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First record of the potential invasive crab *Rhithropanopeus harrisii* (Gould, 1841) in the Uruguayan coast

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ABSTRACT

Abstract: Rhithropanopeus harrisii (Brachyura, Panopeidae) is a small euryhaline and eurythermal omnivorous crab native to the Northwest Atlantic. However, it has become an invasive species in various estuaries and coastal areas far from its original habitat, most likely due to intercontinental shipping. Once it establishes itself in one location, it can spread to neighboring regions via maritime currents. In 1982, this species was found in South America, specifically in Lagoa dos Patos, Brazil. In this study, we present the first record of R. harrisii larvae in Uruguay, located in the coastal marine zone of Laguna de Rocha, approximately 300 km south of Lagoa dos Patos. It was discovered during a study that involved 15 plankton samplings, conducted from February 2016 to February 2017. Notably, the observation of R. harrisii larvae was limited to the coastal zone during the summer and autumn of 2016 (February to April). All observed larvae were in the zoeal stage, and their abundance ranged from three to 185 individuals per 100 m³. The temperature and salinity values recorded during sampling surveys with the presence of R. harrisii ranged from 19 to 22.4 °C and 11.8-32.5 ppt, respectively. The discovery of the larval stage suggests that adults of this species may be reproducing in the eastern coastal zone of Uruguay or nearby regions. The area in which they are currently found could potentially serve as a biological corridor, facilitating their spread to other estuaries of great economic and ecological importance, such as the Río de la Plata, as well as other coastal lagoons and subestuaries in Uruguay and Argentina. Further monitoring studies are necessary to determine whether this species became established in the area. Potential ecological consequences in our region derived from its presence are herein discussed.

Keywords: Exotic species, Southwestern Atlantic, Estuarine crab, Zoea, Early detection

Trade globalization has significantly accelerated the worldwide rate of biological invasions (Mack et al., 2000; Meyerson and Mooney, 2007). The introduction

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of alien and invasive species has had a major impact on coastal and estuarine environments, in particular (Grosholz, 2002; Nehring, 2006). Among the various pathways for species transfer to new biogeographic regions, marine transportation plays a major role (Nehring, 2006; Lodge et al., 2006). Invertebrate organisms, especially those in their larval stage, have been extensively dispersed in this mode of transportation (Minchin and Gollasch, 2003).

Rhithropanopeus harrisii (Brachyura, Panopeidae) is a small euryhaline and eurythermal crab. This omnivorous species is native to the East Coast of North America, specifically the Northwest Atlantic region. It has developed a life strategy that involves larval vertical migrations, allowing it to retain its larvae within estuaries (Cronin, 1982; Forward, 2009). While its native distribution spans from New Brunswick, Canada, to Veracruz, Mexico, R. harrisii is considered an invasive species in various coastal areas far from its origin. It has successfully established populations in Europe, Asia, Central America, and South America (D'Incao and Martins, 1998; Roche and Torchin, 2007; Iseda et al., 2007; Langeneck et al., 2015). This expansion has been facilitated by the species' ability to tolerate a wide range of environmental conditions during both its pelagic (larvae) and benthic (juveniles and adults) phases. Estuaries and port areas have been particularly favorable for its proliferation. Despite being primarily an estuarine crab in its native range, R. harrisii has demonstrated the ability to survive in environments with diverse salinity values, ranging from 0.1 to 35 ppt. It has even managed to colonize freshwater habitats, including lagoons with no direct connection to the ocean (Roche et al., 2009).

