Development and functional morphology of the larval foregut of two brachyuran species from Northern Brazil

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ABSTRACT

Feeding is an important factor for the successful rearing of larvae of the crab species. Further information on the morphological features of the foregut may reveal larval feeding behaviour and/or whether there is a lecithotrophy in some or even in all stages of the larval cycle. In the present study, the structural development of the foregut and their digestive functions were examined in larvae of two brachyurans, Uca vocator and Panopeus occidentalis, reared in the laboratory. During larval development, the foreguts of the larvae in the first and last zoeal stages and in the megalopa stage were microscopically examined, described and illustrated. The zoeal foreguts of both species were well developed, showing specialization with a functional cardiopyloric valve and a filter press. The megalopa stage had a complex and specialized gastric mill similar to that found in adult crabs with the appearance of rigidly calcified structures. These results support the hypothesis that the feeding behaviour of each larval stage is directly related to the morphological structure of the foregut. Such facts strongly indicate that all larval stages of both U. vocator and P. occidentalis need an external food source before completing the larval development in a planktonic environment.

Key words: digestive system, megalopa, Panopeus occidentalis, stomach, Uca vocator, zoea.

INTRODUCTION

Feeding is an important factor that significantly affects the larvae of several brachyuran crab species, although an external food supply may not be necessary in the case of lecithotrophy, where some larvae reach the subsequent larval stage or even complete larval development without external food (Kittaka 1988, Nishida et al. 1990, Kittaka 1994, Anger et al. 1995, Abrunhosa and Kittaka 1997a, b, Nates and McKenney Jr 2000, Anger 2001). Some species, however, may present a facultative lecithotrophy when external food is not available (Anger et al. 1995, Thessalou-Legaki et al. 1999, Anger 2001). Therefore, further information concerning suitable feeding measures for larval rearing is needed, and this is critical for the successful rearing in the laboratory of this crab species.

Examinations of the behaviour and characteristics of larval morphology in species reared under laboratory conditions may contribute to this knowledge, and may also provide an understanding of the complex biological and ecological relationships which occur among these species in the nature (Anger 2001). Detailed morphological studies of the decapod digestive system have demonstrated a close relationship between the morphological characteristics of the digestive system and feeding behaviour (Nishida et al. 1990, Abrunhosa and Kittaka 1997a).

Evidences of food abstinence were observed during the transitory stage (puerulus) in larvae of lobsters belonging to the genera Panulirus, Jasus and Palinurus (Kittaka 1988, 1994, Kittaka and Ikegami 1988, Kittaka 1994, Nates and McKenney Jr 2000, Anger 2001).
In these species, a noticeable morphological change was observed in the digestive system during metamorphosis, which showed as well as a reduction of the mandibles and the setal number in the external feeding apparatus and showed an underdeveloped foregut (Nishida et al. 1990, Wolfe and Felgennhauer 1991, Mikami and Takashima 1993, Lemmens and Knott 1994). Abrunhosa and Kittaka (1997a, b) observed similar facts in king crab species of the genus *Paralithodes* (important fishery resource of the North Pacific) during the megalopal stage. More recently, a similar behaviour was also observed in zoeal stages of the callianissid *Lepidophthalmus siriboia* (Abrunhosa et al. 2006).

In previous studies, the development of the foregut and mouthparts have contributed to the better understanding of the feeding behaviour of a cultured species, which has allowed the identification of an adequate feeding regime and, consequently, has enhanced the survival rate during larval development (Nishida et al. 1990, Minagawa and Takashima 1994, Abrunhosa and Kittaka 1997a). Many studies have focused on the morphological and functional aspects of the foregut in adult decapod specimens (Meiss and Norman 1977, Kunze and Anderson 1979, Suthers and Anderson 1981, Suthers 1984, Skillet and Anderson 1986, Mikami and Takashima 1993, Pinn et al. 1999, Jha and Homechaudhuri 2001, Brosing et al. 2002, Castro and Bond-Buckup 2003), however, very few efforts to investigate these aspects in the larval stages have been undertaken in the past. Therefore, in this present study, the morphology of the larval foreguts of two decapod crabs from the Northern Brazil, *Uca vocator* Herbst 1804 (Ocypodidae) and *Panopeus occidentalis* Saussure 1857 (Xanthidae) were investigated, and the functionality of these organs were studied in detail.

