Hindawi
Aquaculture Research
Volume 2023, Article ID 7409585, 12 pages
https://doi.org/10.1155/2023/7409585

Research Article

Effects of Salinity on the Reproductive Cycle of the Mangrove Oyster *Crassostrea tulipa* in Hatchery Conditions

Simone Sühnel,[1] **Francisco José Lagreze-Squella**,[2] **Jefferson Francisco Alves Legat**,[3] **Angela Puchnick-Legat**,[3] **Åsa Strand**,[4] **Serena Sühnel Lagreze**,[2] **and Claudio Manoel Rodrigues de Melo**[1]

[1]*Federal University of Santa Catarina, Laboratory of Marine Mollusks, Department of Aquaculture, Florianopolis, SC 88061-600, Brazil*
[2]*Federal University of Paraná, Laboratory of Aquaculture Engineering and Shellfish, Centre of Marine Studies, Pontal do Paraná, PR 83255-000, Brazil*
[3]*Brazilian Agricultural Research Corporation, Coastal Tablelands, Aracaju, SE 49025-040, Brazil*
[4]*IVL Swedish Environmental Research Institute, Kristineberg 566, 451 78 Fiskebäckskil, Sweden*

Correspondence should be addressed to Simone Sühnel; ssuhnel@gmail.com

Received 7 December 2022; Revised 14 February 2023; Accepted 21 March 2023; Published 27 April 2023

Academic Editor: Mahmoud Dawood

Understanding the effects of environmental parameters on the reproductive cycle of shellfish is an essential tool for successful hatchery conditioning management and, consequently, for seed supply to the industry. For the tropical oyster, *Crassostrea tulipa*, little is known about the factors affecting the maturation process and reproduction, but there are indications that salinity may be of importance. Two experiments were carried out to evaluate the effects of salinity on the reproductive cycle of broodstock of *C. tulipa*. In the first experiment, oysters were reared in five salinities (40, 35, 30, 25, and 20) for 40 days and gonad histological samples were collected regularly. In a follow up experiment, oysters were reared in variable salinity conditions changing from 40 to 30 or from 20 to 30 over a period of 40 days. Salinity was found to affect the reproductive cycle of *C. tulipa*. Oysters kept in constant salinity of 30 showed a higher condition index compared to animals in other salinities, with most of the animals in the prespawning stage. Salinity variation was also shown to affect *C. tulipa* maturation. The prespawning stage was the most observed reproductive stage in low salinity, and high salinity induced a resting stage in the female animals. The results are considered the first step towards developing a protocol for the conditioning of *C. tulipa* in hatchery conditions.

1. Introduction

Low trophic-level mariculture, including culture of filter feeding bivalves, is recognised as a sustainable means of increasing ocean-based food production for the growing world population [1, 2]. Global aquaculture has shown an increasing production in the last years, with a growth of 38.5% from 2009 to 2019 [3]. Presently (2020), marine bivalve molluscs represent 14.2% of the global aquaculture production at 17.4 million tons, and oysters, the most farmed molluscan species, represent 35.9% of the total bivalve production [3].

In Brazil, there are increasing efforts directed towards the production of low trophic mariculture species. Brazilian oyster production is currently based on farming three oyster species, two of which are native, the mangrove oyster *Crassostrea tulipa* (accepted name by the World Register of Marine Species for *Crassostrea gasar* (Adanson 1757); syn- = *Crassostrea brasiliana*) and *Crassostrea rhizophorae* [4], and the Pacific oyster *Crassostrea gigas* (Thunberg 1793) [5]. The total production of oysters in Brazil has remained stable between 2,100 and 4,800 tons per year over the past 20 years, with an average yearly production over the last 10 years of approximately 3,200 tons [3]. The cultivation of Pacific oysters

is restricted to a few regions in Brazil with colder seawater temperatures (i.e., southern Brazil), since higher temperatures throughout the year can compromise the growth and survival of this species. Consequently, the expansion of the oyster sector in the tropical regions is tightly liked to the development of a technological basis for culture of the native species. The mangrove oyster (*C. gasar*), also known as the bottom oyster and black oyster, occurs along the Brazilian coast from Pará to Santa Catarina [6] and is a promising candidate among the potential native species for oyster culture due to its larger size compared to *C. rhizophorae* [4]. Mangrove oyster (*C. gasar*), a tropical oyster species, is produced at a lower rate, when compared to the Pacific oyster, in nine states of Brazil [7] including Santa Catarina.

Seed supply is essential to any bivalve production, and for the mangrove oyster, it is no exception; seeds are traditionally sourced using sea-based collectors targeting settlement of naturally produced larvae from wild populations [8]. During sea-based collection, a mix of native species is obtained, including the target species (mangrove oyster) and the slow growing *C. rhizophorae*. The two species are difficult to distinguish and can only be distinguished because of their differentiated growth rate, which reduces the economic return to farmers when a part of the production is based on the nontarget species. Alternatively, seeds of the mangrove oyster can be produced using land base hatchery facilities. The key components in any oyster hatchery procedure are the control over the maturation process (conditioning), spawning, larval rearing, and settlement stages, followed by the nursery stages of the produced oyster seed [9]. Presently, there is information about the optimal rearing conditions for larvae in terms of temperature and salinity [10] for this species, but other aspects have been less well explored, and many studies are ongoing to develop efficient hatchery protocols for the mangrove oyster.

The reproductive cycle of bivalves is affected by endogenous and exogenous factors, e.g., temperature, photoperiod, salinity, and food availability [11–15], and, consequently, varies on both spatial and temporal scales [16, 17]. There is evidence that the onset of gametogenesis in temperate environments is largely controlled by temperature [11, 14, 18, 19]. All temperate Pacific oyster populations demonstrate rapid gonadal proliferation when temperatures begin to rise after a cold period, inducing a resting stage [20–22]. In tropical regions, however, temperatures remain constant throughout the year, spawning normally occurs year-round [23], and little is known about the controlling factors that affects spawning. Studies have suggested that salinity acts as an exogenous factor affecting the reproductive cycle [14], especially in tropical regions where salinity shows a large variation (for example, from 0 to 45), but information about its influence on gamete development is scarce. According to Antonio et al. [24], in regions where there is low temperature variation, the relationship between precipitation, salinity, and primary productivity is the determining factor for the production and release of gametes.

The reproductive cycle of the mangrove oyster in natural environments has been studied in Santa Catarina [25, 26], Paraná [27], Maranhão [26], and Pará [28] states. In the Maranhão state (tropical climate), Legat et al. [26] reported constant seawater temperatures between 27 and 30°C but significant fluctuations in salinity, ranging between 5 and 32 throughout the year; all sexual stages, i.e., gametogenesis, prespawning, spawning, and resting (rarer in the north), were observed in adults of the mangrove oyster during the study period. Similar patterns of maturity (occurrence of viable gametes) and resting stages have also been observed in another tropical part of Brazil (Rio Grande do Norte state), where seawater parameters are similar to those described by Legat et al. [26] although with salinities reaching 44 during the dry season (M. Kafensztok, personal communication). In the state of Pará (humid tropical climate), Paixão et al. [28] observed that the decrease in salinity in the rainy season promoted an increase in the number of sexually mature oysters and that, in the months of low rainfall, there is an increase in salinity and the presence of animals in the spawning phase. However, it is not clear how salinity affects oyster gamete development and its interaction (sexual stages and salinity) in conditioning.

The salinity tolerance of adult mangrove oysters has been explored in terms of survival [29, 30], growth [29], and filtration rate [31]. Long-term survival of adult oyster was observed in salinities between 4 and 40, and for short periods, survival was observed in salinities between 2.1–4 and 40–50 [30]. Optimal salinity for fertilisation and larval development of mangrove oyster was 28 in a study developed by Legat et al. [10] which tested the salinities 7, 14, 21, 28, and 35. However, reproductive stages are sensitive to changes in abiotic conditions, and the effects of salinity on the reproductive conditioning and in the maturation and spawning processes of the mangrove oyster in controlled conditions are yet to be determined. This is crucial for the development of efficient hatchery protocols including successful conditioning, especially for regions where salinity is the main exogenous factor changing throughout the year. To explore the effects of salinity on the reproductive cycle of the mangrove oyster in controlled conditions, two experiments were performed, including (i) different salinity values kept at a constant level and (ii) two treatments of increasing and decreasing salinity, respectively. The second experiment was designed to evaluate the effect of changing salinity on the maturation processes. The present study was essential to determinate the effects of constant salinity and salinity variation in the reproductive conditioning of the mangrove oyster and to better understand the effects of salinity in the environmental reproductive cycle.