This species exhibits a pattern of persisting in a particular site for an extended period before initiating an expansion phase towards neighboring regions (Fowler et al., 2013). A notable example is its introduction in Europe, with the first records dating back to 1874 in the Netherlands. In the following years, it was recorded in other parts of the continent, such as the Black Sea, the Atlantic coast, and the Mediterranean Sea. Once established in a new region, i.e., transitioned from a group of new colonist individuals into a self-sustaining population, the species can undergo rapid expansion within that area. A case in point is its colonization of the Baltic Sea, where a population was initially recorded in the southern zone and quickly expanded northward (Gagnon and Boström, 2016; Kotta and Ojaveer, 2012). The success of R. harrisii in spreading and establishing in the Baltic Sea can be attributed to its ability to tolerate brackish waters, which prevail in the region, as well as to the absence of native predatory crab species (Kotta and Ojaveer, 2012; Kottaetal., 2018). In South America, the first observation of this crab occurred in the estuarine region of Lagoa dos Patos (Figure 1A) in 1982 (Tavares, 2011) and was documented for the first time in 1998 (D'Incao and Martins, 1998). Its establishment was confirmed in subsequent years (Rodrigues and D'Incao, 2015; Rodrigues and Fonseca, 2021). The species have also been reported in Sao Paulo and probably in Alagoas (Coelho et al., 2008). However, no reports of this species in other regions of the continent have been recorded.

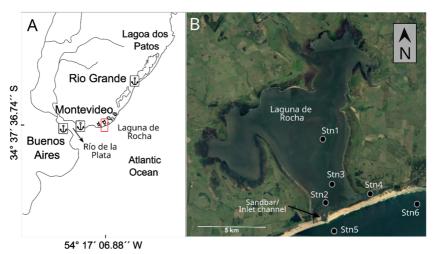


Figure 1. A) Location of Laguna de Rocha and adjacent coastal zone (red box) on the coast of Uruguay in the southwestern Atlantic. The main commercial ports in the region (Río Grande, Montevideo, and Buenos Aires) are indicated. B) The sampling stations in Laguna de Rocha (Stn 1, Stn 2, Stn 3, Stn 4) and in the adjacent coastal waters (Stn 5, Stn 6) are indicated with grey circles. The inlet channel location is indicated with an arrow.

The Atlantic coast of Uruguay serves as a biological corridor for estuarine crabs migrating from the southwestern Atlantic region (e.g., Ituarte et al., 2012). Furthermore, in the southern region of Brazil, along the Uruguayan and northern Argentinian coast, three major international commercial ports are found (Fig. 1 A), as well as several smaller commercial and sports ports. This means that invasive species have multiple alternative routes to enter the region, aside from ocean currents (e.g., Projecto-Garcia et al., 2010). The large estuary of the Río de la Plata is found on the southwestern Uruguayan and northern Argentinian coasts, with several coastal lagoons connected to the sea. Estuaries are located along the Brazilian and Argentinian coasts (Figure 1A). The diverse range of habitats along this coastline (e.g., Giménez et al., 2014; Kandratavicius et al., 2015), coupled with the turbid waters of the Río de la Plata and its discharge plume (Piola et al., 2008; Maciel et al., 2023), create favorable conditions for the establishment of R. harrisii. Consequently, it is reasonable to expect the species to establish and rapidly expand, similar to its expansion in the Baltic Sea (Kotta and Ojaveer, 2012; Nurkse et al., 2015). Although the impacts of this crab in newly colonized areas have not been extensively studied, evidence suggests that it can cause economic losses by damaging pipelines and interfering with gillnet catches when abundance is high (Roche and Torchin, 2007; GISD 2008). It has also been observed to cause changes in benthic invertebrate and phytoplankton communities, as well as in the availability of pelagic nutrients (Kotta et al., 2018).

This work represents the initial documentation of *R. harrisii* in Uruguay, based on the identification of zoeal larvae in the coastal marine area adjacent to Laguna de Rocha, situated approximately 300 km south of Lagoa dos Patos. Our study provides information on the abundance of recorded larvae and describes the prevailing environmental conditions during the observation period. Moreover, we examined the potential implications of this discovery at a regional level.

