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Reproductive cycle of *Crassostrea gasar* cultivated in three different locations at the Estuarine Complex of Paranaguá (PR)

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Abstract

The reproductive cycle is an essential aspect of oyster farming. Understanding the period of each sexual stage aids in planning oyster harvest, seed collection with artificial collectors, and reproductive season for hatchery seed production. In this sense, this work evaluated the reproductive cycle of *Crassostrea gasar* oysters cultivated in three important oyster farming locations in the Estuarine Complex of Paranaguá, Rasa Island, Medeiros, and Ponta Oeste (Mel Island), with monthly oyster sampling in each sampling site. Temperature and salinity were recorded *in loco* at each sampling time. In the laboratory, oysters were identified and measured; tissue samples were collected for molecular identification of species; and histology was conducted for reproductive cycle analysis. Histological examination of the gonadal tissue was performed with slides stained with Harris hematoxylin and eosin. Salinity and temperature data showed no differences between the three sampling sites during the studied period. Molecular analysis showed that the oysters sampled were *C. gasar* (100%). Histological analysis showed intense spawning of *C. gasar* in December and February (26.72 °C) and June (20 °C). There was a resting stage in both males and females during winter. Some oysters (n = 21) were parasitized by *Bucephalus* sp., and it was impossible to determine oyster sex.

Keywords: Mangrove oyster, Oyster farming, Sexual stages, Reproductive cycle, Trematoda parasitism

Oysters of the genus *Crassostrea* (Sacco, 1897) are essential for oyster farming worldwide (Cavaleiro et al., 2006) both due to the nutritional value of their meat and the profitability of its commercialization (Ajana, 1980). In Brazil, the two native species, *Crassostrea gasar,* also known as *C. brasiliana* (Adanson, 1757), and *Crassostrea rhizophorae* (Guilding, 1828), as well as the exotic species Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), are the main species for aquaculture (Souza Sampaio et al., 2017).

Popularly known as mangrove oysters, *C. gasar* forms a wild population in the intertidal and infralittoral regions, rocky shores, or mangrove swamps (Christo and Absher, 2006). Despite being a source of income for the coastal community, a large part of the stock maintained in productive systems still originates from wild populations (Christo et al., 2015). About 85% of the planet's natural oyster beds are extinct (Beck

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et al., 2011). The constant extraction of these filter-feeding bivalves from the natural environment affects the ecosystem's ability to produce water quality and habitat standards for other organisms (Lotze, 2006).

The reproductive behavior of oysters is analyzed by studying their cycle on a time and space scale considering environmental variables such as temperature and salinity (Montanhini-Neto et al., 2013; Castilho-Westphal and Ostrensky, 2017). The species *C. gasar* has excellent potential to leverage aquaculture in Paraná due to satisfactory growth for commercialization and suitability to the weather (Pereira et al., 2003; Cavaleiro et al., 2016; Legat et al., 2017).

The stages of the reproductive cycle of *C. gasar* can be defined as gametogenesis, pre-spawning, spawning, and resting, as described by Legat et al. (2021) and Sühnel et al. (2023), along with descriptions for other bivalve species, such as for the scallop *Nodipecten nodosus* (Sühnel et al., 2010), the oyster *Crassostrea gigas* (Sühnel et al., 2017), and the clam *Anomalocardia brasiliana* (Lagreze-Squella et al., 2018).

Crassostrea. gasar starts gametogenesis at approximately 90 days old, with a height of around 10–20 mm (Legat et al., 2017; 2021). Sühnel et al. (2017) observed that oysters of the species *C. gigas*, after 15 days of cultivation, with 10 mm in height, already had males at the beginning of gametogenesis.

As they do not present external sexual dimorphism, histology has been used as a tool to identify sex and reproductive stages in several studies (Galvão et al., 2000; Ferreira et al., 2000; 2006; Castilho-Westphal et al., 2013; Montanhini-Neto et al., 2013; Gomes et al., 2014; Legat et al., 2021; Sühnel et al., 2023).

In Paraná, oyster seeds for aquaculture are still primarily collected via wild caught (Christo et al., 2015). However, it is necessary to understand this mangrove oyster's reproductive cycle to plan harvest, seed collection with artificial collectors, and reproductive season for hatchery seed production. The sustainability and resilience of oyster farming communities depend on mechanisms that allow managing natural stocks by combining production and conservation. Thus, knowing the reproductive cycle is key for the success of oyster farming. This work evaluated the reproductive cycle of *C. gasar* oysters cultivated in three locations in Laranjeiras Bay, an estuary belonging to the Estuarine Complex of Paranaguá (CEP).

