Facultative secondary lecithotrophy in the megalopa of the shrimp *Lysmata seticaudata* (Risso, 1816) (Decapoda: Hippolytidae) under laboratory conditions

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Certain decapod crustaceans can catabolize internal reserves to undergo partial or full larval development. This feature is termed secondary lecithotrophy, if energy used results from plankton derived organic matter accumulated by earlier larval stages. The present work reports the ability of Lysmata seticaudata megalopa to molt to the first juvenile stage in the absence of food. Unlike previous records of secondary lecithotrophy displayed by non-feeding last larval stages of hermit crabs and spiny lobsters, the megalopa of L. seticaudata retains its feeding capacity. This is the first time such a feature has been reported in decapods, and the term facultative secondary lecithotrophy is proposed. The build up of energy reserves continues during the last zoeal stage of L. seticaudata, with starved zoea IX failing to molt to megalopa. Energy reserves that enable starved megalopa to molt to juvenile seem to be partially depleted, with starved juveniles produced either from starved or fed megalopae being unable to molt to the next juvenile stage. The longer resistance of starved juveniles produced from fed megalopae (nine days), compared to that of starved juveniles produced from starved megalopae (five days), indicates that some energy reserves may pass to the juvenile, not being totally depleted at metamorphosis.

INTRODUCTION

Although the larval stages of decapod crustaceans generally require planktonic food to survive, certain species are able to rely on internal reserves to undergo partial or full larval development. This feature is commonly referred to as lecithotrophy, being considered an adaptation to low or unpredictable food production in the environment where larval development takes place (Anger, 2001). Two different types of lecithotrophy have been recognized in decapod crustaceans: primary lecithotrophy, where the energy used to develop through some or all postembryonic larval stages results from egg yolk reserves (reflecting the degree of female investment in reproduction); secondary lecithotrophy, where the energy used by a late non-feeding larval stage to molt to a juvenile (that resumes feeding activity) results from plankton derived organic matter accumulated by earlier larval stages (Anger, 1989).

Secondary lecithotrophy has been well documented for hermit crab species of the genus *Pagurus*, suggesting that it may be related to a high habitat specialization (e.g. the need for empty shells for hermit crabs

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megalopae to metamorphose) (Anger, 1989; Harvey, 1996). However, the occurrence of secondary lecithotrophy among spiny and slipper lobster species (McWilliam and Phillips, 1997; Booth *et al.*, 2005; George, 2005) cannot be explained by an extreme habitat specialization. Anger (2001) referred that the selective force involved in this phenomenon could be "a very long duration of the onshore transport across the continental shelf of tropical and subtropical regions". Additionally, Anger (2001) notes that in tropical and subtropical waters, "the production of large plankton or small nekton with suitable size and accessibility may be insufficient for the nutrition of the large nektonic pueruli". Nonetheless, despite these hypotheses, a wider and more conclusive explanation for the occurrence of secondary lecithotrophy in decapods is still missing.

If, as suggested by Anger (2001), the selective force for the occurrence of secondary lecithotrophy is the long duration of onshore transport, it is possible that other decapod larvae that attain large sizes and have long larval periods can also display such a feature. Caridean shrimps of the genus *Lysmata* display larval periods that range from 20 to over 150 days and are able to delay metamorphosis through the occurrence of mark-time molting [defined by Gore (1985) as a sequence of molts displayed by a certain larval stage with few morphological changes, despite a considerable increase in larval size] (Calado *et al.*, 2003a). According to Gurney (1942), the long paddle shaped pereiopods displayed by *Lysmata* larvae, as well as their large sizes (giant larvae), suggest that they may be well adapted to dispersal in oceanic waters.

The present work investigates, under laboratory conditions, the existence of secondary lecithotrophy in the megalopa of *Lysmata seticaudata*, with the term megalopa being used *sensu* Williamson (1969), as the equivalent to the larval stage designated by glaucothoe for anomuran crabs and puerulus or nisto for spiny and slipper lobsters, respectively.

