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Original Article

Larvae of *Armases rubripes* (Decapoda, Brachyura) in Amazon oligohaline creeks: larval exportation?

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ABSTRACT

Three oligohaline creeks in the Amazon region with different levels of anthropogenic contamination were monitored quarterly regarding the larval density of *Armases rubripes* and their relationship with temperature, pH, conductivity, salinity, as well as the concentrations of dissolved oxygen, biochemical, oxygen demand, chemical oxygen demand, fecal coliforms, and some dissolved ions, between March 2011 and January 2012. A total of 17,949 *A. rubripes* larvae in the zoea I developmental stage were identified in the creeks, suggesting export to the continental shelf. Larvae were present every four months, but the highest density occurred in the Amazon rainy season (January), indicating the reproductive peak of the species. Furthermore, we found that the greatest larval abundance and distribution occurred in the most preserved creek, with the lowest concentration of nitrogenous compounds and coliforms. The generalized linear model indicated that conductivity and total suspended solids content are positively correlated with larval density whereas salinity and Ca concentration are negatively correlated with larval density. Therefore, the water quality is concluded to be a structuring factor for the larval population of *A. rubripes*.

KEYWORDS

Crustacean, estuary, Guajará Bay, pollution, zoea, zooplankton

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INTRODUCTION

Sesarmid crabs are common in a variety of habitats, including marine, brackish, freshwater, and terrestrial intertidal environments (Abele, 1992; Anger et al., 2008). Currently, the family Sesarmidae comprises 30 living genera (De Grave et al., 2009), of which only *Aratus* H. Milne Edwards, 1853, *Sesarma* Say, 1817, and *Armases* Abele, 1992 occur on the Brazilian coast (Melo, 1996).

Armases rubripes (Rathbun, 1897) is a small crab species that inhabit the tropical and subtropical zones, in Central and South America, and is commonly found in salt marshes, marshes, and mangroves, inhabiting the roots and bases of the vegetation stems (Melo, 1996). During development, the species goes through four larval stages (zoea), including the megalopa stage, described by Díaz and Ewald (1968). There is a single record of the larval ecology of this species in the Amazonian marine region, where zoea occurs throughout the year, but at higher density during high rainfall periods (January to July), indicating a reproductive peak in the rainy season (Lima et al., 2019). According to Lima et al. (2019), little is known about the biology and ecology of this group in Brazil while there is no information available on the adult population in the Amazon region.

In the last twenty years, a greater effort has been dedicated to investigating some aspects of this species, such as morphological (Lima and Oshiro, 2006) and physiological aspects (Santos et al., 2009), sexual maturation, reproductive period (Lima et al., 2006), sexual dimorphism (Marochi et al., 2019), as well as the influence of environmental factors on larval development (Luppi et al., 2003) and morphology (Díaz and Ewald, 1968). However, information about the meroplanktonic phase of its life cycle in a natural environment, the larval ecology, and the effects of pollution on the species remain unknown.

The pollution resulting from the urbanization process greatly affects aquatic environments. Research being conducted on the human action effects on the quality of surface water in Brazilian watersheds has proven that anthropogenic action harms the environment. For example, the removal of riparian vegetation facilitates the entry of pollutants into water bodies causing alterations in the physical-chemical and microbiological quality of water (Sant'Ana et al., 2019; Bega et al., 2021). The effect of anthropogenic influence has already been described for Amazonian prawn *Macrobrachium amazonicum* (Heller, 1862) larvae, which only occur in preserved creeks (Quaresma et al., 2019).