The studies were conducted in CEP, Laranjeiras Bay (25°30' s; 48 °30' w) in three sampling sites: Rasa Island (25°19' S; 48°23' W), Medeiros (25°22' S; 48°27' W), and Ponta Oeste (25°30' S; 48°22' W) (Mel Island). From November 2017 to October 2018, adult oysters (n = 30) were collected monthly per sampling site, totaling 1,080 oysters analyzed in each location. At each sampling time, temperature and salinity were recorded *in loco* using Conductivity, Temperature, and Depth (CTD) sensors.

The oysters used in this study were purchased from farmers in each sampling site, who obtained the seeds or juveniles from the natural environment. In Ponta Oeste and Medeiros, oysters were cultivated in lantern nets hung in long lines (at a density of 60 oysters per floor and 3 m depth of cultivation). On Rasa Island, oysters were grown in the intertidal region directly in the sediment (on bottom culture).

After collection, the oysters were transported in thermal boxes to the Laboratory of Shellfish and Aquaculture Engineering (LEMAqui) at the Center of Marine Studies (CEM) for biometry and tissue sampling. Measurement of oyster height, according to Galtsoff (1964), was performed using a 0.02 mm precision caliper. For tissue collection, oysters were opened, and a transversal section of the soft parts was obtained for histology, as described by Sühnel et al. (2016). A fragment of the muscle tissue was stored in absolute alcohol for molecular species identification.

For histology, after fixation in Davidson's solution for 24 hours, the samples were stored in 70% alcohol until processing. Embedding was conducted in paraffin and then cut to a thickness of 5 µm in a microtome with disposable razors. The cuts were placed on glass slides, stained with Harris Hematoxylin and Eosin (Howard et al., 2004), and, after receiving coverslips, observed under a light microscope (Olympus Optical Co., model

CX40RF 100) to identify sex and reproductive stage. To determine the reproductive stage, gametogenesis, pre-spawning, spawning, and rest were considered, following the criteria defined by Sühnel et al. (2010) for scallop *Nodipecten nodosus* and used later by Lagreze-Squella et al. (2018) with *Anomalocardia brasiliana*, as well as by Legat et al. (2021) and Sühnel et al. (2023) with *C. gasar*.

To identify the oyster species used in the study, DNA was extracted from the adductor muscle tissue using a AccuPrep® Genomic DNA Extraction Kit (Bioneer), following the manufacturer's protocol. Tissues from 30 samples from each point were macerated, resulting in a monthly sample pool per point. DNA concentration and quality were estimated using a spectrophotometer (Eppendorf).

DNA amplification was performed using the Polymerase Chain Reaction (PCR) technique, following the protocol by Ludwig et al. (2001) with adaptations as follows: samples were processed with two specific primers for specific gene 16S *C. rhizophorae* and *C. gasar* (Table 1). The PCR reaction had a final volume of 20 µl, with 2.5U of TaqPlatinum (Invitrogen), 1X of PCR buffer, 1.5 mM of MgCl2, 0.2 mM of dNTPs, 0.4 µM of primer, and 2µg.µl^{-1} of DNA. The final volume was brought to 20 µl with MiliQ water. The amplification reaction was performed in a thermocycler programmed for an initial denaturation at 94 °C for 4 minutes, 32 cycles of denaturation at 94 °C for 20 seconds, annealing at 59 °C for 44 seconds, and strand extension at 72 °C for 30 seconds, followed by a final extension at 72 °C for 1 minute. Afterward, the reaction was evaluated on a 1% agarose gel stained with Syber green (running conditions: 90 V for 50 min.) and visualized on a transilluminator (Locus Biotecnologia L.Pix).

Table 1. Primer used in the PCR for oyster species identification following Ludwig et al. (2011)

Species	Primer Forward	Primer Reverse	Gene
C. gasar	CACTGTCTCTTAGTTCTATG	AAGCCCTTTAGTTAATACGAG	16S
C. rhizophorae	GCCCAGTGCGATATTAAGTC	CGAACAGACCTACTCACT	16S

Temperature and salinity data were analyzed for basic assumptions of analysis of variance (ANOVA) using the Shapiro-Wilk test for normality of error and the Bartlett test for homogeneity of variance in the R statistical programming language. Parametric data were analyzed with Tukey's test for comparisons between sites. Shell height data were tested for basic assumptions of ANOVA using the Bartlett test for homogeneity of variance and Shapiro-Wilk for normality test. Height was analyzed using one-way ANOVA, with the main effects "sampling sites" (Rasa Island, RI; Medeiros, ME; and Ponta Oeste, PO), with pairwise comparisons using the nonparametric Wilcoxon Test.

