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# Dynamics of reproduction in a captive shrimp broodstock: unequal contribution of the female shrimp and a hidden shortage in competent males

S. PARNES, S. RAVIV, D. AZULAY and A. SAGI*
Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel
Tel. +972 (8) 646-1364; Fax: +972 (8) 647-2890; email: sagia@bgu.ac.il

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Summary

The major bottleneck in the breeding in captivity of penaeid shrimp is the fact that females do not spontaneously undergo vitellogenesis, which thus has to be induced endocrinologically by eye-stalk ablation. Partial compensation for the low responsiveness of the females to this treatment is obtained by the use of very large broodstock populations. This old problem was newly approached in the current study by monitoring individual female life-spans and vitellogenic cycles in a small population of the Pacific white shrimp, *Litopenaeus vannamei*. Vitellogenic activity and spawning peaked during the second month of the four-month trial and decreased thereafter due to the sharp fall in the number of reproductively active females. Only about 75% of all the females in the broodstock were reproductively active, and most of them spawned for the first time, or exhibited fully vitellogenic ovaries, during the first two weeks post-ablation. Nevertheless, the best performing females, which comprised less than 20% of the female broodstock, matured more rapidly, exhibiting fully vitellogenic ovaries 4.1±3.4 days after eye-stalk ablation. These animals contributed more than 50% of the total eggs and nauplii produced. The total number of vitellogenic cycles recorded from all reproductively active females was 305, but in less than a third of these cycles were the females observed to be carrying spermatophores or sperm masses. Since females—but not males—in captivity undergo accelerated reproductive cycles due to the endocrine induction, it is suggested that the lack of mating consistently reported from maturation systems may be due to the lack of sufficient ready-to-mate males.

Key words: Pacific white shrimp, *Litopenaeus vannamei*, Crustacea, Decapoda, vitellogenesis, reproduction; maturation

Introduction

Although species of *Penaeus* shrimp are among the most prolific organisms on Earth, very little is known about their reproductive biology in the wild, since there are no direct observations on the behavior and reproduction of these species in their natural habitats: most of our knowledge on reproduction in penaeid shrimp pertains to populations bred in captivity. The following four major bottlenecks in the breeding of shrimp in captivity are among the most stubborn, long-standing

*Corresponding author.

puzzles in the field of penaeid reproduction (Browdy, 1992; Browdy, 1998).

(1) Vitellogenesis does not take place spontaneously — maturation of the ovaries and spawning has to be induced endocrinologically by (unilateral) eye-stalk ablation together with the provision of a rich maturation diet (Bray and Lawrence, 1992; Ogle, 1992; Robertson et al., 1993; Treece and Fox, 1993; Wickins and Lee, 2002; Wyban and Sweeney, 1991).

(2) Only a small proportion of the induced females contribute the larger part of total post-larvae production, while the ovaries of many induced females never mature or mature long after ablation (Bray et al., 1990; Wyban and Sweeney, 1991; Bray and Lawrence, 1992; Ogle, 1992; Robertson et al., 1993; Treece and Fox, 1993; Palacios et al., 1999b; Wickins and Lee, 2002; Arcos et al., 2003b).

(3) Although sub-adult males mature spontaneously, and spermatophores containing mature spermatozoa develop and regenerate after mating, without the need for any special treatment, dietary or otherwise (Ceballos-Vazquez et al., 2003; Ogle, 1992; Parnes et al., 2004), male reproductive capacity is highly variable, and there is no known objective measure that can be used to evaluate it in any particular population (Ceballos-Vazquez et al., 2004; Rosas et al., 1993).

(4) Mating success is usually poor, as shown by the fact that seemingly fully vitellogenic females can be scooped out of the mating tank night after night, and even several times on the same night, and found not to have mated (Alfaro and Lozano, 1993; Chamberlain et al., 1983; Leung-Trujillo and Lawrence, 1987; Pascual et al., 1998).

These bottlenecks necessitate the use of very large broodstocks and prevent efficient selection for desirable traits because the females can spawn only in a single season.