Only zoea I and II of *A. rubripes* were found in Amazonian waters, which suggests a larval export strategy to the sea (Lima et al., 2019). Several behaviors observed in Sesarmidae are also observed in other species such as *Parasesarma tripectinis* (Shen, 1940) and *Parasesarma bidens* (De Haan, 1835) that also export their larvae to the sea (Hsueh, 2002). *Armases roberti* (H. Milne Edwards, 1853) larvae develop in the lower estuary or coastal marine waters (Anger et al., 2006) while *Sesarma curacaoense* De Man, 1892 retains its larvae in the limnetic environment with little dependence on saltwater (Anger and Charmantier, 2000). Thus, this study aims to fill this knowledge gap for *A. rubripes* larvae in the Amazon, investigating whether the contamination of creeks in Guajará Bay can affect the larval occurrence of *A. rubripes*.

We carried out an investigation in three Amazonian oligohaline creeks with different levels of anthropogenic impact, to estimate the larval density and its relationship with varying temperature, salinity, tides, creek portion, and quantity of free ions in the water to verify the occurrence of the dispersal pattern of larval exportation in oligohaline tidal channels.

MATERIALS AND METHODS

Study area

The study was conducted in three Amazonian oligohaline creeks in the Guajará Bay located near the metropolitan region of Belém (Fig. 1). All three creeks have floodplain vegetation, are periodically flooded, and are influenced by meso tides of approximately 4 meters (Gregório and Mendes, 2009). Sites were chosen based on exposure to contaminants besides being exposed to sources of anthropogenic contamination such as sewage and industrial waste.

The Combu Creek, located near the mouth of the Guamá River and the Guajará Bay, has as main drainages the Guamá, Moju, Acará, and Capim Rivers (Gregório and Mendes, 2009). This area is considered

