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Pea crab-oyster interspecific interaction on tropical coast: Brachyuran pinnotherid *Zaops ostreum* (Say, 1817) living in the host bivalve ostreid *Crassostrea brasiliana* (Lamarck, 1819)

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Abstract. In this study, we report the association between the pea crab *Zaops ostreum* (Say, 1817) living in the oyster *Crassostrea brasiliana* (Lamarck, 1819), collected in an estuarine region of Northeast Brazil. Oysters were collected along the mouth of the Santa Cruz Channel, Barra de Catuama (State of Pernambuco), between 2016 and 2017. A total of 224 oysters and 38 crabs were collected. Relative density of *Z. ostreum* was 16.96% (7.14% for males and 9.82% for females) in analyzed oysters. Prevalence during dry season ranged from 6.25% to 15.60%, whereas in rainy season ranged from 3.10% to 12.50%. *Z. ostreum* proportion of females to males was 1:0.73. Most occurrences were female-male couples (79.29%) with no isolated males or triple occupancy being observed. Female-male couples and ovigerous females were detected throughout most of the studied period, possibly indicating a distinct reproduction behaviour on

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tropical areas. This association expands knowledge on the aspects of social behaviour and host range of *Z. ostreum*, remarkably associated with bivalves of the Family Ostreidae.

Keywords: Ecology; Oysters; Pea crabs; Brazilian estuary; Barra de Catuama.

Resumo. ***Interação interespecífica entre ostra e caranguejo-ervilha na costa tropical: pinnotherídeo braquiúro* Zaops ostreum *vivendo no bivalve ostreídeo hospedeiro* Crassostrea brasiliana *(Lamarck, 1819)***. Neste estudo, é registrado a associação entre o caranguejo-ervilha *Zaops ostreum* (Say, 1817) vivendo na ostra *Crassostrea brasiliana* (Lamarck, 1819), coletada em uma região estuarina do nordeste do Brasil. As ostras foram coletadas ao longo da foz do Canal de Santa Cruz, Barra de Catuama (Estado de Pernambuco), entre 2016 e 2017. Um total de 224 ostras e 38 caranguejos foram coletados. A densidade relativa de *Z. ostreum* foi de 16,96% (7,14% para machos e 9,82% para fêmeas) em todas as ostras analisadas. A prevalência durante a estação seca variou entre 6,25% e 15,60%, enquanto na estação chuvosa variou entre 3,10% e 12,50%. A proporção de *Z. ostreum* entre fêmeas e machos foi de 1:0,73. A maioria das ocorrências foi de casais de fêmeas e machos (79,29%), não tendo sido observados machos isolados ou ocupação tripla. Casais de fêmeas e machos e fêmeas ovígeras foram detectados durante a maior parte do período estudado, possivelmente indicando um comportamento de reprodução distinto em áreas tropicais. Essa associação amplia o conhecimento sobre os aspectos do comportamento social e da gama de hospedeiros de *Z. ostreum*, notavelmente associado a bivalves da Família Ostreidae.

Palavras-chave: Ecologia; Ostras; Caranguejos-ervilha; Estuário Brasileiro; Barra de Catuama.

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Introduction

Classification of ecological interactions is one of the main themes in aquatic life studies (Emparanza et al., 2011; Queiroz et al., 2011; Boehs et al., 2012; Lima et al., 2014, 2017; Ferreira-Jr. et al., 2015). In certain ecological associations, crustaceans utilize the body structures of other organisms during development, reproduction and feeding (Baeza and Thiel, 2007; Martinelli Filho et al., 2014; Hanke et al., 2015), occurring either alone or forming couples (Baeza and Thiel, 2007). Crustaceans of the Family Pinnotheridae, commonly known as pea crabs, are often found associated with several organisms (Hultgren et al., 2022), such as tunicates (Hernández and Bolaños, 1995), echinoderms (Queiroz et al., 2011; Lima et al., 2014), molluscs (Sun et al., 2006; Asama and Yamaoka, 2009; Mena et al., 2014; Hanke et al., 2015), as well as in polychaetes tubes (McDermott, 2005, 2009). The association between crabs and oysters is well-known due to aquaculture activities, particularly oyster farming, as the presence of pinnotherids affects the development and growth of these bivalves (Nascimento and Pereira, 1980; Mena et al., 2014; Hanke et al., 2015).