2. Materials and Methods

2.1. Study Organisms and Acclimation. Adult mangrove oyster (*C. tulipa*; 1-year-old, already producing viable gametes) from the broodstock of the Laboratory of Marine Molluscs, Federal University of Santa Catarina (LMM-UFSC), was used. The oysters from the experimental grow-out area of the LMM-UFSC at Sambaqui beach (27°29′20″S; 48°32′26″W) were transported (cooler box) to the LMM hatchery at Barra da Lagoa (27°35′0″S; 48°26′28″W).

Seawater temperature on the day of oyster collection for experiments I and II was 24.5°C and 23.6°C, respectively, with a salinity of 35 at both occasions.

In the hatchery, the oysters were cleaned (fouling removed manually), induced to spawning with air exposure for two hours (stress stimulus for spawning induction), and then placed in suspended lantern nets in an aerated holding tank (15,000 L) filled with treated seawater (filtered to 1 μm and sterilised with UV light) with a temperature of 28°C and a salinity of 35. Gamete suspension obtained by striping one male of the same group of oysters was added to the tank to induce spawning, and after that, the oysters were left undisturbed overnight. Initial inducement of spawning was carried out to reduce nontreatment effects on gonadal maturation and to avoid any unexpected spawning during the experiment. Spawning was verified the following day by visual inspection of the tank and by draining it through a 20 μm screen to retain any larvae or embryos present in the water column; sample analyses were performed in a Sedgewick-Rafter chamber and with a light microscope. Spawning animals were also evaluated in terms of gonadal development by comparing the condition index (CI) of pre- and postspawning-induced oysters and by histological analysis of a subsample of the oysters.

For acclimation to each salinity level after spawning (AS), oysters were placed in experimental units (described below) for three days. The salinity was increased or decreased by 50% from the ambient salinity (35) in relation to the target salinity during the first day and by the remaining 50% during the second day (50% day^{-1}). Adjustment of salinity was carried out by increasing or decreasing the salinity level by one salinity unit every hour to reduce osmotic stress. High salinities were achieved by adding a refined salt (Romani S/A), free of anti-humectant and iodine, to the sump tanks, where the salt was dissolved and distributed to the experimental units for each salinity treatment. Low salinities were achieved by adding freshwater to the sump tanks. Once reaching the desired salinity, animals were acclimated to the system for an additional 24 hours (third day) before each experiment was started. Chlorine from freshwater was neutralised using sodium thiosulfate at a concentration of 5 g 1000 L^{-1}.

2.2. Experimental Semiclosed Recirculation Aquaculture System. Five recirculation aquaculture systems (RAS) (Figure 1) were used for experiment I and three were used for experiment II. Each RAS consisted of one sump tank (350 L plastic tank) with aeration and a submersible pump (Aleas HM-608; 3000 L·h^{-1}) connected to four conditioning tanks (experimental units (EUs); 15 L plastic tank, with final capacity of 11.5 L water). Each EU was supplied with aeration, seawater inflow and outflow, and microalgae. Seawater flow in the EU was adjusted to achieve a water exchange of three times each EU volume per hour (i.e., 0.57 L·min^{-1}). The EU of each RAS was randomly placed in the experimental system. Each RAS was designed to support a maximum of 120 oysters of 70–80 mm length (i.e., 30 oysters per EU). Two interconnected feeding tanks (200 L fiberglass cylindrical-conical tanks) were used to deliver algae to the oysters. In one feeding tank, a submersible pump (Aleas HM-608; 3000 L·h^{-1}) was used to pressurise the microalgae in the feeding line to each EU. The flow of microalgae to each EU was adjusted for continuous delivery over 24 h (i.e., 0.01 L·min^{-1}). Uneaten microalgae were flushed out of each EU, returned with the outflow water to the sump tanks, and then returned to the EUs with the inflow water.

The RAS used in the experiment was, in fact, a semiclosed recirculation aquaculture system (semi-RAS) with a daily exchange of seawater in the sump tank that represented 85% of the total volume in each RAS.

2.3. Experiments and Data Analysis. To evaluate the effect of salinity on oyster maturation (experiment I), 500 oysters (animals selected from 50 to 60 mm, with 58.3 ± 1.3 mm shell height ($n = 12$; [32])) were distributed into the EUs (25 individuals per EU). Each semi-RAS had a specific salinity of 40 (S40), 35 (S35), 30 (S30), 25 (S25), and 20 (S20), and each salinity was consequently replicated in four EUs. The salinities were selected to correspond to the natural salinity variations in the tropical and subtropical region of Brazil. The experiment was conducted over a 40-day period.

In the second experiment (experiment II), 300 oysters (59.3 ± 2.9 mm shell height) were used to test the effects of increasing (S20–30) or decreasing (S40–30) salinities on maturation. A third treatment with a constant salinity (S30) was used as a control. The control treatment was based on the best results from experiment I. In the decreasing salinity treatment (S40–30), salinity was maintained at 40 for 15 days. Then, it was reduced by one salinity unit per day during the daily water exchange in the sump tanks for 10 days until a salinity of 30 was reached, and after that, it was maintained at 30 for an additional 16 days. Similarly, for the increasing salinity treatment (S20–30), salinity was maintained at 20 for 15 days, then it was increased by one salinity unit per day during the daily water exchange in the sump tanks until a salinity of 30 was reached, and after that, it was maintained at 30 for an additional 16 days.

In both experiments, microalgae *Chaetoceros muelleri* (Lemmermann 1898), *Isochrysis galbana* (Parke 1949), and *Diacronema lutheri* (Green 1975) in a 1 : 0.5 : 0.5 ratio, respectively, were used for broodstock feeding. Feeding ration of microalgae was calculated based on 3% of oyster meat dry weight in each microalgae dry weight (DW), in each EU. Microalgae dry weight was obtained by the gravimetric method. For each microalgae species, the cell concentration was counted using a Neubauer chamber, and 30 mL samples ($n = 3$) were filtered through pretreated fiberglass filters (Macherey-Nagel GF-3; diameter 47 mm; pretreatment: filters gently washed in distilled water, dried at 60°C, incinerated in a muffle furnace at 450°C, and weighed), using a vacuum pump (Prismatec, Model 132). Then, filters were dried (60°C for 48 h) and weighed, and each microalgal cell DW was calculated dividing the total DW by the total cell number. The microalgae dry weight was as follows: *C. muelleri*: 52 ± 3 μg 10^{-6} cell, *I. galbana*: 36 ± 0.4 μg 10^{-6} cell, and *D. lutheri*: 32 ± 2 μg 10^{-6} cell.

