported for wild prawn populations.

The freshwater prawn *Macrobrachium rosenbergii* is native to the Indo-West Pacific region (Holthuis 1980), but because it has several characteristics considered to be appropriate for culture, it has been transferred to almost every continent. To date, the culture of *M. rosenbergii* either for commercial purposes or research activities has been reported in a total of 77 countries (New 1995). Prawn farming has considerable economic importance, particularly in Asia (China, Thailand, Vietnam, Taiwan, Bangladesh, and India) and in Latin America (Ecuador and Brazil). World production in 1997 was estimated at 60,995 metric tons (FAO 1999).

Hatchery production of *M. rosenbergii* postlarvae requires reliable sources of egg-bearing females. For many hatcheries located in tropical regions, year-round availability of larvae is guaranteed by the use of egg-bearing females obtained from culture ponds or, within the natural distribution range of this species, from rivers, lakes and estuaries. Under temperate and sub-tropical conditions, however, the usual practice is to select adult males and females during fall harvests and to maintain them indoors in temperature-controlled environments during winter. Unfortunately, although several studies provided valuable information regarding broodstock management (Ling 1969; Wickins and Beard 1974; Chow 1982; Malecha 1983; O'Donovan et al. 1984; Chavez Justo et al. 1991; Damrongphol et al. 1991; Daniels et al. 1992), a more fine-focused approach dealing specifically with the performance of captive *M. rosenbergii* broodstock has not been considered. In addition, to our knowledge no data on the frequency of spawning and rematuration of individual female prawns is available. Furthermore, with the exception of Wickins and Beard (1974), most studies failed to provide information on the reproductive performance of captive stocks.

The present paper reports on the breeding frequency, fecundity, hatching rate, larval output and viability of a pond-reared stock maintained under strictly controlled laboratory conditions for 6 months. The results are discussed in relation to those available from field and laboratory studies.

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TABLE 1. *Composition of the experimental diet (% dry weight).*

Ingredients	%	Ingredients	%
Casein[a]	15	Attractant (protein extract)[f]	3.6
Lobster meal[b]	14	Starch[a]	22.82
Soy protein isolate[c]	8.75	Cellulose[a]	1.82
Squid meal[b]	5	Kappa-carrageenan[a]	5
Shrimp meal[b]	2.5	Astaxanthin[g]	1.25
Crab meal[b]	2.5	Cholesterol[a]	0.6
Threonine[a]	0.8	De-oiled soy lecithin[h]	1
Valine[a]	0.65	Choline chloride[a]	1
Lysine[a]	0.9	Mineral mix[e]	4
Histidine[a]	0.45	Vitamin mix[e]	2
Arginine[a]	1	Ascorbyl polyphosphate[g]	0.27
Leucine[a]	0.4	dl-α-tocopherol acetate[i]	0.04
Iso-leucine[a]	0.16	Butylated hydroxytoluene[i]	0.005
Soybean oil[d]	2.15	Butylated hydroxyanisole[i]	0.005
Fish oil[e]	2.33		

[a] Sigma, USA.
[b] Rieber & Son, Norway.
[c] Protein Technologies International, Belgium.
[d] Vandermoortele, Belgium.
[e] E50, Inve Aquaculture, Belgium.
[f] Primex, Norway.
[g] Roche, Belgium.
[h] Emulpur N, Lucas Meyer, Germany.
[i] Federa, Belgium.

Materials and Methods

Origin of Animals

Adult *M. rosenbergii* obtained from pond-reared sources in Thailand were transported to the Laboratory of Aquaculture, Ghent University, Belgium. After arrival, prawns were gradually acclimated to 28 C. They were maintained in a freshwater recirculation system at 28 C and a 12-h light/12-h dark photoperiod for 2 mo before the beginning of the experiment. During this period they were fed a shrimp broodstock diet (Inve Technologies N.V., Belgium). At the beginning of the experiment, all females were sexually mature and males were of the blue-claw (BC) morphotype (Kuris et al. 1987). Wet weight and total length of the females were 26.2 ± 5.1 g and 14.5 ± 5.7 cm, males were 40.2 ± 5.2 g and 15.5 ± 1.1 cm, respectively.