The genus *Zaops* Rathbun, 1900 comprises three valid species of the Family Pinnotheridae found in West Atlantic waters, among which only *Zaops ostreum* (Say, 1817) is reported on Brazilian coastal environments from Northeast to Southern Brazil (Nascimento and Pereira, 1980; Martins and D'Incao, 1996; Sankarankutty and Ferreira, 2001; Bezerra et al., 2006; Felder et al., 2009; Almeida et al., 2010; Boehs et al., 2012). *Z. ostreum* is characterized by arched-shape orbits, cephalothorax curved on both sides of the carapace, clypeus emarginate at anterior end, and chelipeds covered with white hairs (Say, 1817; Melo, 1996). It is known to be parasitic on bivalve oysters of the genus *Crassostrea* Sacco, 1897, including *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea virginica* (Gmelin, 1791) (Stauber, 1945; Christensen and McDermott, 1958; Beach, 1969; Nascimento and Pereira, 1980; Martins and D'Incao, 1996; Melo, 1996; O'Beirn and Walker, 1999; Sankarankutty and Ferreira, 2001; McDermott, 2009; Almeida et al., 2010; Hanke et al., 2015), which can cause economic losses in aquaculture. *Z. ostreum* has an interspecific relationship with the oysters' species, feeding on the phytoplankton and zooplankton that these oysters filter (McDermott, 1997; Almeida et al., 2010; Hanke et al., 2015). Individuals of *Z. ostreum* can also occur in the pallial cavity of other bivalves, such as *Anomalocardia flexuosa* (Linnaeus, 1767), *Anomia peruviana* d'Orbigny, 1846, *Mytilus edulis* Linnaeus, 1758, and members of the genus *Pecten* Müller, 1776 (Bezerra et al., 2006; Sabry et al., 2011; de Gier and Becker, 2020). *Z. ostreum* females can also establish a negative ecological interspecific relationship with the ostreids (like parasitism), damaging on the host soft parts (Hanke et al., 2015).

Members of the Family Ostreidae are molluscs that commonly occur in shallow tropical waters (Amaral and Simone, 2014), typically forming specific interrelationships with other invertebrates (O'Connor and Newman, 2001; Sabry and Magalhães, 2005; McDermott, 2006; Radashevsky et al., 2006; Herbert et al., 2009; Martinelli Filho et al., 2014; Ferreira Jr. et al., 2015; Hanke et al., 2015). In Northeast Brazil, *Crassostrea brasiliana* (Lamarck, 1819) is one of the main oysters characterized by the rounded to ellipsoidal greenish-brownish shells with non-uniform margin, left valve fixed in substrate (such as rock or roots of mangrove) and adductor muscle on dorsal-posterior region, representing 20% of total oyster size (Amaral and Simone, 2014).

Despite the many studies between pinnotherid crabs and oysters, the ecological relationship of *Z. ostreum* and *C. brasiliana* is still unknown. In this way, this study records the association of *Z. ostreum* and *C. brasiliana* as well as addresses ecological aspects of the interaction on tropical waters from Northeast Brazil.

Materials and methods

Study site

The survey was carried out at Barra de Catuama Beach (Figure 1), which belongs to the estuarine system of Itamaracá Island on the Northern coast of the State of Pernambuco, Northeast Brazil (07° 33' 38" S, 35° 00' 09" W). The estuary receives inputs from several rivers, forming the Santa Cruz Channel (Medeiros and Kjerfve, 1993), which is characterized by a flat topography and a mean depth ranging from 4 to 5 m at low tide (Medeiros and Kjerfve, 1993; Neumann-Leitão and Schwamborn, 2000). The vegetation in the region is represented by *Rhizophora mangle* Linnaeus, 1753, *Avicennia shaueriana* Stapf & Leechman *ex* Moldenke, 1939 and *Laguncularia racemosa* (Linnaeus, 1753) (Neumann-Leitão and Schwamborn, 2000). Adjacent to the Santa Cruz Channel and Barra de Catuama Beach, beach rocks parallel to the shore reduce wave action and hydrodynamics in the region (Medeiros and Kjerfve, 1993).

