# Density of larval and adult forms of the burrowing crustaceans *Lepidophthalmus siriboia* (Callianassidae) and *Upogebia vasquezi* (Upogebiidae) in an Amazon estuary, northern Brazil

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Although thalassinidean shrimps exert considerable influence over the structure of benthic communities no ecological studies have been conducted on the larvae and adults of Lepidophthalmus siriboia and Upogebia vasquezi in the equatorial region. The aim of the present study was to identify the reproductive period of these species in an Amazonian estuary. Monthly collections were performed in the estuary of the Marapanim River (northern coast of Brazil) over the course of one year (August 2006 to July 2007) and adults were obtained from bare sandy substrate and between boulders in the intertidal zone of the estuary at four sites. Thalassinidean larvae were also sorted from plankton samples (200  $\mu$ m mesh) collected monthly at six sites. Larvae of the species Callichirus major, Lepidophthalmus siriboia and Upogebia vasquezi were collected, whereas adults of only the latter two species were found. Upogebia vasquezi was the most abundant species. There was no correlation between larval density and abiotic factors (water temperature, salinity and pH); however, the density of U. vasquezi adults was negatively correlated with salinity in the burrows. Lepidophthalmus siriboia and U. vasquezi reproduce throughout the year in the Marapanim River estuary, with peaks of greater intensity in some reproductive periods of the year, mainly correlated with changes in salinity.

Keywords: estuary, meroplankton, macrobenthos, Amazon

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## INTRODUCTION

Thalassinideans are burrowing benthic decapods, with more than 95% of species inhabiting shallow waters (o to 200 m) in marine and estuarine environments (Dworschak, 2005). These organisms exert considerable influence over the structure of benthic communities via their ability to bioturbate the sediments (Kinoshita et al., 2003), with effects on the infauna and seagrasses in coastal environments (Berkenbusch et al., 2007) and some species are considered pests in oyster aquaculture, reducing the stability of the bottom substrate where oysters are raised (Dumbauld et al., 2006). Moreover, species of commensal invertebrates are often associated with their burrows, such as shrimp from the family Alpheidae (Anker et al., 2001) and larval phoronid primarily (Santagata, 2004).

The infraorder Thalassinidea has been split in two infraorders, Axiidea de Saint Laurent, 1979, that includes *Lepidophthalmus siriboia* Felder and Rodrigues, 1993, and

**Corresponding author:** D.B. Oliveira Email: danybrito@ufpa.br Gebiidea de Saint Laurent, 1979, that includes *Upogebia vas-quezi* Ngoc-Ho, 1989, with a total of 615 thalassinidean species already described (De Grave *et al.*, 2009), distributed along a latitudinal gradient, less diverse at high latitudes and more frequent at low latitudes. Thirty-six per cent of the species are concentrated in the western Indo-Pacific Ocean and 22% are found in the south-eastern Atlantic (Dworschak, 2005), and 43 thalassinidean species are reported for the coast of Brazil (Melo, 1999; Nucci & Melo, 2001).

Many species have been exploited for use as live bait in artisanal and recreational fishing in diverse locations (Pezzuto, 1998; Hodgson *et al.*, 2000; Souza & Borzone, 2003; Contessa & Bird, 2004; Botter-Carvalho *et al.*, 2007), which could lead to the overexploitation of some groups. Moreover, the disturbance created by both the bait-pumping and associated trampling of adjacent mudflat harvesting shrimp, may alter the habitat and influence resident communities (Contessa & Bird, 2004).

Despite their ecological importance, little is known about the biology of many thalassinidean species (Candisani *et al.*, 2001), primarily because of their cryptic lifestyle and difficulties in capturing specimens (Rodrigues, 1976; Coelho *et al.*, 2000). In relation to larval development of thalassinidean species, according to Pohle *et al.* (2011) larval information is available for about one-eighth of the species and one-quarter of known genera. Therefore, studies on the population and reproductive biology of these species, such as those previously carried out by Rodrigues (1976), Dworschak (1988), Tamaki & Ingole (1993), Dumbauld *et al.* (1996), Nates & Felder (1999), Berkenbusch & Rowden (2000), Tamaki & Miyabe (2000), Candisani *et al.* (2001), Kinoshita *et al.* (2003), Botter-Carvalho *et al.* (2007) and Rotherham & West (2009) are important to understand the lifecycle of thalassinideans and the management of this group.