Differences in sex ratios (female and males) and reproductive stages (gametogenesis, initial pre-spawning, spawning, and resting) of females and males were analyzed using the nonparametric permutation t test by linear contrast (Westfall et al., 1999) with sampling site and sampling time (November and December of 2017, January, February, March, April, May, June, July, August, September, and October of 2018)) as explanatory factors. All analyses were performed using R-Studio.

Molecular analysis indicated that 100% of the oysters in this study were of the *C. gasar* species (Figure 1). The final size of the amplified DNA for the species was 390 bp. Oyster height (Table 2) from Rasa Island (82.2 ± 11.4 mm) was significantly ($p < 0.05$) lower in height compared to those from Medeiros (87.2 ± 10.9 mm) and Ponta Oeste (89.2 ± 12.0 mm).

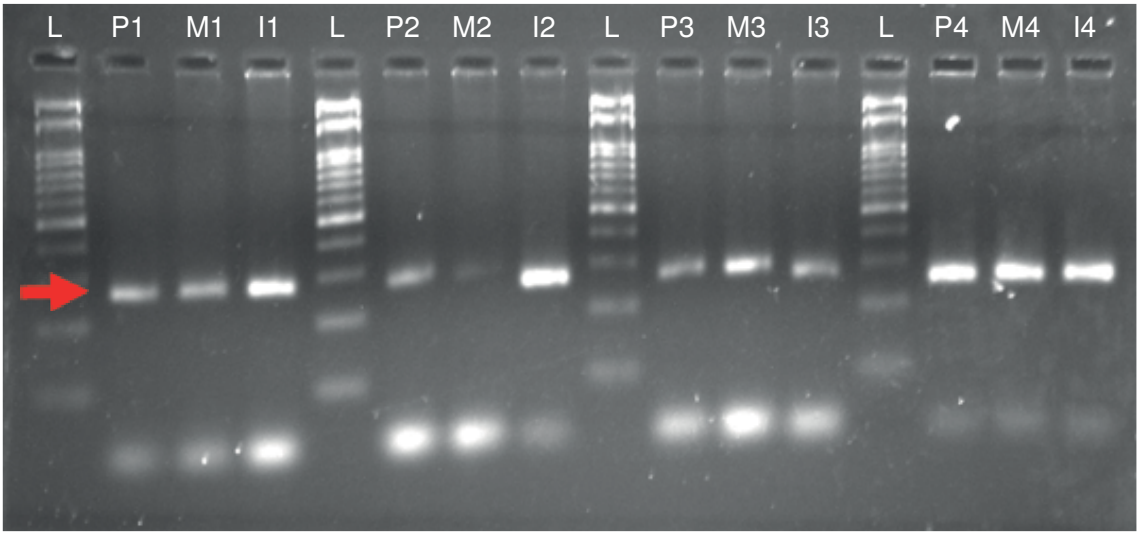

Figure 1. Molecular results of *C. gasar* species identification with Agarose gel electrophoresis, arrow indicates the 390-base pair (bp). The letters at the top represent Ladder (L), Ponta Oeste (P1, P2, P3, P4), Medeiros (M1, M2, M3, M4), and Rasa Island (I1, I2, I3, I4)

Table 2. Mean height (± standard deviation) of oysters from the sampling sites, Rasa Island, Medeiros, and Ponta Oeste (Mel Island), in each sampling time. Different letters show statistical differences in mean shell height between sites

Year	Sampling time (month)	Rasa Island	Sampling site Medeiros	Ponta Oeste
		(mm)	(mm)	(mm)
2017	Nov	98.6 ± 9.8	91.1 ± 12.4	91.3 ± 13.4
	Dec	91.2 ± 8.3	92.9 ± 9.6	90.8 ± 14.4
2018	Jan	80.0 ± 8.1	88.5 ± 10.2	84.7 ± 6.5
	Feb	78.3 ± 8.5	82.0 ± 8.0	94.1 ± 10.3
	Mar	81.0 ± 7.1	82.4 ± 8.6	78.0 ± 8.0
	Apr	75.0 ± 6.4	78.5 ± 8.4	89.4 ± 10.5
	May	83.3 ± 8.7	89.6 ± 10.0	92.4 ± 9.8
	Jun	80.1 ± 7.9	94.8 ± 13.5	85.2 ± 9.4
	Jul	79.0 ± 8.1	80.7 ± 10.2	85.2 ± 9.4
	Aug	80.4 ± 8.7	87.6 ± 10.7	92.2 ± 10.2
	Sep	90.6 ± 12.0	89.1 ± 6.5	101.6 ± 12.8
	Oct	69.1 ± 8.1	89.8 ± 6.6	85.5 ± 9.1
Mean		82.2 ± 11.4[a]	87.2 ± 10.9[b]	89.2 ± 12.0[b]

The sea water temperature (Figure 3) in the three sampling sites did not vary significantly during the study period, with a mean of 24 ± 0.4 ºC in Rasa Island, 25 ± 0.2 ºC in Medeiros, and 25 ± 0.2 ºC in Ponta Oeste. The salinity showed a significant difference ($p < 0.05$) between the sampling sites over time, with means of 21 ± 0.4 in Rasa Island, 23 ± 0.3 in Medeiros, and 28 ± 0.3 in Ponta Oeste.

