ELSEVIER

Aquaculture 190 (2000) 103–118

Aquaculture

www.elsevier.nl/locate/aqua-online

Role of ecdysteroids and methyl farnesoate in morphogenesis and terminal moult in polymorphic males of the spider crab *Libinia emarginata*

Guiomar Rotllant *, Peter Takac [1], Lei Liu [2], Grant L. Scott, Hans Laufer

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA

Received 10 January 2000; received in revised form 23 March 2000; accepted 30 March 2000

Abstract

Here we examine morphogenesis and terminal anecdysis in male morphotypes of the spider crab *Libinia emarginata* through eyestalk removal (ablation). Methyl farnesoate (MF), an unepoxidated form of juvenile hormone (JH III) and hydroxyecdysone, a moulting hormone, were measured in attempt to understand these processes and to determine the physiological characteristics of each morphotype, to be able to induce maturation by hormonal treatment and improve crustacean culture. Following arrival at the laboratory, ablated small-claw unabraded small-carapace males (SUM) moulted within 30 days to a juvenile and control SUM moulted within 75 days to a mature morphotype with large claws. Following eyestalk removal small-carapace abraded males (SAM), large-carapace unabraded large-claw (LUL) and large-carapace abraded large-claw (LAL) males never moulted even after 60 days, while the other ablated morphs moulted in 25–35 days. Significant increase of MF levels was observed in SUM, SAM and LUL de-stalked animals compared to intact control group, while no differences were observed in LAL individuals. In fact, LAL crabs presented the highest level of MF up to 2.23 ng/ml. Ten days prior to moulting MF increased 4-fold, and the ecdysteroids increased 15-fold in the haemolymph of ablated SUM and 25% in their testes. Ecdysteroid levels in the haemolymph of the SAM, LUL and LAL

* Corresponding author. Present address: Institut de Ciències del Mar, C.S.I.C. Passeig Joan de Borbó, s/n, E-08039 Barcelona, Spain.

E-mail addresses: guio@icm.csic.es (G. Rotllant), uzaetaka@savba.savba.sk (P. Takac), leiliu@uiuc.edu (L. Liu), laufer@uconnvm.uconn.edu (H. Laufer).

[1] Present address: Institute of Zoology, Slovak Academy of Sciences, Dubravska cesta, 9, SK-842 06 Bratislava, Slovak Republic.

[2] Present address: W.M. Keck Center for Comparative and Functional Genomics, University of Illinois at Urbana-Champaign, 330 Edward R. Madigan Laboratory, 1201 W. Gregory Drive, Urbana, IL 61801, USA.

PII: S0044-8486(00)00388-4

morphotypes remained low (around 5 ng/ml), and none of these animals moulted. The results strongly suggest that: (1) SUM are able to moult, and represent the juvenile stage, (2) SAM, LUL and LAL are terminally moulted and may represent the reproductive population, (3) circulating ecdysteroids induce moulting in juveniles, (4) high levels of ecdysteroids in the testes of SAM, LUL and LAL suggest a gonadal function, and (5) circulating MF controls morphogenesis of juveniles (SUM) and seems to stimulate gonads in adults.

Keywords: Spider crab; Morphogenesis; Terminal moult; Ecdysteroids; Methyl farnesoate

1. Introduction

While many species of decapod crustaceans continue to moult and grow throughout their lives, others, like spider crabs (Majidae) undergo an allometric differentiation of the chelae when they moult to maturity and become anecdysic (Teissier, 1933, 1935; Hartnoll, 1963; Conan and Comeau, 1986; Conan et al., 1988; Comeau et al., 1991). However, some authors propose that the large species of spider crabs such as *Chionoecetes*, continue to moult after reaching the large claw form. This is based on the finding of morphometrically mature males that had well developed second carapaces, indicating imminent moulting (Somerton, 1981; Dawe et al., 1991), and from long-term captivity experiments that allow morphometrically mature male *Chionoecetes bairdi* to moult after 26–27 months of captivity (Paul and Paul, 1995).

The terminal moult is not necessarily a maturational moult, because spermatophores may be present in the sperm duct of male spider crabs prior to this moult. In *Libinia emarginata*, Hinsch (1972) reported that males with a carapace length (CL) of 19 mm had sperm that is morphologically indistinguishable from that of mature males. The different male morphotypes of the spider crab present differences in the size and weight of the reproductive system, morphological features of the carapace and reproductive behaviour (Homola et al., 1991; Sagi et al., 1994).

Crabs are anecdysic after they have undergone a terminal moult, and are therefore incapable of additional moulting. It has been proposed that the Y-organs may degenerate or become permanently inhibited following this moult. Carlisle (1957) noted a reduction in the size of the Y-organs after the moult to puberty in *Maia squinado*, and this gland is not present after the onset of sexual maturity in *Pisa tetradon* (Vernet-Cornubert, 1958, 1960). In *Acanthonyx lunulatus*, histology of the Y-organs revealed that these degenerate completely after the terminal moult (Chaix et al., 1976). In the isopod *Sphaeroma serratum* the concentration of 20-hydroxyecdysone and the size of the Y-organs also decrease after the onset of sexual maturity (Charmantier et al., 1977; Charmantier and Trilles, 1979). Furthermore, eyestalk removal (ablation) initiates ecdysis in juvenile *P. tetradon* and *L. emarginata* but not in the adults (Vernet-Cornubert, 1960; Hinsch, 1972). Generally, haemolymph ecdysteroid content is higher in young crabs than in mature males (Cormier et al., 1992; Laufer et al., 1993). In addition, ecdysteroids have been identified in the ovaries and eggs of *Parapenaeus fissurus* (Jeng et al., 1978), *Penaeus monodon* (Young et al., 1993), *Palaemon serratus* (Spindler et al., 1987), *Macrobrachium rosenbergii* (Wilder et al., 1991; Young et al., 1991),

Homarus americanus (Lisk, 1961; Couch et al., 1987), *H. gammarus* (Goudeau et al., 1990), *Nephrops norvegicus* (Fairs et al., 1989) and *A. lunulatus* (Chaix and De Reggi, 1982), and in the testes of *H. americanus* (Burns et al., 1984) and *L. emarginata* (Laufer et al., 1993).

Bilateral extirpation of the stalked eyes not only resulted in an accelerated moult but has been shown to delay metamorphosis in larvae by the production of extra larval stages in shrimp, *Palaemon macrodactylus* (Little, 1969) and *Palaemonetes varians* (Le Roux, 1984); the lobster *H. americanus* (Charmantier et al., 1988); and intermediate stages in crabs, *Rhithropanopeus harisii* (Costlow, 1968), and *Sesarma reticulatum* (Costlow, 1966; Freeman and Costlow, 1980). The endocrine control of morphogenesis in crustaceans is not well understood, but evidence suggests that inhibitory factors from the sinus glands in the eyestalks are involved.