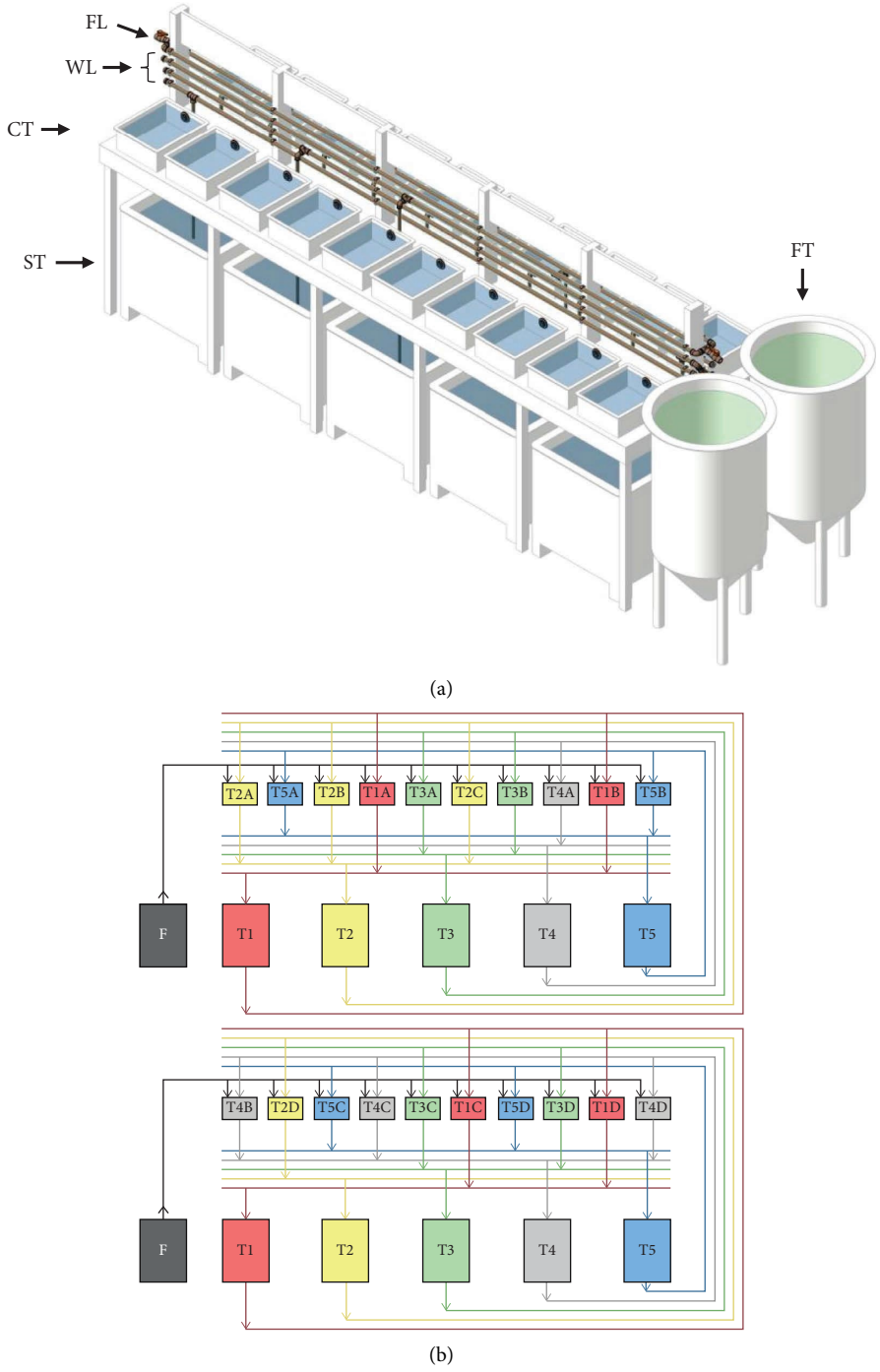

FIGURE 1: Semiclosed recirculation aquaculture system (semi-RAS) used in the conditioning experiments. (a) Illustration of the 5 semi-RAS installed in a wood rack. FL: feeding line; WL: water line; CT: conditioning tank; ST: sump tank; FT: feeding tank. (b) Graphical design of feed flow and seawater recirculation in each semi-RAS used. F: feed; T1, T2, T3, T4, and T5 each semi-RAS system. Design: Serena Sühnel Lagreze.

Microalgae *C. muelleri, I. galbana,* and *D. lutheri* were cultured in separate units (100 L plastic bags) in a semi-continuous system with 50% of the culture volume harvested every 4 days. After each harvest, the bags were refilled with filtered (0.22 μm) and sterilised (UV) seawater as well as Conway media (1 mL·L^{-1}).

Daily maintenance routines for both experiments included a daily batchwise seawater exchange of the sump tank and feed tank. Both the sump and feeding tanks were cleaned with a lemon solution (two lemons blended in 1 L of freshwater and diluted in 3 L of freshwater) before they were refilled (sump tank: refilled with new seawater adjusted to the specific salinity of each treatment and feeding tanks: refilled with microalgae). After maintenance, the flow of seawater and feed in each EU was adjusted to the initial settings. Salinity was measured with a refractometer.

The seawater temperature of each treatment was registered in the sump tanks before and after seawater exchange and remained constant during the 40 days of experiment I ($24.1 \pm 0.5^{\circ}$C and $24.1 \pm 0.3^{\circ}$C, respectively) and the 40 days of experiment II ($24.1 \pm 0.5^{\circ}$C and $24.1 \pm 0.3^{\circ}$C, respectively).

To monitor oysters for physical signs of meat growth in both experiments, the oyster condition index (CI) was evaluated at the beginning of the experiment (T0; after spawning induction, AS) ($n = 12$) and after 40 days (T40) in each treatment ($n = 3$ from each EU). For only experiment I, an additional sample of oysters ($n = 12$) was also collected before the spawning induction for CI evaluation. At each sampling occasion, total oyster live weight (g) was determined. Dry tissue weight (g) and dry shell weight (g) were measured by drying the meat and shells separately in an oven (60°C) for 48 h. Condition index was calculated Crosby and Gale [33]: [dry tissue weight (g)/(total live weight (g)−dry shell weight (g))] × 100.

To evaluate the oyster maturation status by histology, 12 oysters were sampled before spawning induction, after spawning induction (in the beginning of the experiment; T0/AS), and for all treatments, after 10, 20, 30, and 40 days (T10, T20, T30, and T40, respectively) in experiment I and after 7, 14, 24, 31, and 40 days (T7, T14, T24, T31, and T40, respectively) in experiment II (i.e., during stable salinities of S20 or S40: T0, T7, and T14, during the transition period (T24), and during stable salinity of S30: T31 and T40). At each sampling, for each treatment, the oysters were opened and a 2 mm cut was taken, according to standard procedures [34]. The cut was fixed in Davidson's solution for 24 hours and then transferred to ethanol (70%). Oyster tissues for CI were dehydrated with a series of increasing concentration ethanol treatments and included in paraffin. Five micrometer sections were cut, mounted on glass slides, and stained with hematoxylin-eosin. Histological analysis involves the sex identification (sex ratio analysis) and sexual stage classification of each animal according to the method described in Sühnel et al. [35] and Legat et al. [26] considering four stages: gametogenesis (GA), prespawning (PS), spawning (SP), and resting (RE). Animals in reabsorption and/or glycogen accumulation phases were classified in the resting stage. To better understand the effect of salinity on the maturation process of females and males, prespawning and spawning stages were subdivided into initial and advanced, according to the oocytes and follicle development stage.

2.4. Statistical Analysis. For both experiments, shell height and condition index (CI) data were tested for the basic assumptions of analysis of variance (ANOVA), using the Kolmogorov–Smirnov test for normality of errors and the Brown–Forsythe test for homogeneity of variance. Height was analysed using two-way ANOVA, with the main effects "salinity treatment" (constant salinities: S20, S25, S30, S35, and S40 for experiment I and constant salinity S30 and salinity variation S20–30 and S40–30 for experiment II), "sampling time" (T0, T10, T20, T30, and T40 for experiment I and T0, T7, T14, T24, T31, and T40 for experiment II), and the interaction between "salinity treatment" × "sampling time." Pairwise comparisons of salinity treatments and sampling time means were carried out using Tukey's test ($p < 0.05$).

Condition index was subjected to one-way ANOVA to analyse the effect of treatments (before spawning induction (BS), after spawning induction (AS; T0), and salinity treatments (S20, S25, S30, S35, and S40) at T40 for experiment I and AS (T0) and salinity treatments (S20–30, S40–30, and S30) at T40 for experiment II), followed by comparison of treatment means using Tukey's *t*-test ($p < 0.05$).

Differences in sex ratios (female and males) and reproductive stages (gametogenesis, initial prespawning and advanced prespawning, initial spawning, advanced spawning, and resting) of females and males were analysed using the nonparametric permutation *t* test by linear contrast [36] with treatment and sampling time as explanatory factors. All analyses were performed using Statistical Analysis System software [37].

2.5. Ethical Approval. According to Brazilian legislation, authorization for the use of invertebrates, including oysters, is not required for scientific experiments.

3. Results

3.1. Growth Analysis. In experiment I, there was no difference in oyster height between treatments or sampling times, and there was no significant interaction between treatment and time (Table 1). The final average oyster size at T40 was 54.1 ± 3.2 mm ($n = 60$).