Sex ratio (female: males) in Rasa Island was 3.3 M: 1 F, in Medeiros 2.3 M: 1 F, and in Ponta

Oeste 3.1 M: 1 F. Percentage of indeterminate sex (Table 3) increased from June to September for oysters from Rasa Island and Medeiros and from July to September for oysters from Ponta Oeste. Animals with indeterminate sex were due to the absence of gametes in the resting stage and to the presence of germ cells in the gametogenesis stage, without sex definition.

Table 3. Percentage of animals with indeterminate sex and number of parasitized animals with *Bucephalus* sp. (in brackets, the total number of animals analyzed) in Rasa Island (IR). Medeiros (ME) and Ponta Oeste (PO).

Year	Sampling time (month)	Indeterminate sex (%)			Number of parasitized animals with *Bucephalus* sp.		
		IR	ME	PO	IR	ME	PO
2017	Nov	0	3.3	3.3	0(30)	1(30)	1(30)
	Dec	0	0	6.7	0(30)	1(30)	1(30)
2018	Jan	10.0	0	16.7	2(30)	0(30)	1(30)
	Fev	6.7	0	3.3	1(30)	0(30)	1(30)
	Mar	6.7	3.3	3.3	0(30)	0(30)	1(30)
	Apr	10.0	13.3	3.3	0(30)	0(30)	1(30)
	May	26.7	6.7	6.7	0(30)	1(30)	0(30)
	Jun	43.3	60.0	0	2(30)	0(30)	0(30)
	Jul	50.0	73.3	20.0	2(30)	1(30)	1(30)
	Aug	73.3	83.3	86.7	1(30)	0(30)	1(30)
	Sep	86.7	73.3	46.7	0(30)	0(30)	1(30)
	Oct	0	0	3.3	0(30)	0(30)	0(30)

Histological analysis of *C. gasar* samples revealed the four reproductive stages: gametogenesis, pre-spawning, spawning, and rest (Figure 2).

Histology analysis of oysters from Rasa Island (Figure 3) showed the female pre-spawning stage pick in the late spring and summer (November and January with 100% and 61% of the females, respectively) and at the end of summer and autumn (March, April, and May, with 79%, 89%, and 42%, respectively). Spawning picks were observed in the summer (December and February, with 76 and 100% of the females, respectively) and in the winter (in June and July, with 67% and 50% of the females, respectively). The resting stage in the females started in April (6%), increasing until August (100%). Gametogenesis in females was observed in March to May in less percentage, with 100% in September and 41% in October. Males showed pre-spawning picks in the summer, with 100% of the animals in December and February and less percentage in the Autumn (April, 42%). A male spawning pick was observed in March (100%) and spring (August and October, with 100 and 86%, respectively). The resting stage in males was followed in May (50%) and June (75%) and in less percentage in October (14%). Gametogenesis in males was observed only in July (67%) and in September (100%)