Morphogenesis and reproduction in crustaceans seem to be regulated by a juvenile hormone (JH), as in insects. The JH-like compound, methyl farnesoate (MF), synthesized and secreted by the mandibular organ (MO) functions in adult crustaceans in a manner similar to its function in adult insects, that is JHs seem to stimulate reproduction in both males and females (Laufer et al., 1987; Laufer et al., 1988; Homola et al., 1991). Synthesis of MF in MOs is negatively regulated by factors from sinus glands (SG) (Laufer et al., 1987, 1989). These factors are called mandibular organ inhibiting hormones (MOIHs), and they have recently been isolated from the sinus gland, sequenced and cloned in *L. emarginata* (Liu and Laufer, 1996; Liu et al., 1997a,b) and in *Cancer pagurus* (Wainwright et al., 1996).

The present study uses eyestalk ablation to induce moulting. Ecdysteroids and MF levels were measured to determine which male morphotypes in *L. emarginata* are anecdysic and sexually mature.

2. Material and methods

2.1. Animals

L. emarginata were collected in two locations, off the coast 2 km south of Woods Hole, MA and 1 km north of Vineyard Haven harbour, Martha's Vineyard, during the summers of 1993 and 1994; and 2–4 km off shore from New London, CT during the summer of 1995. Male morphotypes were identified and selected according to the length of their carapace (CL), small (CL $<$ 60 mm) or large (CL $>$ 60 mm); the condition of their exoskeleton, abraded (old shell) or unabraded (new shell); and claw (propodus) lengths (PL), small (PL $<$ CL) or large (PL $>$ CL). Hence, the following nomenclature was used: SUM = small-claw, unabraded, small-carapace male, with flexible exoskeleton covered with a scaly epicuticle; LUL = large-carapace, unabraded, large-claw male; LAL = large-carapace, abraded, large-claw male; and SAM = small-carapace, abraded, males, with heavily calcified exoskeleton. A total of 1402 animals were measured (carapace and propodus length), weighed and tagged, then held in tanks in the facilities of the Marine Biological Laboratory (Woods Hole, MA), or in the Marine Biological Laboratory of the University of Connecticut (Noank, CT). They were maintained separately in individual plastic cages on a shallow sea-table supplied with fresh running

seawater at ambient temperature (18 ± 2°C) and salinity (33.0 ± 1 ppt). The crabs were fed with fresh or frozen squid every other day ad libitum.

The animals were divided into two groups: intact eyestalk controls (742), and eyestalk-ablated (660). Eyestalks were removed with forceps after the crabs had been chilled on ice for 30 min; then the wounds were cauterized immediately to reduce mortality due to possible infection and bleeding. Eyestalk removal caused around 20% of mortality. Haemolymph samples and testes were taken from animals that were sacrificed on day 1 of the experiment and at various intervals up to day 73. Another two groups, one with intact eyestalks and the other with eyestalks ablated, were maintained until moulting occurred.

2.2. Reproductive index (RI)

Body weight was measured to ± 0.01 g. The testes, vas deferens, and accessory gland (Homola et al., 1991) were dissected and weighed separately to ± 0.01 g to determine their wet weight. The reproductive indices were calculated by dividing the sum of the components of the reproductive system by the body weight and multiplying by 100.

2.3. Haemolymph levels of MF

Prior to dissection, 2 ml samples of haemolymph were taken from the base of walking legs, using a 5-ml syringe with an 18-gauge needle. The samples were transferred to 15-ml borosilicate tubes containing ice-cold acetonitrile (5 ml) and 4% NaCl (2 ml). The samples were vigorously mixed, and then stored at -20°C if they were not extracted immediately. Prior to extraction, an aliquot (15–25 ng) of *cis–trans* MF, a nonbiologically active isomer, was added to each tube as an internal standard (Sagi et al., 1991a). The samples were extracted twice with 500 μl of hexane. The hexane fractions were pooled, then stored in tightly capped 1.5-ml amber glass vials at -20°C. Before analysis by High Performance Liquid Chromatography (HPLC), each sample volume was reduced by evaporation under nitrogen to approximately 100–250 μl. The whole sample was then injected into a Waters HPLC system (model 501 pump and Waters 486 absorbance detector set at 214 nm). *Cis–trans* and all-*trans* MF, the biological isomers, were separated using normal phase with a 5-μm, 100-A Microsorb-MV silica column, 4.6 mm × 25 cm (Rainin Instrument), and a running solvent of 1% diethyl ether in hexane (flow rate of 1.5 ml/min, 1000–1500 psi). Elution times for *cis–trans* and all-*trans* MF were determined by running a mixture of the isomers as external standards. Peak areas were calculated with Maxima 820 software. The amount of MF present in each sample was determined by comparing the peak area of the internal standard (*cis–trans* MF), which represented a known amount, to the peak area of the all-*trans* MF.

2.4. Radioimmunoassay of ecdysteroids

Radioimmunoassays were carried out as described by Chang and O'Connor (1979). The antibody was produced by Dr. Walter E. Bollenbacher and was described by

Soumoff et al. (1981). β-ecdysone 2-hemisuccinate was conjugated to thyroglobulin and the antiserum raised against this conjugate had a strong binding affinity for 20-hydroxyecdysone. The cross-reactivity was as follows: 50% competition occurs at about 250 pg for 25-deoxyecdysone, 325 pg for ecdysone, 600 pg for 20-hydroxyecdysone and $>$ 10,000 pg for 3-dehydroecdysone (Ernest S. Chang, personal communication).

A total of 10 μl of fresh haemolymph without pre-treatment were used, while 100 mg (wet weight) of testes were extracted twice with 1 ml of 80% ethanol (the insoluble material was removed by centrifugation ($1000 \times g$), then 40 μl of the supernatant was added to an Eppendorf tube and dried in a rotary concentrator). The aqueous haemolymph or the dry testes extracts were mixed with or dissolved in, respectively, 100 μl borate buffer (0.1 M boric acid, 0.1 M sodium tetraborate, 0.075 M NaCl, pH 8.4) containing approximately 12,000 dpm [^{3}H] α-ecdysone (82.80 Ci/mmol; New England Nuclear). Next, 100 μl of borate buffer containing a polyclonal ecdysteroid antiserum was added. Then the samples were incubated at 37°C for 30 min. The reaction was stopped by the addition of 200 μl of ice cold (4°C) saturated ammonium sulphate in borate buffer solution, followed by precipitation (20 min). Samples were then centrifuged for 20 min at $2300 \times g$, the supernatants were aspirated off, and the pellets rinsed in 50% saturated ammonium sulphate. The resuspended pellets were incubated and centrifuged, then the supernatant was removed as before. Rinsed pellets were then resuspended in 30 μl water and 1 ml of scintillation fluid (Packard) was added. After mixing, the samples were placed in standard scintillation vials and activity was counted in a scintillation counter. A standard curve was constructed by adding concentrations of 20-hydroxyecdysone (Sigma) from 25 to 4 ng to a series of incubation tubes. The resulting values correspond to the ecdysteroids detected by the antibody and they are expressed in 20-hydroxyecdysone equivalents. The background represents less than 3%. The recovery was over 80% and was tested with a water sample for the haemolymph and with muscle tissue for the testes.