This study area encompasses Laguna de Rocha (LR), a brackish coastal lagoon, and its adjacent coastal zone in Uruguay, located at coordinates 34° 38' S - 54° 17' W (Figure 1). LR is part of a

series of lagoons situated along the Uruguayan and southern Brazilian coasts. With a surface area of 72 km², LR is characterized by its shallow nature, with an average depth of 1 m during the study period. It intermittently connects to the ocean via a channel that opens on the sandbar once or multiple times a year. These periodic connections result in the continuous interchange of water between the lagoon and the sea (Rodríguez-Gallego et al., 2015). The adjacent coastal zone is influenced by the plume of the Río de la Plata, resulting in typical water salinity values of around 30 ppt. However, the salinity can sometimes decrease significantly, ranging from 7 to 25 ppt (Machado et al., 2021). The water characteristics in the coastal zone, along with their associated biological communities, are influenced by various meteorological and oceanographic processes such as coastal upwelling, fluvial discharges, and wind patterns. These processes exhibit seasonal and interannual variations, impacting the coastal zone's dynamics and its ecosystems (Machado et al., 2013; Martínez and Ortega, 2015; Trinchin et al., 2019).

LR, along with its adjacent coastal zone, holds a significant ecological value, being designated part of the National System of Protected Areas and recognized as Ramsar Site. One of the distinguishing features of this lagoon and coastal area is the limited human impact, characterized by the absence of coastal roads that cross the lagoon and a low housing population density. This preservation has contributed to the conservation of the natural environment. The study area is home to various crab species, with a notable presence in both LR and the adjacent coastal zone. Among these species, Neohelice granulata (mud crab), Cyrtograpsus angulatus (rock crab), and Callinectes sapidus (blue crab) are particularly dominant. These species differ from others by developing a larval export strategy wherein the adults primarily inhabit the lagoon while the larvae reside in the coastal zone (Bas et al., 2009; Rodríguez and Luppi, 2020). The ecological significance of the first two species in this region of the continent lies in their role as bioturbators and as a crucial food source for seabirds (Botto et al., 2005). On the other hand, the blue crab holds notable commercial importance within the local artisanal fishery operating in the lagoons (Fabiano and Santana, 2006).

The study that found the presence of *R. harrisii* was conducted from February 2016 to February 2017. The sampling design comprised 15 plankton samplings, encompassing four sites within LR and two sites in the adjacent coastal area (Figure 1), except for S1 to S4, where sampling in LR was limited to two sites (see Table 1). The primary objective of the study was to investigate the community composition of fish and decapod larvae (Machado et al., 2021; Machado, 2022). Field trips occurred approximately every 45-60 days, but the sampling frequency was increased to 7-15 days when the

channel on the sandbar was open (Table 1). Measurements of *in situ* water temperature (°C) and salinity (ppt) were recorded at each site and sampling. Quantitative larval sampling was carried out using a conical net with a diameter of 65 cm and a mesh size of 500 μ m in the sea. In the lagoon, an epibenthic sledge trawl measuring 1 m x 0.8 m, with a 500 μ m mesh, was used. Flowmeters affixed to the nets were used to estimate the volume of filtered water. Zoeae abundance was quantified as 100 m³. The samples were preserved in 4% formalin and subsequently counted and identified under a microscope with taxonomic keys (Connolly, 1925; Santos and González-Gordillo, 2004).

Table 1. Sampling surveys conducted from February 2016 to February 2017 (S1 to S15) at Laguna de Rocha and its adjacent coastal waters. Dates, sandbar state (open/closed), and presence (P) or absence (A) of *Rhithropanopeus harrisii* is indicated. The hyphen (-) indicates missing samplings survey due to adverse weather conditions for navigation and the asterisk (*) indicates samplings including only Stn 1 and Stn 2 sites. Stn: sampling sites.

Samplings surveys	Laguna de Rocha	Coastal waters	Sandbar state	R. harrisii P/A			
S1	02/16/16 *	02/26/2016	Closed	P: Stn 5, Stn 6			
S2	03/30/2016 *	03/31/2016	Closed	P: Stn 5, Stn 6			
S3	04/21/2016 *	04/22/2016	Open	P: Stn 6			
S4	04/30/2016 *	05/05/2016	Open	А			
S5	06/13/2016	06/15/2016	Open	А			
S4	07/28/2016	08/09/2016	Open	А			
S7	10/06/2016	10/07/2016	Open	А			
S8	10/14/2016	-	Open	А			
S9	10/21/2016	10/22/2016	Closed	А			
S10	10/29/2016	-	Open	А			
S11	11/04/2016	-	Open	А			
S12	11/14/2016	11/15/2016	Open	А			
S13	11/29/2016	11/30/2016	Open	А			
S14	12/15/2016	12/21/2016	Open	А			
S15	02/14/2017	02/03/2017	Closed	A			