In the current study, we investigated the reproductive efficiency of a small breeding population of the Pacific white shrimp, *L. vannamei*, through individual monitoring of males and females alike. A particular effort was made to record all the vitellogenic cycles. The results of this study were compared with other studies on captive broodstock populations with the aim of reaching a deeper understanding of reproduction dynamics in this species and hence of providing solutions to some of the puzzles described above.

Materials and Methods

Experimental maturation system

The experimental maturation system was situated in a greenhouse at Ben-Gurion University of the Negev, Beer-Sheva, Israel. This recirculating seawater system consisted of a maturation tank and a water treatment facility, fitted with aerobic and anaerobic biofilters, a particulate sand filter and a foam fractionator. The system was filled with 7 m^3 of seawater, and its temperature was maintained at 29±1ºC. A photoperiod of 14 h light and 10 h dark, with 30 min at the beginning and at the end of the light period simulating sunrise and sunset, was imposed.

Shrimp broodstock (about one year old and with weights of about 39 g and 43 g for male and female shrimp, respectively) was purchased from a specific pathogen-free producer (SIS-Shrimp Improvement Systems Inc., Florida). Each shrimp was eye tagged with a numbered, colored plastic ring for individual identification. The shrimp were fed an enriched maturation diet of fresh frozen sandworms and squid, chopped and mixed with paprika, and shrimp maturation pellets, at ~25% of the wet body weight made up of 9–10% of sandworms (given immediately after the daily inspection), 15–16% of squid and 1–2% of pellets. The spawning system comprised 60-l black polyethylene tanks. For the spawning tanks, fresh synthetic seawater was prepared (and maintained at 28±1ºC) each time a female was found to have mated.

Experimental procedure

After an acclimation period, ovarian maturation was induced by tying the nonlabeled eyestalk. Thereafter, for the next four months, females were monitored daily for signs of ovarian development. Females with fully developed ovaries were captured, identified and examined for the presence of a spermatophore/sperm-mass. A mated female was transferred to a separate spawning tank and left to spawn without any further disturbance for a few hours. Thereafter, the female was examined again, and if the animal had spawned, it was returned to the maturation system. The number of eggs in the spawn was evaluated volumetrically.

Results

Female reproductive activity and egg and nauplii production

Of the 41 female shrimp, 31 underwent vitellogenesis (75.6 %) and exhibited mature ovaries. Of these, 23 spawned for the first time or were observed with fully vitellogenic ovaries during the first week after ablation, five during the second week, two during the third week and only one thereafter, i.e., 90.3% spawned for the first time and/or exhibited fully vitellogenic ovaries during the first two weeks post-ablation. Five of the 31 females