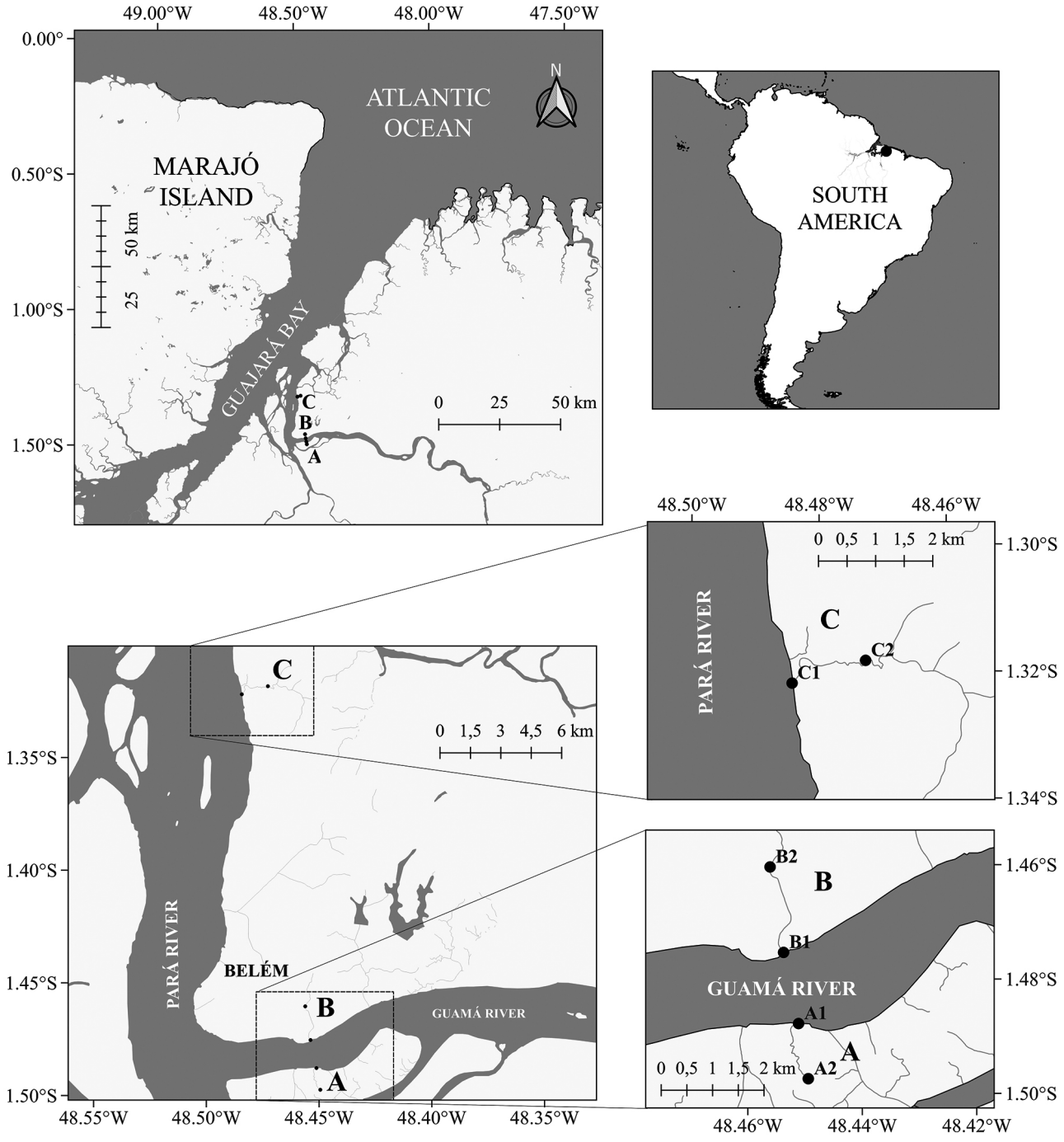

Figure 1. Study area and sampling locations. Combu (**A**): reference site, Tucunduba (**B**) and Mata-Fome (**C**): various sources of anthropogenic contamination. Downcreek (1) and 2 km into the mid-downcreek (2) of the creek towards the upstream.

an Environmental Protection Area since 1997 (SEMA, 2008), and was chosen because it is a reference site, and a relatively protected environment. It has the least direct impact and the largest native coastal vegetation among the studied creeks. The Tucunduba Creek, located on the opposite bank of the Combu Creek, crosses an urban area and is constantly flooding due to the rainfall regime, receiving untreated effluents, garbage disposal and organic waste that are carried to the sea (Souza et al., 2016). The Tucunduba area had an intense migratory flow of human inhabitants, mainly during the 20th century, which generated a mass occupation causing social and environmental problems (Azevedo et al., 2020). Also, the Mata Fome

Creek has the same anthropogenic impact resulting from the occupation of its banks for over three decades; families have occupied the area irregularly since the 1980's (Ravena-Cañete et al., 2009) causing contamination by domestic effluents. In the latter area, water is inappropriate for human consumption, as several potability parameters are outside those recommended by Brazilian legislation.

Data collection

Sampling took place quarterly, from March 2011 to January 2012, covering the two Amazon seasonal periods, rainy (January and March) and less rainy (June and September). Superficial plankton hauls were carried out countercurrent, lasting 3 minutes, downcreek (1) and 2 km into the mid-downcreek (2) in the main channel of each creek towards the upstream (A, B, and C; Fig. 1), during flood and ebb tides. For this, we used conical-cylindrical plankton nets, with a 300 µm mesh (2 m long with a 60 cm diameter net opening) and coupled flowmeter.

Simultaneously to the collection of biological material, the abiotic parameters of temperature (T) (°C), salinity (Sal), pH, conductivity (Cdt) (µs/cm^3), total dissolved solids (TDS), and dissolved oxygen (DO) (mg/L) were measured in situ using a multiparametric probe (YSI), while transparency (Tra) was estimated by a Secchi disk. In addition, 1 L of water was collected to measure turbidity (Tb), total suspended solids (TSS), and chemical oxygen demand (COD) by spectrophotometry (UV-VIS; DR2400 from Hach). The nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), ammonium (NH_4), phosphorus (PO_4), sulfate (SO_4), sodium (Na), calcium (Ca), total iron (Fe), free chlorine (Cl), magnesium (Mg), potassium (K), bromine (Br), and lithium (Li) ions were determined by ion chromatography (ICS Dual 2000 Dionex, USA), while the biochemical oxygen demand (BOD) was evaluated by respirometry (APHA/AWWA/WEF, 2005). The concentrations of total and thermotolerant *Escherichia coli* coliforms were measured using the Collilert-18®/Quanti-Tray®/2000 chromogenic substrate method of the IDEXX Laboratories, Inc.© following the manufacturer's recommendations and the 'Standard Methods for the Examination of Water and Wastewater' (APHA/AWWA/WEF, 2005) and US EPA (2010).

