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Reproduction and molt in previously spawned and first-time spawning red-claw crayfish *Cherax quadricarinatus* females following eyestalk ablation during the winter reproductive-arrest period

Amir Sagi [a,*], Rami Shoukrun [a], Tal Levy [b], Assaf Barki [b], Gideon Hulata [b], Ilan Karplus [b]

[a] *Department of Life Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva, 84105, Israel*
[b] *Department of Aquaculture, Institute of Animal Science, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan, 50250, Israel*

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Abstract

The effect of unilateral eyestalk ablation on reproduction, molting and quality of offspring was investigated in *Cherax quadricarinatus* females during the reproductive-arrest period (winter). At the end of the Autumn, females that had spawned for the first time had relatively uniform, early vitellogenic ovaries, while most females that had spawned previously had ovaries containing two distinctly sized and coloured oocyte populations. These included one population of small oocytes, similar to oocytes found in first-time spawners, while the other comprised of large oocytes. Winter reproduction of females that had spawned previously was not affected by eyestalk ablation, whereas significantly increased spawning activity was observed in eyestalk-ablated first-time spawners. Both females that had spawned previously and first-time spawners that were eyestalk ablated molted significantly more frequently than intact females. The size and number of offspring were not affected by eyestalk ablation. These results show that small synchronous oocyte populations were affected by eyestalk ablation while large oocytes were not affected; females with single-sized-oocyte ovaries tended to be more receptive to the treatment. Unilateral eyestalk ablation may present a solution for induction of spawning only in young females and perhaps in synchronized broodstock. © 1997 Elsevier Science B.V.

Keywords: Reproduction; Spawning; Molt; Eyestalk ablation; *Cherax quadricarinatus*; Decapoda; Crustacea

* Corresponding author. E-mail: sagia@bgumail.bgu.ac.il

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1. Introduction

The red-claw crayfish, *Cherax quadricarinatus*, is a large, freshwater crustacean from northeast Australia and Papua-New Guinea. This species has recently attracted considerable attention as a potential crop in temperate-zone aquaculture, as a result of its fast growth and its tolerance to a wide range of temperatures from 5°C to 42°C (Ackefors, 1994). Preliminary experiments in Israel have demonstrated good survival at temperatures as low as 6°C (M. Zoran, personal communication). *C. quadricarinatus* can be raised to market size (70–100 g) in 6–9 months (Jones, 1990, 1995a,b,c; Karplus et al., 1995; G. Zohar, 1995 personal communication). The crayfish can weigh 250–300 g at two years of age and can reach a maximum weight of 400–600 g (Ackefors, 1994). Sexual maturity is attained within 7–9 months (Rouse et al., 1991).

To maximize production under temperate-zone aquaculture conditions, it is necessary to exploit the full potential of the spring-summer growout season. To achieve this goal, nursed juveniles that have been hatched during the winter should be stocked in growout ponds early in spring. Studies of a large broodstock population of *C. quadricarinatus*, under temperate zone conditions in Israel, showed a seasonal pattern of spring-summer breeding with a decrease (almost to cessation) of spawning during the winter (Barki et al., 1997). It has thus been suggested that an efficient culture strategy for this crayfish in temperate zones will require the induction of spawning during the winter reproductive-arrest period.

In commercial hatcheries of stringently controlled crustacean species, notably penaeids, vitellogenesis and spawning are stimulated by removal of the endocrine complex in the eyestalk, comprising the X-organ and the sinus gland, which is responsible for secretion of gonad-inhibiting hormone (GIH) (Quackenbush, 1991). Unilateral eyestalk ablation results in gonad maturation and spawning in several penaeid species (Santiago, 1977; Primavera, 1978; Choy, 1987).

The hormone-mediated process of gonad maturation is characterized by rapid deposition of yolk in the oocyte, with a concomitant increase in oocyte diameter (Eastman-Reks and Fingerman, 1985; Quackenbush, 1989). In *C. quadricarinatus*, gonad maturation is characterized by the development of two distinctly sized, and often distinctly pigmented, oocyte populations (Sagi et al., 1996). It is possible that oocyte development is regulated differentially by neurohormones, enabling one oocyte population to rapidly increase in diameter in preparation for spawning while the other remains relatively dormant.