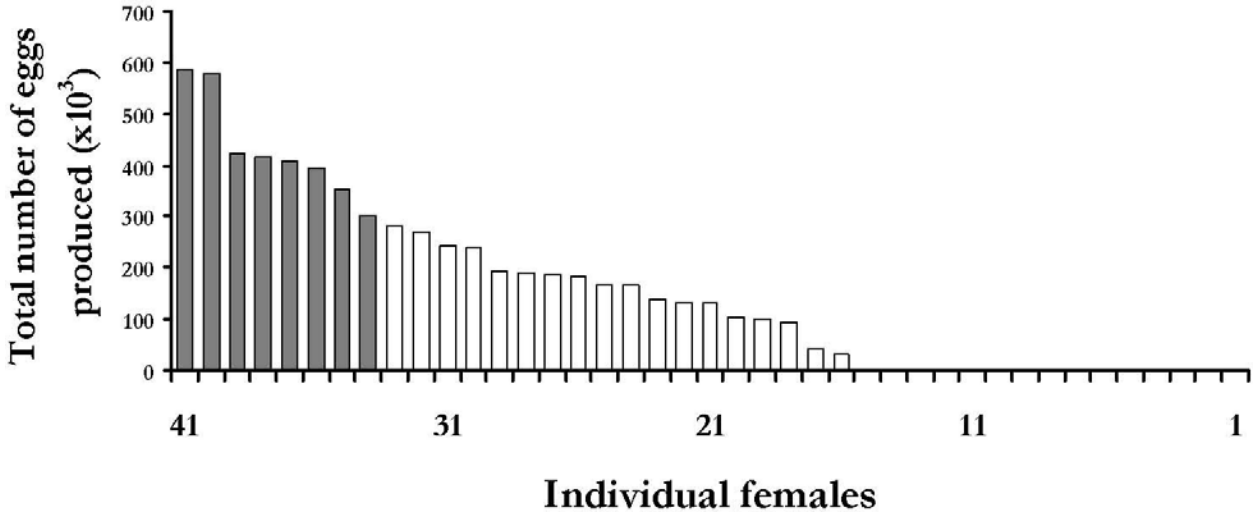

Fig. 1. Reproductive output, i.e., total spawned eggs, for each *L. vannamei* female in the broodstock (n = 41). Grey bars represent the eight top-performing females, which together spawned over 50 % of the eggs produced by all 31 reproductively active females. Vacant places on the X-axis represent females for which spawning was never recorded.

that developed mature ovaries were never observed with an attached spermatophore.

Fig. 1 summarizes the individual reproductive output in terms of egg production of all 41 females in the broodstock. Fifteen females did not undergo vitellogenesis or were never found to have mated. The eight best performing females (dark-gray bars) comprised less than 20% of all the females in the broodstock, but together they produced 50% of total reproductive output of the broodstock in terms of numbers of spawns, egg and nauplii (Table 1). For these eight females, the average time from eyestalk ablation to first ovarian maturity was 4.1±3.4 days, while the average value for all the active females (light and dark gray bars) was 6.0 ± 4.4 days. The average numbers of eggs/spawn and nauplii/spawn were $8.2\times10^4\pm3.8\times10^4$ and $3.2\times10^4\pm 2.8\times10^4$, respectively (Table 1). The females for which 10 or more vitellogenic cycles were recorded together produced more than 90% of the nauplii (this group included the eight best performing females).

Fig. 2, which presents the relationship between the reproduction output and female survival, shows that the number of vitellogenic cycles peaked during the second month, and that of recorded spawns, during the third month of the experiment. There was a steady decrease from 31 to 10 in the number of reproductively active females during the first three months of the experiment due to mortality. The 10 females that survived until the end of the experiment were those with the best reproduction performance. These animals underwent vitellogenic cycles continuously throughout the four months of the experiment. Thereafter, the vitellogenic process halted completely, but the animals seemed to be healthy and active and lived for at least six months after the completion of the experiment.

Interrelationship between female molt cycles, vitellogenic cycles and spawns

A female was regarded as "spent" if she spawned in the spawning system or was found with spent ovaries within 24 h of being observed with fully developed ovaries. A review of the 38 cases of consecutive spawns or vitellogenic cycles in our broodstock revealed that there were 11 cases in which a female was found with fully developed ovaries, spawned, and was then observed again with a maturing ovary within 24 h; the numbers of cases for which a maturing ovary was again observed after 48, 72 or 96 h were 11, 12 and 4, respectively. Among the 38 cases, there were 12 cases of consecutive spawns with only 72 h between the spawns, and 8 with 96 h between spawns. One female spawned three times in a row, with 96 h between the first and second spawns and 72 h between the second and third spawns (Fig. 3, top panel). Such closely occurring reproductive events most probably took place within the same molt cycle, as was confirmed by the fact that after such a reproductive sequence, the female was not detected with ripe ovaries again for the next five to six days, during which time it apparently went through an ecdysis event.

The average life span of the 31 reproductively active females was 98±26 days. Assuming an average molt cycle duration of 14 days per female and two vitellogenic cycles in each molt cycle, a conservative calculation of the total number of vitellogenic cycles of all

Table 1. Analysis of female reproductive output data from a reproduction experiment with *L. vannamei* shrimp

	Total	Females with 10 or more recorded vitellogenic cycles*		Eight top performing females*	
No. of analyzed females	41	19	(46.3%)	8	(19.5%)
Recorded vitellogenic cycles	305	237	(77.7%)	108	(35.4%)
Recorded spawns	91	79	(86.8%)	49	(53.8%)
Eggs produced ($\times 10^6$)	6.35	5.36	(84.4%)	3.23	(50.8%)
Nauplii produced ($\times 10^5$)	8.98	8.08	(90.1%)	4.49	(50.0%)

*Numbers in parentheses are percentages of the total numbers.

