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Relation between Physiological Variations and Economic Feasibility in Farmed Mangrove Oysters (*Crassostrea gasar*) in Subtropical Estuary

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HIGHLIGHTS

- Evaluation of physiological changes in mangrove oysters (*Crassostrea gasar*) in subtropical estuarine environments.
- Assessment of mortality, metabolic rate, and ammonia excretion at varying salinities (0-35 g L^{-1}) and temperatures (15, 20, 25, 30°C).
- Potential for significant advancement in the region through small-scale family oyster farming.

Abstract: This study evaluates the physiological changes in mangrove oyster *Crassostrea gasar* in adult individuals cultivated in subtropical estuarine environments. To assess mortality at different salinities, 0 to 35 g L^{-1} concentrations were used. The same salinities were also employed to evaluate the metabolic rate and ammonia excretion. Furthermore, the oysters were exposed to different temperatures (15, 20, 25, and 30°C) to assess the metabolic rate and ammonia excretion. The mean mortality significantly decreased after a salinity of 4 g L^{-1}. Another objective was to verify, based on the survival conditions found in the different salinities tested, 63% in salinities between 5 and 10 g L^{-1}, 72% for salinity between 11 and 19 g L^{-1}, and 81% for salinities between 20 and 30 g L^{-1}, the economic viability of cultivating the mangrove oyster, practiced in the region, in 0.1 ha. The financial indicators of Gross Revenue and Operating Profit, as well as the profitability indicators of Gross Margin and Profitability Index, showed positive values in all three proposed survival conditions. Due to the particularities described here, in all tested conditions, at the selling price of a dozen oysters of US$ 4.00, there was economic viability with profitability always higher than the minimum attractiveness rate of 10.5%.

Keywords: Ammonia excretion; estuary; mortality; oxygen consumption; oyster farming.

INTRODUCTION

The mangrove oyster *Crassostrea gasar* (Adanson 1757) is a vital resource for artisanal extractive activities and small seafood farmers in the Cananeia estuary complex on the south coast of São Paulo. This region is one of the largest natural banks of this species in Brazil. Research indicates that the exploitation of this mollusk began in the 1940s for subsistence, evolving into a commercial venture by the 1970s [1,2].

Since the 1960s, numerous studies have focused on developing cultivation technologies for the mangrove oyster in Cananeia. By the 2000s, research had shifted towards estimating natural stocks and proposing sustainable management measures [1]. In 1995, oyster fattening, a method derived from full-cycle cultivation, was introduced to local communities. This technique involves "seeding" adult oysters until they reach a commercial size, which takes about six months [3,4].

Oyster fattening increases reproductive activity, as oysters reach sexual maturity at around 20 mm [5]. This method not only provides environmental benefits but also enhances income for producers. However, despite the profitability of full-cycle cultivation, its long duration of approximately 22 months has hindered its adoption [3]. The shorter, six-month fattening period is more appealing to local communities.

Despite extensive research, the influence of salinity on the physiological aspects of oysters in Cananeia remains understudied. Most studies have focused on distribution models in relation to salinity, while the physiological responses to different salinity levels, such as filtration rate and oxygen consumption, have not been thoroughly investigated [6].

Salinity significantly affects the development, growth, and survival of bivalve mollusks. It is a crucial factor in determining their distribution and can be easily measured and controlled. Changes in salinity can lead to various physiological responses, including shifts in metabolic rates and ammonia excretion, yet few studies have addressed these aspects in a subtropical estuarine environment [7].

Understanding the metabolic response of mangrove oysters to salinity and temperature gradients can offer valuable insights for natural bank management and aquaculture practices. This study aims to analyze the oxygen consumption, ammonia excretion, and survival of adult *C. gasar* oysters under different salinity and temperature conditions. It also seeks to assess the economic viability of fattening cultivation based on survival rates at varying salinities.

MATERIAL AND METHODS

Analysis of physiological variations in *Crassostrea gasar*

All experiments were conducted at the Fisheries Institute facilities situated at the Cananeia base on the southern coast of the state of São Paulo, Brazil (Figure 1). The oysters were sourced from local producers. Prior to the analyses, DNA samples confirmed that the individuals belonged to the species *C. gasar.* Additionally, preliminary tests were carried out to establish salinity and temperature gradients, oyster density, oyster sizes, and the analysis.