The water temperature in the study area exhibited the characteristic seasonal fluctuations commonly observed in subtropical regions, ranging from 8.2 to 28.6 °C. As expected for a shallow system, LR recorded both the maximum and minimum values (Table 2). Compared to the coastal zone, LR showed lower or equivalent salinity values, ranging from 1 to 32.5 ppt, whereas the coastal zone exhibited a range of 7 to 32.5 ppt.

Following the opening of the channel across the sandbar in April 2016, a decrease in salinity was observed in both LR and the adjacent zone. This decline continued until reaching the minimum value in each site (Table 2). Subsequently, during the spring season, salinity values in both areas gradually recovered, surpassing 15 ppt. More details about the environmental conditions during the study are shown in Machado et al. (2021). **Table 2.** Temperature (°C), salinity (ppt), and *Rhithropanopeus harrisii* abundance (100 m³) in Laguna de Rocha (Stn 1, tn 2, Stn 3, Stn 4) and in the coastal waters (Stn 5, Stn 6) from February 2016 to February 2017 (S1 to S15). *R. harrisii* was only recorded in the coastal waters. The relative abundance of *R. harrisii* in comparison to the total zoea abundance in the respective sample is provided between brackets. The gray columns denote that the sandbar was closed.

		Summer	Autum	Autumn Spring												
		02/16	03/30	04/21	04/30	06/13	07/28	10/06	10/14	10/21	10/29	11/04	11/14	11/29	12/15	02/14
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Temperature (°C)	Stn 1	28.6	21.6	18.4	11.8	8.5	15.3	17.2	18.5	15.1	14.1	17.5	20.0	22.0	18.6	24.4
	Stn 2	23.5	22.1	18.2	13.7	8.2	10.4	17.8	18.6	15.9	14.1	17.9	21.0	21.6	19.5	24.5
	Stn 3	-	-	-	11.7	8.6	10.2	18.7	19.5	15.9	14.5	18.2	20.8	20.7	20.0	24.8
	Stn 4	-	-	-	11.6	9.2	10.5	19.1	18.4	17.4	14.0	19.3	22.9	25.5	20.0	25.5
	Stn 5	22.4	21.1	19.0	14.6	10.8	11.7	11.8	-	14.3	-	-	18.3	18.0	21.1	22.6
	Stn 6	22.4	21.3	19.0	14.6	11.1	12.0	11.7	-	14.7	-	-	18.4	18.2	20.7	22.6
Salinity (ppt)	Stn 1	9.4	16.4	1.2	7.4	8.3	8.4	15.7	15.8	14.3	23.1	18.0	19.3	26.0	27.7	16.9
	Stn 2	16.6	16.7	1.1	5.0	7.5	8.7	15.6	17.3	15.1	15.9	16.9	18.5	29.2	26.7	17.4
	Stn 3	-	-	-	8.8	7.3	5.0	16.0	17.0	15.4	23.1	16.9	20.0	29.3	27.5	17.0
	Stn 4	-	-	-	8.2	7.0	8.3	16.6	17.6	15.0	23.1	21.0	23.3	30.4	30.4	17.4
inity	Stn 5	32.5	21.1	11.8	6.8	17.3	20.6	32.3	-	32.4	-	-	28.0	31.7	29.3	31.8
Sali	Stn 6	32.5	21.5	12.0	6.5	18.5	24.6	33.3	-	32.5	-	-	28.8	31.9	30.5	31.9
	Stn 5	1.5 (1)	215 (4)	7 (29)	0	0	0	0	-	0	-	-	0	0	0	0
Abundance (100 m ⁻³)	Stn 6	4.1 (2)	155 (12)	0	0	0	0	0	-	0	-	-	0	0	0	0

Zoeae of *R. harrisii* were identified and characterized by distinct morphological features (Figure 2). Carapace bore rostral, dorsal, and lateral spines, with the rostral spine surpassing twice the length of the cephalothorax. The antennae exhibit a length equivalent to that of the rostral spine. The second pleonite presents a small dorso-lateral process, while the third pleonite lacks such a process. The fourth pleonite was marked by a short postero-lateral process, while the fifth displays an elongated postero-lateral process. The telson is bifurcated, each fork featuring a dorsal spine, while lateral spines are absent.