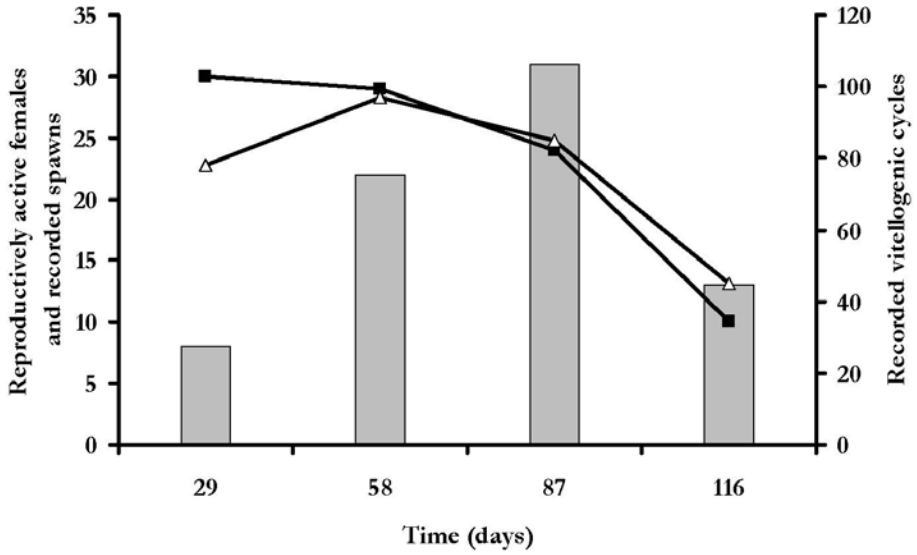

Fig. 2. Reproductive activity of female broodstock. The X-axis represents the 116 days of the experiment divided into four equal time periods, each of 29 days. The data plotted were summed up at the end of each time period (at 29, 58, 87 and 116 days). Plotted on the left Y-axis: ■, number of reproductively active females; grey bars, number of recorded spawns. Plotted on the right Y-axis: Δ, number of recorded vitellogenic cycles.

31 females will give about 430 such events. The actual total number of vitellogenic cycles observed in this study was 305.

Male–female interaction

Of the 26 male shrimp in this experiment, all survived the entire experiment and were never observed to develop melanization of their spermatophores. The number of reproductively active females was 30, 29 and 24 at the end of the first, second and third months, respectively. Thus, throughout the experiment the ratio of males to reproductively active females was approximately 1:1. The active females were examined for the presence of a compound spermatophore or sperm mass more than 900 times throughout the experiment (an average of 28±14 examinations per female). On only 91 occasions did the examination reveal that a female had mated and in all of them a spawn was recorded. Sometimes, females with fully vitellogenic ovaries did not mate for three, four or five consecutive days (as observed in 36, 9, and 3 cases, respectively), as was shown by examinations for the presence of a compound spermatophore or sperm mass that covered several days, including two to four times during the night (Fig. 3, middle panel).