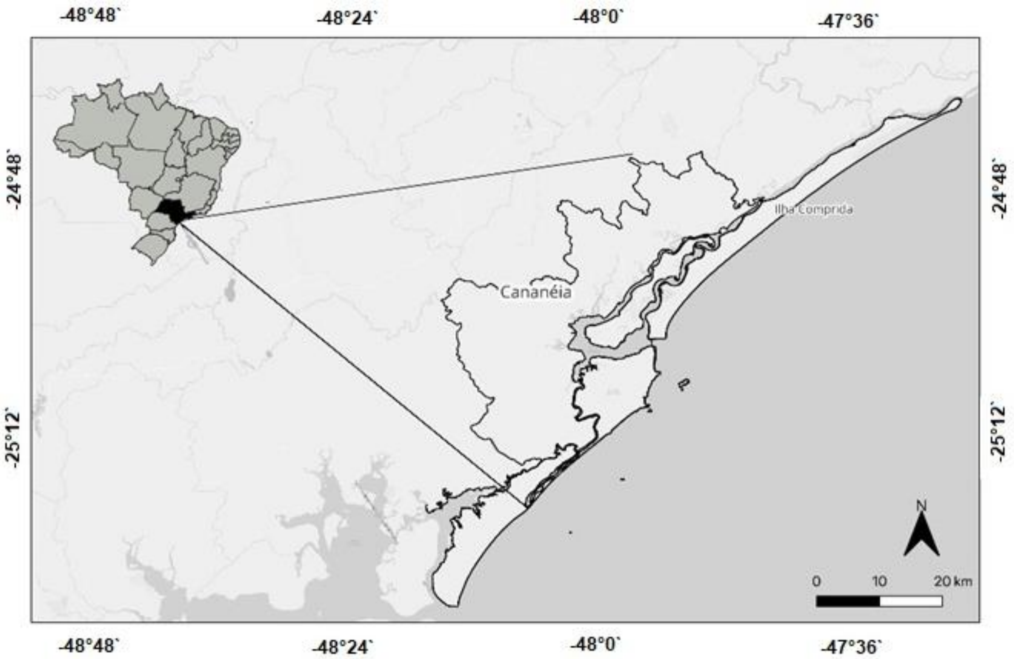

Figure 1. Location of the farms of the mangrove oyster *Crassostrea gasar,* Cananeia complex estuary, in the Brazilian state of São Paulo.

The oysters were acclimated in glass respirometers for a duration of 3 hours with continuous water circulation to mitigate handling-induced stress. Subsequently, the water flow was halted, and the respirometers were sealed for a 24-hour assessment of specific oxygen consumption and specific ammonia excretion in a known volume of water. To investigate the impact of temperature, a salinity of 25 g L^{-1} and temperatures of 15, 20, 25, and 30°C were employed. To explore the influence of salinity on oysters, concentrations of 1, 2, 3, 4, 5, 10, 20, 25, 30, and 35 g L^{-1} were utilized at 20°C. To measure salinity, the multiparameter probe KR86021 with an accuracy of 0.1 ppt was used.

Previous experiments were carried out with oysters at salinities of 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 35 g L^{-1}. Subsequently, salinities of 0, 1, 2, 3, 4, 5, 10, 20, 30, and 35 g L^{-1} were defined for the experiment, through a completely randomized design, with six treatments and three replicates, totaling 18 experimental units consisting of glass cylindrical containers with a useful volume of 3 liters. The containers were previously washed and sterilized, filled with water, and maintained under gentle and continuous aeration.

The salinity was measured using a refractometer with a precision of 0.05. The respirometer was built in our laboratory, along with the entire system for measuring oxygen consumption and ammonia excretion.

Oysters were initially brushed and encrustations (epibionts) were removed, and the oysters were allowed to acclimate for 6 days at a salinity of 18 g L^{-1} in 3 L containers with aeration and complete water change daily, at a density of 6 oysters per container.

The water used in the experiment came from the Cananeia estuary, stored in tanks that, through evaporation, reached a salinity above 35 g L^{-1} and were later diluted to the desired salinities. For each salinity, 3 glass containers with 3 L of water and constant aeration were used, containing 6 oysters per container, totaling 180 oysters in 30 containers. The average sizes of the oysters were: in height, 62.19 (± 1.35) mm in length; 24.43 (± 0.85) mm wide.