Experimental Conditions

Prawns were maintained in three separate recirculation units, each containing one 150-L holding tank and one 40-L biological filter. To allow the individual follow up of females, the holding tank was divided into nine compartments, each housing one *Macrobrachium* adult (total of six females and two males per tank). The ninth compartment contained the water evacuation pump. Approximately 20% of the water was exchanged daily after the removal of wastes and uneaten feed. During the experiment, the levels of NH_4^+-N, NO_2-N and NO_3^--N were kept under 0.2, 0.06 and 100 mg/L, respectively. Freshwater was maintained at 28.7 ± 0.8 C and the photoperiod was set at 12-h light/12-h dark (Chavez Justo et al. 1991). Three 18-W fluorescent lamps placed 0.9 m above the water level of each holding tank provided a light intensity of 600 lx. An additional group of males was kept under similar conditions. These prawns replaced any male that died during the course of the experiment.

A formulated diet (Table 1) containing 44% crude protein and 9% total lipids was

offered in excess twice a day (0930 and 1800). Every morning excess feed and wastes were siphoned out, and aerated and pre-heated freshwater was added to refill the experimental units. At the same time, the occurrences of moults and mortality were recorded.

Evaluation Parameters

Ovarian development, moulting and spawning events were checked daily over 180 d. Ovarian development was classified according to colour, size, and outline of the ovaries as described by Chang and Shih (1995). Provided that the newly moulted female had developed ovaries, it was isolated with a hard-shelled male for mating during 3 h. Seven days later, eggs were manually removed from the abdomen of the female. The total (somatic) wet weight of the females was then assessed after blotting. Similarly, prior to weighing, the excess surface water from the egg clutch was removed by blotting. Three egg sub-samples were weighed and counted to determine the total number of eggs per clutch.

Moults were classified as non-reproductive moults, if during the intermoult period no ovarian development occurred, or pre-mating moults, if mating and egg laying followed. The duration (D; in d) and the instantaneous growth rate ($G = (\ln W_2 - \ln W_1)/(T_2 - T_1)$; where W_1 and W_2 = weight at time T_1 and T_2, respectively) of the intermoult periods preceding non-reproductive and pre-mating moults were evaluated.

Egg Hatchability

Hatching rates were estimated *in vitro* in 200-mL glass cones. A single egg clutch, weighed to contain 100–200 eggs, was added to the hatching cones. Natural (33 g/L) seawater diluted to 6 g/L with deionised water was exchanged daily with pre-heated water. Water temperature was 28.2 ± 1.1 C and gentle aeration was provided. The eggs were not disinfected throughout the incubation period. The hatching rate was determined by counting the number of larvae and dead eggs approximately 24 h after hatching.

Larval Viability

Larval viability was estimated by culturing newly hatched larvae for 8 d. The larviculture set-up consisted of six 20-L cylinder-conical tanks connected to a single recirculation system containing a settlement sump and a submerged biological filter with a volume of 125 L. An upwelling system with a water flow rate of 0.33 L/min was used and gentle aeration was applied to all rearing tanks. Fluorescent lamps provided 750 lx at the water surface for 12 h/d. One thousand newly hatched larvae from each spawn were transferred into one tank (density of 50 per L). Sea water was diluted to 12 g/L with deionised water and maintained at 28.0 ± 1.0 C. *Artemia franciscana* nauplii (Great Salt Lake strain) were offered at a density of 10–15/mL from day 2 to day 7. On day 8, the culture was terminated and the surviving larvae were counted.

Statistical Analyses

Duration of the intermoult periods, the respective growth rates, and reproductive performance parameters were analysed by analysis of variance (one-way ANOVA) and, where appropriate, Tukey's honest significant difference (HSD) test was applied. Differences were considered to be significant at 5%. In an attempt to identify the factors responsible for differences in reproductive performance, regression analysis of female size against the number of eggs produced per spawning event and per female body weight was also performed. All data are presented as means ± SD.

Results

From the 18 females stocked, three deaths were recorded, which represented a survival rate of 83.3%. Males suffered a higher mortality rate. From the six males initially stocked, five died during the course of the experiment, resulting in a survival

TABLE 2. *Reproductive performance of* M. rosenbergii *females maintained in captivity for 180 d according to their spawning order. Within rows, superscript letters indicate significant differences* ($P < 0.05$).