Lepidophthalmus siriboia (Callianassidae) and Upogebia vasquezi (Upogebiidae) are found on the north-eastern coast of the State of Pará (northern Brazil) in the present study. Descriptions of the larval morphology of *L. siriboia* are available (Abrunhosa *et al.*, 2005), but there are no ecological studies on the larval and adult forms of these species in the equatorial region. The aim of the present study was to determine the reproductive period of thalassinidean species in the Marapanim River estuary in the State of Pará (northern Brazil), using data on the density of larval and adult forms.

## MATERIALS AND METHODS

## Collection of juvenile and adult thalassinideans

Adult shrimp sampling was carried out monthly from August 2006 to July 2007, perpendicular to the shoreline together with sediment from bare sand and between boulders from the intertidal region. Four sites were established (two on each bank of the Marapanim River, east and west) in two areas comprising the upper and lower portion of the mesolittoral

region. In each site, there were three replicates of 0.5 m<sup>2</sup> quadrat placed randomly, totalling 16 monthly samples (4 sites (A1 and A2 on western bank; B1 and B2 on eastern bank)  $\times$  2 microhabitats (bare sand and between boulders)  $\times$  2 areas (upper and lower portion of the mesolittoral region)  $\times$  12 months), totalling 192 samples, with three sub-samples each (Figure 1).

Subsamples were demarcated with a polyvinyl chloride (PVC) quadrat (0.5 m in length by 0.5 m in width). Burrowing shrimp were captured by digging in each quadrat, with a PVC suction tube ('yabby pump'), with 0.9 m in length and 0.05 m in diameter. The five first centimetres of the substrate were collected following the removal of rock fragments. Samples were sieved over a 3 mm mesh and washed in running water from the estuary for the separation of the organisms and retained shrimp were stored in labelled recipients, which were initially kept in ice and subsequently fixed in 70% alcohol until analysis.

Aliquots of water were removed from the burrows with a syringe (3 ml), for the determination of salinity using an optical refractometer. Data on total and mean monthly precipitation were obtained from the Brazilian National Water Agency (Agência Nacional de Águas, 2007). All material was identified at the species level using identification keys from studies carried out by Melo (1999) and Rodrigues & Pezzuto (1999) at the Laboratory of Fish Biology and Management of Aquatic Resources of the Universidade Federal do Pará (Brazil).

The total length (TL, from the tip of the rostrum to the posterior margin of the telson) measure was taken using a digital caliper (nearest 0.01 mm) and the density was expressed as number of individuals/100<sup>2</sup>. The Kruskal–Wallis test was



Fig. 1. Geographical location of study area with indication of sampling sites of adult thalassinideans in the Marapanim River estuary (Pará, northern Brazil), August 2006 to July 2007; A1 and A2, western bank; B1 and B2, eastern bank; detail in upper left corner: schematic of sampling location in intertidal regions.

## Collection of thalassinidean larvae

Zooplankton samples were collected from six sites distributed in two areas (A and B) in shallow waters (bank of the main channel) of the estuary (A1:  $0^{\circ}38'12''S$  and  $47^{\circ}38'74''W$ ; A2:  $0^{\circ}40'35''S$  and  $47^{\circ}38'31''W$ ; A3:  $0^{\circ}42'38''S$  and  $47^{\circ}41'23''W$ ; B1:  $0^{\circ}36'14''S$  and  $47^{\circ}35'15''W$ ; B2:  $0^{\circ}40'35''S$  and  $47^{\circ}36'29''W$ ; B3:  $0^{\circ}43'43''S$  and  $47^{\circ}39'35''W$ ).

Area A corresponded to the western bank of the Marapanim River, on which the city of Marapanim and the fishing communities of Araticum, Aracumirim and Alegria are located. Area B corresponded to the eastern bank of the river, which has virtually no urban communities. Sites A1, A2 and A3 were aligned with the position of Sites B1, B2 and B3 on the opposite margin of the estuary, except when sand banks or rocks impeded the exact alignment of sites. The establishment of these sites was based on the gradient of salinity in the estuary, considering three zones: Zone I (A1 + B1), nearest to the open sea; Zone II (A2 + B2), intermediate; and Zone III (A3 + B3), innermost portion of the estuary with lowest salinity. Three distinct climatic periods were considered: dry season (August to December), transition periods (January, June and July) and rainy season (February to May).

Twelve monthly field expeditions were carried out between August 2006 and July 2007, encompassing the characteristics of the dry season, transition periods and rainy season. Zooplankton sampling was performed during the daytime outgoing tide, totalling 72 samples (6 sites  $\times$  12 months), with two replicates per site.