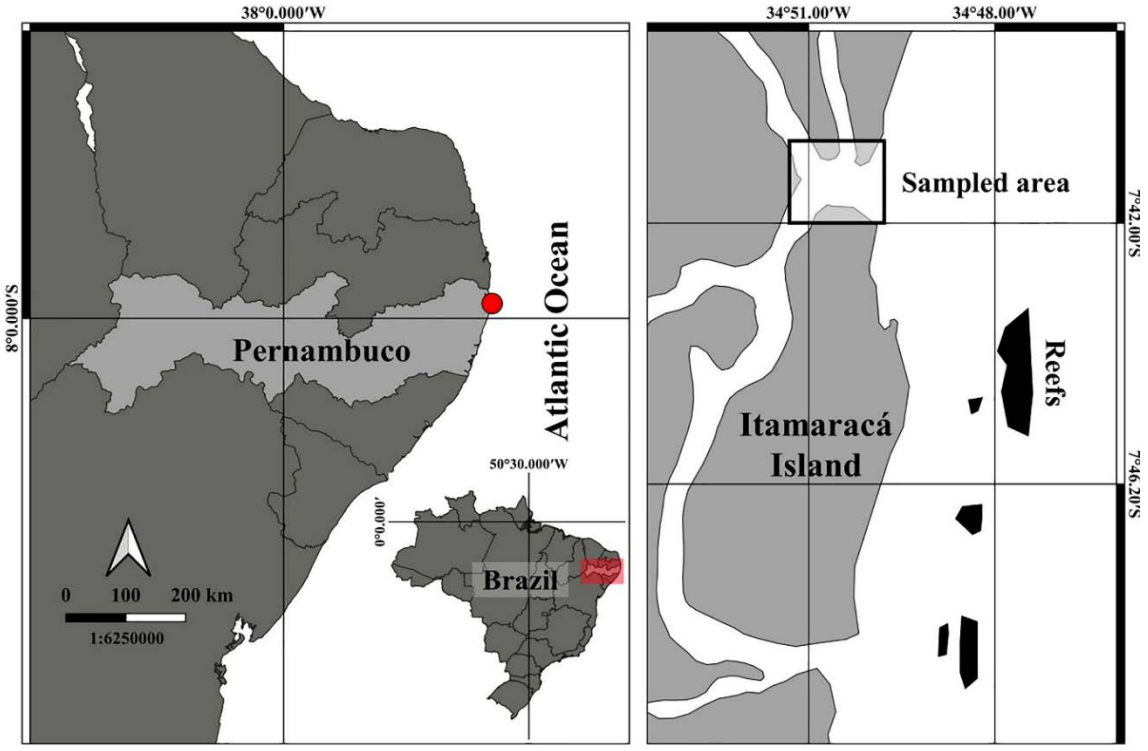

Figure 1. Study site at Barra de Catuama Beach, located at mouth of Santa Cruz Channel, State of Pernambuco, Northeast Brazil.

Sampling and laboratory procedures

Sampling was performed between June 2016 and April 2017, considering April to August the rainy season and September to March the dry season (Table 1). Oysters attached to wooden pillars along the mouth of the Santa Cruz Channel were randomly collected at low tide in the intertidal zone with the aid of spatulas, with standardization of 32 oysters per sample. Organisms were stored in a cooler filled with ice and sent to Enzymology Lab of the Biochemistry Department, Federal University of Pernambuco (Brazil). Identification of the oysters was based on Amaral and Simone (2014). All individuals were opened and checked macroscopically for the presence of the pea crabs (Figure 2). Crabs internally associated with each oyster were counted, photographed, removed and identified based on de Melo (1996).

All organisms were deposited in the Coleção de Metazoários Aquáticos e Terrestres (COMAT [Aquatic and Terrestrial Metazoan Collection]), Unidade Acadêmica de Ciências Exatas e da Natureza (UACEN [Academic Unit of Exact and Natural Sciences]), Centro de Formação de Professores (CFP [Professor Education Centre]) from the Universidade Federal de Campina Grande (UFCG [Federal University of Campina Grande]) in the Municipality of Cajazeiras, Paraíba, Northeast Brazil. The material was collected under IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) permit number (SISBIO 32198), Brazilian Ministry of Environment.