The aim of the present study was to investigate the efficacy of eyestalk ablation in the induction of gonad maturation and spawning in *C. quadricarinatus* broodstock during the winter reproductive-arrest period under temperate-zone conditions. The effect of unilateral eyestalk ablation in females having ovaries containing differently sized oocyte populations was also investigated.

2. Materials and methods

2.1. Experimental animals

Two types of *C. quadricarinatus* female were used in this study. The first group consisted of females that had been held in breeding tanks for over a year and had

spawned at least once prior to the experiment. This group was designated 'previously spawned' females. The second group consisted of approximately 7-month-old females, approaching sexual maturity, that had been harvested from experimental earthen ponds after a growout period of five months. Since the latter population did not contain berried females, these specimens were considered not to have spawned previously and were thus termed 'first-time spawners'.

To study the structure and status of the ovaries at the beginning of the reproductive-arrest period, 10 previously spawned females and 12 first-time spawners were dissected at the end of the fall (last week of October). The previously spawned females weighed 110.9 ± 8.1 g and had carapace length of 54.8 ± 2.0 mm. The first-time spawners weighed 37.4 ± 4.5 g, and their carapace length was 38.5 ± 1.5 mm.

For the winter experiment, 50 previously spawned and 50 first-time spawners were collected in mid-October. Half of the females of each type were subjected to unilateral eyestalk ablation (destalked) at the beginning of November. The other half were left intact and served as the control. All the females were divided into groups of five animals, each group containing females of the same type and treatment. Each group of females was held with one mature male (weighing about 90 g). The previously spawned females weighed 65.4 ± 4.4 g and had a carapace length of 49.3 ± 0.9 mm. The data for the first-time spawners were 54.8 ± 1.9 g and 43.9 ± 0.4 mm for weight and carapace length, respectively. Each female was individually marked with colored plastic bands glued to its carapace.

2.2. Holding facilities

The animals were held in four separate recirculating water systems, one for each treatment. Each system consisted of five 120-l tanks, equipped with shelters and maintained under an ambient light regime. The temperature ranged between a minimum of 26 ± 0.5°C and a maximum of 28 ± 0.7°C. The mean dissolved oxygen level was 75% saturation, and ammonium and nitrite levels did not exceed 0.5 and 0.05 ppm, respectively. The animals were fed artificial pellets (37% protein, enriched with calcium), fish flesh, ground carrots and green leaves. A recirculating water system of 22 glass aquaria was used for housing berried females during the hatching period.

2.3. Procedures

Unilateral eyestalk ablation was performed using surgical scissors, and the wound was cauterized with liquid nitrogen. The experiment began in the first week of November and continued to the third week of January. The tanks were checked daily for molts. Two days after molting, the marking on the carapace was renewed. Females were checked for the presence of eggs once a week. The day at which a female was first observed to be berried was considered as the spawning date (with a maximal error of 6 days); thus, cumulative spawning data are expressed by week. Berried females were transferred to a hatching aquarium when eye spots were first visible in the embryos (stage 5, Jones, 1990, 1995a), and observed daily to determine the date of release of the young. Release date was defined as the first day a juvenile was observed walking

independently on its mother's exoskeleton or on the bottom of the aquarium. Ten days later, the young were separated from the mother and counted. A random sample of 20 juveniles was weighed (± 0.001 g), and the female was returned to her original tank. Comparisons between treatments were analyzed using the Mann–Whitney U Test.

Females sampled in the Autumn and a representative sample of females taken at the end of the experiment were weighed and dissected. Their gonads were removed and weighed (± 0.001 g). The gonadosomatic index (GSI) was calculated as the percentage of gonad weight out of total body weight. Oocyte diameter was measured under a light microscope using an objective micrometer (± 0.01 mm). Mean oocyte diameter ($\pm$s.e.) was calculated from a sample of at least 15 oocytes per oocyte population, and the difference between populations was tested by one-way ANOVA, $P \leq 0.05$.