METHOD

Larvae of *L. seticaudata* were hatched in the laboratory from wild ovigerous shrimps collected during the month of July 2006 in Sagres (southwestern coast of Portugal), using baited traps described by Calado and Narciso (2004). The most active larvae (those displaying pronounced positive phototactic responses) from five different ovigerous shrimps were selected in groups of 400 larvae and were transferred to three cylindrico-spherical 20 L rearing tanks (larval density of 20 larvae L^{-1}) modified from those described by Calado *et al.* (2003b). Larvae were cultured using the protocols that maximized survival and settlement synchronism described in detail by Calado *et al.* (2005). Artificial seawater was prepared using freshwater purified using reverse osmosis and mixed with the salt Crystal Sea® produced by Marine Enterprises International®, following the instructions of the manufacturer. Salinity was maintained at 35 ± 1‰ and temperature was kept at 24 ± 1°C through a heating/cooling system. Ammonia and nitrite were monitored daily and maintained below detectable levels. Nitrate and pH showed average values ($\pm$ standard deviation, SD) of 3 (± 2.5) mg L^{-1} and 8.2 (± 0.1), respectively. The tanks were illuminated from above with fluorescent light, with a photoperiod of 14 h light: 10 h dark. *Lysmata seticaudata* larval stages were identified according to the descriptions provided by Calado *et al.* (2004).

The following experiments were performed exclusively using individual rearing techniques in 200 ml Petri dishes, in order to exclude cannibalism as a potential food source, with their water temperature being kept stable at 24 ± 1°C with the help of a water bath:

Experiment 1: ability of zoea IX to molt to megalopa in the absence of food

After detecting the first larvae at the eighth zoeal stage, larval development was monitored every hour and 60 larvae were selected immediately after molting to the ninth zoeal stage. Selected larvae at the ninth zoeal stage were removed from the culture tanks, observed under a stereomicroscope for translucent guts (assuring no larval prey had been ingested) and each one was placed under starvation in a Petri dish with 200 mL of 1 μm filtered artificial seawater. The Petri dishes were inspected daily, 100% of their water being replaced by fresh 1 μm filtered artificial seawater, with survival and ability to molt to megalopa also being recorded.

Experiment 2: ability of megalopa to molt to juvenile in the presence or absence of food

Larvae remaining in the 20 L culture tanks were allowed to continue their development and were monitored every hour, in order to detect newly metamorphosed megalopae. One hundred and twenty of those newly metamorphosed megalopae were selected in two groups of 60 individuals each and were also observed under a stereomicroscope for translucent guts (again to assure no larval prey had been ingested). Each megalopa of group 1 was placed under starvation in a Petri dish with 200 mL of 1 μm filtered artificial seawater.

The megalopa of group 2 were placed individually in a Petri dish with 200 mL of 1 μm filtered artificial seawater and 50 newly hatched *Artemia* nauplii.

The Petri dishes were inspected daily, with 100% of their water being replaced by new 1 μm filtered artificial seawater, and 50 newly hatched *Artemia* nauplii being added to each group 2 megalopae. Survival and ability to molt to the juvenile stage was recorded daily for all megalopae. Additionally, the number of uneaten larval prey and gut coloration of group 2 megalopae were also monitored under a stereomicroscope.

Experiment 3: ability of first stage juvenile to molt to second stage juvenile in the presence or absence of food

Immediately after molting, juveniles produced from megalopa under starvation (see Experiment 2) were divided into two equal groups: group A juveniles were starved in Petri dishes (under the same conditions as earlier described for megalopa in Experiment 2), in order to evaluate the existence of any further energetic reserves that enabled them to molt to the second juvenile stage; each group B juvenile was placed in a Petri dish with 200 mL of 1 μm filtered artificial seawater and 50 newly hatched *Artemia* nauplii to verify if they resumed their feeding activity and molted to the second juvenile stage.

Juveniles produced from megalopa in the presence of food (see Experiment 2) were also immediately divided into two equal groups: each group C juvenile was starved in a Petri dish, to verify if they were able to molt to the second juvenile stage, while each group D juvenile was placed individually in a Petri dish with 200 mL of 1 μm filtered artificial seawater and 50 newly hatched *Artemia* nauplii. All Petri dishes were inspected daily, with 100% of their water being replaced by new 1 μm filtered artificial seawater, and 50 newly hatched *Artemia* nauplii being added to groups B and D juveniles. Survival and ability to molt to the second juvenile stage was recorded daily for all groups.

RESULTS

Experiment 1: ability of zoea IX to molt to megalopa in the absence of food

None of the larvae at the ninth zoeal stage were able to metamorphose to megalopa when placed under starvation and all died after 4 or 5 days (12 and 88%, respectively).

Experiment 2: ability of megalopa to molt to juvenile in the presence or absence of food

All 60 megalopae of group 1 placed under starvation were able to successfully molt to the juvenile stage after 3, 4 or 5 days (20, 45 and 35%, respectively). All megalopae of group 2 placed in the presence of *Artemia* nauplii were also able to molt to the juvenile stage after 3, 4 or 5 days (25, 45 and 30%, respectively). In group 2 megalopae, between 98 and 100% of all newly hatched *Artemia* nauplii were ingested daily by the megalopae, with all of them displaying an orange-brownish colored gut when observed under a stereomicroscope.