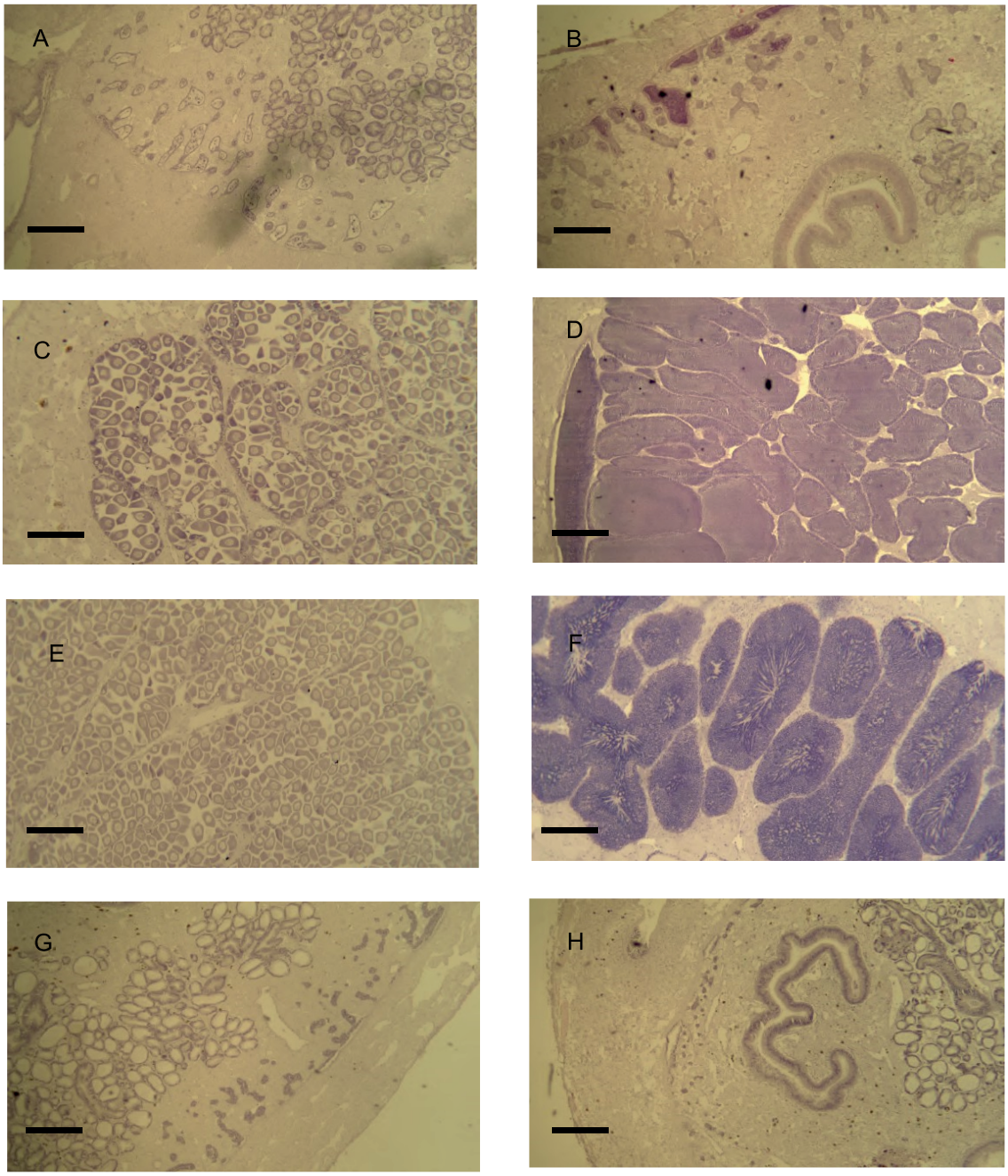

Figure 2. Reproductive stages of the mangrove oysters *C. gasar* females and males (left and right columns, respectively), where A and B: Gametogenesis; C and D: Pre spawning; E and F: Spawning; and G and H: Rest. Bars represent 200 µm

Oysters from Medeiros (Figure 3) showed a female pre-spawning stage pick in the spring (November with 100% of the females) and in the summer (January with 65%), followed by a peak in the autumn (March, April, and May, with 50, 93 and 75%, respectively). Spawning peaks were observed in the summer (December and February, with 100% of females) and winter (June and July, with 50 and 20% of the females, respectively). The resting stage in the females started in March (33%), increasing in June (50%) and July (80%). Gametogenesis in females was observed in March

and May in less percentage, rising to 100% in the spring (August and September). Males showed pre-spawning from November to May, with a peak in December (67%), February (100%), and April (58%). Males in the spawning stage were observed from November to May, with high percentage in January (67%), March (64%), May (80%), and October (100%). The resting stage in males was observed in March (27%), April (17%), June (50%), and September (50%). Gametogenesis in males was observed only in April (8%), June (50%), and September (50%).