At the end of the expeditions, we obtained a total of 144 samples (3 creeks × 4 months × 2 creek portions × 2 tides × 3 subsamples), with an initial volume of 500 mL each, fractionated with a Folsom-type subsampler in 125 mL aliquots. From these aliquots, *A. rubripes* larvae were dissected and identified according to their morphological characteristics such as the antenna, antennula, maxillipeds and telson under a Zeiss Axio Scope A1 microscope (Carl Zeiss, Oberkochen, Germany) following the methodology of Díaz and Ewald (1968).

Statistical analysis

Relative abundance (%) and larval density (larvae/100 m^3) were calculated for the Combu, Tucunduba, and Mata-Fome Creeks; 1 – downcreek and 2 – mid-downcreek (Fig. 1); and flood and ebb tides for the periods of March, June, September, and January. Larval density (larvae/100 m^3) was estimated by dividing the number of larvae in each sample by the volume of water filtered through the plankton net. The filtered volume was calculated using the number of rotations of the flowmeter coupled to the net opening, from the difference in the digits observed at the beginning and end of each drag, given by: $V = A \times R \times C$, where: A = net opening area (m^2), R = number of flow meter rotations during dragging (final digit – initial digit), and C = measurement factor after calibration.

To verify the variation of larval density in the creeks, creek portions, and tides in the studied months, we used the non-parametric Kruskal-Wallis test since the dataset did not meet the normality and homoscedasticity criteria. The Generalized Linear Model (GLM) was performed to verify the influence of variables on the larval density of the species. The Gamma distribution family with log link function was used (Zuur et al., 2009) because the response variable is characterized as a set of positive continuous data, and the simulated envelope method for model validation (Moral et al., 2017). Both analyses were performed using R (R Core Team, 2018).

RESULTS

Environmental variables

The water temperature in the creeks varied between a minimum of 25 °C (March 2011) and a maximum of 29.8 °C (June 2011). Average salinity also fluctuated over the months; the lowest salinity (0.01) was recorded in March 2011 and the highest (0.4) in January 2012, but the values were predominantly close to 0. The concentration of total suspended solids (TSS) varied from 11 mg/L in January 2012 to 380 mg/L in March 2011. The variation observed for the abiotic factors is summarized in Appendix 1 (Tab. A1).

The highest concentrations of total and thermotolerant coliforms and *E. coli* was determined to be in Tucunduba Creek (3,945, 1,339, and 684 mg/L, respectively), followed by Mata Fome (1,467, 646, and 304 mg/L), and Combu with the lowest concentration (1,333, 335, and 111 mg/L). Furthermore, it is noteworthy that almost all these values exceed the limit of 200 mg/L established by Resolution No. 357 (CONAMA, 2005).

Because the NO_2, NH_3, NH_4, PO_4, Na, SO_4, Mg, K, Br, and Li ions were not detected in all samples and did not meet the model balancing assumption, they were not included in the GLM.

Larval density

A total of 17,949 specimens of *A. rubripes* larvae were identified in the three creeks studied, all in the zoea I developmental stage. Other larval stages of zoea and the megalopa were not found. The highest mean larval density was found in Combu Creek (736/100 m^3) (KW-H = 60.13; $P < 0.01$) where larvae were present in creek portions and tidal conditions in all studied months (Tab. 1, Fig. 2). Larval density peaked during ebb tide in January, with no significant difference between upcreek and downcreek. The mean larval density in Combu Creek is twice the density recorded in the Mata Fome Creek (324/100 m^3) and thirty-five times the density of Tucunduba Creek (21/100 m^3) (Fig. 3). In Tucunduba, no significant difference in larval density was observed between creek portions and tides in the studied months, while in Mata Fome, larvae were found only close to downcreek (Tab. 2).