In experiment II, no difference in oyster size was observed between treatments, and there was no significant interaction (treatment x time, Table 1). However, oysters from treatments S40–30, S20–30, and S30 sampled at T40 (65.4 ± 3.6 mm) were significantly (Tukey's test; $p < 0.05$) larger than oysters at T0 (59.3 ± 3.6 mm) (Table 1).

3.2. Condition Index. In experiment I, the condition index (Table 1 and Figure 2(a)) of the oysters was significantly (Tukey's test; $p < 0.05$) higher before the spawning induction (BS) than after (AS/T0). In a salinity of 30, the CI at T40 (19.3 ± 3.9) was significantly (Tukey's test; $p < 0.05$) higher than the CI at T0 (AS; 7.9 ± 1.1), as well as those for all other salinity treatments at T40 (S40: 7.4 ± 1.2; S35: 8.5 ± 1.9; S25: 10.8 ± 3.0; and S20: 7.1 ± 1.2). The second highest CI at T40 was observed at S25, which was significantly (Tukey's test; $p < 0.05$) higher compared to AS/T0 and to the CI of oysters held at S20 and S40 but was not significantly different from the CI of oysters at S35. The CI in treatment S35 did not differ significantly from the CI in S20 or S40 at T40.

In experiment II, the CI (Figure 2(b) and Table 1) of oysters at T0 (AS, 13.1 ± 2.3) was significantly (Tukey's test; $p < 0.05$) higher than at T40, for all tested salinities. There were no differences in the CI of oysters between different salinity treatments at T40 (S40–30: 9.8 ± 1.3; S20–30: 9.9 ± 1.7; S30: 9.7 ± 1.4).

Table 1: Statistical analysis of the effects of treatment (different salinities: S40, S35, S30, S25, and S20 for experiment I and S40–30, S20–30, and S30 for experiment II) and sampling time (T0, T10, T20, T30, and T40 for experiment I and T0, T7, T14, T24, T31, and T40 for experiment II) on shell height and condition index (CI) of mangrove oyster *Crassostrea tulipa*.

Experiment	Variable	Factor	*F*	Df	*p*
I	Height	Treatment	0.91	4	0.4595
		Sampling time	0.65	3	0.5830
		Interaction ($T \times S$)	1.77	12	0.0525
	CI	Treatment	97.42	6	**<0.0001**
II	Height	Treatment	0.08	2	0.9226
		Sampling time	4.83	4	**0.0010**
		Interaction ($T \times S$)	0.43	8	0.9027
	CI	Treatment	10.20	3	**<0.0001**

Significant results (ANOVA, $p < 0.05$) are marked in bold.

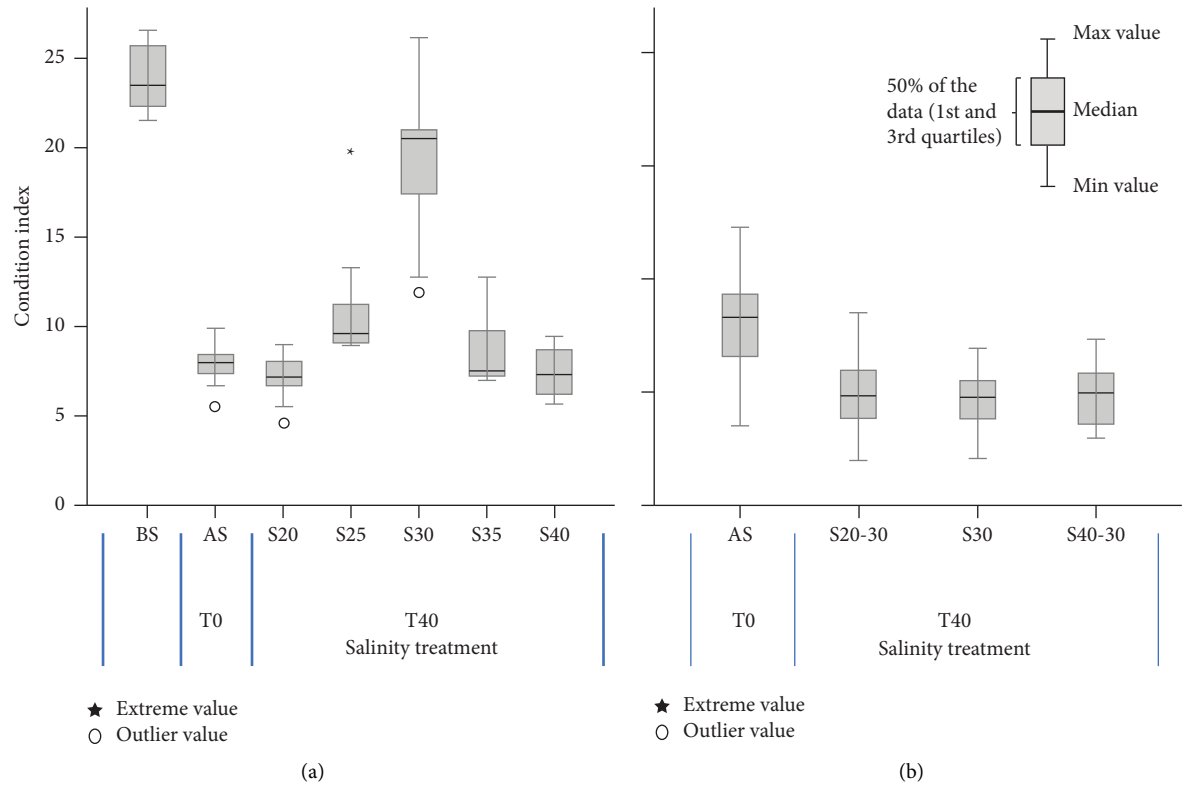

Figure 2: Condition index of mangrove oyster *Crassostrea tulipa*. (a) Condition index of oyster before spawning (BS) and after spawning (AS/T0) induction and after 40 days (T40) in each tested salinity (40 (S40), 35 (S35), 30 (S30), 25 (S25), and 20 (S20)) in experiment I. (b) Condition index of oyster after spawning induction (AS/T0) and after 40 days (T40) in each tested salinity (S40–30, S20–30, and S30) in experiment II. Boxes represent quartiles, the thick line within boxes represents the median, the whiskers show nonoutlier range, and open circles represent outliers.

3.3. Sex Ratio. The sex ratio of oysters in experiments I and II did not differ between sampling times or treatments. In experiment I, males and females showed a sex ratio proportion of 1.4 : 1 (M : F). Approximately 11% of the animals displayed an indeterminate sex and 1% were identified as hermaphrodites. In experiment II, males and females were identified in equal proportions (1 : 1, M : F). Approximately 1% of the animals displayed an indeterminate sex and 1% were observed to be hermaphrodites. Equal proportions of females and males were observed in all treatments and at all sampling times, except for treatment S40 at T30 (experiment I), where 75% of the animals were males and 25% indeterminate. Hemocyte infiltration associated with gamete absorption was observed in all females in the resting stage.

3.4. Sexual Stage

3.4.1. Female and Male Sexual Stages before and after Spawning Induction. In experiment I, before spawning induction (BS), females were in the advanced prespawning

stage (86%) and initial spawning stage (14%), and all males were in the advanced prespawning stage. After spawning induction (AS/T0), females were all in advanced spawning stage and males were in initial spawning (75%) and in advanced spawning (25%).

In experiment II, before (BS) and after (AS/T0) spawning induction, females were all in the gametogenesis stage, while males were primarily in the gametogenesis and initial prespawning stages (BS: 86% in gametogenesis and 14% in initial prespawning; AS/T0: 83% in gametogenesis and 17% in initial prespawning).

3.4.2. Female Sexual Stages during the Experiments. In experiment I, all maturation stages except spawning (i.e., gametogenesis, prespawning, and resting) were observed in females (Figure 3(a)). After 10 days (T10) of conditioning, all females were in the resting stage in all treatments. After this time, females in treatment S40 remained in the resting stage until T40. In treatment S35, females transitioned from a dominance of resting stage (75% at T20) to gametogenesis (100% at T40) over time. Females in treatment S30 displayed a classical maturation pattern, transitioning from the resting stage (100% in T10) to gametogenesis at T20, T30, and T40 (60, 100, and 50%, respectively) and to the initial prespawning stage at T40 (50%). In S25 and S20, females displayed different proportions of resting and gametogenesis over time, except for S20 at T20, where 20% of the females were in advanced prespawning.