Data analyses

Prevalence per sampling event (P = percentage of the number of infected hosts per number of hosts examined in the sampling event), Relative Density (RD = total number of parasitic individuals on the hosts per total number of hosts [infected + uninfected] in the sample), and Mean Intensity (MI = total number of parasitic individuals on the hosts per

number of infected individuals of the host species in the same sample) were calculated with methodology based on Margolis et al. (1982) and Mergo Jr. and Crites (1986).

Bar Chart plot with number of infected oysters during rainy and dry seasons was carried out through the PAST statistical software, version 3.25 (Hammer et al., 2001). Through this same software, a parametric t-test analysis was applied, assuming data normality (Shapiro Wilk test, $p > 0.05$, $W = 0.99$ and 0.81 for rainy and dry seasons, respectively), to verify differences in pea crabs among the sampled seasons. Carapace Width (CW) of the crabs and oysters' size were measured using digital callipers. Graphs for representation of the results were produced in Excel and PowerPoint Microsoft Office™ software.

Table 1. Occurrence of *Zaops ostreum* individuals (including ovigerous females) in the *Crassostrea brasiliana*, considering 32 examined oysters per sampling event, with a total of 224 analyzed specimens, considering April to August the rainy season and September to March the dry season. Prevalence (P) represents the percentage of oysters with crabs in each sampling event; Relative Density (RD) is the percentage of crabs individuals per total oysters in the sample; and Mean Intensity (MI) is the number of crabs in the sample per the number of oysters with crabs in the same sample, based on Margolis et al. (1982) and Mergo Jr. and Crites (1986) methodology.

Collection date (MM/DD/YYY)	Prevalence (P) of infected oysters per sampling event	Non-ovigerous females	Ovigerous females	Males	Crabs per sample / Relative Density (RD)	Couples' rate / Sex ratio	Mean Intensity (MI)
06/18/2016	4 / 12.50%	2	2	3	7 / 21.88%	75% / 1F:0.75M	1.75
08/20/2016	2 / 6.25%	2	-	2	4 / 12.50%	100% / 1F:1M	2.00
09/17/2016	4 / 12.50%	-	4	-	4 / 12.50%	0% / 1F:0M	1.00
10/15/2016	2 / 6.25%	-	2	2	4 / 12.50%	100% / 1F:1M	2.00
12/18/2016	4 / 12.50%	-	4	4	8 / 25.00%	100% / 1F:1M	2.00
02/12/2017	5 / 15.63%	4	1	4	9 / 28.13%	80% / 1F:0.8M	1.80
04/29/2017	1 / 3.13%	-	1	1	2 / 6.25%%	100% / 1F:1M	2.00
Total	22 (9.82% in 224 total oysters)	8	14	16	38 (16.96% of total crabs in 224 total oysters)	79.29% (average rate) 1F:0.73M	1.79 (average MI)