Thalassinidean larvae were collected in horizontal neuston hauls. Sampling was carried out for three minutes at a velocity of approximately one to 1.5 knots using conical-cylindrical plankton net with a mesh size of 200  $\mu$ m. A previously calibrated Hydrobios flow meter was coupled to the mouth of the net to calculate the volume of water filtered during the sampling. Samples were fixed in 4% buffered formaldehyde. The following abiotic factors were also determined during the sampling using a multiparameter YSI analyser: water temperature (°C), hydrogen potential (pH) and salinity.

In the laboratory, zooplankton samples were divided into smaller aliquots with a Folsom Plankton Splitter. A volume of 250 ml was defined for sorting and identification of thalassinidean larvae. The larvae were analysed using a Zeiss optical stereomicroscope and Leica optical microscope with a micrometric grid. Organisms were identified to the lowest possible taxa, based on descriptions contained in previous studies (Sandifer, 1973; Ngoc-Ho, 1981; Nates *et al.*, 1997; Strasser & Felder, 1999; Santos & González-Gordillo, 2004; Abrunhosa *et al.*, 2005). The megalops and juveniles of *L. siriboia* collected in the zooplankton samples were identified according to Abrunhosa *et al.* (2005).

#### RESULTS

Temperature was higher between August and December, reaching its lowest value in February  $(27.5^{\circ}C)$  and rising

once again from March to July. The pH ranged from 7.5 to 9.0, with the highest values recorded from January to March. The salinity of the water and burrows was higher in drier months (August to December), with an intermediate value in January and lower values in more rainy months (February to July) (Figure 2). Although the mean temperature throughout the study was similar, the variation in this factor was significant between climatic periods: dry season—August to December, transition periods—January, June and July, and rainy season—February to May (H = 46.5; P < 0.0001). Salinity and pH also varied significantly (H = 117.5; P = 0.03 and H = 7.1; P < 0.0001, respectively).

Larvae of Callichirus major (Say, 1818), Lepidophthalmus siriboia and Upogebia vasquezi were collected, whereas only adult specimens of the latter two species were collected. Table 1 displays the number of individuals and density of each taxon identified according to stage of development. Upogebia vasquezi was the most abundant species in the meroplankton, accounting for 92% of the total, followed by L. siriboia (5%) and C. major (3%). The first zoea stage was the most frequent in the samples of the three thalassinidean species. Upogebia vasquezi was also the dominant species among the adults, accounting for 82% of the samples, whereas L. siriboia accounted for the remaining 18%. The density of thalassinidean larvae was not significantly correlated to abiotic factors (temperature, salinity and pH). The density of adult U. vasquezi was negatively correlated with the salinity recorded in the burrows (Table 2). The mean density of L. siriboia adults was highest in June, whereas mean density of the larval form was highest in February (Figure 3). Upogebia vasquezi adults were abundant from January to April, with the greatest abundance of larvae in December (Figure 3).

The smallest *L. siriboia* adult measured was a female with 14.31 mm TL and the largest was an ovigerous female with 55.01 mm TL. The smallest *U. vasquezi* adult measured was a male with 10.96 mm TL and the largest was an ovigerous female with 61.50 mm TL (Figure 4). *Lepidophthalmus siriboia* megalops and juveniles were collected on zooplankton samples and were not measured. The unidentified sex individuals are probably still juveniles, but they were grouped in 'others' (Figure 5).

Peak density of L. siriboia and U. vasquezi larvae occurred in months in which there were ovigerous females or in consecutive months following the presence of ovigerous females in the estuary. Ovigerous L. siriboia females were collected in the months of September, November, February, April, May, June and July, with the greatest frequency registered in June. In the months following those in which ovigerous females were collected (August, October, December and July), there were small peaks in larval density, with the greatest frequency of L. siriboia larvae recorded in February (Figure 5A). Ovigerous U. vasquezi females were collected from December to July, with the exception of April. Larval peaks for this species were recorded in December, January, February and July, coinciding with the months of occurrence of ovigerous females. Small peaks in larval density were also recorded in August, October and November, but no ovigerous females were collected in these months (Figure 5B). Most of the larvae present in zooplankton samples were Stage 1 zoeae and peaks in their abundance coincided with the presence of ovigerous females (February for L. siriboia; January, February and July for U. vasquezi; Figure 6).



**Fig. 2.** Abiotic factors recorded monthly during collection of thalassinidean larvae and adults in the Marapanim River estuary (Pará, Brazil) from August 2006 to July 2007; (A) temperature; (B) pH; (C) salinity—recorded concomitantly to collection of larvae; (D) salinity recorded in burrows of adult thalassinideans. KW-H, Kruskal–Wallis test values plus *P* values. Different letters symbolize significant differences with  $\alpha = 0.05$ .