Spawning order	I	II	III	IV	V
Number of spawns retained	18	17	12	11	5
Eggs per spawning event	40,466 b ± 7,963	46,428 ab ± 11,724	50,229 ab ± 10,435	47,604 ab ± 9,588	57,238 a ± 15,803
Eggs per female weight (eggs/g)	1,466 ± 160	1,424 ± 246	1,528 ± 163	1,338 ± 239	1,519 ± 193
Hatching rate (%)	83.2 ± 14.4	82.4 ± 18.9	86.3 ± 8.6	87.3 ± 8.1	97.7 ± 2.2
Larval survival to day 8 (%)	86.1 ± 9.3	89.0 ± 6.8	89.1 ± 9.6	92.3 ± 6.1	89.8 ± 5.4

rate of only 16.7%. A high mortality of males was also observed during the acclimation period. A few days preceding death, males appeared weak, lethargic, stopped feeding, and were unable to maintain body posture. Epibiont fouling of the carapace was also noticed.

The experimental design enabled us to individually follow the reproductive cycle of *M. rosenbergii* females during at least six consecutive intermoult periods. Females took an average of 27.5 ± 4.7 d to moult.

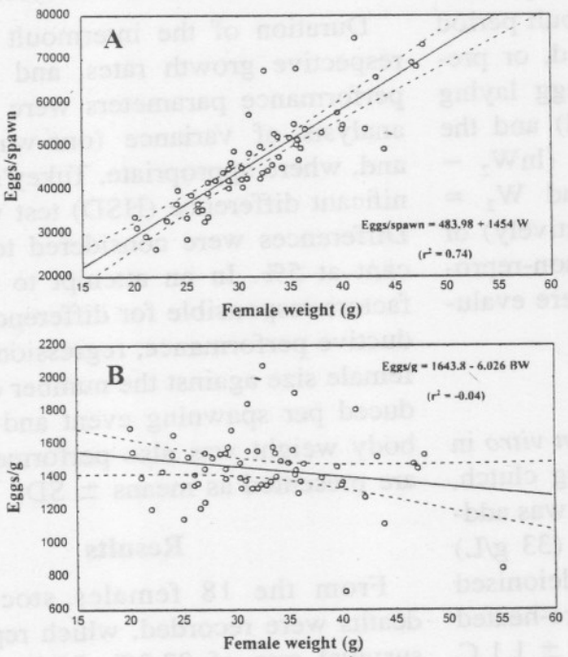

FIGURE 1. *Regression lines and respective 95% confidence limits between female body weight (W) and number of eggs per spawning event (A), and number of eggs per female body weight (B).*

Although no significant difference in the duration of the intermoult periods in relation to the preceding moult was detected (26.8 ± 4.8 and 28.0 ± 4.3 d for the non-reproductive and pre-mating moults, respectively), the instantaneous growth rate was significantly higher in non-reproductive moults (4.43 ± 1.97 mg/d) than in pre-mating moults (2.61 ± 1.62 mg/d).

From a total of 126 moulting events recorded, 76 of them (60.3%) were associated with reproduction, i.e., an egg clutch was observed on the abdomen of the females 1 or 2 d after moulting. The average number of spawns per female during the experimental period was 4.2 (± 1.2), whereas five females were able to spawn five times during the course of the experiment (Table 2). However, the females did not retain all clutches. From the 76 clutches observed, 13 (17.1%) were lost 1 to 3 d after spawning.

The number of eggs per spawning event varied considerably between individuals, ranging from 26,587 to 74,775 eggs (mean of 46,512 ± 11,220 eggs). The number of eggs per spawn increased significantly with female size (Fig. 1A) and spawning order (Table 2). The relationship between the number of eggs per spawning event (NES) and female size (W; in g) was found to be NES = 484 + 1,454 W (r^2 = 0.74). The regression between female size and egg production efficiency (number of eggs per female weight; eggs/g) resulted in a low relationship coefficient (r^2 = −0.04; Fig. 1B).

The number of eggs per female weight was not significantly affected by spawning order (Table 2).