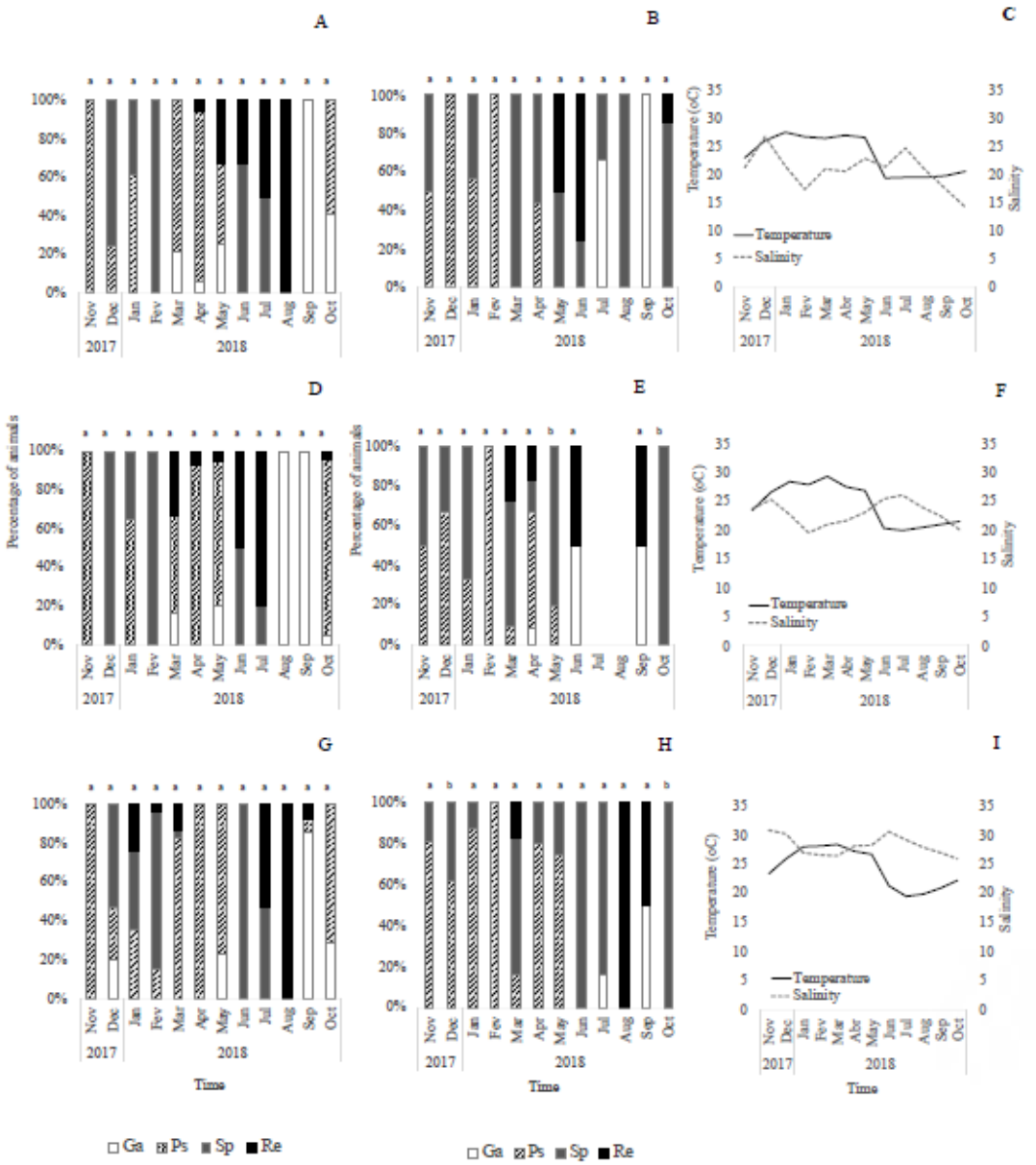

Figure 3. Sexual stage of mangrove oyster *Crassostrea gasar* females and males from Rasa Island (A and B, respectively) from Medeiros (D and E, respectively) and from Ponta Oeste (G and H, respectively). Temperature and salinity for Rasa Island (C), Medeiros (F), and Ponta Oeste (I) at each sampling time. Ga = gametogenesis; Ps = pre-spawning; Sp = spawning; Re= resting

Oysters from Ponta Oeste (Figure 3) showed a female pre-spawning stage pick in the spring (November with 100% of the females) decreasing until February, when an increase occurred during the autumn (March, April, and May, with 88, 100, and 77%, respectively). Female spawning peaks were observed in the summer (December to February) and winter (June and July). The resting stage in the females started in January (24%), increasing in July (53%) and August (100%). Gametogenesis in females was observed in December and May in less percentages, rising in the spring (September and October in 86 and 27%, respectively). Males showed pre-spawning stage from November to May, with 100% of the males in February. Males in the spawning stage were observed from November to October, except in February, August, and September, with 100% in June and October. The resting stage in males was observed in March (17%), August (100%), and September (50%). Gametogenesis in males was observed only in July (17%), September (50%), and October (50%).

Statistical analysis between sampling sites showed no significant difference in female oysters' reproductive stages in each sampling time. For males, differences in the reproductive stage were observed in December, May, and October. In December, males' sexual stage from Rasa Island were different ($p < 0.05$) from Ponta Oeste; in May, males from Medeiros were different ($p < 0.05$) from Rasa Island and Ponta Oeste; and in October, males from Rasa Island were different ($p < 0.05$) from Medeiros and Ponta Oeste.