3. Results

Most of the ovaries dissected from previously spawned females at the end of the Autumn contained two distinctly sized oocyte populations ($P < 0.05$). The small oocytes were whitish to pale orange/pale green, had an average diameter of 0.68 ± 0.58 mm, and changed very little in size with an increase in GSI ($P = 0.377$, $r = 0.336$). The large oocytes reached an average diameter of as much as 2.70 ± 0.55 mm (Fig. 1A). Unlike the small oocytes, the diameter of the large oocytes correlated strongly with the increase in GSI ($P = 0.001$, $r = 0.892$), and their colour varied through orange, orange brown, green and olive dark-green. In two females, three distinctly sized (with diameters of 0.52 ± 0.03, 1.05 ± 0.03 and 1.85 ± 0.05 mm) and distinctly colored (white, olive-green and orange, respectively) oocyte populations were observed. In contrast, ovaries of first-time spawners dissected at the end of the fall contained oocytes that were more homogeneous in color (pale orange) and diameter. In some cases, two distinctly sized oocyte populations ($P < 0.05$) were observed, with the large oocyte group reaching an average diameter of up to 0.85 ± 0.13 mm (Fig. 1A).

Winter spawning of previously spawned females was not affected by eyestalk ablation (Fig. 2A). Among previously spawned females, both intact and destalked, approximately one third mated and spawned during the experimental period (November through January). On the other hand (Fig. 2B), a difference in cumulative spawning was observed between intact and destalked first-time spawners as early as the third week of December (six weeks after eyestalk ablation); the difference became statistically significant at the fourth week of December (χ^2; $P < 0.05$). Intact first-time spawners did not spawn until the second week of January (4%), while destalked females started spawning a month earlier, and 50% of the population had spawned by the second week of January. By the end of the experimental period, most of the destalked females had spawned (74%).

In both previously spawned and first-time spawners, molting events were significantly more frequent (χ^2; $P < 0.05$) in destalked females than in intact animals (Fig. 3). Among destalked previously spawned females, 48% molted, starting four weeks after the ablation (Fig. 3A), while only 22% of the first-time spawners molted, starting in the seventh week of the experiment (Fig. 3B and Table 1).

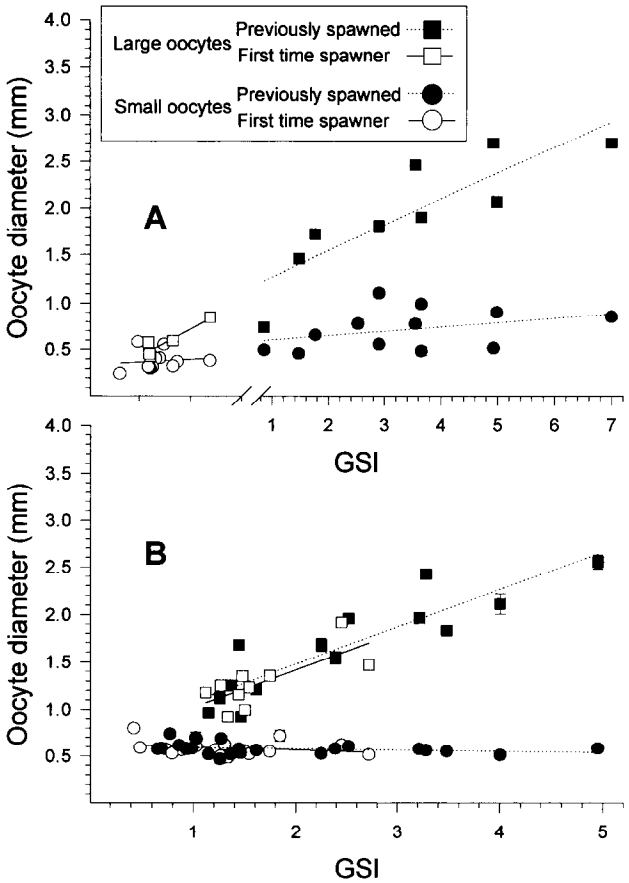

Fig. 1. Different oocyte populations in the ovaries of previously spawned *C. quadricarinatus* females and first-time spawners at the beginning (A) and end (B) of the experimental period. Error bars represent s.e. Each ovary containing two oocyte populations is represented by two data points, one for the large population and one for the small.

No case of a single female that both molted and spawned was observed during the experimental period. Females that neither spawned nor molted were most frequent among the intact first-time spawners (72%) and intact previously spawned females (67%). Such a phenomenon was much less frequent among destalked females, being observed in 4% of first-time spawners and 14% of previously spawned animals (Table 1).