Discussion

The results of this study for female reproduction are in keeping with a number of well-known phenomena that are consistently reported from trials for penaeid reproduction, mainly for *L. vannamei*. One such phenomenon, a short latency period—often as little as three days—from unilateral eyestalk ablation to complete ovarian development and first spawn has been reported to be significantly shorter in the better spawners (Arcos et al., 2003b; Arcos et al., 2004; Bray and Lawrence, 1992; Palacios et al., 1999a). Our findings of 4.1± 3.4 days for the best-performing females vs. 6.0±4.4

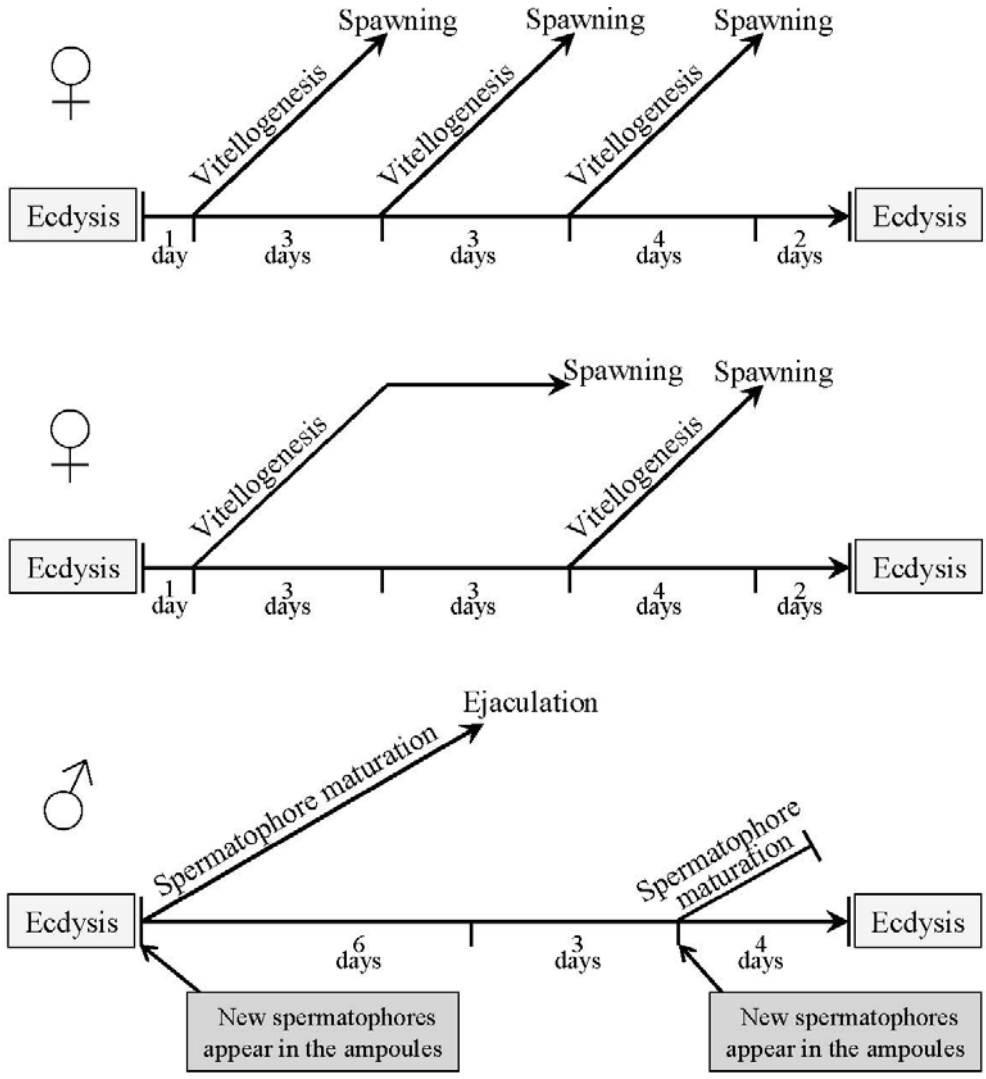

Fig. 3. Schematic diagram describing the reproductive dynamics of two top-spawning females and a male *L. vannamei* in a captive breeding population. The upper panel shows the data for a 13-day intermolt period for a representative eyestalk-ablated vitellogenic female weighing 43 g. This female spawned three times in a row with only three days between the first and second and between the second and third spawns. The middle panel shows a similar intermolt period for a female found with fully vitellogenic ovaries but not mating for three consecutive days (as shown by the absence upon examination of attached spermatophores). On the 7th day of this intermolt period, examination of this animal showed that it had spawned. Thereafter, it underwent vitellogenesis and spawned for the second time. The bottom panel shows a 13-day intermolt track of a representative 40 g male typical of a captive breeding population. Overlaid on this track are literature-based considerations regarding male spermatophore dynamics during the intermolt period (see discussion). New translucent spermatophores arrive at the terminal ampoules on the night of ecdysis and take at least six days to mature and turn pearly white in color in the ampoules. This is probably the earliest time that a male can mate successfully. Then, it takes three to four more days for a new pair of spermatophores to appear inside the ampoules. This new pair of spermatophores will not be able to complete the maturation process and will be degraded before the next ecdysis.

days for the other females are indeed in keeping with these observations. The decline in the reproductive output of the broodstock as a whole after three to four months that was obtained in this study is also a known characteristic of captive shrimp breeding and serves to emphasize the need to remove poor spawners from the broodstock (Bray and Lawrence, 1992).

The finding that the ovaries of some females matured several times in a single molt cycle are in keeping with studies that reported that the number of spawns produced by a female shrimp in captivity in a single molt cycle ranged between 2 and 6, depending on the species and whether the female was intact or eye-stalk ablated (Bray and Lawrence, 1992; Browdy and