In experiment I, statistical analysis of female sexual stages showed significant differences ($p < 0.05$) in maturity related to treatment (independent of time, permutation t test) and to sampling time (independent of treatment, permutation t test). Specifically, treatment S40 was different ($p < 0.05$) from S30 and S20, as were T0 from T10, T30, and T40 and T10 from T20, T30, and T40 (Figure 3(a)).

Similarly, in experiment II, gametogenesis, prespawning, and resting stages were observed in females (Figure 4(a)). In S40–30, the proportion of females in gametogenesis decreased with time from T7 to T31 (67, 50, 25, and 25%, respectively). The proportion of females in the resting stage increased from 17% at T14 to 75% at T24 and T31 and decreased to 50% at T40. The initial prespawning stage was observed at T7 (33%) and T14 (33%), but it decreased to 25% at T40. The advanced prespawning stage was only observed at T40 (25%). In S20–30, the maturation process was rapid, with a dominance of advanced prespawning at T14 (100%), T24 (67%), T31 (80%), and T40 (100%), except for T7, when the initial prespawning was the only stage found. For S30, a similar pattern to S20–30 was observed but with some variations: initial prespawning stage at T7 (88%), T14 (13%), T24 (29%), T31 (25%); advanced prespawning stage at T7 (13%), T14 (75%), T24 (57%), T31 (63%), and T40 (100%); resting at T14 and T24 (13 and 14%, respectively); and gametogenesis at T31 (13%).

Statistical analysis of the female sexual stages showed significant differences ($p < 0.05$) in maturity related to time (independent of treatment, permutation t test) but not between treatments. For time, maturation stages at T0 and T7 did not differ, but both differed ($p < 0.05$) from T24, T31, and T40.

3.4.3. Male Sexual Stages. In experiment I, all maturation stages (gametogenesis, prespawning, spawning, and resting) were observed in males (Figure 3(b)). In treatment S40, males transitioned into the advanced spawning stage at T10 (60%), followed by a peak in the initial prespawning stage at T20 (67%), then resting at T30 (56%), and subsequently a peak at the prespawning stage at T40 (60%). In treatment S35, males remained in the spawning stage at T10 (72% initial and 14% in advanced spawning). After this, the prespawning stage developed and ranged from 51% (38% initial and 13% in advanced) at T20 to 63% (38% initial and 25% in advanced) at T30 and to 43% (29% initial and 14% in advanced) at T40. The resting stage increased from 25% at T20 to 43% at T40. In treatment S30, all males were in gametogenesis at T10 and then the prespawning stage became dominant from T20 (71%; 14% initial and 57% in advanced) to T40 (63%; 13% initial and 50% in advanced). The resting stage was only observed at T20 and T30 in a low proportion of males, 29 and 20%, respectively. In treatment S25, males transitioned from high proportions in gametogenesis at T10 (88%) to high proportions in the initial prespawning stage at T40 (75%). The resting stage was only observed at T20 and T40 in low proportions (29 and 25%, respectively). Males in S20 displayed a varied pattern of maturation and transitioned between gametogenesis, initial prespawning, and resting at various times, with elevated proportions in gametogenesis at T10 (50%) and T30 (67%), initial prespawning and resting stages (each 40%) at T20, and gametogenesis again at T40 (50%).

Statistical analysis of the male sexual stages in experiment I showed no significant differences over time or between treatments (permutation t test).

In experiment II, gametogenesis, prespawning, and resting were observed in males (Figure 4(b)). In S40–30, males transitioned from a predominance in the initial prespawning stage at T7 (100%) to the advanced prespawning stage at T40 (75%). In S20–30, the initial prespawning stage dominated at T7 after which the advanced prespawning stage dominated during the rest of the sampling periods and reached 100% at T40. At S30, a similar pattern was observed with a dominance of the initial prespawning stage at T7 (100%), after which this stage decreased while the advanced prespawning increased until T31 (75%), after which the initial prespawning stage increased again (60%).

Statistical analysis of the male sexual stages in experiment II showed significant differences ($p < 0.05$) in maturation stages between sampling times (permutation t test), where sexual stage at T0 was different from T31 and T40, and that at T7 was different from T31.

4. Discussion

In this study, it was demonstrated that salinity affects the maturation process of the mangrove oyster (*C. tulipa*),

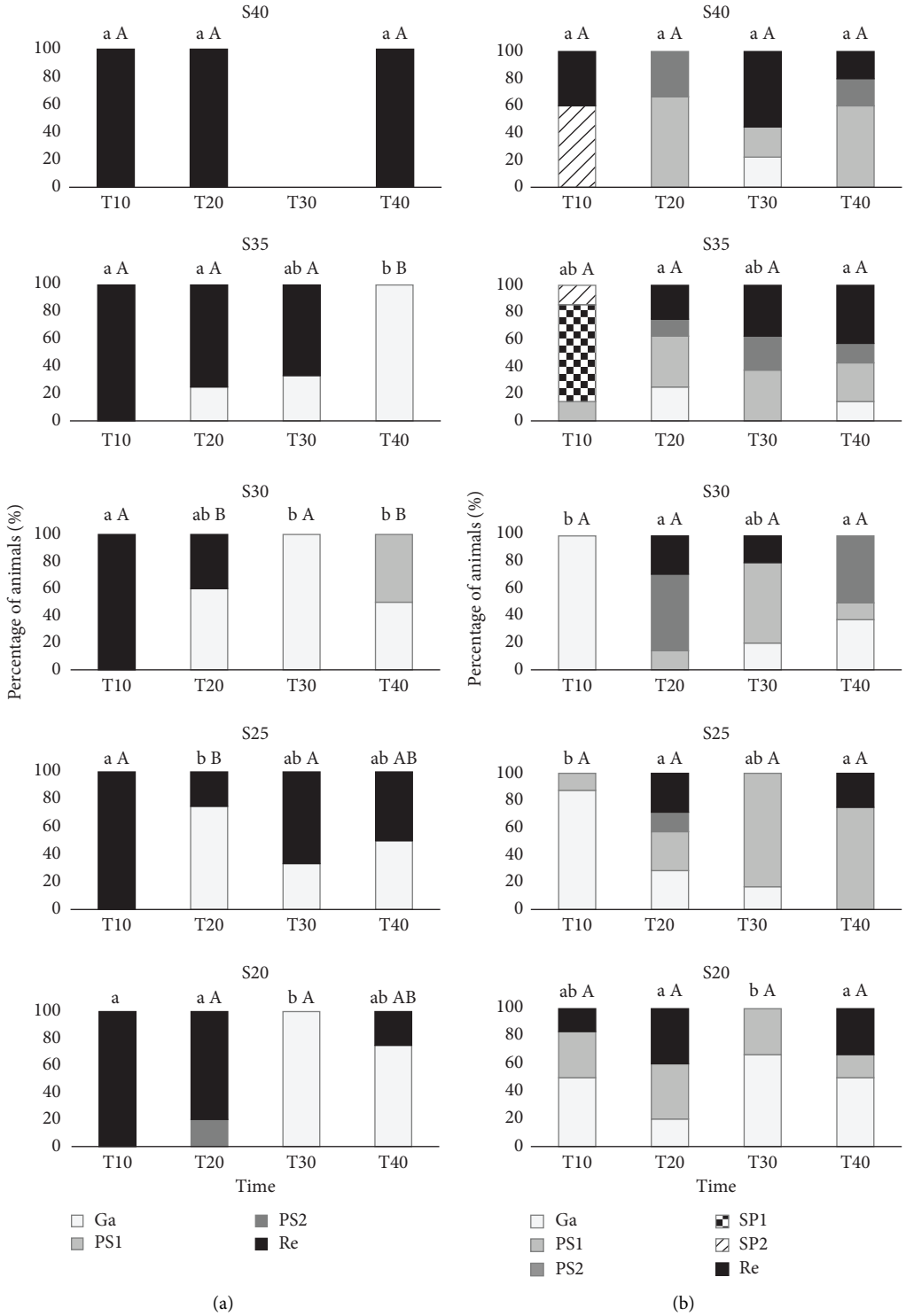

FIGURE 3: Sexual stage of mangrove oyster *Crassostrea tulipa* females (a) and males (b) matured in laboratory in experiment I with constant salinities of 40 (S40), 35 (S35), 30 (S30), 25 (S25), and 20 (S20) after 10 (T10), 20 (T20), 30 (T30), and 40 (T40) days. Ga = gametogenesis; PS1 = initial prespawning; PS2 = advanced prespawning; SP1 = initial spawning; SP2 = advanced spawning; Re = resting. Lower letters show differences ($p < 0.05$) between times in each treatment; capital letters show differences ($p < 0.05$) between treatments in each time by permutation t test.