Detailed morphological descriptions of these two were previously reported by Ingle (1985) and Rieger (1999) for *P. occidentalis* and *U. vocator*, respectively. Biogeographically, adults of both species are found in mangroves and in the estuarine areas along the coast of the Americas (Atlantic South). In Brazil, these species occur in mangrove areas from state of Pará, northern Brazil, to the state of Santa Catarina, southern Brazil (Melo 1996, Dicle et al. 2010).

**MATERIALS AND METHODS**

**LARVAL REARING**

Larvae were obtained from egg-berried females of *U. vocator* and *P. occidentalis* collected one day before hatching in the mangroves of the Caeté river estuary (Northern Brazil). The females were transported to laboratory where they were washed and kept individually in aquaria (5 L) filled with seawater and constant aeration. The crabs were maintained in ambient temperature (26°C), salinity 30, pH 8.0 and without food until larval release.

After hatching, the zoea larvae were transferred to plastic recipients filled with 150 mL of seawater for culture. The larvae were reared at a density 20 ind./recipient in constant conditions of temperature (26 ± 1.5°C), salinity (30), pH (8.0) and in photoperiod regime (12:12 h light:dark cycle) until the megalopal stage. Larvae were fed daily with rotifers *Brachionus plicatilis* (50 ind./mL), *Artemia* nauplii (5 ind./mL) and microalgae *Thalassiosira* sp (initial concentration about 30 cel/mL$^4$). The recipient seawater was changed at every two days, and the larvae were monitored daily for mortality or moults to subsequent stages.

**LARVAL FOREGUT DISSECTION**

For both species, at least 10 individuals of the first (ZI), last zoeal stage (ZIV for *P. occidentalis* and ZV for *U. vocator*) and megalopa were sampled and used for dissection and illustrations. The methodology applied was similar to that accomplished by Abrunhosa et al. (2003). The samples were fixed in formalin solution 10% (v/v) for a 24 hours period. Then, they were immersed in aqueous solution of potassium hydroxide 5% (KOH) (p/v) and heated to 80°C for 30 minutes. Later, the samples were washed with distilled water and immersed in ethylic alcohol 70% + glycerine solution (1:1).

**ILLUSTRATION AND DESCRIPTIONS OF THE FOREGUT**

After the treatments described above, the larvae of the respective stages were placed in a slide with ethylic alcohol 70% + glycerine solution (1:1). The larvae were dissected under an optic microscopy (ZEISS). Fine needles (BD Ultra-Fine®, 12.7 × 0.33 mm) were used for dissection. The foreguts were extracted and transferred to another slide where they were stained with a...
solution of methylene blue 1%. The foreguts were observed and illustrated in lateral view, aided by a micrometer disc. The terminology used for descriptions of the foregut follows Meiss and Normann (1977), Nishida et al. (1990), Abrunhosa and Kittaka (1997a) and Abrunhosa et al. (2003).

RESULTS

LARVAL CULTURE

The complete larval development of *U. vocator* was constituted of 5 zoeal and 1 megalopal stages, while the one of *P. occidentalis* was composed by 4 zoeal and 1 megalopal stages. In *U. vocator*, the larval survival rate until the megalopa stage was 90%, and the larval development period from hatching to megalopa ranged from 10 to 12 days. For *P. occidentalis*, the larval survival rate was 100%, and the development period until the megalopa stage was about 12 days. The survival rates and development periods of each zoeal stage of *U. vocator* and *P. occidentalis* are shown in the Tables I and II, respectively.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Survival rate (%) and intermolt period (days) of <em>Uca vocator</em> larvae reared in the laboratory.</th>
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<tr>
<td>Larval stage</td>
<td>Survival (%)</td>
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<tr>
<td>Zoaa</td>
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<td>Zoae</td>
<td>98</td>
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<td>Zoae</td>
<td>90</td>
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<td>Megalopa</td>
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*The experiment ended in megalopa and they did not moult to the first juvenile.*

MORPHOLOGY OF THE LARVAL FOREGUTS

*Uca vocator*

The foreguts of the first and last zoeal stages of *U. vocator*: showed a cardiopyloric valve separating the cardiac from the pyloric chamber, as well a filter press that becomes specialized during the larval development. There is a degree of specialization of the foregut along the larval development where the number of setae and size are increased. After the metamorphosis to megalopal stage, a considerable morphological change in the foregut was observed with the appearance of ossicles of the gastric mill and lateral teeth.