Samocha, 1985; Coman and Crocos, 2003; Emmerson, 1980, 1983). We must have missed many of the spawns that occurred in the maturation tank (305 vitellogenic cycles vs. 91 recorded spawns), since in at least eight cases, fully vitellogenic females that were examined twice within 2 h were found to have spawned upon the second examination. This finding is quite common in shrimp hatcheries and is the reason that the water in maturation tanks is monitored for presence of eggs or nauplii (Bray and Lawrence, 1992; Misamore and Browdy, 1996). However, to the best of our knowledge, the present study presents the first report in open thelycum species of the number of vitellogenic cycles per molt cycle.

Perhaps the most puzzling finding of our study was that a relatively small fraction of the female broodstock contributed 50% of the total production and that the ovaries of a substantial number of females never matured. This enigmatic phenomenon and the fact that individual female performance is extremely variable has been noted in a number of studies (Table 2; Arcos et al., 2004; Arcos et al., 2003b; Arcos et al., 2003a; Bray and Lawrence, 1998; Bray et al., 1990; Hoang et al., 2003; Holtschmit and Romero, 1991; Palacios and Racotta, 2003; Palacios et al., 1999b; Palacios et al., 1999a; Racotta et al., 2003; Wyban and Sweeney, 1991). Table 2 shows that the average fraction of non-active females exceeds 30% and that, on average, less than 20% of the females contributed more than 60% of the reproductive output of the whole broodstock. These figures pertain to different species from both wild and captive populations and in both eyestalk ablated and intact females. Despite the universality of this phenomenon, it has been treated on an individual level only in two studies—the present one and that of Wyban and Sweeney (1991), who showed a genetic basis for the variability among *L. vannamei* females. In shrimp maturation systems, the problem of a small number of females producing the greater part of the broodstock is addressed by culling females, but it seems to us that such a procedure can be efficient only if the females are individually monitored (Browdy, 1992; Robertson et al., 1993; Wyban and Sweeney, 1991).

In contrast to females, sub-adult males mature spontaneously and develop spermatophores (Ceballos-Vazquez et al., 2003; Ogle, 1992; Parnes et al., 2004), but as with females, adult male reproductive capacity is highly variable (Ceballos-Vazquez et al., 2004). Among other factors, lack of spermatophore adhesiveness is believed to contribute to the lack of mating reported from many breeding trials (Alfaro and Lozano, 1993; Ceballos-Vazquez et al., 2004; Chamberlain et al., 1983; Leung-Trujillo and Lawrence, 1987; Pascual et al., 1998; Rosas et al., 1993). Adhesiveness is conferred by extracellular materials that are laid down on the spermatophore forming in each of the two ampoules (Bauer and Cash, 1991; Chow et al., 1991; King, 1948; Malek and Bawab, 1974; Ro et al., 1990). After ejaculation the two spermatophores have to be attached to one another and then to adhere to the female abdomen. The molding and maturation processes of the spermatophores inside the ampoule thus appear to be extremely important.