2.5. Data analysis

The arithmetic means and standard errors for RIs, and MF and ecdysone levels detected in different experimental groups were determined from 7–10 individuals in each group. The significance of the differences found between the control group and de-eyestalked group was determined by Student's *t*-test at the $p < 0.05$ probability levels (Sokal and Rohlf, 1969) and Sigma Plot software.

3. Results

3.1. Moulting observations

Eyestalk intact control SUM animals moulted within 75.75 ± 2.23 days of the beginning of the experiment in early July, and ablated SUM moulted within 31.71 ± 2.38

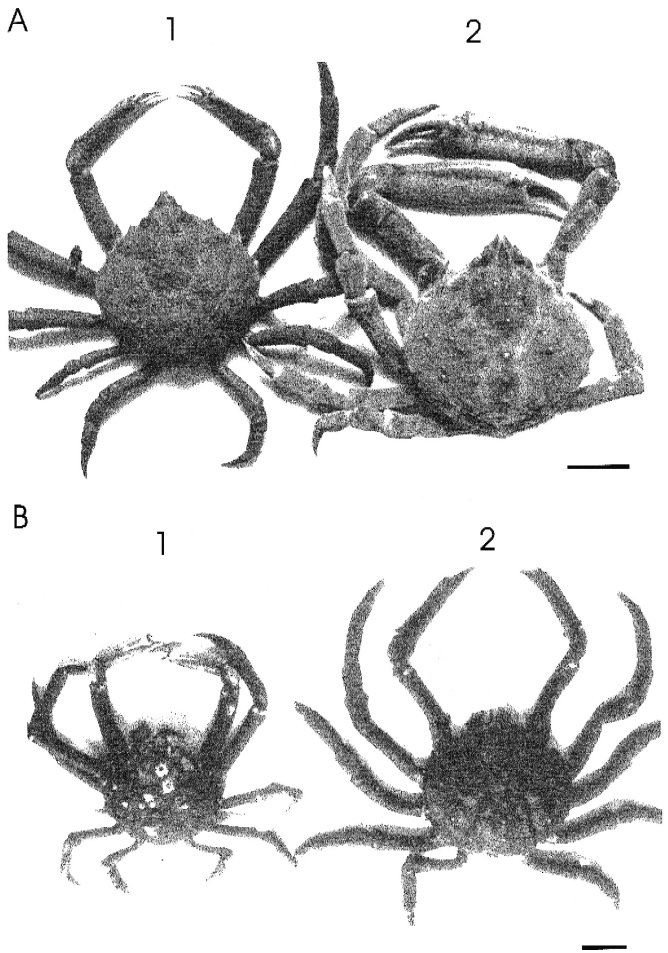

Fig. 1. Small unabraded male (SUM) of *L. emarginata* before moulting (1) and after moulting (2). (A) Intact control SUM. (B) Eyestalk-ablated SUM. Note that control SUM moulted in a large claw animal (mature) while ablated SUM moulted in a small claw animal (juvenile). Bars represent 3 mm.

days of the beginning of the experiment. All intact SUM moulted into an animal that presented a large claw (Fig. 1A) while all ablated SUM moulted into a crab that showed a small claw (Fig. 1B). None of the SAM, LUL and LAL males moulted, even the males in which the eyestalk had been removed for 2 months.

3.2. Reproductive index

The RIs of intact and ablated morph males of *L. emarginata* in each group of sampling ($n = 6–10$) were calculated (Fig. 2). No significant differences were observed

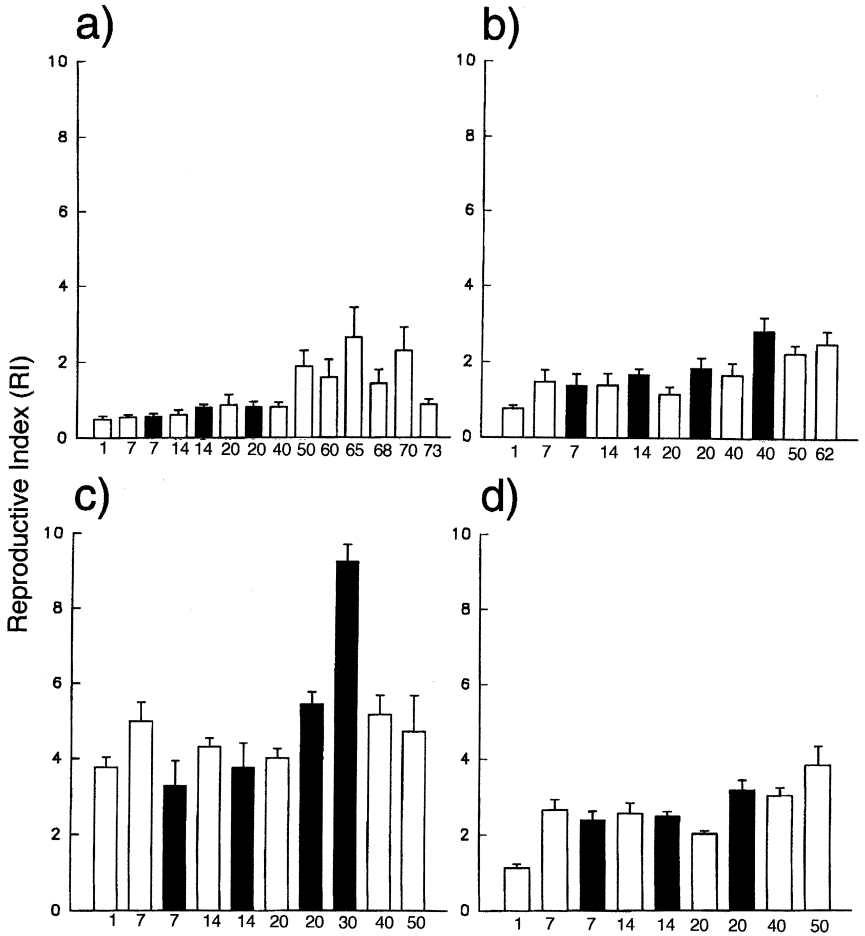

Fig. 2. The RI in four types of *L. emarginata* males. (a) Small unabraded male (SUM), (b) large unabraded large claw male (LUL), (c) small abraded male (SAM), and (d) large abraded large claw male (LAL). Animals were divided into two groups: intact eyestalk controls (white columns) and eyestalk ablated (black columns), and they were sacrificed on day 1 of the experiment and at various intervals up to day 73. The bars represent mean ± SEM of 6–10 values. No significant differences were observed between control and ablated animals.

between controls and ablated animals. The RIs for SUM are shown in Fig. 2a, and low values [0.80–2.64] were observed. The LUL had similar RI values [0.76–2.84] as shown in Fig. 2b. The SAM and LAL RIs were significantly higher, with values of [3.28–9.24] for the SAM (Fig. 2c) and [1.14–3.84] for the LAL (Fig. 2d).