The water in all the containers was daily renewed in its entirety, and the containers were properly washed at each change. Oysters were observed daily to identify and remove dead individuals. Mortality was observed when oysters with open valves showed no reaction to the touch of the shell. Through daily monitoring throughout the experiment, the ambient temperature of the water was recorded, with an average of 19.9°C (±1.3°C) for the first 30 days of the experiment.

The respirometers were shielded with a screen to isolate the organisms from any potential disturbances in the laboratory environment. The difference in oxygen concentrations measured at the beginning and end of the confinement period was utilized to calculate the specific oxygen consumption ($mLO_2\, g^{-1}\, h^{-1}$) over the course of 3 hours, taking into account the respirometer volume and the wet weight of the organism. The animals were taken out of the shell, followed by weighing, immediately after the completion of the experiments. In order to mitigate the impact of low oxygen concentrations and metabolite accumulation on metabolic processes, the duration of the experiments was regulated to ensure that the final oxygen concentration remained above 70% of the initial concentration. Dissolved oxygen was determined using Winkler's method [8].

Samples for specific ammonia excretion analysis were collected immediately after the metabolic rate analysis. Ammoniacal nitrogen was quantified using the modified Solórzano method [9] by Strickland and Parsons [10]. The difference in ammonia concentrations measured at the beginning and end of the confinement period was utilized to calculate the specific ammonia excretion (mg $g^{-1}\, h^{-1}$). The respirometer volume, wet mass of the organism, and duration of confinement were taken into account in the calculation. Although the oysters did not receive any additional food during the experimental period, only feeding on nutrients provided by the daily water changes

Mortality and Specific oxygen consumption and specific ammonia excretion data were analyzed for normality of distribution using the Shapiro-Wilk test and homoscedasticity of variances using the Levene test. As the results were normal and homoscedastic, the differences between the means of the treatments were evaluated by means of analysis of variance (ANOVA) followed by Tukey's test of multiple comparisons, with a significance level of $p<0.05$ [11].

Economic feasibility in farmed mangrove oysters (*Crassostrea gasar*)

According to the specificities of this small-scale activity practiced in the region, for the present study, the cultivation area encompassed 1,000 m^2 (0.1 ha), with capacity for 20 trays of 10 m^2 each, supported by cement stakes and crosspieces, with two polypropylene plastic screens installed on them [3]. The initial density in the tray was 25 dozen m^{-2}, using oysters with an average height of 40 mm extracted from mangroves [3,4], and the survival rate at the end of the 6-month cycle ranged from 63% for salinity between 5 and 10 (Condition A), 72% for salinity between 11 and 19 (Condition B), and 81% for salinities between 20 and 30 (Condition C). Table 1 presents the main production factors for oyster farming, under the three conditions tested, in the estuary of Cananeia, southeastern coast of Brazil.

Table 1. Parameter assumptions for the cultivation of mangrove oyster (*Crassostrea gasar*), in 0.1 ha, on the Cananeia region, southeastern coast of Brazil, May 2024.

Item	Unit	Quantity
Initial tray density	Dozen m^{-2}	25
Production cycle	Month	6
Condition A Survival rate	%	63
Condition B Survival rate	%	72
Condition C Survival rate	%	81
Trays (10 m^2)	Nº	20
Initial number of oysters $cycle^{-1}$	Dozen	5000
Final number of oysters $cycle^{-1}$ (Condition A)	Dozen	3150
Final number of oysters $cycle^{-1}$ (Condition B)	Dozen	3600
Final number of oysters $cycle^{-1}$ (Condition C)	Dozen	4050
Cycles per year $^{-1}$	Nº	2
Final number of oysters $year^{-1}$ (Condition A)	Dozen	6300
Final number of oysters $year^{-1}$ (Condition B)	Dozen	7200
Final number of oysters $year^{-1}$ (Condition C)	Dozen	8.100
Boat	Hour $year^{-1}$	360

Source: Research data.

Production Cost

Investment structures, operating expenses, financial and profitability indicators were based on methodologies already applied and validated in different recent studies of economic feasibility of marine aquaculture projects [12,13,14]. Prices for inputs and commercialization were primarily surveyed from local suppliers and producers.