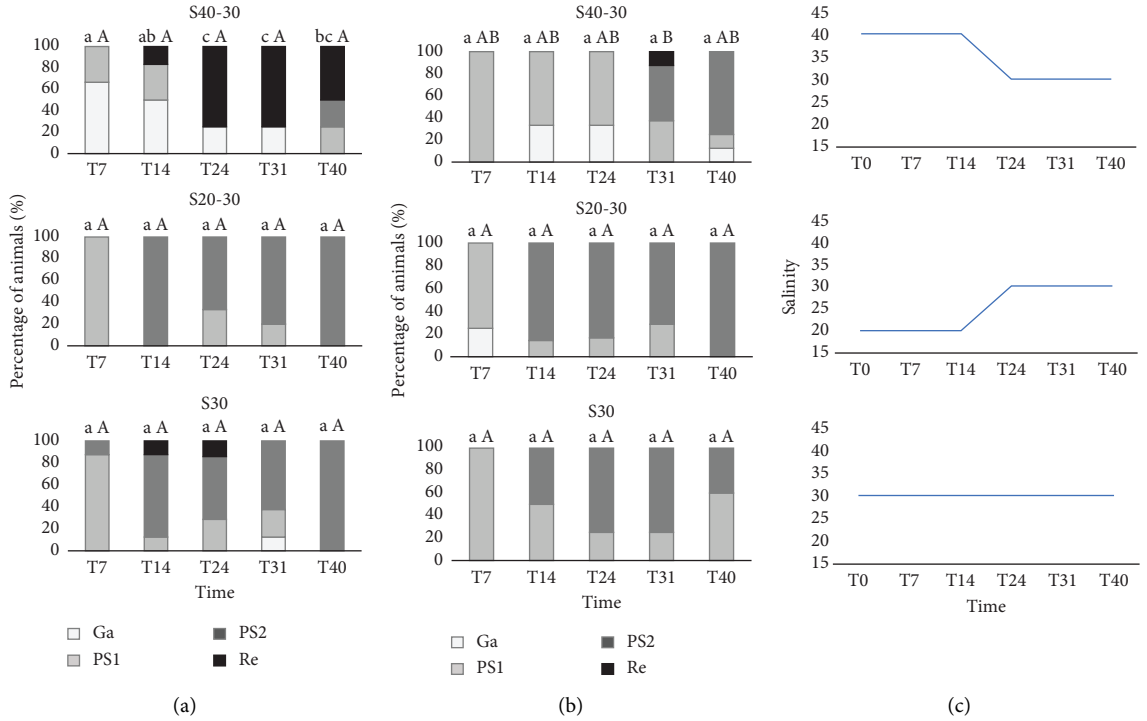

FIGURE 4: Sexual stage of mangrove oyster *Crassostrea tulipa* females (a) and males (b) matured in laboratory in experiment II, with salinity (c) variation from 40 to 30 (S40–30) and from 20 to 30 (S20–30) and constant at 30 (S30), after 7 (T7), 14 (T14), 24 (T24), 31 (T31), and 40 (T40) days. Ga = gametogenesis; PS1 = initial prespawning; PS2 = advanced prespawning; Re = resting. Lower letters show differences ($p < 0.05$) between times in each treatment; capital letters show differences ($p < 0.05$) between treatments in each time by permutation *t* test.

where constant salinity of 30 and 25 or increasing salinity from 20 to 30 resulted in the best maturation of the oysters. Salinities higher than 35 had a significant detrimental effect on gonadal development. This pattern was especially pronounced in female oysters who were induced to a resting stage by high salinities (S40).

The salinities tested in the present study were within the tolerance range (4–40) of mangrove oyster as described by Horodesky et al. [30] and in accordance with the natural range observed in the culture areas of this species [26, 38]. Although bivalves tend to close their valves at stressful salinity levels [39, 40], oysters in the present study did not demonstrate valve closing when exposed to high (S40) or low (S20) salinities, suggesting that animals acclimated to tested salinities. Visual evaluation of the presence of pseudofaeces and faeces in all experimental units indicated that the animals were filtering particles and they were ingesting the diet offered during the experimental period. Also, the increase in shell height in experiment II indicates that water provides conditions for the animals to grow in shell. However, more studies are needed to evaluate if (and if so how) the tested salinities affect *C. tulipa* feeding and energy requirements.

Salinity affects different physiological processes in bivalves, such as heart rate [41, 42], excretion rate [43, 44], respiration rate, clearance rate, and absorption efficiency [44]; however, salinity tolerance limits are species-dependent [14]. As osmoconformers [45, 46], marine and estuarine bivalves have little or no osmotic regulation capacity in their hemolymph. Instead, these species regulate cell volume and maintain isosmotic cells by adjusting the concentrations of intracellular free amino acids and other small organic molecules [14]. Regulation of intracellular and extracellular ionic concentrations requires energy, which is acquired from the hydrolysis of the phosphoanhydride bonds in adenosine triphosphate (ATP) and adenosine diphosphate (ADP) [47]. This energy demand for osmotic regulation may explain the induction to a resting stage of female oysters at high salinities (i.e., salinity 40) that were observed in the present study and also the slow maturation process in salinity treatment S35, where 100% of oysters reached the gametogenesis stage after only 40 days of conditioning.

In the experiment of constant salinity exposure, the highest percentage of both females and males in prespawning stage was observed in salinity 30, suggesting that, at this salinity, animals can expend energy into maturation processes. In accordance, the condition indices of oysters after 40 days of conditioning were higher in salinity treatments S30 and S25 than in the lower (S20) or higher (S35 and S40) salinity treatments. Thus, salinities of 30 and 25 are suggested as the optimal salinities for conditioning *C. tulipa* under hatchery conditions. In addition, a salinity of 20 also affected the maturation process of *C. tulipa* positively, with gametogenesis initiating after 30 days of conditioning; however, the maturation response in this salinity (S20) was slower, compared to S25 and S30. At a salinity of 24, after 78 days of mangrove oyster conditioning, Gomes et al. [25] observed more individuals in spawning stages than in

a salinity of 34; this finding also suggested that average salinities (between 20 to 30) are preferred for this species.

In agreement with the results of this study, maturation processes of mangrove oysters in the field and in tropical environments of Brazil have also been reported to be affected by salinity, as demonstrated by Paixão et al. [28] and Antonio et al. [24] in the north (Pará state) and in the northeast (Maranhão state), respectively. Paixão et al. [28] observed more adult *C. gasar* in maturing and mature stages during low salinity conditions (rainy and rainy-dry periods), compared to high salinity conditions (dry periods). Similarly, Antonio et al. [24] observed the highest percentage (28.6%) of female *C. rhizophorae* oysters in the resting stage at high salinities (i.e., salinity of 42).

The maturation process in different salinities also differed between males and females. At salinity treatment S40, the 40 days of conditioning were not enough for females to start the maturation process. In contrast, males started the maturation process after 20 days. Similarly, at all other salinities, gametogenesis and prespawning started earlier in males (T10) than in females (T20), and males transitioned further in the maturation process compared to females. This may be explained by the lower energy demand of forming sperm compared to oocytes, as suggested by Hayward and Gillooly [48] who, when analysing different group of animals, observed that the cost of egg production is higher (3.5 orders of magnitude) than sperm production. The later response of females at salinity treatment S40 could be related to the higher energy demand for osmotic regulation and, consequently, to the lower availability of energy and nutrients for gametogenesis. Nevertheless, little is known about the physiology of molluscs in hypersaline habitats (salinity of 40 or above) [49].