3.3. MF levels

A significant increase in MF levels was observed in SUM, SAM and LUL de-stalked animals compared to intact control group, while no differences were observed in LAL

individuals. LAL crabs presented the highest level of MF [1.67–2.23 ng/ml], which was only reached by LUL and SAM morphs when they were ablated (Fig. 3). The MF level measured in SUM crabs was the lowest: levels in intact animals were [0.10–0.21 ng/ml] of MF and they increased significantly in the SUM-ablated group on day 7 (0.75 ± 0.15 ng/ml hemolymph), on day 14 (0.54 ± 0.13 ng/ml), and on day 21 (0.89 ± 0.08 ng/ml) (Fig. 3a). The MF level measured on day 1 in LUL crabs was (0.56 ± 0.15 ng/ml) and remained low in intact animals [0.17–0.57 ng/ml]. A significantly higher MF level was measured in the LUL-ablated group on day 7 (1.63 ± 0.19 ng/ml), on day 14 (1.46 ± 0.21 ng/ml) and on day 20 (0.75 ± 0.20 ng/ml) (Fig. 3b). MF titers measured in SAM crabs were higher than in SUM and LUL animals. The MF level in control SAM increased from day 1 (0.51 ± 0.06 ng/ml) to day 50 (0.95 ± 0.22 ng/ml). Significantly higher MF levels in de-stalked SAM crabs than in control animals on day 1 were measured on day 7 (2.02 ± 0.44 ng/ml) and on day 14 (2. 22 ± 0.44 ng/ml) and the highest MF titre was measured on day 20 (2.27 ± 0.36 ng/ml) (Fig. 3c).

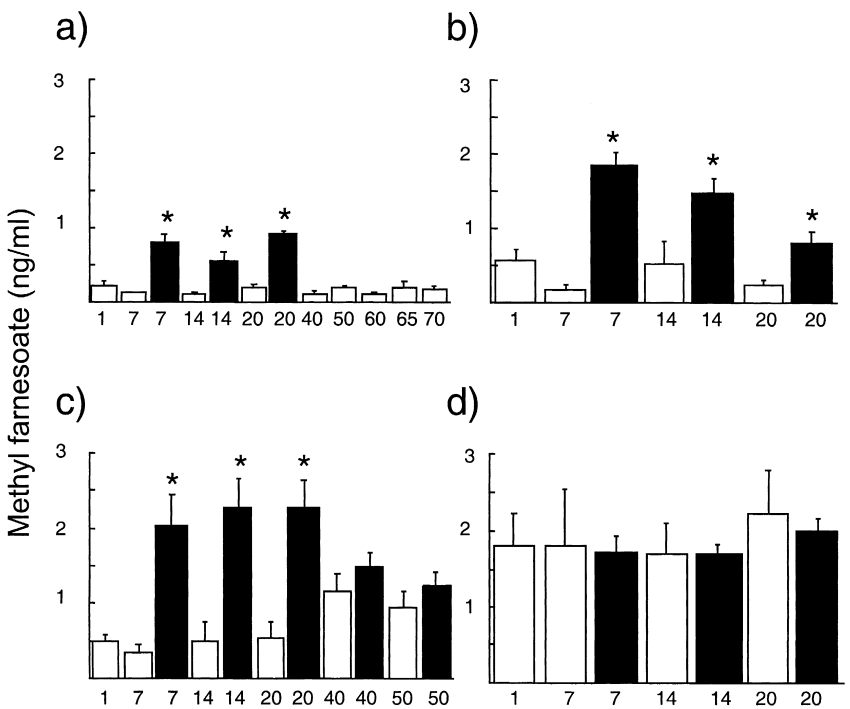

Fig. 3. Circulating levels of methyl farnesoate (ng/ml) of four types of *L. emarginata* males. (a) Small unabraded male (SUM), (b) large unabraded large claw male (LUL), (c) small abraded male (SAM), and (d) large abraded large claw male (LAL). Animals were divided into two groups: intact eyestalk controls (white columns) and eyestalk ablated (black columns). Haemolymph samples were taken from animals that were sacrificed on day 1 of the experiment and at various intervals up to day 70. The bars represent mean ± SEM of 6–10 values. Significance of differences between control and ablated animals are shown by an asterisk ($p < 0.05$).

3.4. Ecdysteroid levels

Ecdysteroid levels were measured in the haemolymph (Fig. 4) and in the testes (Fig. 5). In the haemolymph, ecdysteroid levels in control SUM were low [1.23–15.56 ng/ml] until day 68, they peaked on day 70 to 32.44 ± 14.26 ng/ml, and then decreased on day 73 (10.59 ± 6.08 ng/ml), which was just before moulting (day 75). In the ablated SUM, the ecdysteroid levels increased more quickly than in controls. By