The Gamma model results showed that conductivity and content of total suspended solids are positively correlated with larval density, whereas salinity and calcium are negatively correlated with larval density (Tab. 3), indicating that these factors are determinants for the larval density of *A. rubripes*.

Table 1. Descriptive statistics for *Armases rubripes* larval density in the Combu creek. N = number of samples, Max = maximum density, Med = median, 25 % = 1st quartile, 75 % = 3rd quartile. The minimum density in all treatments was zero.

	N	Max	Med	25 %	75 %
Downcreek	22	2375.44	87.46	44.90	322.00
Mid-downcreek	19	8363.85	267.03	75.45	548.49
Flood tide	22	2375.44	75.45	27.69	172.90
Ebb tide	19	8363.85	291.92	87.46	1000.37
Total	41	8363.85	128.37	54.90	412.38

Table 2. Results of the Kruskal-Wallis test for the *Armases rubripes* larval density measured in the creeks, creek portions, and tides in the studied months. Values in bold denote statistically significant differences for α = 5 %.

Creek	Months	Portion	Tide			
	KW	p	KW	p	KW	p
A - Combu	**9.60**	**0.02**	0.16	0.68	2.39	0.12
B - Tucunduba	3.12	0.37	0.008	0.92	1.72	0.18
C - Mata Fome	–	–	**5.70**	**0.01**	0.04	0.83

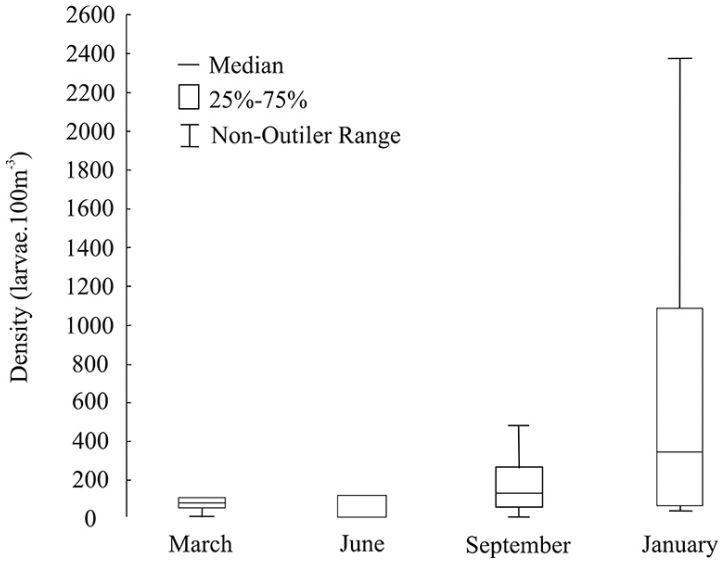

Figure 2. *Armases rubripes* larval density in the Combu Creek over the studied months.