#### DISCUSSION

# Physical factors and adults

A number of authors report the influence of physical factors, such as temperature, over patterns of growth, distribution and abundance among thalassinidean species (Thessalou-Legaki, 1990; Pezzuto, 1998; Botter-Carvalho *et al.*, 2007; Rotherham & West, 2009). Other authors also report temperature to be a determinant of the reproductive period among thalassinidean species (Wooldridge & Loubser, 1996; Botter-Carvalho *et al.*, 2007). Moreover, in the case of *Callianassa filholi* Milne-Edwards, 1878, the availability of food sources has been reported to be a determinant of the reproductive period (Berkenbusch & Rowden, 2000). In the Marapanim River estuary, temperature exerted no significant influence over the density of *L. siriboia* and *U. vasquezi* adults and was not a determinant of reproductive activity for these species.

# Physical factors and ovigery

Patterns in the frequency of ovigerous females and abundance of larvae and adults in this study demonstrate that Lepidophthalmus siriboia and Upogebia vasquezi reproduce throughout the year in the estuary of the Marapanim River (Pará, Brazil). The peak of greatest reproductive intensity was in June for *L. siriboia*, whereas peaks of greater

 Table 1. Number of individuals and total density of thalassinidean species collected from the Marapanim River estuary (Pará, Brazil) according to stage of development; N, number of individuals; D, total density expressed in number of larvae/100 m<sup>3</sup> for larval stages and number of ind./100 m<sup>2</sup> for adults (relative density).

Family	Species	Stage of development	N	D
Callianassidae	Callichirus major	Zoea I	32	80.9
		Zoea II	8	18.7
		Zoea III	12	30.0
		Zoea IV	4	9.6
	Lepidophthalmus siriboia	Zoea I	24	195.3
		Megalop	12	45.8
		Juvenile	4	9.0
		Adult	114	456.0
Upogebiidae	Upogebia vasquezi	Zoea I	1298	4538.8
		Zoea II	20	50.8
		Zoea III	8	17.8
		Adult	537	2148.0

Species/factors	Temperature		рН		Water salinity		Burrow salinity	
	R	Р	R	Р	R	Р	R	Р
Larvae								
Callichirus major	0.5	0.12	-0.1	0.77	0.3	0.39	-	-
Lepidophthalmus siriboia	0.1	0.66	-0.3	0.32	0.3	0.43	-	-
Upogebia vasquezi	0.3	0.38	-0.3	0.33	0.6	0.06	-	-
Adults								
L. siriboia	-	-	-	_	_	-	-0.3	0.37
U. vasquezi	-	-	-	-	-	-	-0.7	0.01*

 Table 2. Spearman's correlation coefficient (R) for larval density (number of larvae/100 m<sup>3</sup>) and adult density (number of ind./100 m<sup>2</sup>) of thalassinidean species in relation to abiotic factors (temperature, pH, salinity and salinity in burrows); \*significant results (P<0.05).</th>

reproductive intensity occurred in January, June and July for *U. vasquezi*. Rodrigues (1976) collected ovigerous females of *Callichirus major* Say, 1818 at Santos Bay, Brazil, during March, June, July, November and December, suggesting reproduction all over the year. Candisani *et al.* (2001) found berried females of *Upogebia noronhensis* Fausto-Filho, 1969 throughout the year, suggesting reproduction is continuous at Ubatuba, south-eastern Brazil.

## Physical factors and larvae

In coastal and estuarine environments, decapod larvae are subjected to temporal and spatial variability in salinity, undergoing osmotic stress, which can reduce growth and survival rates (Anger, 2001; Torres et al., 2002). Since that larval stages of crustaceans species in estuaries, undergo development under this range of environmental conditions, Paula et al. (2001) studied the temperature and salinity effects on the larval development of Upogebia africana Ortmann, 1894, in relation to survival and duration of larval stages. Also Newman et al. (2006) investigated the influence of temperature on the larval development of two Upogebia species. In the Marapanim River estuary larval density of U. vasquezi was significantly greater in the dry season and transition periods, in which the salinity of the water was greater (mean: 28.5  $\pm$  4.3 in dry season and 18.2  $\pm$  3.3 in transition periods) in comparison to the rainy season (mean:  $13.5 \pm$ 3.7), therefore low salinity is not favourable to the development of the larvae of this species. Most of laboratory rearing of *Upogebia* species larvae occurs in salinity range of 30-35 (Konishi, 1989; Siddiqui & Tirmizi, 1995; Shy & Chan, 1996; Melo & Brossi-Garcia, 2000). However, the significant negative correlation between the density of *U. vasquezi* adults and salinity in the burrows (R = -0.7; P < 0.05) is an indication that the adults may be more euryhaline than larvae.