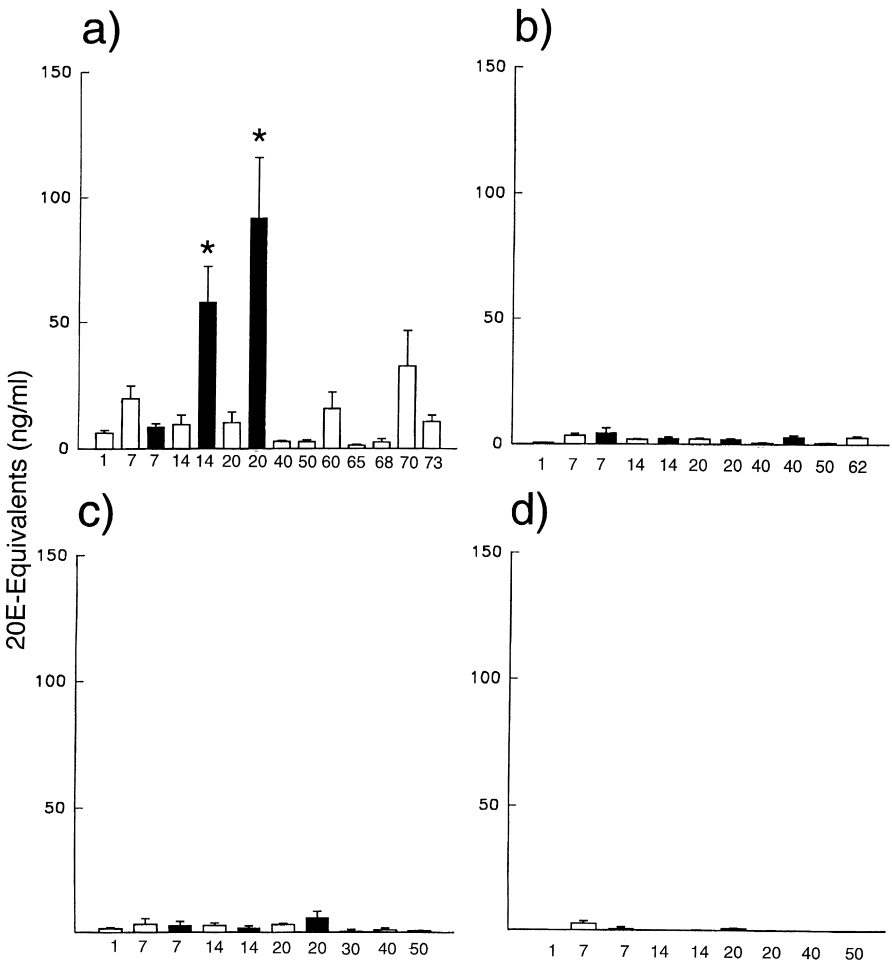

Fig. 4. Circulating levels of ecdysteroids, expressed as 20-hydroxyecdysone equivalents (ng/ml), in the haemolymph of four types of *L. emarginata* males. (a) Small unabraded male (SUM), (b) large unabraded large claw male (LUL), (c) small abraded male (SAM), and (d) large abraded large claw male (LAL). Animals were divided into two groups: intact eyestalk controls (white columns) and eyestalk ablated (black columns). Haemolymph samples were taken from animals sacrificed on day 1 of the experiment and at various intervals up to day 73. The bars represent mean ± SEM of 6–10 values. Significance of differences between control and ablated animals are shown by an asterisk ($p < 0.05$).

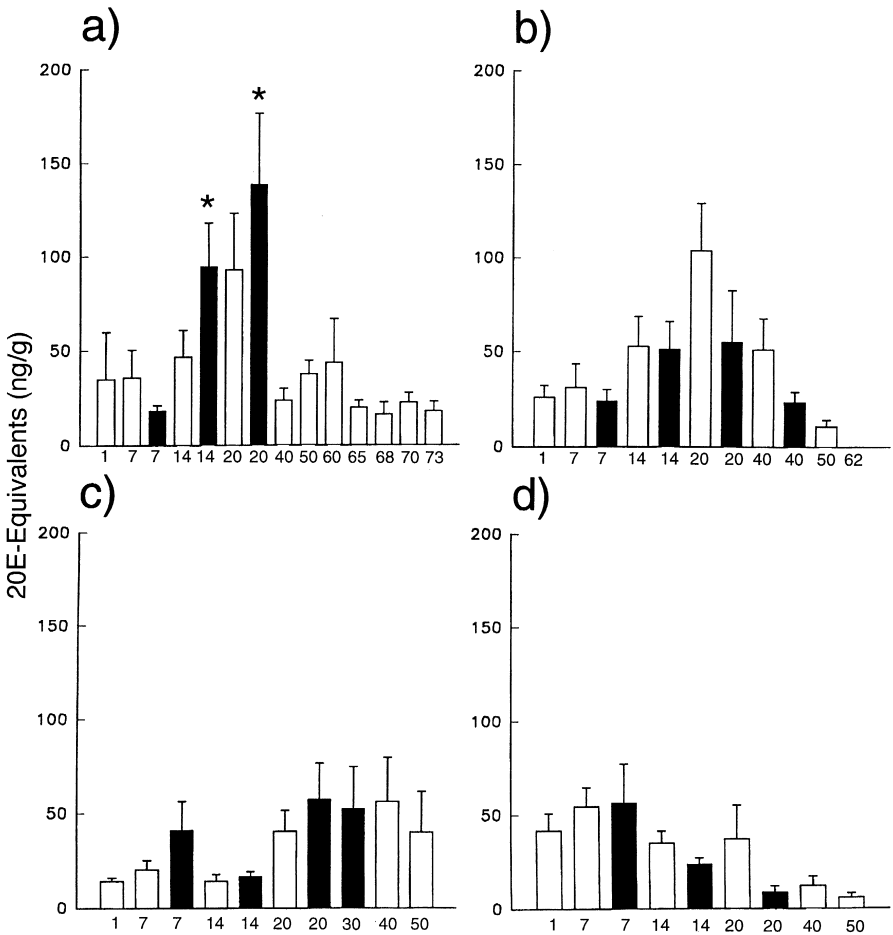

Fig. 5. Ecdysteroid levels expressed as 20-hydroxyecdysone equivalents (ng/g), in the testes of four types of *L. emarginata* males. (a) Small unabraded male (SUM), (b) large unabraded large claw male (LUL), (c) small abraded male (SAM), and (d) large abraded large claw male (LAL). Animals were divided into two groups: intact eyestalk controls (white columns) and eyestalk ablated (black columns). Testes were taken from animals sacrificed on day 1 of the experiment and at various intervals up to day 73. The bars represent mean ± SEM of 6–10 values. Significance of differences between control and ablated animals are shown by an asterisk ($p < 0.05$).

day 7, the haemolymph ecdysteroids had increased to 8.60 ± 1.22 ng/ml; by day 14 to 58.17 ± 14.31 ng/ml; and by day 20 to 91.73 ± 24.22 ng/ml (Fig. 4a). All the other morphotypes (LUL, SAM and LAL) had basal levels of ecdysteroids in the haemolymph during the entire study [0.37–6.63 ng/ml] (Fig. 4b,c and d).

In the testes, ecdysteroid concentrations increased significantly in ablated SUM (day 7, 17.89 ± 2.97 ng/g; day 14, 94.56 ± 23.30 ng/g; and day 20, 138 ± 37.64 ng/g). In the control SUM, the range of ecdysteroid levels was [15.88–46.88 ng/g] (Fig. 5a). No significant differences in gonad ecdysteroids were observed between intact and ablated

individuals of the three morphotypes (LUL, SAM and LAL), and the amounts detected were similar to those of the control SUM [5.84–54.94 ng/g] (Fig. 5b,c and d).

4. Discussion

We tested the effect of eyestalk removal on moult induction in four male morphotypes of the spider crab *L. emarginata*. As has been shown before, eyestalk ablation in crabs that are still growing, like the SUM, reduced the intermoult period. Ablation failed to induce moulting in the other morphotypes (LAL, LUL and SAM), suggesting that they have lost the ability to moult and are anecdysic. Males with abraded exoskeletons have been anecdysic for at least a year demonstrated by the presence of epibionts on the exoskeleton and their epicuticle is worn away. The exoskeletons of unabraded morphs, which have recently moulted, are covered by a thick velvety epicuticle (Homola et al., 1991). LUL are unabraded morphs (newly moulted) but, as described by Aldrich (1974), they are morphologically mature males. The morphological allometry also made it possible to recognise small size mature crabs. Small crabs appear to live for several years, as evidenced by several layers of barnacles in rocky habitats, while large crabs in the mud were never found with more than a few scattered barnacles, an indication that they may not have lived as long as the small males. Thus, Aldrich (1974) proposed two reproductive strategies: small size (and small number of larvae in the case of females) coupled with several reproductive years, and large size coupled with a shorter reproductive period. He also observed that the smallest mature males mated with mature females when large crabs were absent. Aldrich (1974) suggested that all morphologically and behaviourally mature morphs, either large claw or small abraded, were terminally anecdysic.