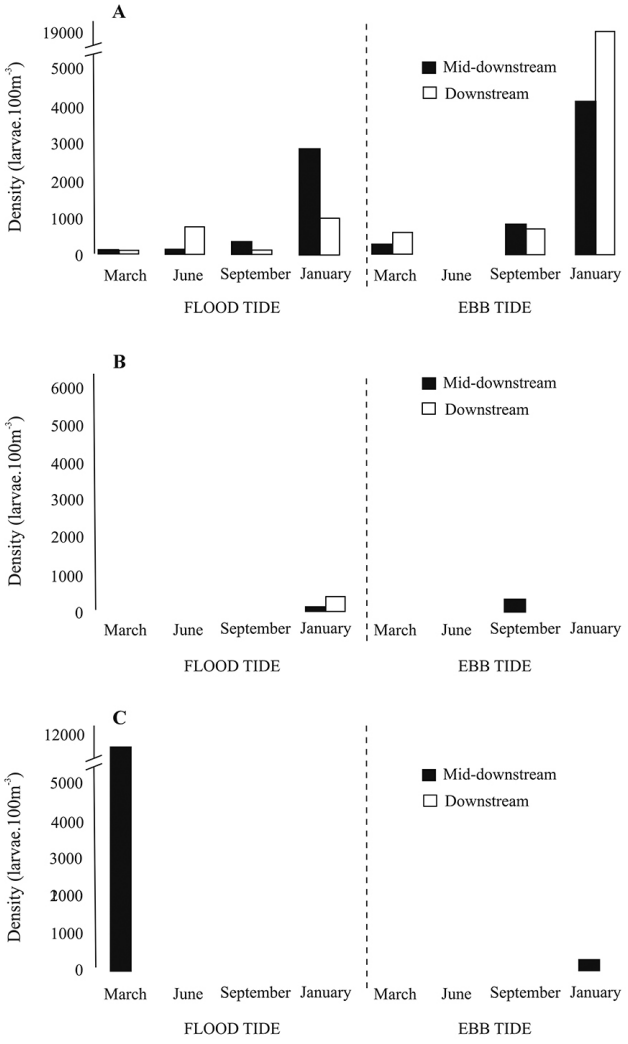

Figure 3. *Armases rubripes* larval density in the Combu, Tucunduba, and Mata Fome Creeks.

Table 3. Estimate of coefficients, standard error, and probabilities (p) of Gamma continuous distribution model. Values in bold denote statistically significant differences for α = 5 %. T = temperature, pH = potential of hydrogen, Cdt = conductivity, TDS = total dissolved solids, Sal = salinity, DO = dissolved oxygen, Tra = transparency, TSS = total suspended solids, COD = chemical oxygen demand, NO_3 = nitrate, Ca = calcium, Cl = free chlorine.

	Estimate	Standard Error	t	p
Intercept	26.211	4.781	5.483	<0.001*
T (°C)	0.545	0.332	1.639	0.106
pH	-0.096	0.367	-0.262	0.794
Cdt (μs/cm^3)	**0.162**	**0.079**	**2.044**	**0.045***
TDS (mg/L)	-0.292	0.157	-1.864	0.066
Salinity	**-8.127**	**3.912**	**-2.077**	**0.041***
DO (mg/L)	0.076	0.158	0.482	0.631
Tra	0.027	0.016	1.654	0.102
TSS (mg/L)	**0.025**	**0.012**	**2.067**	**0.042***
COD (mg/L)	-0.004	0.008	-0.493	0.623
NO_3 (mg/L)	-0.029	0.033	-0.876	0.384
Ca (mg/L)	**-0.127**	**0.035**	**-3.678**	**<0.001***
Cl (mg/L)	0.000	0.002	-0.124	0.902

DISCUSSION

Armases rubripes larvae in the zoea I developmental stage present in the three creeks suggest that the species spawning occurs in oligohaline environments, and its occurrence in the four months matches the continuous seasonal reproduction suggested by Lima et al. (2006; 2019) who researched Brachyura larvae in southeastern Brazil and other coastal regions over one year.

In our study, we verified that larval density increased significantly in January. On the Amazonian Continental Shelf, the highest larval density of *A. rubripes* was also recorded for that month (Lima et al., 2022), which corresponds to the rainy season in the Amazon region and, therefore, seems to indicate the reproductive peak of the species. As it has also been observed and reported for *Aratus pisonii* (H. Milne Edwards, 1837), the increasing reproductive activity during the high rainfall period can be advantageous since the observed increase in nutrients and productivity, favors larval development (Conde and Díaz, 1989).