At the end of the experimental period, first-time spawners had a wider range of oocyte diameters than at the beginning of the experiment (compare Fig. 1A and B). In first-time spawners, large oocytes reached diameters of 1.9 ± 0.05 mm at the end of the experimental period, while previously spawned females had a larger oocyte diameter of up to 2.55 ± 0.07 mm (Fig. 1B). The size of the small oocytes did not exceed

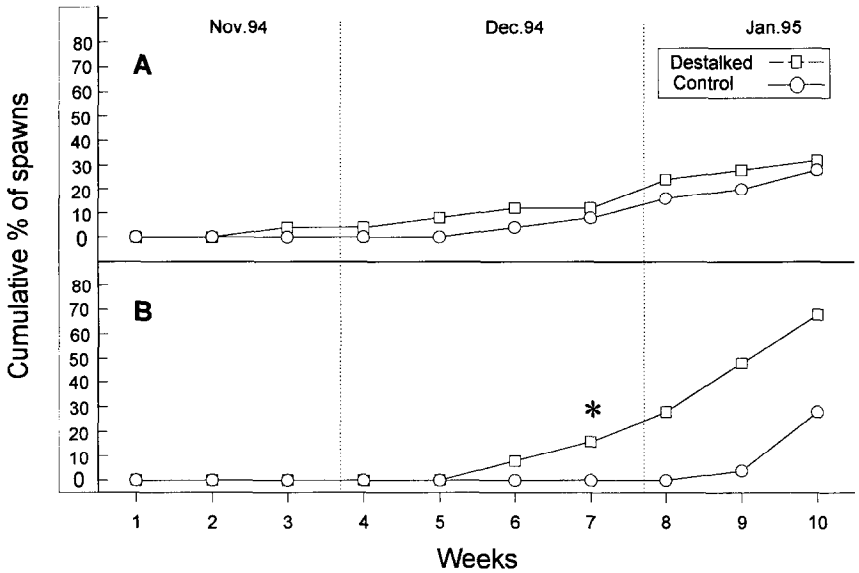

Fig. 2. Cumulative % of spawns during the winter in intact *C. quadricarinatus* females versus destalked previously spawned animals (A) and destalked first-time spawners (B). * Significantly different at $P < 0.05$ from that week onward.

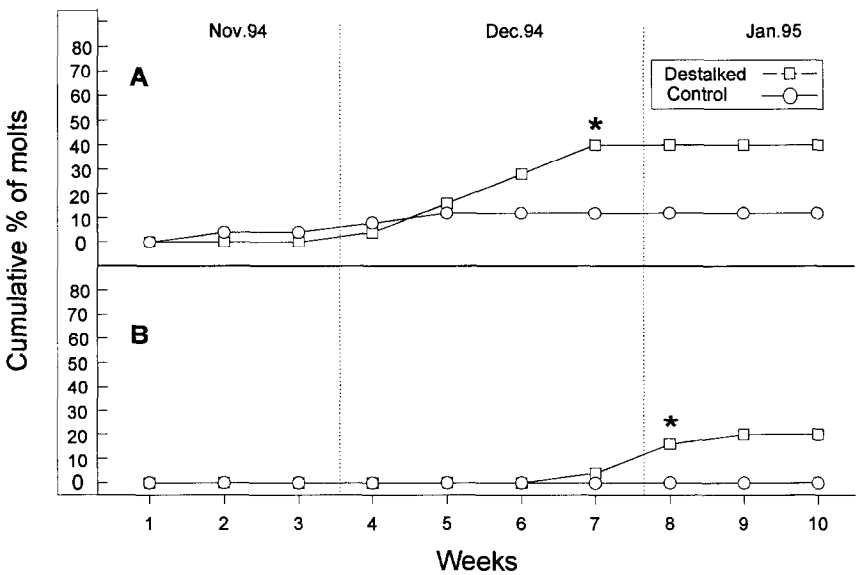

Fig. 3. Cumulative % of molts during the winter in intact *C. quadricarinatus* females versus destalked previously spawned animals (A) and first-time spawners (B). * Significantly different at $P < 0.05$ from that week onward.

Table 1
Summary of reproduction and molting events in first-time spawners and previously spawned *C. quadricarinatus* females that underwent eyestalk ablation or remained intact

Treatment	Spawning and/or molting	First-time spawners		Previously spawned	
		n	%	*n*	%
Eyestalk ablation	Spawned	17	74	8	38
	Molted	5	22	10	48
	Neither	1	4	3	14
Intact	Spawned	7	28	7	29
	Molted	0	0	3	13
	Neither	18	72	14	58

0.80 ± 0.02 mm in the two types of female, in both destalked and control animals. Previously spawned females had a wider range of GSIs; while the highest GSI observed in first-time spawners was 2.72, the GSI of previously spawned females reached as much as 4.95. Both first-time spawners and previously spawned females that had a GSI smaller than 1.1 spawned up to 48 h prior to being sampled.