In concurrence with the stable salinity treatments, salinity variation over time also affected the maturation process of the mangrove oyster (*C. tulipa*). Oysters conditioned in low salinity (S20) followed by an increase to salinity treatment S30 showed a rise in the prespawning stage from T14 to T40. In contrast, the exposure to high salinity (S40) followed by a decrease in salinity (S30) induced oysters to change to the resting stage when salinity started to decrease. These results demonstrate, for the first time, that high salinity (S40) may negatively affect the maturation process in the mangrove oyster (*C. tulipa*) in laboratory conditions. This is an important finding for the development of efficient hatchery protocols for the species, especially in areas where local salinity varies throughout the year according to the rainy season, as in Pará [38] and Maranhão [24, 26] states. In addition, salinity variation supported a rapid transition from the gametogenesis stage (at T0) to initial prespawning stage after 7 days (S40–30, S20–30, and S30) and to the advanced stage after 7 days for S30 and 14 days for S20–30. In comparison, maturation in a stable and optimal salinity (S30) required 40 days in conditioning for females and males to reach an equivalent maturation status. This is the first report of mangrove oyster (*C. tulipa*) achieving the prespawning stage in such a short period of conditioning. It is worth mentioning that the transitioning time into the prespawning condition differed between the S30 treatments in experiments I and II, which were performed in March (summer end) and in November (spring end), respectively. In a study developed with mangrove oyster (*C. gasar*) cultivated in the same region where the broodstock for the present study were obtained, Gomes et al. [25] observed that in March, animals were in resting stage, and in November, they were in late growth stage. This corroborates the existence of seasonal rhythms in the maturation process, which should be explored further.

The effects of salinity were also observed on the condition index (CI). The condition index of animals exposed to salinity variation was also affected by salinity 30, where after 16 days (from T24 to T40) of exposure to salinity 30 in the treatments with salinity variation (S40–30 and S20–30) and constant salinity (S30), there was no difference in the condition indices of oysters, suggesting synchronism in the oyster CI at this salinity. After 40 days of conditioning, a decline of the CI was observed in experiment II and can be related to the use of reserve material (glycogen) from gamete production. At T0, histological analysis showed that oysters were in gametogenesis with a lot of connective tissue in the gonad, and at T40, they had less connective tissue with a lot of gametes in the prespawning stage.

In conclusion, this study illustrates, for the first time, the effects of different salinity regimes on the maturation process of the mangrove oyster (*C. tulipa*) in laboratory semiclosed recirculation aquaculture systems. The results also show the effects of salinity on the maturation process of the commercially valuable mangrove oyster that is relevant to the maturation processes of other oyster species in tropical regions. The results suggest that conditioning of the mangrove oyster at salinities of 25–30 will enhance the maturation process, salinities greater than 35 may induce a resting stage, and the effects of salinity on the maturation process differ between males and females. Moreover, the results constitute the first step towards developing a protocol for reproductive conditioning of the mangrove oyster in hatchery conditions and highlight the need to explore seasonal variations in maturation of this species more thoroughly. Development of reliable conditioning and hatchery protocols may support an expansion of the oyster industry in northern and northeastern Brazil with associated socioeconomic and environmental benefits.

Data Availability

The data used to support the findings of this study are available from the Zenodo repository under the name of the title of this article and from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

All authors were responsible for obtaining, processing, and analysing the data and preparing the manuscript.

Acknowledgments

The authors wish to thank the Brazilian National Council for Scientific and Technological Development (CNPq) (grant no. 108798/2017-8) for the Senior Postdoctorate scholarship awarded to Dra. Simone Sühnel and for the Productivity scholarship awarded to Dr. Claudio Manoel Rodrigues de Melo and the Brazilian Agricultural Research Corporation (Embrapa) (SEG 22.16.05.023.00.00). The authors also thank Dra. Aimê Rachel Magenta Magalhães for histology equipment at Laboratory of Experimental Malacology of Federal University of Santa Catarina and Christopher Langdon for presubmission reviews. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 818173, through the AquaVitae Project.

References

[1] D. L. Aksnes, P. Holm, M. Bavinck et al., "Food from the oceans: how can more food and biomass be obtained from the oceans in a way that does not deprive future generations of their benefits?" *Evidence Review Report No. 1. SAPEA, Science Advice for Policy by European Academies*, SAPEA, Berlin, Germany, 2017.

[2] R. Hilborn, J. Banobi, S. J. Hall, T. Pucylowski, and T. E. Walsworth, "The environmental cost of animal source foods," *Frontiers in Ecology and the Environment*, vol. 16, no. 6, pp. 329–335, 2018.

[3] Fao, "Global aquaculture production Quantity (1950 - 2020," 2022, https://www.fao.org/fishery/statistics-query/en/aquaculture/aquaculture_quantity.

[4] S. W. Christo and T. M. Absher, "Reproductive period of *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea brasiliana* (Lamark, 1819) (Bivalvia: Ostreidae) in Guaratuba Bay, Paraná, Brazil," *Journal of Coastal Research*, vol. 39, pp. 1215–1218, 2006.

[5] J. F. A. Legat, A. Puchnick-Legat, F. H. D. S. Fogaça, C. R. Tureck, S. Sühnel, and C. M. R. D. Melo, "Growth and survival of bottom oyster *Crassostrea gasar* cultured in the Northeast and South of Brazil," *Boletim do Instituto de Pesca*, vol. 43, pp. 172–184, 2017.

[6] A. G. C. D. Melo, E. S. Varela, C. R. Beasley et al., "Molecular identification, phylogeny and geographic distribution of Brazilian mangrove oysters (*Crassostrea*)," *Genetics and Molecular Biology*, vol. 33, no. 3, pp. 564–572, 2010.

[7] A. R. G. Macedo, S. Sühnel, C. A. M. Cordeiro et al., "Growth and survival of the native oyster *Crassostrea gasar* cultured under different stocking densities in two grow-out systems in tropical climate," *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, vol. 73, no. 4, pp. 893–901, 2021.

[8] A. C. Smaal, J. G. Ferreira, J. Grant, J. K. Petersen, and Ø. Strand, *Goods and Services of marine Bivalves*, Springer Nature Switzerland, Heidelberg, Germany, 2019.

[9] M. M. Helm, N. Bourne, and A. Lovatelli, "Hatchery culture of bivalves. A practical manual," *FAO Fisheries Technical Paper No. 471*, FAO, Rome, Italy, 2004.

[10] J. F. A. Legat, A. Puchnick-Legat, C. H. A. D. M. Gomes, S. Sühnel, and C. M. R. D. Melo, "Effects of salinity on fertilization and larviculture of the mangrove oyster, *Crassostrea gasar* in the laboratory," *Aquaculture*, vol. 468, pp. 545–548, 2017.

[11] C. Fabioux, A. Huvet, P. Le Souchu, M. Le Pennec, and S. Pouvreau, "Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock," *Aquaculture*, vol. 250, no. 1-2, pp. 458–470, 2005.

[12] S. Dridi, M. S. Romdhane, and M. Elcafsi, "Seasonal variation in weight and biochemical composition of the pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia," *Aquaculture*, vol. 263, no. 1-4, pp. 238–248, 2007.

[13] J. Normand, M. Le Pennec, and P. Boudry, "Comparative histological study of gametogenesis in diploid and triploid pacific oysters (*Crassostrea gigas*) reared in an estuarine farming site in France during the 2003 Heatwave," *Aquaculture*, vol. 282, no. 1-4, pp. 124–129, 2008.

[14] E. Gosling, *Marine Bivalve Molluscs*, John Wiley & Sons, Ltd, Hoboken, NJ, USA, 2015.

[15] D. Matias, S. Joaquim, A. M. Matias, and A. Leitão, "Reproductive effort of the European clam *Ruditapes decussatus* (Linnaeus, 1758): influence of different diets and temperatures," *Invertebrate Reproduction and Development*, vol. 60, no. 1, pp. 49–58, 2016.

[16] M. Enríquez-Díaz, S. Pouvreau, J. Chávez-Villalba, and M. Le Pennec, "Gametogenesis, reproductive investment, and spawning behavior of the pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependent strategy," *Aquaculture International*, vol. 17, no. 5, pp. 491–506, 2009.

[17] M. Dutertre, P. G. Beninger, L. Barillé et al., "Temperature and seston quantity and quality effects on field reproduction of farmed oysters, *Crassostrea gigas*, in Bourgneuf Bay, France," *Aquatic Living Resources*, vol. 22, no. 3, pp. 319–329, 2009.