The occurrence of zoea I exclusively raises the hypothesis of larval export to the adjacent platform since no subsequent larval stages were found in the creek portions or the tides over the studied months. Likewise, the hypothesis that these larvae migrate to more saline waters to complete the development of the larval cycle was recently confirmed by Lima et al. (2022), who found all zoea stages of the species (I – IV) in waters of the Amazonian continental shelf up to 158 km from the coast, and the megalopa up to 83 km from the coast, so that they possibly return to the channels only after reaching the megalopa or juvenile developmental stages (Montú et al., 1990; Schubart and Diesel, 1998). There is no information on the population structure of the adult population, so the discussion for this region remains limited.

Similar to *A. rubripes*, other sesarmids such as *A. roberti* and *A. angustipes* (Dana, 1852) have a similar ontogenetic migration pattern, which includes incubation in freshwater, transport of larvae downcreek, development of later zoea in coastal waters, and final re-migration of juvenile megalopae and crabs to the limbic habitats of adults (Anger et al., 2008). Additionally, laboratory experiments also indicate that the species *Armases ricordi* (H. Milne Edwards, 1853) (Diesel and Schuh, 1998) and *Parasesarma catenatum* (Ortmann, 1897) (Paula et al., 2003) export their larvae from the estuary to the sea.

Another important point is that the highest larval density and distribution occurred in Combu Creek, which has a lower concentration of nitrogen compounds and fecal coliforms and does not show a varying concentration of these compounds, unlike Tucunduba Creek, where high concentrations of nitrite and coliforms were recorded during the ebb tide (Souza et al., 2016). Likewise, a similar abundance pattern

has been observed and reported for *M. amazonicum* larvae in the same creeks, where the highest larval density occurred in Combu Creek, with the presence of early and final stages of development, as the species perform larval retention (Quaresma et al., 2019). This supports the hypothesis of larval exportation for *A. rubripes*, as there is no retention of larvae in the upper parts of the creeks.

Therefore, water quality is a structuring factor for the *A. rubripes* population, so the high degree of anthropogenic contamination in addition to the lack of native vegetation cover does not favor the development and permanence of larvae, as verified in the Tucunduba and Mata Fome Creeks. The degradation of the riparian forest increases the flow of pollutants into the water, which may be related to the urbanization of the Tucunduba and Mata-Fome sites, thus demonstrating the effects of urbanization and pollution on water quality (Ternus et al., 2011; Durigon et al., 2015).

In our study, despite the low variation of salinity, the density of *A. rubripes* zoea I correlated negatively with water salinity, which may be because zoea I hatch in a salinity below 5, and tolerance to increased saline concentration is acquired only in the later developmental stages. This is supported by the fact that the highest density of zoea I larvae was found in Combu Creek, which showed the lowest salinity variation ($\bar{x}$ = 0.016, ± SD = 0.008). This is further corroborated by Lima et al. (2019) who found zoea II in salinity > 14, and by Lima et al. (2022), who found all larval stages (zoea I–IV and megalopa) in salinities between 10 and 36. In general, zoea and megalopa larvae of the *Armases* species have a good osmoregulatory capacity that varies according to the lifestyle of the different species (Anger et al., 2008). The family Sesarmidae has numerous examples of adult and larval adaptations associated with evolutionary lifestyle transitions, which have allowed this group to succeed in adaptive radiation in limbic and terrestrial environments (Frusher et al., 1994; Schubart et al., 1998).

Crabs tend to prefer releasing their larvae during spring flood tides at night (Morgan and Christy, 1995). However, no difference was observed between flood and ebb tides in this study. Since the sampling in this study was conducted during the daytime, it was not possible to observe any nycthemeral variation. Additionally, for some crab species, other environmental conditions, such as osmotic stress and predation, have a more significant role in larval release (Gueron et al., 2023). Another hypothesis is that *A. rubripes* may exhibit high phenotypic plasticity regarding larval release in relation to tidal rhythms (Christopher et al., 2008), thereby increasing their reproductive success.