*Zoaa* (Fig. 1)

**Foregut** – Simple, chitinous and lacking – hard structures (total length 1.1 mm).

**Cardiac chamber** – Little larger than pyloric chamber; cardiac wall lacking setae; numerous fine setae on the floor base; cardiopyloric valve redounded and robust bearing many setae on its extension and showing strong setae on the posterior portion.

**Pyloric chamber** – About 2/5 cardiac chamber size; fine setae on the posterior portion of the cardiac roof; filter press large and well-developed occupying all the inferior portion of the chamber; numerous setae on the inter-ampullary ridge.

*Zoae V* (Fig. 2)

**Foregut** – More complex than stage I and lacking – rigid structures (total length 1.8 mm).

**Cardiac chamber** – Cardiac roof showing fine setae on the roof with row of elongate and fine setae in the anterior portion; posterior portion with numerous rigid and long setae; cardiac wall lacking setae; cardiopyloric valve redounded and large with strong setae almost in all of its extension.

**Pyloric chamber** – About 2/5 cardiac chamber size; fine setae on the posterior portion of the cardiac roof; filter press specialized, occupying all inferior portion of the chamber; inter-ampullary ridge with rows of elongate and medium setae; ampullary net well-developed and functional.

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Megalopa (Fig. 3)

**Foregut** – Showing a complex gastric mill, oesophageal valve and other rigid structures (total length 3.8 mm).

**Cardiac chamber** – Enlarged dorso-ventrally by plates and gastric mill ossicles (about 3 times the pyloric chamber); cardiac floor showing almost vertically; numerous ossicles of gastric mill present: mesocardiac, pterocardiac; zygocardiac (supporting the lateral teeth), urocardiac (supporting medial tooth), propyloric, exo-

**Pyloric chamber** – Fine setae on the posterior portion of the roof; filter press specialized bearing a well-developed and functional ampullary net; inter-ampullary ridge with rows of elongate setae; some ossicles pres-
DEVELOPMENT AND FUNCTIONAL MORPHOLOGY OF THE LARVAL FOREGUT

Fig. 3 – *Uca vocator*. Megalopa foregut in lateral view (right side). Scale bar = 0.4 mm. Abbreviations: app = anterior pleuropyloric ossicle (a pair); c = cardiac chamber; cpv = cardiopyloric valve; exp = exopyloric (a pair); fp = filter press; ia = inferior ampullary ossicle; ir = interampullary crista; ilc = anterior lateral cardiac ossicle; lcp = lateral ossicle of the cardiopyloric valve; lt = lateral teeth (a pair); mp = middle pleuropyloric ossicle (a pair); msc = mesocardiac ossicle; oes = oesophagus; p = pyloric chamber; ov = oesophageal valve; pam = pre-ampullary ossicle; pec = pectineal ossicle (a pair); pip = posterior inferior pyloric ossicle; pop = pos-pectineal ossicle (a pair); pp = pleuro-pyloric ossicle (a pair); ppc = pre-pectineal (a pair); prp = propyloric ossicle; ptc = pterocardiac ossicle; py = pyloric ossicle; sd = subdentate ossicle (a pair); up = uropyloric ossicle; urc = urocardiac ossicle; zyc = zygocardiac ossicle (a pair).

ent: uropyloric, anterior pleuropyloric, posterior pleuropyloric; middle pleuropyloric, pre-ampullary; inferior pyloric, anterior inferior and posterior pyloric ossicle (other ossicles as illustrated in Fig. 6).

*Panopeus occidentalis*

Zoea I (Fig. 4)

Foregut – Simple and chitinous (total length 0.72 mm).

**Cardiac chamber** – Showing numerous setae on the cardiac floor; cardiac wall lacking setae; cardiopyloric valve redound and robust with firm and erect setae on the posterior portion.

**Pyloric chamber** – Pyloric wall lacking setae; pyloric floor showing setae in all portion; filter press well-developed and filling all inferior portion of the chamber; inter-ampullary ridge rows of numerous setae.

Zoea IV (Fig. 5)

Foregut – more developed compared to zoea I stage, but similar in shape; lacking rigid structures (total length 3 mm).

Cardiac chamber – Cardiac floor with a row of elongate setae; cardiac wall with few setae on the posterior portion; cardiopyloric valve redound, large and holding erect and strong setae on the posterior portion; cardiac floor with fine setae in the posterior portion.