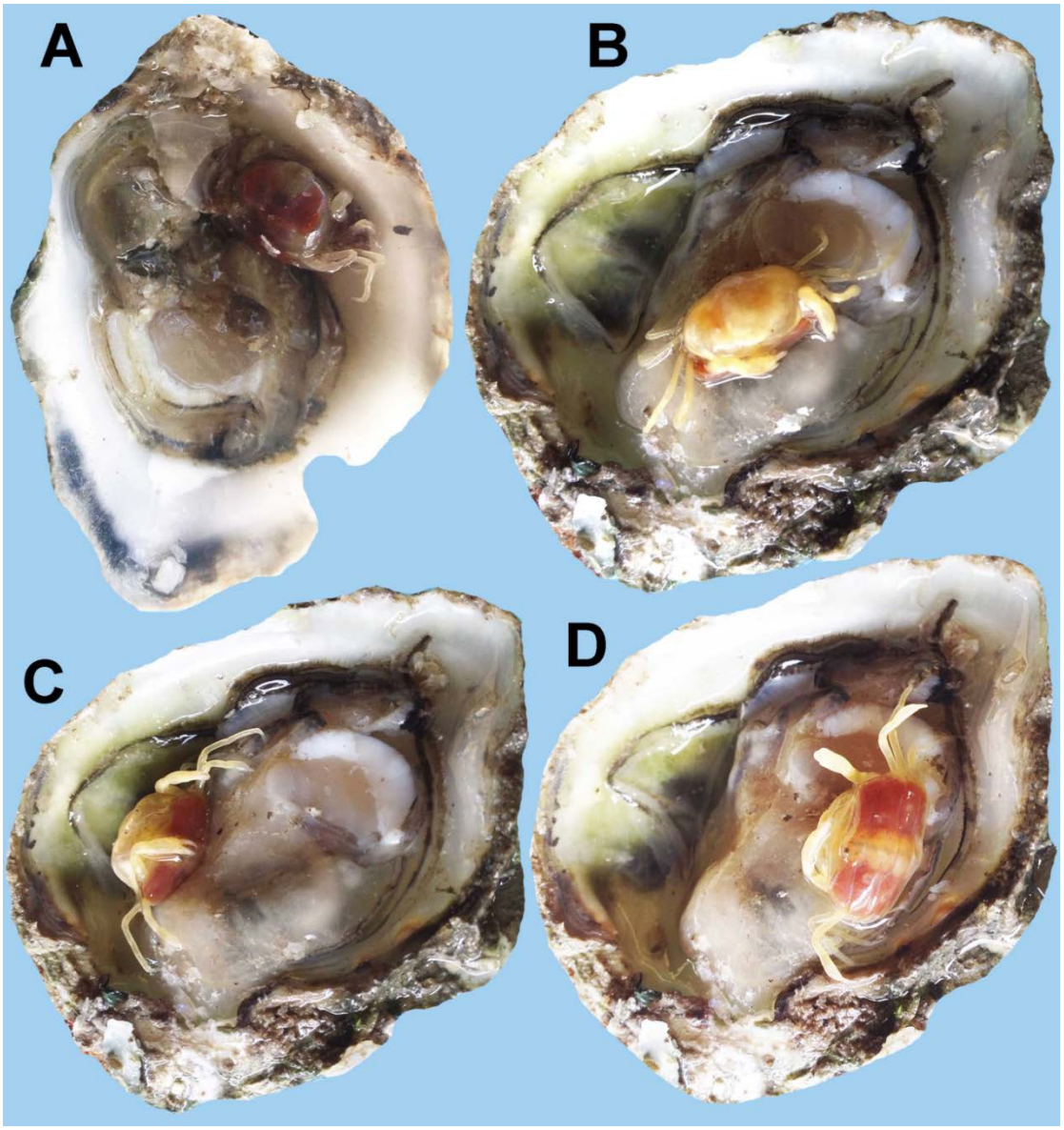

Figure 2. Ovigerous females of *Zaops ostreum* associated with *Crassostrea brasiliana* collected at Barra de Catuama Beach, State of Pernambuco, Northeast Brazil: **A**. CW = 5.3 mm; **B-C**. CW = 5.6 mm.

Results

A total of 224 individuals of *C. brasiliana* were collected during seven surveys carried out at Barra de Catuama (NE Brazil) in dry and rainy seasons. Of these, 22 specimens of *C. brasiliana* presented *Z. ostreum* in the pallial cavity (Figures 2-3, Table 1), totalizing 38 pea crabs found. Of those 22 were females-F (14 ovigerous) and 16 males-M, representing a Relative Density (RD) of 16.96% (7.14% for males and 9.82% for females; ranging from 6.25% to 28.13% per sampling event) in all oysters analysed. Prevalence was 9.82%, ranging from 3.13% to 15.63% per sampling event, average female-male couples' rate was 79.29%, with an average sex ratio of 1F:0.73M and an average Mean Intensity (MI) of 1.79 (Figure 3a, Table 1).