In summary, the zoea I larvae of the *A. rubripes* semiterrestrial crab hatch in brackish environments, and the water quality of these water bodies has a great influence on larval density. Furthermore, the zoea stages are most certainly exported to the Amazon Continental Shelf searching for more stable conditions for their development, which should be further investigated in the region near the mouth of the Amazon River and adjacent shelf.

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ADDITIONAL INFORMATION AND DECLARATIONS

Author Contributions

Conceptualization and Design: JMML. Performed research: YRGSB, FAL, JMML. Acquisition of data: YRGSB, JMML. Analysis and interpretation of data: YRGSB, JMML. Preparation of figures/tables/maps: YRGSB, FAL, ABGB. Writing - original draft: FAL, ABGB. Final version: YRGSB, JMML.

Consent for publication

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All study data are included in the article and/or supplementary material

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Study permits

All experiments conducted in this study complied with current applicable Brazilian state and federal laws (process DIFAP/ IBAMA # 02018.008516 94-2005)

APPENDIX 1

Table A1. Descriptive statistics of abiotic factors in the Combu, Tucunduba, and Mata Fome creeks. T = temperature, pH, Cdt = conductivity, TDS = total dissolved solids, Sal = salinity, DO = dissolved oxygen, Tra = transparency, Tb = turbidity, TSS = total suspended solids, COD = chemical oxygen demand, NO_2 = nitrite, NO_3 = nitrate, NH_3 = ammonia, NH_4 = ammonium, PO_4 = phosphorus, SO_4 = sulphate, Na = sodium, Ca = calcium, Fe = total iron, Cl = free chlorine, Mg = magnesium, K = potassium, Br = bromine, Li = lithium, BOD = biochemical oxygen demand. Min = minimum, Max = maximum, SD = standard deviation.

	Min	Max	Average	SD	Median	Quartiles 25 – 75 %
T (ºC)	11	28	21.16	2.07	21.08	20.41–22
pH	5.52	8.86	6.61	0.5	6.54	6.29–6.83
Cdt (µs/cm^3)	13	636	127.5	146.8	40	28–266
TDS (mg/L)	7	318	64.5	74.34	20	14–133.5
Salinity	0.1	0.4	0.06	0.07	0.02	0.01–0.13
DO (mg/L)	2.35	9.79	5.82	1.71	5.9	5–6.9
Tra	5	70	27.58	13.38	23	18–36.5
Tb	11	261	54.84	43.54	48.5	18–82
TSS (mg/L)	11	380	48.93	49.69	39	23–59
COD (mg/L)	1	225	36,77	38.06	25	16.5–43
NO_2 (mg/L)	0	0.47	0.08	0.12	0.009	0–0.14
NO_3 (mg/L)	0	40.4	2.86	4.98	1.2	0.46–3
NH_3 (mg/L)	0	8.45	1.47	1.93	0.76	0.1–2
NH_4 (mg/L)	0	10.26	1.78	2.34	0.93	0.1–2.4
PO_4 (mg/L)	0	2.36	0.47	0.53	0.34	0.04–0.65
SO_4 (mg/L)	0	79.74	9.64	12.51	4.6	2.7–13
Na (mg/L)	2.82	80.16	15.22	12.72	13.57	7–18.1
Ca (mg/L)	0	55.0	11.0	13.13	5.46	2–16.63
Ferro (mg/L)	0	2.34	0.32	0.42	0.1	0.02–0.64
Cl (mg/L)	0	764.8	55.87	142	13.47	10.1–27.3
Mg (mg/L)	0	5.58	2.32	1.5	2	1–3.1
K (mg/L)	0	15.39	3.3	2.76	2.18	1.5–5.1
Br (mg/L)	0	8.29	1.18	1.61	0.8	0–1.61
Li (mg/L)	0	0.2	0.01	0.02	0.01	0–0.02
BOD (mg/L)	1	27	7.42	4.81	7	3–10