Although there were no significant differences in the density of *L. siriboia* larvae and adults between months and there were also no significant correlation to abiotic factors, the larvae were caught at salinities ranging from 20 to 32, with the occurrence of only three larvae at a salinity of 8. The larval development under laboratory conditions of both *L. siriboia* (Abrunhosa *et al.*, 2005) and *U. vasquezi* (Oliveira *et al.*, unpublished data) occurs at higher degrees of salinity, as for other thalassinidean species, such as *Callianassa tyrrhena* (Petagna, 1792), which reaches its maximal development in the laboratory at a salinity of 29 or higher (Thessalou-Legaki, 1990). Moreover, the spawning of callianassid species is also favoured at salinities greater than 20 (Botter-Carvalho *et al.*, 2007).

Decapod larvae are generally present in zooplankton communities of coastal waters throughout the year and their presence is commonly associated with the reproductive period and spawning of adults (Fehlauer & Freire, 2002). In tropical estuaries, the spawning of these crustaceans can occur throughout the year, contrasting with the pattern found in temperate estuaries (Dittel & Epifanio, 1990), in which the majority of decapods release their larvae in a particular period of the



Fig. 3. Mean density  $\pm$  standard error of *Lepidophthalmus siriboia* and *Upogebia vasquezi* in larval and adult forms in the Marapanim River estuary (Pará, Brazil) from August 2006 to July 2007.



Fig. 4. Mean of total length for females (F), ovigerous females (OF), indeterminate sex (IND) and males (M) of *Lepidophthalmus siriboia* and *Upogebia vasquezi*. Vertical bars, standard deviation.

year, when environmental conditions are favourable (Gonçalves *et al.*, 2003). For example, Paula (1987) encountered two peaks of decapod larval abundance at São Torpes bay, south-western Portugal. Moreover, specific taxa may exhibit distinct seasonality.

Species that reproduce in an estuarine environment generally have a complex lifecycle that involves mechanisms of larval retention and/or exportation, depending on the natural habitat of the species in the adult phase (Wooldridge & Loubser, 1996). In the present study, U. vasquezi and L. siriboia larvae were found at higher salinities, which may indicate that these larvae develop in the open sea and return to the estuary in the final stages of development for settlement. This larval exportation mechanism is very important to the reproductive success for U. vasquezi, as the adults occur in greater abundance at lower salinities, which are incompatible with the larval development of the species. This reproductive strategy has also been reported for U. africana, which reaches its reproductive peak in summer, exporting the hatched eggs to the open sea, with the re-invasion of the post-larvae in the estuarine environment (Wooldridge & Loubser, 1996). The absence of larval stages Zoea II and Zoea III for L. siriboia in plankton during the present study also supports the hypothesis that these larvae are not retained in the Marapanim River estuary like Petrolisthes armatus (unpublished data), suggesting different mechanisms of larval transportation among sympatric decapods.

In the Marapanim River estuary, *C. major* larvae were also caught, especially under conditions of high salinity (20 to 33), with only one larva caught at a salinity of 18. Unlike *L. siriboia* and *U. vasquezi*, which occurred in both larval and adult forms, no *C. major* adults were caught. The relatively long duration of the plankton stage of the genus *Callichirus* contributes to the greater dispersion of larvae. Adult populations are distributed throughout the intertidal open coastline, especially in locations made up mostly of silica sand



Fig. 5. Relationship between total larval density (number of larvae/100 m<sup>3</sup>) and frequency (%) of adults collected per month in the Marapanim River estuary (Pará, Brazil) with representation of ovigerous females, non-ovigerous females and others (category includes males and juveniles); (A) Lepidophthalmus siriboia; (B) Upogebia vasquezi.



Fig. 6. Monthly density (number of larvae/100 m<sup>3</sup>) of different larval stages of *Lepidophthalmus siriboia* and *Upogebia vasquezi* collected in the Marapanim River estuary (Pará, Brazil).

(Rodrigues & Shimizu, 1997). It is possible that this preference for a silica substrate/sediment on the part of species of *Callichirus* is not compatible with sedimentological characteristics of the Marapanim River estuary. This would explain the absence of adults in the A1, A2, B1 and B2 samples, as muddy sediments predominate at these sites. Granulometric analysis of the sediment in which the thalassinidean species were collected in the Marapanim River estuary are being carried out in order to elucidate the type of sediment preferred by the species in the region.

There is little knowledge on the dynamics of thalassinidean larvae in the natural environment at the different latitudes at which the species are found, especially in tropical regions, where the greatest number of species from this group occur. Thus, the data presented here make a fundamental contribution to the knowledge on the life history of these species.

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