Animals parasitized with *Bucephalus* sp. were observed in all sampling sites with low prevalence (Table 3). In Rasa Island, parasitized animals were observed in January, February, June, July, and August. In Medeiros, parasitized animals were observed only in November, December, May, and July. In Ponta Oeste, at least one animal was parasitized in all months except in May, June, and October.

In this study, the species *C. gasar* was the only species found in all samples from the three sampling sites, suggesting that farmers are producing *C. gasar.* Since most of the oysters for farming come from wild populations, this predominance of *C. gasar* species cultured can be due to the criterion of farmers collecting oysters from mangrove areas or the predominance of this species in the extraction area. The use of molecular analysis to identify oyster species has been applied considering the reliability of the results (Ignacio et al., 2000; Legat et al., 2009; De Melo et al., 2010; Ludwig et al., 2011), mainly due to morphological similarity between this species (Ignacio et al., 2000). Lapègue et al. (2002) and Ignacio et al. (2000), via molecular analysis, identified the presence of *C. gasar* in Guaratuba Bay and the Estuarine Complex of Paranaguá (South of Brazil). Following the methodology described by Ludwig et al. (2011), the comparison of sequences in the GenBank database revealed that the original article specified exclusive amplification for *C. gasar* or *C. rhizophorae*. In this study, the same primer was used, amplifying only the DNA corresponding to the *C. gasar* species. On the other hand, the primer intended for *C. rhizophorae* did not amplify any of the samples analyzed, providing evidence of the species used in the study.

The similarity in the reproductive cycle of oysters from the three studied sites could be related to the lower temperature variation observed between the studied sites. Besides the salinity variation between sites surveyed in this study, salinity below 20 registered in February in Rasa Island and Medeiros could explain the female spawning event of 100% oysters in that month. Although salinity did not reach 20 in Ponta Oeste in February, a partial group of oysters in the spawning stage were observed. Ponta Oeste is a site more exposed to marine water, consequently with higher salinities throughout the year compared to Rasa Island and Medeiros, which are in the innermost part of the bay and exposed to freshwater from the mountain around the CEP.

The main factors for regulating the reproduction of *C. gasar* are abiotic factors, such as temperature and salinity (Gosling, 2003; Lopes et al., 2013; Montanhini-Neto et al., 2013; Castilho-Westphal and Ostrensky, 2017; Sühnel et al. 2023). Temperatures from 18 °C to 26 °C can induce spawning of *C. gasar* (Pereira et al., 2001; Ramos et al., 2014), and salinities from 15‰ to 35‰ are

tolerable for the survival of the species, showing euryhaline behavior (Funo et al., 2015). Under controlled conditions, the development of *C. gasar* larvae in salinity 28 was found to be the best pattern for normal larvae (Legat et al., 2017a), while salinity 25 to 30 has shown better outcomes for broodstock to promote gonad development (Sühnel et al., 2023).

Moreover, abiotic factors can influence the proportion of males and females in the wild. The higher number of males compared to females of *C. gasar* found in this work are different from data observed for another bay (Guaratuba Bay) in Paraná (approximately 50 km distance from sampling sites), where Castilho-Westphal et al. (2013) observed a higher proportion of females compared to males. The higher proportion of males observed in this study could be related to the lower salinities registered in the sampling sites. In regions with low salinity ranging from 12 to 25, the sex ratio of males is higher than that of females, whereas in salinity variations from 25 to 32‰, the ratio is reversed (Galvão, 2000). According to Guo et al. (1998), the proportion of females and males is also related to the age of the animals, with young populations showing a predominance of males and mature populations of females.

Along the presence of males and females in this study, we also found oysters with indeterminate sex, as observed by other authors (Christo and Absher, 2006; Castilho-Westphal et al. 2013; Ramos et al., 2014; Legat et al., 2021; Sühnel et al., 2023). Christo and Absher (2006), via the condition index, analyzed empty gonads in species without gonadal tissue covering the digestive gland, defining the oysters in these conditions as indeterminate sex. In this work, the indeterminate sex was due to rest, the beginning of gametogenesis, in which germinal tissue was insufficient for precise sex definition or parasitism. Gomes et al. (2014) observed that winter showed the highest rate of indeterminate animals in the rest period.