Rhithropanopeus harrisii larvae were observed exclusively in the adjacent coastal zone, specifically

at Stn 5 and Stn 6, in three out of the 15 samplings. These occurrences were recorded during the summer and early autumn of 2016 (Table 2). The recorded temperature and salinity values during these positive R. *harrisii* samplings ranged from 19 to 22.4 °C and 11.8 to 32.5 ppt, respectively. The zoeae abundance varied from 1.5 to 215 individuals per 100 m³. No differences were found in larval abundance between the two sampled sites in the coastal zone (data not shown). The maximum abundance of *R. harrisii* larvae was observed just before the opening of the sandbar, coinciding with a salinity level of 22 ppt in the coastal zone. However, following the autumn sandbar break,

the abundance notably decreased and was absent in subsequent samplings throughout the study period. Compared to other dominant species, the abundance of *R. harrisii* zoeae remained relatively low, ranging from 1% to 29% of the total abundance of brachyuran zoeae (Table 2).

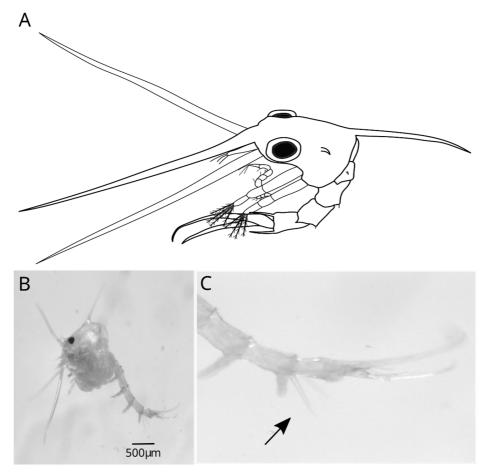


Figure 2. *Rhithropanopeus harrisii* (Gould, 1841) zoea. A) General scheme of zoea I– lateral view. Redraw from Connolly (1925). B) and C) Photographs of zoea III collected in the adjacent coastal waters of Laguna de Rocha. The arrow in C) shows the long postero-lateral process on the fifth pleonite.

According to the morphological features, the zoeae found in this study belonged to *R. harrisii*. Within the Panopeidae family, *R. harrisii* is the only species found in Brazil and Uruguay (13 species hold larval descriptions). Notably, it is distinguished by the absence of a dorso-lateral process on the third pleonite and the presence of an elongated postero-lateral process on the fifth pleonite (Figure 2). Despite the lack of adult reports in Uruguay, specimens that likely belong to this species have been stored as unidentified in the scientific collection of the Museo Nacional de Historia Natural in recent years (Scarabino personal communication). While the zoeae differ in

appearance, adult external morphological features may closely resemble those of other Panopeidae members, such as *Dyspanopeus sayi*.

The presence of *R. harrisii* larvae suggests two possible scenarios. The species may be reproducing along the Atlantic coast of Uruguay or they may have been transported by currents from neighboring regions like Lagoa dos Patos, although this latter scenario is less probable. Despite the low absolute and relative abundance of *R. harrisii* compared to other crab species, and its sole record in the larval stage, this discovery serves as an early indication of the species' presence in this area (e.g. Reaser et al. 2020). To ascertain its establishment in Uruguay, further investigations are imperative, specifically focusing on evaluating the benthic phase. Previous research highlights *R. harrisii* juveniles and adults prefer coastal and brackish environments, particularly those featuring high spatial heterogeneity, such as aquatic vegetation and reefs, as opposed to bare bottoms (Kotta and Ojaveer, 2012; Aarnio et al., 2015).