The X-organ sinus gland complex in the eyestalk synthesises two groups of peptides, the Crustacean Hyperglycemic Hormone (CHH) neuropeptide family and the chromatophorotropines (Van Herp and Soyez, 1997). The primary physiological roles of the CHH, Gonad-Inhibiting Hormone (GIH), Moult-Inhibiting Hormone (MIH) and MOIH are now recognised. Nevertheless, a multifunctional role of these peptides is quite clear (Van Herp, 1998). With regard to a regulation of moulting, the MIH acts mainly by inhibiting the synthesis of ecdysteroids by the Y-organ, but CHH also has an inhibitory effect on the synthesis of this hormone, although CHH was much less effective than MIH in this respect (Webster, 1998). Then, eyestalk ablation removes the source of inhibition of the Y-organs, resulting in increased ecdysteroid levels in the haemolymph (Chang, 1997). In *L. emarginata*, eyestalk ablation only increased ecdysteroids in SUM while in LUL, LAL and SAM ecdysteroid levels in the haemolymph remained basal. The concentrations of ecdysteroids in the haemolymph of juvenile crabs were higher than those found in morphometrically mature animals in *C. opilio* males (Cormier et al., 1992) and in *L. emarginata* females (Laufer and Deak, 1995). In addition, Vernet-Cornubert (1960) and Hinsch (1972) observed for *P. tetraodon* and *L. emarginata*, respectively, that ablation of the eyestalks results in ecdysis in juvenile individuals, but not in adults. These results, plus the moulting capacities of SUM, suggest that this morphotype may be a juvenile form, while the abraded and large claw males, in which

moulting was not observed, may be mature morphotypes with different reproductive strategies, as suggested by Aldrich (1974). The low concentrations of ecdysteroids observed in the anecdysic morphotypes are necessary for basal growth or regeneration as suggested by Hopkins (1992) in *Uca pugilator*, or for repairing cuticular damage as proposed by Halcrow and Steel (1992) in *C. opilio*. Since the Y-organs degenerate or are permanently inhibited in anecdysic forms (Carlisle, 1957; Chaix et al., 1976), the higher levels of ecdysteroids observed in the testes of the mature morphotypes could be synthesised in the testes themselves. Ecdysteroids have been found in ovaries, eggs and testes of prawns, lobsters and crabs (Chang et al., 1976; Chaix and De Reggi, 1982; Laufer et al., 1988; Goudeau et al., 1990; Laufer and Deak, 1995). They have been shown to enhance cell proliferation in primary cultures of lobster testes (Brody and Chang, 1989), and stimulate DNA synthesis in the testes of the freshwater prawn *M. rosenbergii* (Sagi et al., 1991b), possibly during early phases of spermatocyte proliferation.

Eyestalk ablation removes the source of inhibition not only of the Y-organs but also of the MO. In fact, eyestalk removal has been shown to increase MF hemolymph titers in juvenile or adult spider crab *L. emarginata* (Laufer et al., 1986, 1987), and *H. americanus* and *Orconectes virilis* (Tsukimura et al., 1989).

This report presents evidence of a relationship between the levels of MF in the hemolymph and morphogenesis of small-claw, small-carapace, unabraded male of the spider crab *L. emarginata*. Control SUMs that had low levels of MF are moulted into males with large claws while ablated SUMs, whose levels of MF were 4-fold those of the controls prior to ecdysis, moulted in a male with small claws. Because allometric differentiation of the chelae at maturity has been described in Majid crabs (Teissier, 1933, 1935; Hartnoll, 1963; Conan and Comeau, 1986; Conan et al., 1988; Comeau et al., 1991) these results indicate that SUM morphotype may represent the juvenile population. SAM, LUL and LAL never moulted and presented higher levels of MF, suggesting that they are mature morphotypes and the removal of their eyestalks enhances testis growth. Eyestalk removal causes hypertrophy and changes in the ultrasructure of the MO in larvae (Le Roux, 1983) and adults (Byard et al., 1975; Bazin, 1976; Hinsch, 1977), further showing that the MO is stimulated by ablation. However, eyestalk ablation did not produce an increase in MF level in LAL crabs, and this morphotype presented the highest levels of MF. Sagi et al. (1994) propose that LAL are the primary reproductive males with high circulating and synthetic rates of MF.

Recently, Laufer et al. (1998) demonstrated that the administration of MF in the crayfish *Procambarus clarkii* stimulates ovarian maturation. Treatment with hormones to induce reproduction should be done, taking the different morphotypes into account. Hence, the physiological characterisation of these morphotypes represents important information for the successful manipulation of growth and reproduction of this crustacean species in culture.

5. Conclusion

L. emarginata presents a clear polymorphism in males, and from the four morphotypes studied, we can conclude that: (1) SUM are able to moult, and represent the

juvenile stage, (2) SAM, LUL and LAL are terminally moulted and may represent the reproductive population, (3) circulating ecdysteroids induce moulting in juveniles, (4) high levels of ecdysteroids in the testes of SAM, LUL and LAL suggest a gonadal function, and (5) circulating MF controls morphogenesis of juveniles (SUM) and may stimulate gonads in adults as observed in insects.

Acknowledgements

The authors thank Dr. E.S. Chang (Bodega Marine Laboratory, Bodega Bay, CA) for the gift of the ecdysteroid antiserum that was produced by Dr. W.E. Bollenbacher (University of North Carolina Chapel Hill). The research reported here was supported by the Sea Grant College Program (NOAA) and by a grant from the Spanish Government to the first author.

References

Aldrich, J.C., 1974. Allometric studies on energy relationships in the spider crab *Libinia emarginata* (Leach). Biol. Bull. 147, 257–273.

Bazin, F., 1976. Mise en évidence des caractéres cytologiques des glandes steroidogenes dans les glandes mandibulaires et les glandes Y du crabe *Carcinus maenas* (L.) normal et épedonculé. C.R. Acad. Sci. Paris, Ser. D 282, 739–741.

Brody, M.D., Chang, E.S., 1989. Development and utilization of crustacean long-term primary cell cultures: ecdysteroids effects in vitro. Int. J. Invertebr. Reprod. Dev. 16, 141–147.

Burns, B.G., Sangalang, G.B., Freeman, H.C., McMenemy, M., 1984. Isolation and identification of testosterone from the serum and testes of the American lobster, *Homarus americanus*. Gen. Comp. Endocrinol. 54, 429–432.

Byard, E.H., Shivers, R.R., Aiken, D.E., 1975. The mandibular organ of the lobster, *Homarus americanus*. Cell Tissue Res. 162, 13–22.

Carlisle, D.B., 1957. On the hormonal inhibition of molting in decapod crustacea: II. Terminal anecdysis in crabs. J. Mar. Biol. Assoc. U. K. 36, 291–307.

Chaix, J.C., De Reggi, M., 1982. Ecdysteroid levels during ovarian development and embryogenesis in the spider crab *Acanthonyx lunulatus*. Gen. Comp. Endocrinol. 47, 7–14.