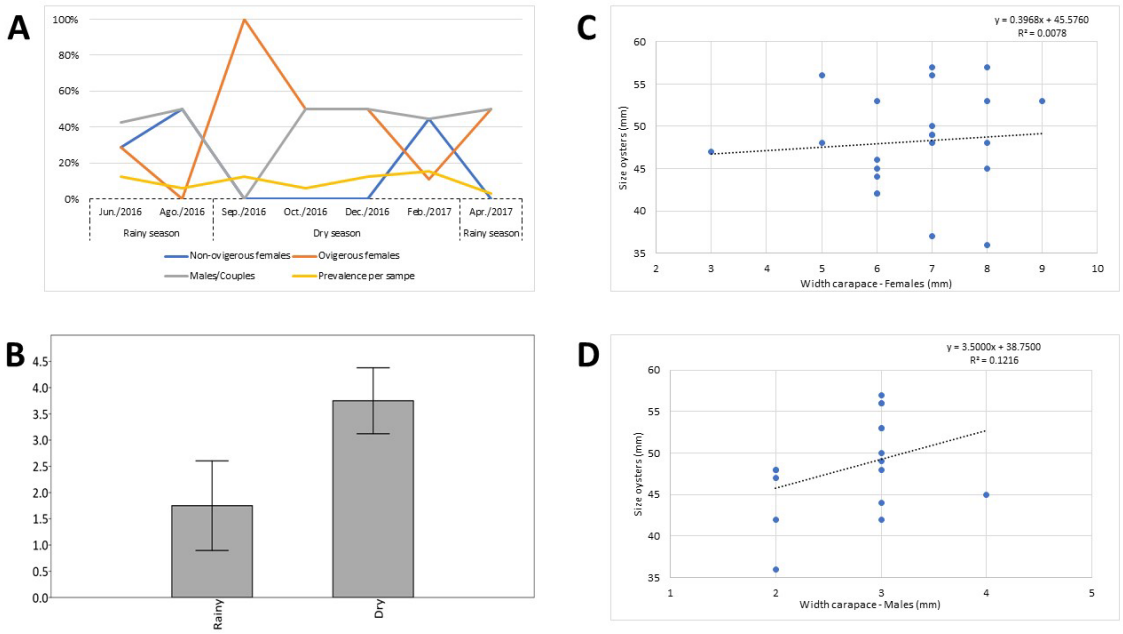

Figure 3. **A**. Percentage of non-ovigerous females, ovigerous, males and couples of *Zaops ostreum* pea crabs in each *Crassostrea brasiliana* oysters during the analyzed period. April to August represent the rainy season and September to March are the months of dry season. **B**. Bar Chart plot: Number of crabs individuals per sampling event during rainy and dry season, including standard error. **C**. Relation between size oysters (mm) and width female crabs' carapace (mm). **D**. Relation between size oysters (mm) and width male crabs' carapace (mm).

On the rainy season, in June 2016, seven pea crabs were collected (RD = 21.88%), three female-male couples per oyster (two ovigerous and one non-ovigerous) and an isolated non-ovigerous female. Prevalence (P) was 12.50% with a couples' rate of 75% (sex ratio of 1F:0.75M), and Mean Intensity (MI) of 1.75. In August 2016, two non-ovigerous female-male crabs couples were found (P = 6.25%, RD = 12.50%, 100% of couples' rate, sex ratio 1F:1M, and MI = 2.00). In April 2017, only one ovigerous female-male couple was found (100% of couples' rate, 1F:1M), therefore these values were counted, P = 3.13%, RD = 6.25%, and MI = 2.00 (Figure 3a, Table 1).

During the dry season, in September 2016 a total of four ovigerous pea crabs were sampled (one per oyster), with values of P = 12.50%, RD = 12.50%, 0% of couples' rate (1F:0M), and MI = 1.00. In October 2016, two oysters with an ovigerous female-male couple in each were observed (100% of couples' rate, 1F:1M), constituting P = 6.25%, RD = 12.50%, and MI = 2.00. At the end of 2016 in December, eight crabs were found, being four ovigerous females-males couples in four oysters (100% of couples' rate, 1F:1M), with P = 12.50%, RD = 25.00%, and MI = 2.00. Finally, in February 2017 was the month with more crabs detected (nine), being four non-ovigerous females, one ovigerous, and four males in five oysters, meaning P = 15.63%, RD = 28.13%, 80% of couples' rate (1F:0.8M), and MI = 1.80 (Figure 3a, Table 1).