The absence of *R. harrisii* larvae in the coastal zone after the opening of the sandbar in autumn 2016 suggests potential impacts of climatic and hydrological events on the species' populations. This observation bears relevance when managing sandbar openings in the Laguna de Rocha protected area. Conversely, the intermittent connection between LR and the sea may explain the absence of R. harrisii larvae in LR. While limited estuary-sea connectivity can hinder the spread of exotic species (Garside et al., 2014), observations in places like Culvert Miraflores Lake, in Panama, where direct oceanic connection is absent, imply a broader colonization potential for the species. Moreover, LR hosting other estuarine-marine invasive species indicates that intermittent sea connection may not entirely prevent marine alien invasions (Vidal et al., 2021).

The potential impacts of R. harrisii on local species and ecosystems require consideration, drawing insights from previous studies. In the Baltic Sea, where R. harrisii was introduced, adverse effects on the benthic invertebrate community, increased pelagic nutrient availability, and phytoplankton bloom frequency were observed (Kotta et al., 2018). High abundances of *R. harrisii* juveniles and adults lead to elevated consumption rates of benthic invertebrates like bivalves, polychaetes, and amphipods (Aarnio et al., 2015), which play a crucial role in energy transfers within and between benthic and pelagic habitats (Griffiths et al., 2017; Milessi et al., 2010; Bergamino et al., 2018). Given the composition and ecological role of benthic invertebrates in regional estuaries (e.g., Giménez, 2006), coupled with nutrient levels in water and sediments (Rodríguez-Gallego et al., 2017; Tuduri et al., 2021), the expansion of R. harrisii may

induce analogous shifts in benthic invertebrates and nutrient dynamics.

The presence of *R. harrisii* in South American estuaries could potentially alter the prevailing crab larvae structure. Unlike species with larval export strategies such as Neohelice granulata and Cyrtograpsus angulatus, R. harrisii exhibits behaviors favoring estuarine habitation. The establishment of *R. harrisii* could lead to an increase in larval abundance, consequently competing with other crab species' larvae for food resources, encompassing nauplii, copepods, and other meso- and microzooplankton (Jeffs and O'Rorke, 2020). Moreover, it could also induce competition between organisms reliant on the same prey items, especially fish larvae susceptible to larval-period starvation, thus affecting its recruitment (Hjort, 1914; Cushing, 1969). Consequently, anticipating and monitoring the potential consequences of R. harrisii establishment is imperative, as it could have major impacts on the estuarine food web.

The detection of *R. harrisii* on Uruguay's Atlantic coast serves as an early warning for invasive species management. Swift action and early detection are critical for successfully eradicating aquatic invasive species (Reaser et al., 2020; Roche et al., 2009). Taking proactive measures, like employing trapping methods for capturing adults and controlling potential vectors such as ballast water or nautical activities, can mitigate the spread of both adults and larvae to other coastal regions. However, the feasibility and potential unintended consequences of chemical eradication methods should be thoroughly evaluated (Bax et al., 2002). Some experts contend that eradicating this crab in open environments may be challenging (Kotta et al., 2018). Regardless of the chosen eradication strategy, continuous monitoring of R. harrisii in the Laguna de Rocha protected area, encompassing various life stages (larval and adult), is essential. Given the designation of numerous coastal lagoons and adjacent seas, such as national and international marine protected areas, along with the significance of Río de la Plata in sustaining fishing activities for Uruguay and Argentina, extending monitoring efforts

to these estuarine systems is crucial to safeguard ecological integrity and economic interests.

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AUTHOR CONTRIBUTIONS

- IM: Conceptualization, Methodology. Formal analysis, Investigation, Resources, Writing- Original draft, Project administration, Funding acquisition.
- SP: Investigation, Writing Review & Editing.
- RV: Investigation, Writing Review & Editing, Visualization.
- DC: Conceptualization, Methodology, Resources, Writing-Review & Editing, Funding acquisition.
- LRG: Conceptualization, Methodology, Resources, Writing-Review & Editing, Supervision.

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