Pyloric chamber – Filter press specialized and complex, filling all inferior portion of the chamber; inter-ampullary ridge well-developed with rows of medium setae.

Megalopa (Fig. 6)

Foregut – Showing a complex gastric mill; oesophageal valve and rigid structures (total length 3.5 mm).

Cardiac chamber – Enlarged dorsolaterally by the gastric mill ossicles; cardiac floor disposed vertically; cardiopyloric valve specialized and robust; gastric mill ossicles present, such as: mesocardiac, pterocardiac, zygocardiac (supporting the lateral teeth), urocardiac (supporting medial tooth), propyloric, exopyloric and pyloric; other ossicles are observed: prepectineal, pectineal, exopyloric, subdentate, inferior lateral cardiac and lateral cardiopyloric; valve cardiopyloric robust and specialized, bearing firm setae on the superior portion (other ossicles as illustrated in Fig. 6).
DISCUSSION

In the literature, the majority of the reports on larval development and metamorphosis forms (glaucothoe, puerulus, decapodid and megalopa) of decapods are only limited to the descriptions of the general appendages, and detailed information is rather limited regarding the functional morphology of mouthparts, foregut and digestive gland during the larval development. However, the results of these investigations have significantly contributed to improve the larval culture of decapod species (Kittaka 1994, Abrunhosa and Kittaka 1997a, b).

Studies about the functional morphology have demonstrated, for example, that there is an expressive reduction of the larval mortality due to non addition of food in cases of lecithotrophic behaviour, which occurs at some stages or along the entire larval development sequence (Kittaka 1988, Kittaka and Ikegami 1988, Abrunhosa and Kittaka 1997a, b).
Detailed analyses of the foreguts in the zoeal and megalopal stages of \textit{U. vocator} and \textit{P. occidentalis} showed similarities between these two species in both their structures and functions. The foreguts of the zoeal I stage are morphologically simple, but bearing specific setae and a filter press that is apparently functional. The same pattern were observed in the zoea I larvae. The foreguts of the last zoeal stages, (zoea V for \textit{U. vocator} and zoea IV for \textit{P. occidentalis}) also lacked gastric mill apparatus however, an increase in the degree of specialization of the foregut was also observed, which showed an increment of setae, but the main difference is in the observance of the cardiopyloric valve and the complexity of the filter press. Therefore, the last zoeal stage of both species shows an increasing in the ability to process small particles of food. These observations corroborate with those of previous studies with crab larvae (Shinkarenko 1979, Factor 1982, 1989, Minagawa and Takashima 1994, Abrunhosa et al. 2003).

The cardiac chambers of the zoeal stages do not bear gastric teeth strongly indicating that they may not be capable of grinding hard food. However, these larvae are able to feed on soft food particles, such as \textit{Artemia} nauplii, rotifers, microalgae and other zooplanktonic species available in the aquatic environment. Similarities with the morphological features of the zoea I foregut of decapods were also observed in several other species (Factor 1982, 1989, Nishida et al. 1990, Abrunhosa and Kittaka 1997a, b, Abrunhosa and Melo 2002, 2008, Abrunhosa et al. 2003, Melo et al. 2006). However, some first larvae of the callianissid \textit{Lepidophthalmus siriboia} have been reported showing only few minute setae in the cardiac and pyloric chambers, as well as a reduced filter press (Abrunhosa et al. 2006). These facts, strongly suggested a non-feeding behaviour of zoea larvae of this species.
The foregut is an important component of the digestive system for break down food items into fine particles. The elaboration of the gastric mills placed in the cardiac chamber is necessary to the development of the proper digestion of substantial foodstuffs, and also for the mixing of digestive enzymes from the midgut gland (Icely and Nott 1992). Many works listed in the literature show the megalopa of brachyuran crabs as having well-developed mandibles and setose mouthparts (Perrins 1973 for gerynoids; Gore et al. 1982, Webber and Wear 1981 for majids, Abrunhosa et al. 2003, 2006 for ocyopodids).

Significant changes in both morphological and anatomical features of the foregut take place during the metamorphosis of U. vocator and P. occidentalis from zoea to megalopa stages. These foreguts undergo remarkably morphological changes where newly moulted megalopaæ bear a highly complex foregut. Such moulting brings about changes in the feeding apparatus that is necessary for the processing substantial food in their natural environment.

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