In manually ejaculated males, it was found that spermatophores appear again in the ampoules only after the subsequent molt event (Heitzmann et al., 1993). The new spermatophores that reach the ampoule on the night of ecdysis are translucent and remain so for at least the first six days postmolt (Heitzmann et al., 1993). Their color then becomes pearly (6–12 days postmolt) and finally white by the end of the intermolt period (10–14 days postmolt). This sequence of events probably means that the spermatophores are naturally fully mature only 12–14 days after ecdysis, i.e., only once in each molt cycle. Even if we assume that a pearly color indicates spermatophore maturity, the earliest time that a male could mate successfully would be six days postmolt (Fig. 3, bottom panel), and even in such case, it would not be able to mate again until after the subsequent molt event, since three to four more days are required for a new pair of spermatophores to appear inside the ampoules (Leung-Trujillo and Lawrence, 1991). These spermatophores will not be able to complete their maturation process and will be degraded before the next ecdysis (Parnes et al., 2006). This means that the male starts each intermolt period with a new pair of spermatophores that still have to mature inside the ampoules and thus will be able to mate successfully only once in a molt cycle.

As discussed above, in captivity the female, but not the male, experiences an accelerated reproduction rate, and therefore the traditional ratio (1:1) of females to males in breeding populations is probably not optimal. Some authors have even suggested that it is economically advantageous to use a ratio of two males per three females (Bray and Lawrence, 1992; Robertson et al., 1993), while concomitantly pointing out that: “lack of mating has been a persistent and confusing problem in numerous reproduction trials, and was the source of a great deal of speculation”. When Wyban and Sweeney (1991) isolated their best spawners and obtained 450% more reproductive output, they reported that this was also the result of 50% more mating. In this case it is reasonable to assume that with the males being available only for the best spawners, the total reproductive output of the broodstock would be higher.

Table 2
Summary of the literature regarding the biased distribution of captive penaeid female reproductive output

Percentage of reproductively non-active females	Percentage of females that together yielded ≥50% of total production	Percentage of total reproductive output of the broodstock	Species	Source
—	9.5	50	*L. vannamei*	(Wyban and Sweeney, 1991)
24*	—		*L. vannamei*	(Palacios and Racotta, 2003)
48*	19	57	*L. vannamei*	(Arcos et al., 2003b)
11.1	24 ablated	70	*L. vannamei*	(Palacios et al., 1999a)
57.9	18 unablated	64		
13	25 wild		*L. vannamei*	(Palacios et al., 1999b)
27	15 pond reared			
25*	23.5[a]	65	*L. stylirostris*	(Bray et al., 1990)
30	20[a]	67.7	*Penaeus monodon*	(Bray and Lawrence, 1998)
44*			*L. vannamei*	(Arcos et al., 2004)
10–40	—		*Fenneropenaeus merguiensis*	(Hoang et al., 2003)
36.6	19.5	54.4	*L. vannamei*	This study
30.6 ± 13.8	**19.3 ± 4.9**	**61.9 ± 7.2**		Average for ablated females alone

*All percentages marked with an asterisks were supplied by the sources cited. All other data were calculated from those presented in the sources cited.

An examination of our findings shows that potentially the most competent females can spawn between 12 and 18 times over a three-month captive spawning season. In contrast, the males, whose spermatophores mature at a natural rate, can mate successfully only about six times during the same period of time. Thus, the many instances of females carrying ripe ovaries but not mating for three or more days in this study may be attributed, at least in part, to the absence of males ready for mating. It is therefore evident that in this regard two research avenues should be pursued: endocrinological induction of the males, which could shorten the time required for spermatophore maturation inside the ampoules, or replacement of males without spermatophores in their ampoules with spermatophore-bearing animals. These procedures, if carefully documented, may also contribute to our understanding of penaeid male reproductive biology regarding the spermatophore maturation schedule and dynamics.

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