The intermittent reproduction of *C. gasar* was described as characteristic of the species (Nascimento, 1991; Galvão et al., 2000; Christo and Absher, 2006; Castilho-Westphal et al., 2013; Gomes et al., 2014; Legat et al., 2021). Christo et al. (2015), evaluating the reproductive cycle of *C. brasilian*a (*C. gasar*) using the condition index in marketed oysters in the CEP, observed that spawning females occurred intensely in late spring, summer, and June and intermittently in the other months studied. The spawning percentage was matched with the months showing temperatures of 30°C and 19°C (June), showing that the thermal shock can be an indication to stimulate the release of gametes, which can also be observed in the work of Christo et al. (2015). In the region of Cananéia (São Paulo), Galvão et al. (2000) found the highest spawning rates during spring (25°C), in which the temperature increase influenced the release of gametes. Lenz and Boehs (2011) also point out that the rainy seasons, corresponding to the months when temperature increases and salinity decreases, oysters in the spawning stage are more prevalent. The summer on the coast of Paraná is known for the warming of seawater and high rainfall (average of 2,500 mm) (Lana et al., 2001); similarly, in this study, we found increased temperature and decreased salinity during January, February, and March.

In addition to environmental factors, reproductive strategies also vary according to latitude. In northeastern Brazil, characterized by low latitudes, *C. gasar* has an intermittent reproductive cycle, being in the spawning stage throughout the year with few individuals in the resting stage (Paixão et al., 2013; Legat et al., 2021). In higher latitudes, such as Paraná and Santa Catarina, the cycle remains intermittent, with a prevalence of spawning during spring and summer and periods of rest in winter (Castilho-Westphal et al., 2013; Gomes et al., 2014).

Parasites can also impact the reproductive strategies of oysters. In this work, we found parasitism in oysters by the trematoda *Bucephalus sp*., also found in the analysis of the reproductive cycle of *C. brasiliana* (Galvão, 2000) and of the mussel *Perna perna* (Da Silva et al., 2002; Carneiro-Schaefer et al., 2017). The trematode uses bivalve organisms as host intermediaries in its life cycle (Marchiori et al., 2010), using the animal's lipid and glycogen reserves to meet its nutritional needs, resulting in the depletion of

the host's follicles. Corroborating arguments by Galvão et al. (2000), the oysters parasitized in this work did not present any germ cells, which could infer that the animals were sterilized. An essential factor for the parasite's presence is the seawater's temperature. According to Da Silva et al. (2002), colder temperatures lead to a higher concentration of infestation by *Bucephalus* sp. The authors mention that during the study period, about 60% of infested animals were found in June, July, August, and September, when the temperature was around 20°C.

Studies on the reproductive cycle of oysters and their genetic variation are extremely important for aquaculture and the preservation of the wild population (Legat et al., 2009), as well as investigating the impact of parasitism on the animal's life. On the Paraná coast, the capture of oysters is easily accessible due to the presence of natural stocks, which generated the intense exploitation of this resource (Erse and Bernardes, 2008).

Spawning periods observed in this study are essential for seed collectors using artificial collectors to plan their strategies. Additionally, understanding the reproductive cycle is necessary to know the appropriate moment for spawning induction in the hatchery for seed production. With this, it is expected that the extractivism of wild populations will decrease, contributing to the resilience of the oyster communities in the mangroves on the coast of Paraná. Considering the studied period, this study suggests that November and January are the best months to deploy artificial spat collectors in the studied area. More studies are needed to understand the competition between other species colonizing the spat collectors in these two periods to minimize unwanted species such as barnacles and invasive species. For hatchery operations, the results indicate that broodstock can be collected from the wild or farms in October for conditioning at temperatures above 25 °C for early spawning or collected in December and February to spawn straight from the environment without any conditioning process. To protect wild populations, fishing of adult oysters should be restricted from November to March or resource management should be implemented to avoid stock depletion.

Future studies are suggested to identify the species of *Bucephalus* observed in oyster *C. gasar* to better understand its relationship with the host. Moreover, we suggest evaluating seed collection in artificial collectors and spawning peaks in the natural environment. This work brings essential information for the sustainable management of oyster farming in CEP.

Finally, at the latitude of the Paranaguá Estuarine Complex, the reproductive cycle of *C. gasar* is intermittent, with intense spawning in the warm months, January to March. The gonadal resting stage is observed in both males and females during the studied months, with high quantities during winter. Pre-spawning peaks were observed in November and autumn for females and in the summer for males. Artificial spat collectors should be installed for collecting seeds from November to March. For hatchery production, broodstock can be induced in November and February or conditioned in controlled conditions during October for early spawning.

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AUTHOR CONTRIBUTIONS

T.S.A: Conceptualization; Investigation; Methodology, Analysis; Writing – original draft; Writing – review & editing.

E.Z.A: Methodology; Writing – review & editing.

S.S.: Reproductive analysis, Supervision; Writing – review & editing.

R.L.P.: Supervision; Resources; Writing – review & editing.

F.J.L.: Supervision; Resources; Funding Acquisition; Writing – review & editing.

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