Chaix, J.C., Trilles, J.P., Vernet, G., 1976. Dégénérescence de l'organe Y chez les mâles pubères d'*Acanthonyx lunulatus* Crustacea Decapoda Oxyrhyncha). C.R. Acad. Sci. Paris, Ser. D 283, 523–525.

Chang, E.S., O'Connor, J.D., 1979. Arthropod molting hormones. In: Joffe, B.M., Joffe, H.R. (Eds.), Methods of Hormone Radioimmunoassay. Academic Press, New York, pp. 797–814.

Chang, E.S., Sage, B., O'Connor, J.D., 1976. The qualitative and quantitative determinations of ecdysones in tissues of the crab, *Pachygrapsis crassipes*, following molt induction. Gen. Comp. Endocrinol. 30, 21–33.

Chang, E.S., 1997. Chemistry of crustacean hormones that regulate growth and reproduction. In: Fingerman, M., Nagabushanam, R., Thompson, M.F. (Eds.), Recent Advances in Marine Biotechnology vol. 1 Oxford and IBH Publishing, New Delhi, Calcutta, pp. 163–178.

Charmantier, G., Ollé, M., Trilles, J.P., 1977. Évolution du taux d'ecdystérone, dégénérescence des organes Y et sénescence chez les mâles pubères de *Sphaeroma serratum*. C. R. Acad. Sci. Paris, Ser. D 285, 1487–1489.

Charmantier, G., Trilles, J.P., 1979. La dégénérescence de l'organe Y chez *Sphaeroma serratum* (Fabricius, 1987) (Isopoda, Flabellifera): étude ultrastructurale. Crustaceana 36, 29–38.

Charmantier, G., Charmantier-Daures, M., Aiken, D.E., 1988. Larval development and metamorphosis of the American lobster *Homarus americanus* (Crustacea, Decapoda): effect of eyestalk and juvenile hormone injection. Gen. Comp. Endocrinol. 70, 319–333.

Comeau, M., Conan, G., Robichaud, G., Jones, A., 1991. Life history patterns and population fluctuations of snow crab *Chionoecetes opilio* in the fjord of Bonne Bay on the west coast of Newfoundland, Canada from 1983 to 1990. Can. Tech. Rep. Fish. Aquat. Sci. 1817, 73.

Conan, G., Comeau, M., 1986. Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio*. Can. J. Fish. Aquat. Sci. 43, 1710–1719.

Conan, G., Comeau, M., Cormier, R., 1988. Reply to Donaldson and Johnson. Can. J. Fish. Aquat. Sci. 45, 1501–1503.

Cormier, R.J., Fraser, A.R., Bailey, R.F.J., Raymond, N., 1992. Hemolymph ecdysone concentrations as a function of sexual maturity in the male snow crab (*Chioecetes opilio*). Can. J. Fish. Aquat. Sci. 49, 1619–1623.

Costlow, J.D., 1966. The effect of eyestalk extirpation on metamorphosis of megalops of the blue crab, *Callinectes sapidus* Rathbun. Comp. Endocrinol. 3, 120–130.

Costlow, J.D., 1968. Metamorphosis in crustaceans. In: Etkin, W., Gilbert, L.I. (Eds.), Metamorphosis: A Problem in Developmental Biology. Appleton-Century-Crofts, New York, pp. 3–41.

Couch, E.F., Hagino, N., Lee, J.W., 1987. Changes in estradiol and progesterone immunoreactivity in tissues of the lobster, *Homarus americanus*, with developing and immature ovaries. Comp. Biochem. Physiol. A 85, 765–770.

Dawe, E.G., Taylor, D.M., Hoenig, J.M., Warren, W., Ennis, G.P., Hooper, R.G., Donaldson, W.E., Paul, A.J., Paul, J.M., 1991. A critical look at the idea of terminal molt in male snow crab (*Chionoecetes opilio*). Can. J. Fish. Aquat. Sci. 48, 2266–2275.

Fairs, N.J., Evershed, R.P., Quinlan, P.T., Goad, L.J., 1989. Detection of unconjugated and conjugated steroids in the ovary, eggs, and hemolymph of decapod crustacean *Nephrops norvegicus*. Gen. Comp. Endocrinol. 4, 199–208.

Freeman, J.A., Costlow, J.D., 1980. The molt cycle and its hormonal control in *Rhithropanoeus harristii* larvae. Dev. Biol. 74, 479–485.

Goudeau, M., Lachaise, F., Carpentier, G., Goxe, B., 1990. High titers of ecdysteroids are associated with secretory process of embryonic envelopes in the European lobster. Tissue Cell 22, 269–281.

Halcrow, K., Steel, C.G.H., 1992. Cuticular repair in the mature male snow crab, *Chionoecetes opilio* (Majidae; Crustacea): relation to ecdysteroids. Can. J. Zool. 70, 314–319.

Hartnoll, R.G., 1963. The biology of Manx spider crabs. Proc. Zool. Soc. London 141, 423–496.

Hinsch, G.W., 1972. Some factors controlling reproduction in the spider crab, *Libinia emarginata*. Biol. Bull. 143, 358–366.

Hinsch, G.W., 1977. Fine structural changes in the mandibular gland of the male spider crab, *Libinia emarginata*. (L.), following eyestalk ablation. J. Morphol. 154, 307–316.

Homola, E., Sagi, A., Laufer, H., 1991. Relationship of claw form and exoskeleton condition to reproductive system size and methyl farnesoate in the male spider crab, *Libinia emarginata*. Invertebr. Reprod. Dev. 20, 219–225.

Hopkins, P.M., 1992. Hormonal control of the molt cycle fiddler crab *Uca pugilator*. Am. Zool. 32, 450–458.

Jeng, S.S., Wan, W.C.M., Chang, C.F., 1978. Existence of an estrogen-like compound in the ovary of the shrimp *Parapenaeus fissurus*. Gen. Comp. Endocrinol. 16, 211–214.

Landau, M., Laufer, H., Homola, E., 1989. Control of methyl farnesoate synthesis in the mandibular organ of the crayfish *Procambarus clarkii*: evidence for peptide neurohormones with dual functions. Invertbr. Reprod. Dev. 16, 165–168.

Laufer, H., Landau, M., Borst, D., Homola, E., 1986. The synthesis and regulation of methyl farnesoate, a new juvenile hormone for crustacean reproduction. In: Porchet, M., Andries, J.C., Dhainaut, A. (Eds.), Advances in Invertebrate Reproduction vol. 4 Elsevier, Amsterdam, pp. 135–143.

Laufer, H., Borst, D.W., Baker, F.C., Carrasco, C., Sinkus, M., Reuter, C.C., Tsai, L.W., Scooley, D.A., 1987. Identification of a Juvenile hormone-like compound in a crustacean. Science 235, 202–205.

Laufer, H., Borst, D.W., Foley, T.A., Landau, M., 1988. Ecdysteroid titres in vitellogenic *Libinia emarginata*. J. Insect. Physiol. 34, 615–617.