From the 63 spawns incubated *in vitro,* four groups of fertilised eggs were discarded because of high accumulation of debris on the surface of the eggs probably due to bacterial and/or fungal infections, and five did not hatch due to the lack of aeration overnight. The remaining 54 groups of eggs resulted in viable larvae. Mean hatching rates were consistent and always above 82%. Egg hatchability did not vary significantly with spawning order (Table 2). Survival of larvae after 8 d of culture was also relatively high (Table 2), ranging from 70.0% to 100% (overall mean of 89.1% ± 8.0%).

Discussion

Development in sexually mature *M. rosenbergii* females involves the physiological processes of moulting, somatic growth, and ovarian development. If somatic growth dominates development, intermittent, non-reproductive moults take place. When ovarian development is emphasised, a pre-mating moult is followed by mating and egg laying (Ling 1969) and, as a consequence, somatic growth is sacrificed at the expense of ovarian development. Accordingly, a decline in the growth rate of females entering sexual maturity is often reported (Ra'anan et al. 1990; Chavez Justo et al. 1991) and agrees with our observation that intermoult periods followed by egg laying had decreased growth rates.

In the natural environment, *M. rosenbergii* may spawn 3 to 4 times per year (Ling 1969) or more than 4 times (Rao 1991). Wickins and Beard (1974) reported that one female maintained in captivity spawned 4 times in 170 d, indicating the reproductive potential of this species when maintained under appropriate rearing conditions. This is in line with our results, where females demonstrated a capacity to breed up to 5 times over 180 d. Differences in female size between these studies probably partially explain the differences in breeding frequency, but one can assume that stable, optimal environmental conditions and a more balanced nutrition may also play a significant role. Furthermore, the fact that the eggs were removed may also have a positive impact on the performance of the females, possibly through an increase in breeding frequency (Pandian and Balasundaram 1982; Damrongphol et al. 1991).

Within a group of sexually mature *M. rosenbergii* females, not all moulting events are associated with reproduction. Damrongphol et al. (1991) stated that most mature females emphasise ovarian development, but not all moults are followed by a receptive period. Similarly, in our study females set priority for reproduction, and 60.3% of the moults were followed by mating and egg laying. This is in accordance with Graziani et al. (1993), who observed that 60.6% of the moults of captive *Macrobrachium carcinus* females were followed by egg laying, and Pandian and Balasundaram (1982), who reported that 61% of *M. nobilii* females spawned following a moult. However, the occurrence of consecutive breeding is known to vary seasonally. Wild *M. rosenbergii* females from India showed peak reproductive activity during the summer months of August to October (Rao 1991). Similarly, 90% of *M. rosenbergii* females collected in Israeli ponds during the warmer breeding season were observed to carry eggs (O'Donovan et al. 1984).

Some females dropped the eggs 1–3 d after spawning, indicating the lack of fertilisation (Ling 1969; Wickins and Beard 1974). The reasons for this are not clearly understood, but it might be that the time span between the pre-mating moult and mating was not adequate. Ling (1969) reported that newly moulted females are receptive to males from 3 to 6 h after the pre-mating moult, while Chow (1982) found that receptivity for artificial insemination lasts for 10 to 15 h following a pre-mating moult. It is possible that when females and males were placed together the receptive

period had already occurred. Other possible explanations include the disturbance caused by human presence or the incapacity of some males to successfully mate or to produce quality sperm.

A high mortality of BC males was observed in this study. Prior to death, males appeared weak, displayed a lethargic behaviour, their carapaces were covered with epibionts, and they were unable to maintain body posture. Similar symptoms have also been reported by Daniels (1993). Brock (1993) suggested that the mortality of pond-reared BC males might be due to a terminal anecdysis termed "terminal growth." In its advanced stage of development, males with "terminal growth" would present symptoms similar to those presented by our BC males. Daniels et al. (1992) recommended that both sexually active BC males and orange-claw (OC) males should be stocked to offset the mortality rate of males that occur over a long holding period in captivity. Though OC males have no immediate reproductive role, they will eventually turn into a BC male, which represents the final stage of male development.