[18] R. Mann, "Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* Grown at sustained elevated temperatures," *Journal of the Marine Biological Association of the United Kingdom*, vol. 59, no. 1, pp. 95–110, 1979.

[19] B. L. Bayne, *Biology of Oysters*, Academic Press, London, UK, 2017.

[20] C. Ruiz, M. Abad, F. Sedano, L. O. Garcia-Martin, and J. López, "Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain," *Journal of Experimental Marine Biology and Ecology*, vol. 155, no. 2, pp. 249–262, 1992.

[21] M. Kobayashi, E. E. Hofmann, E. N. Powell, J. M. Klinck, and K. Kusaka, "A population Dynamics Model for the Japanese oyster, *Crassostrea gigas*," *Aquaculture*, vol. 149, no. 3-4, pp. 285–321, 1997.

[22] J. Chávez-Villalba, J. Barret, C. Mingant, J. Claude Cochard, and M. Le Pennec, "Autumn conditioning of the oyster *Crassostrea gigas*: a new Approach," *Aquaculture*, vol. 210, no. 1-4, pp. 171–186, 2002.

[23] G. L. Mackie, "Bivalves," in *The Mollusca, Volume 7: Reproduction*, A. S. Tompa, K. M. Wilbur, N. H. Verdonk, and J. A. M. Van den Biggelaar, Eds., pp. 351–418, Academic Press, Cambridge, Massachusetts, 1984.

[24] I. Antonio, A. Sousa, T. Lenz, I. Funo, R. Lopes, and M. Figueiredo, "Reproductive cycle of the mangrove oyster, *Crassostrea rhizophorae* (Bivalvia: Ostreidae) cultured in a macrotidal high-salinity zone on the Amazon mangrove

coast of Brazil," *Acta Amazonica*, vol. 51, no. 2, pp. 113–121, 2021.

[25] C. H. A. M. Gomes, F. C. Silva, G. R. Lopes, and C. M. R. Melo, "The reproductive cycle of the oyster *Crassostrea gasar*," *Brazilian Journal of Biology*, vol. 74, no. 4, pp. 967–976, 2014.

[26] J. F. A. Legat, A. Puchnick-Legat, S. Sühnel, A. L. M. Pereira, A. R. M. Magalhães, and C. M. R. Melo, "Reproductive cycle of the mangrove oyster, *Crassostrea gasar* (Adanson, 1757), in tropical and temperate climates," *Aquaculture Research*, vol. 52, pp. 991–1000, 2021.

[27] G. G. Castilho-Westphal, F. P. Magnani, and A. Ostrensky, "Gonad morphology and reproductive cycle of the mangrove oyster *Crassostrea brasiliana* (Lamarck, 1819) in the Baía de Guaratuba, Paraná, Brazil," *Acta Zoologica*, vol. 96, pp. 99–107, 2013.

[28] L. Paixão, M. A. Ferreira, Z. Nunes, F. Fonseca-Sizo, and R. Rocha, "Effects of salinity and rainfall on the reproductive biology of the mangrove oyster (*Crassostrea gasar*): Implications for the collection of broodstock oysters," *Aquaculture*, vol. 380-383, pp. 6–12, 2013.

[29] I. C. S. A. Funo, Í. G. Antonio, Y. F. Marinho, and A. O. Gálvez, "Influência da salinidade sobre a sobrevivência e crescimento de *Crassostrea gasar*," *Boletim Do Instituto De Pesca Impact Factor*, vol. 4, pp. 837–847, 2015.

[30] A. Horodesky, G. G. Castilho-Westphal, N. Cozer, V. G. Rossi, and A. Ostrensky, "Effects of salinity on the survival and histology of oysters *Crassostrea gasar* (Adanson, 1757)," *Bioscience Journal*, vol. 35, pp. 586–597, 2019.

[31] A. E. Sutton, K. Yankson, and A. Wudah, "The effect of salinity on particle filtration rates of the west African mangrove oyster," *JYI*, vol. 4, pp. 55–59, 2012.

[32] P. S. Galtsoff, *The american oyster, crassostrea virginica gmelin. Fish bull*, United States Department of the Interior, Washington, DC, USA, 1964.

[33] M. P. Crosby and L. D. Gale, "A review and evaluation of bivalve condition index methodologies with a suggested standard method," *Journal of Shellfish Research*, vol. 1, pp. 233–237, 1990.

[34] D. W. Howard, E. J. Lewis, B. J. Keller, and C. S. Smith, *Histological Techniques For Marine Bivalve Mollusks And Crustaceans*, NOAA Technical Memorandum NOS NCCOS 5, Charleston, South Carolina, 2004.

[35] S. Sühnel, F. Lagreze, M. Bercht et al., "Sexual stages of the female portion in the scallop *Nodipecten nodosus* (Linné, 1758) and astaxanthin quantity in each stage," *Brazilian Journal of Biology*, vol. 70, no. 3, pp. 651–658, 2010.

[36] P. H. Westfall, R. D. Tobias, D. Rom, R. D. Wolfinger, and Y. Hochberg, *Multiple Comparisons and Multiple Tests Using the SAS System*, SAS Institute Inc, Cary, NC, USA, 1999.

[37] Statistical Analysis System (SAS), "SAS: Statistical Analysis System-Getting Started with the SAS Learning Edition," *SAS Institute*, North Carolina, 2005.

[38] L. F. S. Oliveira, M. A. P. Ferreira, L. Juen et al., "Influence of the proximity to the ocean and seasonality on the growth performance of farmed mangrove oysters (*Crassostrea gasar*) in tropical environments," *Aquaculture*, vol. 495, pp. 661–667, 2018.

[39] S. E. Shumway, P. A. Gabbott, and A. Youngson, "The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin-positive substances in the adductor muscles of eight species of bivalve molluscs," *Journal of Experimental Marine Biology and Ecology*, vol. 29, no. 2, pp. 131–150, 1977.

[40] R. A. Elston, D. P. Cheney, B. F. Macdonald, and A. D. Suhrbier, "Tolerance and response of Manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) to low salinity," *Journal of Shellfish Research*, vol. 3, pp. 667–674, 2003.

[41] C. E. Braby and G. N. Somero, "Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*)," *Journal of Experimental Biology*, vol. 209, no. 13, pp. 2554–2566, 2006.

[42] G. Sarà and M. de Pirro, "Heart beat rate adaptations to varying salinity of two intertidal Mediterranean bivalves: the invasive *Brachidontes pharaonis* and the native *Mytilaster minimus*," *Italian Journal of Zoology*, vol. 78, pp. 193–197, 2011.

[43] J. A. Allen and M. R. Garrett, "The excretion of ammonia and urea by *Mya arenaria* L. (Mollusca: Bivalvia)," *Comparative Biochemistry & Physiology*, vol. 39, pp. 633–642, 1971.

[44] C. Resgalla Jr, E. D. S. Brasil, and L. C. Salomão, "The effect of temperature and salinity on the physiological rates of the mussel *Perna perna* (Linnaeus 1758)," *Brazilian Archives of Biology and Technology*, vol. 50, no. 3, pp. 543–556, 2007.

[45] L. H. Mantel and L. L. Farmer, "Osmotic and ionic regulation," in *Internal Anatomy and Physiological Regulation*, L. H. Mantel, Ed., pp. 53–161, Elsevier, Amsterdam, Netherlands, 1983.

[46] E. H. Larsen, L. E. Deaton, H. Onken et al., "Osmoregulation and excretion," *Comprehensive Physiology*, vol. 4, no. 2, pp. 405–573, 2014.

[47] C. Hauton, "Physiological responses: effects of salinity as a stressor to aquatic invertebrates," in *Stressors in the marine Environment: Physiological and Ecological Responses; Societal Implications*, M. Solan and N. M. Whiteley, Eds., pp. 3–24, Oxford University Press, Oxford, UK, 2016.

[48] A. Hayward and J. F. Gillooly, "The cost of sex: quantifying energetic investment in gamete production by males and females," *PLoS One*, vol. 6, no. 1, Article ID ik, 2011.

[49] L. Deaton, "Osmotic and ionic regulation in molluscs," in *Osmotic and Ionic Regulation Cells and Animals*, D. H. Evans, Ed., pp. 107–133, CRC Press Taylor & Francis Group, Boca Raton, FL, USA, 2009.