In the dry season, 25 pea crabs were found (P = 11.16%), almost double the value observed in the rainy period (13 crabs: P = 5.80%) (Table 1), however no significant differences were detected between these periods (p = 0.18, t = 1.51). Similarly, infected oysters were double in the dry season (total 15, ranging from two to five per sample), compared to rainy season (total seven, from zero to four) (Figure 3b, Table 1).

No apparent injuries on the soft parts of *C. brasiliana* were observed. Furthermore, no isolated males, male-male or female-female couples and no three individuals occurred in the same oyster. Analysing the crabs' sexes-rate among themselves, it was observed that during the rainy season the percentage of males was close to 50.00% (42.86% to 50.00%), which were more accompanied by non-ovigerous females in 2016 (28.57% to 50.00%), and only accompanied by ovigerous ones in the last studied month of the rainy season (April 2017). On the other hand, the beginning of the dry season, there was a sudden decrease of males (0%) at observed only in September 2016, with a predominance of ovigerous females throughout this seasonal period, with a percentage of couples ranging from 0% to 50.00% (Figure 3a).

Carapace Width (CW) ranged from 3.00 to 9.00 mm (average 6.68 mm) among females (Figure 3c) and 2.00 to 4.00 mm (average 2.75 mm) among males (Figure 3d); besides that, size oysters ranged from 36.00 to 57.00 mm (average 48.23 mm). The tendency lines comparing CW females-size oysters and CW males-size oysters demonstrated that as the oysters' size increases the crabs (both male and female) are larger, demonstrating a positive correlation of growths between parasites and hosts, representing an equation of $y = 0.3968x + 45.5760$, $R^2 = 0.0078$, for CW females-size oysters (Figure 3c), and $y = 3.5000x + 38.7500$, $R^2 = 0.1216$ (Figure 3d), for CW males-size oysters.

Discussion

Ecological interactions involving the pea crab *Z. ostreum* and other species of the family Ostreidae have been previously discussed, mainly in association with *C. rhizophorae* and *C. virginica* (Stauber, 1945; O'Beirn and Walker, 1999; Sankarankutty and Ferreira, 2001; Bezerra et al., 2006; Sabry et al., 2011; Hanke et al., 2015; De Gier and Becker, 2020). Studies detected that *Z. ostreum* may provoke harms/injuries in soft-parts-oyster of *C. rhizophorae* and *C. virginica* during the feeding behaviour (Hanke et al., 2015), particularly in gills/branchial chambers, including demibranchs, and on the adductor muscle (Stauber, 1945; Christensen and McDermott, 1958), recognizing a disharmonious relationship with *Z. ostreum* acting as parasite in the oysters (Nascimento and Pereira, 1980). In this study, no injuries to the soft parts were observed (particularly in the gills) on oysters containing *Z. ostreum*, so the assessment of the relationship between *Z. ostreum* and *C. brasiliana* requires further studies.

In this study, 22 individuals of *C. brasiliana* were infected with *Z. ostreum*, representing a Prevalence of 9.82% on total 224 analysed oysters (ranging from 3.13% to 15.63% per sampling event). On these, 38 pea crabs were detected throughout the entirety of the survey, indicating 16.96% of total RD (from 6.25% to 28.13 per sample; 7.14% for males and 9.82% for females) (Table 1). In the dry season, 25 crabs were found (P = 11.16%, ranging from 6.25% to 15.63%), almost double the value observed in the rainy period (13 crabs: P = 5.80%, from 3.13% to 12.50%). Lower values were recorded at Jacuruna River (State of Bahia, Northeast Brazil) by Nascimento and Pereira (1980) for prevalence of *Z. ostreum* in *C. rhizophorae* (8.00%, in around to total 52 pea crabs in 650 analyzed oysters), but with a higher peak prevalence between the months of September and October (20.30%, in larger oysters), during samplings performed in dry season (September/1977 to January/1978). As in tropical zones the analysis is carried out by the rainy and dry periods, without delimiting the four usual annual seasons.