The efficiency of egg production, i.e., the number of eggs produced per female unit body weight, may be age and/or size dependent and is generally assumed to increase with female size (Malecha 1983). In our study, the efficiency of egg production was found not to be significantly affected by female size. Costa and Wanninayake (1986) and Rao (1991) reported that in wild populations of *M. rosenbergii* from Sri Lanka and India, respectively, smaller females produced a higher number of eggs per unit body weight. A similar trend has also been demonstrated in the shrimp *Penaeus monodon* (Villegas et al. 1986). In contrast, Patra (1976), Ang and Law (1991) and Cavalli et al. (2000) found that the number of eggs per body weight increased with *M. rosenbergii* female size. Therefore, as several studies present contrasting results, the efficiency of egg production clearly remains a subject of investigation. Essential subjects on this line of studies are, for instance, to investigate environmental, genetic, and nutritional factors that might cause different patterns of body size-fecundity relationships. The efficiency of egg production in females of different size classes will surely depend on several of those factors.

From a reproductive perspective, the selection of smaller females for hatchery use might be considered an advantage, as they have a higher moulting frequency, hence a higher breeding frequency, and perhaps a more efficient egg production. However, even if smaller females were more efficient in terms of egg production per body weight, in a hatchery situation the production of eggs per unit time is crucial. Consequently, hatchery operators usually select the larger females for reproduction because fecundity is linearly related with female weight, as clearly demonstrated in several studies (Patra 1976; Malecha 1983; Costa and Wanninayake 1986; Ang and Law 1991; Rao 1991).

While the number of freshly laid eggs per female represents its "realised reproduction," the number of eggs in late stages of development is closer to the "actual reproduction," i.e., the number of larvae produced. The latter is usually lower among carideans (Corey and Reid 1991). For captive *M. rosenbergii,* Wickins and Beard (1974) suggested that egg losses during *in vivo* incubation could amount to 31% of the eggs initially deposited in the brood chamber. Malecha (1983) observed that egg-carrying females maintained in ponds had a lower fecundity than those maintained in the laboratory. Ang and Law (1991) also demonstrated that under pond conditions females present a decrease in the number of eggs with incubation time. Egg losses were considered to be partially due to their consumption by females, to the continual sloughing off of dying eggs due to epizootic infestations and to the loose nature of the larger grey eggs, which would render them more prone to physical losses. Preventing the decrease in egg numbers by

manually collecting them after spawning followed by *in vitro* incubation might increase the number of larvae released from an egg clutch. For instance, our results show that around 1,450 eggs can be produced for each gram of female weight. Considering a hatching rate of 86%, it would be possible to obtain around 1,250 larvae per gram of female body weight. This procedure would mean a substantial increase over the 1,000 or 450 larvae per gram of female weight proposed by New and Singholka (1982) and Malecha (1983), respectively. The advantages of applying this methodology consist not only in the potential increase in the number of larvae produced, but also in relieving females from the task of incubation, which increases their reproductive output by an increased breeding frequency, as demonstrated for *M. nobilii* (Pandian and Balasundaram 1982) and *M. rosenbergii* (Damrongphol et al. 1991).

However, the use of *in vitro* incubation might also present some constraints. Although eggs of *Macrobrachium* have already been successfully incubated under various systems and conditions (Balasundaram and Pandian 1981; Mathavan and Murugadass 1988; De Caluwé et al. 1995; Das et al. 1996; Cavalli et al. 1999), the risk of microbial infections increases because non-viable eggs, which are usually lost during *in vivo* incubation (Malecha 1983; Ang and Law 1991), are retained in *in vitro* incubation. Furthermore, anti-microbial secretions, thought to be produced by the incubating females (Fisher 1983), are not present. Recently, however, a protocol for the disinfection of *M. rosenbergii* eggs using formaldehyde, hydrogen peroxide and a mixture of antifungal/antibiotic products has been made available (Caceci et al. 1997). It still remains to be experimentally demonstrated whether these larvae are as viable as those from *in vivo* incubation. Caceci et al. (1997) found that larvae from *in vitro* hatching were morphologically indistinguishable from those hatched out naturally. The high larval survival rates observed in this study are also an indication of the viability of the larvae obtained through *in vitro* incubation.

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