The population of large oocytes in destalked first-time spawners that molted and did not spawn during the experimental period had a significantly smaller mean diameter

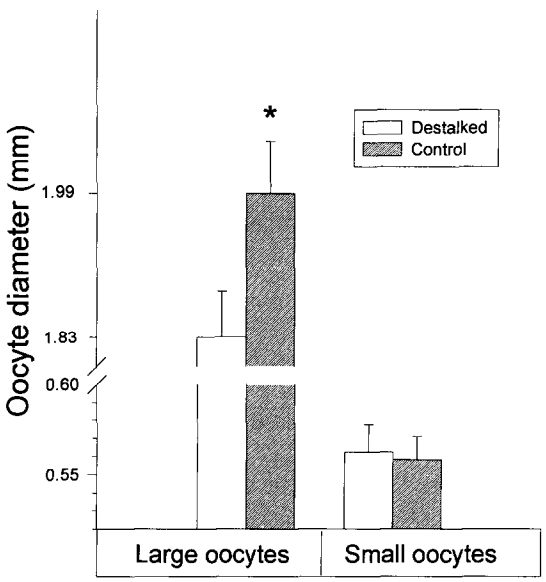

Fig. 4. Oocyte diameter in two distinctly sized oocyte populations in destalked versus control first-time spawners *C. quadricarinatus* females that did not spawn during the experimental period. All the destalked females that did not spawn molted during the experimental period. * Significantly different at $P < 0.05$.

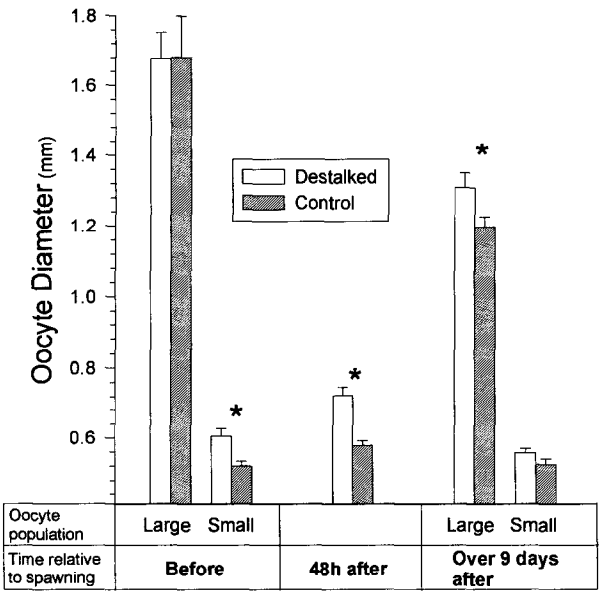

Fig. 5. Oocyte diameter of two distinctly sized oocyte populations in destalked versus control previously spawned *C. quadricarinatus* females before and after spawning. * Significantly different at $p < 0.05$.

than that in the controls (Fig. 4). The diameter of oocytes in destalked previously spawned females was significantly larger than that of the control in small oocytes prior to spawning, in oocytes 48 h after spawning, and in large oocytes more than 9 days after spawning (Fig. 5).

Number and weight of offspring and the period between spawning and releasing the juveniles were not affected by eyestalk ablation, for both the previously spawned group and the first-time spawners (Table 2).

Table 2
Number and size of offspring and time to release, in first-time spawners and previously spawned *C. quadricarinatus* females that were either destalked or left intact

Treatment	Group	*n*	No. of juveniles	Juvenile weight (mg)	Time to release (days)
Eyestalk ablated	First-time spawners	8	235.5 ± 132.5	24.5 ± 4.1	38.4 ± 3.9
	Previously spawned	4	443.7 ± 212.4	23.1 ± 4.6	42.3 ± 3.6
Intact	First-time spawners	10	182.0 ± 132.2	22.2 ± 4.8	34.7 ± 4.0
	Previously spawned	6	321.2 ± 108.3	23.2 ± 3.6	39.5 ± 4.1

Time of release is expressed as the interval between the first time a female was observed berried until release of the first juvenile. The maximal error in estimating spawning date was 6 days. No significant difference was found in any of the columns using the Kruskal Wallis test.