In temperate region surveys, such as in United States, lower *Z. ostreum* prevalence values were reported by O'Beirn and Walker (1999) at Wassaw Sound/Georgia, on *C. virginica* (range 1.00%-8.00%), as well as in the study by Hanke et al. (2015), conducted on the mussels *Geukensia demissa* (Dillwyn, 1817) and *Brachidontes exustus* (Linnaeus, 1758) in Hewletts Creak (1.70%-3.70%). Other surveys in Atlantic USA observed

differences salinity-related, (i) in Delaware Bay was detected a positive effect between the increase of salinity with crabs infestation (from 2.00% to 40.00% in salinity variation from 6 up to 20 ppt) and gill damage (Flower and McDermott, 1952); (ii) otherwise, Beach (1969) verified that salinity had little effect on the distribution of *Z. ostreum* throughout Beaufort sampling points, with salinity varying 20 to 36 ppt (prevalence rate ranging from 0.00% to 40.00%). In addition, Stauber (1945) and Christensen and McDermott (1958) found high level of infestation (up to 80.00% and 60.00%, respectively) by *Z. ostreum* in the oyster *C. virginica* on Delaware Bay and Bay Shore Channel.

According to the conceptual model developed by Baeza and Thiel (2007) about the mating systems of symbiotic crustaceans, adult females of pinnotherid species living with molluscs might be characterized as sedentary, whereas males probably roam a large range of oysters and visit different hosts to copulate with multiple females during reproductive periods, forming polygamous relationships (Christensen and McDermott, 1958). Ovigerous females of *Z. ostreum* were found throughout most of the sampling period at Barra de Catuama Beach (this study), with no males recorded in September (end of rainy season).

During the dry season occurred a high decrease of non-ovigerous females (no non-ovigerous females found from September to December), indicating a reproductive peak during this season. Contrasting with the results obtained by Sankarankutty and Ferreira (2001) in *C. rhizophorae* at Macau Estuary (State of Rio Grande do Norte, Northeast Brazil), most of our occurrences were female-male couples (mean 79.29%) without isolated males and triple occupancy, while the previous authors reported lower female-male couples' rate (28,83%), as well as isolated males and triple occupancy (Sankarankutty and Ferreira, 2001). Nonetheless, no female-male couples were recorded on September at Barra de Catuama (this study) and Macau Estuary (Sankarankutty and Ferreira, 2001). These results obtained in tropical environments highly contrast with results obtained by Christensen and McDermott (1958) at the Bay Shore Channel (USA), were reproductive seasons seem be rather accentuated, with distinct periods for double occupancy and prolonged declines on male abundances after copulation.

In addition, females crab size is directly associated with the host oyster size, with a positive relationship between carapace width and the valve length of oysters (this study, figures 3c-d), which has also been found by Christensen and McDermott (1958) regarding the relationship of *Z. ostreum* and *C. virginica*.

Conclusions

This study reports the association between *Z. ostreum* and *C. brasiliana*. Relative density of *Z. ostreum* was 16.96% (7.14% for males and 9.82% for females) in all analyzed oysters. Females were notably wider in relation to males, which can make it easier to get into oysters for reproduction (Baeza and Thiel, 2007). Besides that, a positive relation between carapace and shells sizes was verified; therefore, in this way, the larger the oysters are the larger the crabs are.

We observed female-male couples (except September 2016) and ovigerous females (except August 2016) throughout all studied period, possibly indicating a distinct reproduction behaviour on tropical areas, e.g. Sankarankutty and Ferreira (2001); differing the findings observed in some North Atlantic studies (Stauber, 1945; Christensen and McDermott, 1958).

No damage was detected in molluscs-soft-parts, however more in-depth studies are necessary for confirming these findings, mainly on Brazilian coasts (and other tropical areas), to enable a better understanding of this new association between *Z. ostreum* and *C. brasiliana*.

Studies on the South Atlantic coast, particularly in Northeast Brazil, related to the association with oysters are scarce. Given this circumstance, in this survey, the occurrence of *Z. ostreum* living in the *C. brasiliana* along the Barra de Catuama Estuary (State of Pernambuco) contributes to the diversity-ecological studies in this region.

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Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Conflict of interests

The authors disclose that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this manuscript.

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