Experiment 3: ability of first stage juvenile to molt to second stage juvenile in the presence or absence of food

Newly metamorphosed juveniles produced from starved megalopa and kept under starvation (group A, $n = 30$) were unable to successfully molt to the second juvenile stage and all died after 5 days. All juveniles produced from starved megalopae and provided with larval prey (group B, $n = 30$) successfully fed and molted to the second juvenile stage after 5 days. Juveniles produced from fed megalopae but kept under starvation (group C, $n = 30$) failed to molt to the second juvenile stage and died after 9 days. All juveniles produced from fed megalopae and provided with food (group D, $n = 30$) ingested the *Artemia* nauplii and molted to the second juvenile stage after five days.

DISCUSSION

The experimental results show that the megalopa of *L. seticaudata* displays secondary lecithotrophy, successfully molting to the first juvenile stage in the absence of food. However, unlike other non feeding decapod larvae previously shown to display secondary lecithotrophy (Anger, 1989; McWilliam and Phillips, 1997), the megalopae of *L. seticaudata* retain their feeding capacity, being able to capture motile prey items (e.g. *Artemia* nauplii). This is the first time such feature has been recorded in larval decapods, and we suggest it to be termed as facultative secondary lecithotrophy.

The build up of energetic reserves, stored as lipids in the R cells of the hepatopancreas (according to Anger, 1991), continues to occur during the last zoeal stage of *L. seticaudata*, since newly molted zoea IX placed under starvation died before molting to megalopa. The energetic reserves that enable starved megalopa to molt to

the juvenile stage seem to be partially depleted during this process [see Nishida *et al.* (1995) for a description of structural changes in the hepatopancreas], with starved juveniles produced either from starved or fed megalopa being unable to molt to the next juvenile stage. Nevertheless, the longer resistance of starved juveniles produced from fed megalopae (nine days), when compared with that of starved juveniles produced from starved megalopae (five days), seems to indicate that some energy reserves are passed from the fed megalopae to the juveniles, not being totally depleted during metamorphosis.

Recently, Bauer (2006) suggested that *Lysmata* species that aggregate in groups and generally display shorter larval periods (such as *L. seticaudata*) may have evolved from ancestral relatives that live in pairs in coral reefs and display longer larval periods [e.g. *L. debelius* (Bruce, 1983) and *L. amboinensis* (De Man, 1888)]. *Lysmata seticaudata* occurs in temperate waters, displays the shortest larval development period and the lowest number of zoeal stages so far recorded in the genus (Calado *et al.*, 2004). In this way, facultative secondary lecithotrophy may also occur among other species of *Lysmata* with longer larval periods and inhabiting the oligotrophic waters of coral reefs. It is highly unlikely that facultative lecithotrophy in *L. seticaudata* has evolved from ancestors with a fully lecithotrophic last larval stage in oligotrophic waters, since the "barriers" to losing feeding ability are far less than those to regaining it (Strathmann, 1978, 1985). Although the existence of secondary lecithotrophy in *L. debelius* and *L. amboinensis* has never been investigated, culture trials of these species have demonstrated that the last zoeal stage delays the molt to megalopa, through the occurrence of mark-time molting, until suitable larval diets are provided (personal observations). For that reason, and as suggested for spiny lobsters (Lemmens, 1994, 1995; McWilliam and Phillips, 1997), it seems that only after reaching a critical level of stored energy will the last zoeal stage molt to the megalopa. Additionally, lower critical energy levels also appear to be required for the last zoeal stage of *L. seticaudata* to molt to megalopa, since it can perform such molt while fed exclusively on newly hatched *Artemia* nauplii, a diet clearly unsuitable for the reef species *L. debelius* and *L. amboinensis* (Calado *et al.*, 2003a).

In conclusion, although some compromises between swimming and feeding have been suggested for the larval form of marine invertebrates (Grünbaum and Strathmann, 2003; Strathmann and Grünbaum, 2006), the external morphology of *L. seticaudata* megalopa (described in detail by Calado *et al.*, 2004) does not seem to be different from that of other hippolytid shrimps. The present results reinforce the idea that developmental patterns among decapod crustaceans are certainly more complex and less generalized than often supposed. Future studies should address the existence of secondary lecithotrophy in other *Lysmata* species, namely those from coral reefs. Additionally, the existence of secondary lecithotrophy should also be investigated among other caridean and stenopodidean genus displaying long larval periods, allowing a wider examination of the existence of this feature among decapod crustaceans.

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