4. Discussion

Two different oocyte development pathways have been described for crustacean ovaries. In one, the ovary contains an oocyte population that is homogeneous in size and stage (O'Donovan et al., 1984), while in the other the ovary may contain oocyte populations of different sizes and stages (Morrissy, 1970, 1975; Schuldt, 1993; Sagi et al., 1996). In *C. quadricarinatus* it was observed that only some of the oocytes in the ovary were involved in the uptake of yolk protein, thus increasing in diameter and changing in pigmentation, and eventually culminating in a spawn (Sagi et al., 1996). Shortly after spawning, most females seemed to posses small, synchronous, either growing or static oocyte populations. The induction of spawning events in destalked first-time spawners and anatomical observations in destalked previously spawned females suggest that the effect of eyestalk ablation was limited to the population of small oocytes at the time of ablation and did not affect the population of large oocytes. It is suggested that only the former may be affected by endocrine intervention, irrespective of the presence of large oocytes.

GIH is produced and secreted from the X-organ/sinus gland complex in the eyestalk of crustaceans (De Kleijn et al., 1992; Rotllant et al., 1993), and ablation of the eyestalk thus generally affects the vitellogenic process (Wilder et al., 1994). The presence of specific receptors for yolk proteins in the oocyte membranes of crayfish (Jugan and Van Herp, 1989) may suggest that vitellogenin uptake is regulated through variation in the expression of vitellogenin receptors during oocyte development. Receptor-mediated endocytosis of vitellogenin has been described in several insects (e.g. Sappington et al., 1995). However, it seems that in *C. quadricarinatus* this effect is limited to a specific group of early vitellogenic oocytes, as was the case for the single population of oocytes in the pre-vitellogenic ovaries of first-time spawners and a similar population of ovaries in females that had spawned previously. The nature of this differential regulation needs to be studied further. In insects, juvenile hormones stimulate vitellogenesis and the patency of ovary tissue for uptake of yolk protein (reviewed in Bellés, 1995). There is supporting evidence that the crustacean juvenile hormone-like compound, methyl farnesoate, may have a role in regulating reproduction (Laufer and Sagi, 1992). The recent discovery of an eyestalk-born neuropeptide with a mandibular organ inhibitory effect (MOIH) (Liu and Laufer, 1996; Wainwright et al., 1996) may suggest that the stimulation of the small oocytes, as observed by us, caused by the removal of the eyestalk, represents an indirect effect through mandibular organ hyperaction.

Functional neuropeptides isolated from crayfish have been shown to posses molt inhibitory (Terauchi et al., 1996) or gonad inhibitory (Aguilar et al., 1992) activity. Our study on the effects of eyestalk ablation on molt and reproduction suggest their existence in *C. quadricarinatus*. Despite the fact that in crustaceans both gonad-inhibiting hormone (Rotllant et al., 1993) and molt-inhibiting hormone (Webster and Dircksen, 1991) are secreted from the same endocrine eyestalk complex, no female in our study underwent both spawning and molting following eyestalk ablation during the experimental period. In first-time spawners that molted, oocyte population growth was retarded compared to non-spawning control females. These facts strongly suggest an antagonistic nature of reproduction versus somatic growth (molt) in this species, in which a

reproductive (pre-copulation) molt does not occur. In many crustacean species, the period of somatic growth is temporally separated from that of reproductive growth (Adiyodi, 1985). In some cases, specific seasons are set apart for reproduction and molt (Webb, 1977). *C. quadricarinatus* represents a case in which there is no strict temporal separation at the population level. On the other hand, the molting event in each crayfish was clearly separated from reproduction, suggesting, at the individual level, a strict endocrine regulation of the partition between these two energy-demanding metabolic events.

In terms of hatchery management, the straight-forward approach of unilateral eyestalk ablation to induce spawning (Primavera, 1978; Hansford and Marsden, 1995) may be suitable only for young (relatively small) *C. quadricarinatus* females and not for *C. quadricarinatus* broodstock populations comprising large females more than one year old. On the other hand, since the present observations indicate that it is the small homogeneous oocyte population that is affected by the ablation, a possible solution to this hatchery management problem might be found in broodstock synchronization.

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