Laufer, H., Wainwright, G., Young, N.J., Sagi, A., Ahl, J.S.B., Rees, H.H., 1993. Ecdysteroids and juvenoids in two male morphotypes of *Libinia emarginata*. Insect Biochem. Mol. Biol. 23, 171–174.

Laufer, H., Deak, P., 1995. Ecdysteroid in maternal and embryonic *Libinia emarginata*. Invertebr. Reprod. Dev. 28, 121.

Laufer, H., Biggers, W.J., Ahl, J., 1998. Stimulation of ovarian maturation in the crayfish *Procambarus clarkii* by methyl farnesoate. Gen. Comp. Endocrinol. 111, 113–118.

Le Roux, A., 1983. Réactions de L'organe mandibulaire à l'ablation des pédoncules oculaires chez les larves et les juvéniles de *Palaemonetes varians* (Leach) (Decapoda, Natantia). C. R. Acad. Sci. Paris 296, 697–700.

Le Roux, A., 1984. Quelques effects de l'ablation des pédoncules oculaires sur les larves et les premiers stades juvéniles de *Palaemonetes varians* (Leach) (Decapoda, Palaemonidae). Bull. Soc. Zool. Fr. 109, 43–60.

Lisk, R.D., 1961. Estradiol-17β in the eggs of the American lobster, *Homarus americanus*. Can. J. Biochem. Physiol. 39, 659–663.

Little, G., 1969. The larval development of the shrimp, *Palaemon macrodactylus* Rathbun, reared in the laboratory, and the effect of eyestalk extirpation on development. Crustaceana 17, 69–87.

Liu, L., Laufer, H., 1996. Isolation and characterization of sinus gland neuropeptides with both mandibular organ inhibiting and hyperglycemic effects from the spider crab *Libinia emarginata*. Arch. Insect Biochem. Physiol. 32, 375–385.

Liu, L., Laufer, H., Wang, Y., Hayes, T., 1997a. Neurohormone regulating both methyl farnesoate synthesis and glucose metabolism in a crustacean. Biochem. Biophys. Res. Commun. 237, 694–701.

Liu, L., Laufer, H., Gogarten, P.J., Wang, M., 1997b. CDNA cloning of a mandibular organ inhibiting hormone from the spider crab *Libinia emarginata*. Invertbr. Neurosci. 3, 199–204.

Paul, A.J., Paul, J.M., 1995. Molting of functionally mature male *Chionoecetes bairdi* Rathbun (Decapoda: Majidae) and changes in carapace and chela measurements. J. Crustacean Biol. 15, 686–692.

Sagi, A., Homola, E., Laufer, H., 1991a. Methyl farnesoate in the prawn *Macrobrachium rosenbergii*: synthesis by the mandibular organ in vitro, and titers in the hemolymph. Comp. Biochem. Physiol. 99B, 879–882.

Sagi, A., Karp, L., Milner, Y., Cohen, D., Kuris, A.M., Chang, E.S., 1991b. Testicular thymidine incorporation in the prawn *Macrobrachium rosenbergii*: molt cycle variation and ecdysteroids effects in vitro. J. Exp. Zool. 259, 229–237.

Sagi, A., Ahl, J.S.B., Danaee, H., Laufer, H., 1994. Methyl farnesoate levels in male spider crabs exhibiting active reproductive behavior. Horm. Behav. 28, 261–272.

Sokal, R.R., Rohlf, F.J., 1969. In: Introduction to Biostatistics. Freeman, San Francisco, CA, pp. 1–368.

Somerton, D.A., 1981. Regional variation in the size maturity of two species of tanner crab (*Chionoecetes bairdi* and *C. Opilio*) in the eastern Bering sea, and its use in defining management subareas. Can. J. Fish. Aquat. Sci. 38, 163–174.

Soumoff, C., Horn, D.H.S., O'Connor, J.D., 1981. Production of a new antiserum to arthropod moulting hormone and comparison with two other antisera. J. Steroid Biochem. 14, 429–435.

Spindler, K.D., Van Wormhoudt, A., Sellos, D., Spindler-Barth, M., 1987. Ecdysteroid levels during embryogenesis in the shrimp *Palaemon serratus* (Crustacea: Decapoda): quantitative and qualitative changes. Gen. Comp. Endocrinol. 66, 116–122.

Teissier, G., 1933. Étude de la croissance de quelques variants sexuels chez *Macropodia rostrata* L. Bull. Biol. Fr. Belg. 64, 401–444.

Teissier, G., 1935. Croissance des variants sexuels chez *Maia squinado*. Trav. Stn. Biol. Roscoff 13, 93–130.

Tsukimura, B., Martin, M., Frinsko, M., Borst, D.W., 1989. Measurement of methyl farnesoate (MF) levels in crustacean hemolymph. Am. Zool. 29, 49A.

Van Herp, F., 1998. Molecular, cytological and physiological aspects of the crustacean hyperglycemic hormone family. In: Coast, G.M., Webster, S.G. (Eds.), Recent Advances in Arthropod Endocrinology. Cambridge Univ. Press, Cambridge, pp. 53–70.

Van Herp, F., Soyez, D., 1997. Reproductive biology of invertebrates. In: Adiyodi, K.G., Adiyodi, R.G. (Eds.), Arthropoda–Crustacea vol. VIII Oxford and IBH Publishing, New Delhi, pp. 247–275.

Vernet-Cornubert, G., 1958. Biologie générale de *Pisa tetraodon* (Pennant). Bull. Inst. Oceanogr. (Monaco) 1113, 1–52.

Vernet-Cornubert, G., 1960. Influence de l'ablation des pédoncules oculaires sur la mue, la ponte et les caractères sexuels externes de *Pisa tetraodon* (Pennant). Bull. Inst. Oceanogr. (Monaco) 1186, 1–24.

Wainwright, G., Webster, S., Wilkinson, M.C., Chung, J.S., Rees, H., 1996. Structure and significance of mandibular organ inhibing hormone in the crab, *Cancer pagurus*. J. Biol. Chem. 271, 12749–12754.

Webster, S.G., 1998. Neuropeptides inhibiting growth and reproduction in crustaceans. In: Coast, G.M., Webster, S.G. (Eds.), Recent Advances in Arthropod Endocrinology. Cambridge Univ. Press, Cambridge, pp. 33–52.

Wilder, M.N., Okumura, T., Aida, K., 1991. Accumulation of ovarian ecdysteroids in synchronization with gonadal development in the giant freshwater prawn, *Macrobrachium rosenbergii*. Zool. Sci. 8, 919–927.

Young, N.J., Webster, S.G., Jones, D.A., Rees, H.H., 1991. Profile of embryonic ecdysteroids in the decapod crustacean, *Macrobrachium rosenbergii*. Invertbr. Reprod. Dev. 3, 201–212.

Young, N.J., Webster, S.G., Rees, H.H., 1993. Ecdysteroid profiles and vitellogenesis in *Penaeus monodon* (Crustacea: Decapoda). Invertbr. Reprod. Dev. 24, 107–118.