The cost structure considered in this study was:

a. Effective Operational Cost (EOC), which included expenses such as: land lease, permanent labor for oyster collection and structure maintenance. In the cultivation management, the owner's remuneration was considered as one monthly minimum wage prevailing in the country of US$ 282.00 (May 2024); fuel and maintenance of the boat used for oyster collection and cultivation; infrastructure expenses and occupancy fee for the Brazilian Navy (SPU);
b. Total Operational Cost (TOC), which corresponds to the EOC plus costs of labor social charges, using for this calculation 40% of the labor cost [14], financial charges, and depreciation of the boat, tools, and equipment;
c. Total Production Cost (TPC), which is the sum of the TOC plus costs related to annual depreciation of facilities and annual interest on the invested capital [13].

Investment Return and Profitability Indicators

For the economic analysis of the activity, a time horizon of ten years was considered, with the investment fully applied in year zero [14].

It is known that to reduce the inaccuracies of an economic-financial analysis, it is necessary to jointly assess the results obtained in various indicators. Among the profitability indicators, the Internal Rate of Return (IRR), the Net Present Value (NPV), and the Payback Period (PP) were used. The Internal Rate of Return (IRR) is an important indicator of economic viability, usually used to compare it to a Minimum Attractiveness Rate (MAR) in decision-making. The MAR is defined as the expected return if the capital were invested in another low-risk fixed income activity or the average market return to be analyzed. For this study, a MAR of 10.5% was used, an interest rate defined by the Brazilian government for the period under study. Thus, the venture will only be economically viable if the IRR is higher than the proposed MAR [15].

The Net Present Value (NPV) is the amount that the entrepreneur would have at the end of the project's time horizon, but adjusted to the present value [13]. The Payback period (PP) is the time required in years for the investment to pay off, or to recover the initial capital invested, which together with the results of IRR and NPV makes it possible to opt, with a lower margin of error, whether the investment and cost proposal is the most appropriate, ensuring the best decision-making by the entrepreneur.

Additionally, a cost indicator in terms of dozens of oysters produced, called the Breakeven Point (BP), was considered, which determines the minimum production necessary to cover the cost, given a selling price per dozen oysters (SPdz), where: BP = TOC/SPdz.

Other profitability evaluation indicators adopted in this study were described [12,13,14]:

a. Gross Revenue (GR): it is the product of the yield of the production multiplied by the selling price per dozen oysters practiced in the market;
b. Operating Profit (OP): difference between GR and TOC. This indicator measures short-term profitability, showing the financial and operational conditions of the activity. Thus, we have: OP = GR - TOC;
c. Gross Margin (GM): margin in relation to TOC, that is, the result obtained after the producer bears the operational cost, considering a certain selling price per dozen oysters and the productivity of the production system for the activity. Formally, we have: GM = (GR - TOC)/TOC x 100;
d. Profitability Index (PI): relationship between OP and GR, in percentage. An important indicator that shows the available revenue rate of the activity after payment of all operating costs. So: PI = (OP/GR) x 100;
e. Cash Flow (CF): it constitutes the algebraic sum of the inflows (gross revenue) and the expenses incurred during the activity cycle. It is a tool that enables identifying a net financial flow each year, which will be used for IRR calculation [14].

The cash flow allows showing the monetary situation of the activity and constitutes the result to cover other fixed costs, risks, and return on capital for a time horizon of 10 years. For its calculation, expenses related to the initial investment in the first year (considered year zero) and the effective operational cost-plus financial charges, labor social charges, and annual interest on the capital invested were considered [13,14]. Estimates were made based on two selling prices per dozen oysters produced (US$ 3.00 and US$ 4.00), a range of values commonly practiced by oyster producers in the local market.

RESULTS

Survival in relation to salinity gradient

Although the oysters did not receive any additional food during the experimental period, only feeding on nutrients provided by the daily water changes. The mean mortality significantly decreased after a salinity of 4 g L^{-1}. The mean survival for *C. gasar* significantly increased up to salinity 20 g L^{-1} and then decreased again up to salinity 35 g L^{-1} (Figure 2). No statistical differences were observed in the survival means between salinities from 5 to 10 g L^{-1}, 11 to 19 g L^{-1} and 20 to 30 g L^{-1} ($p<0.05$).

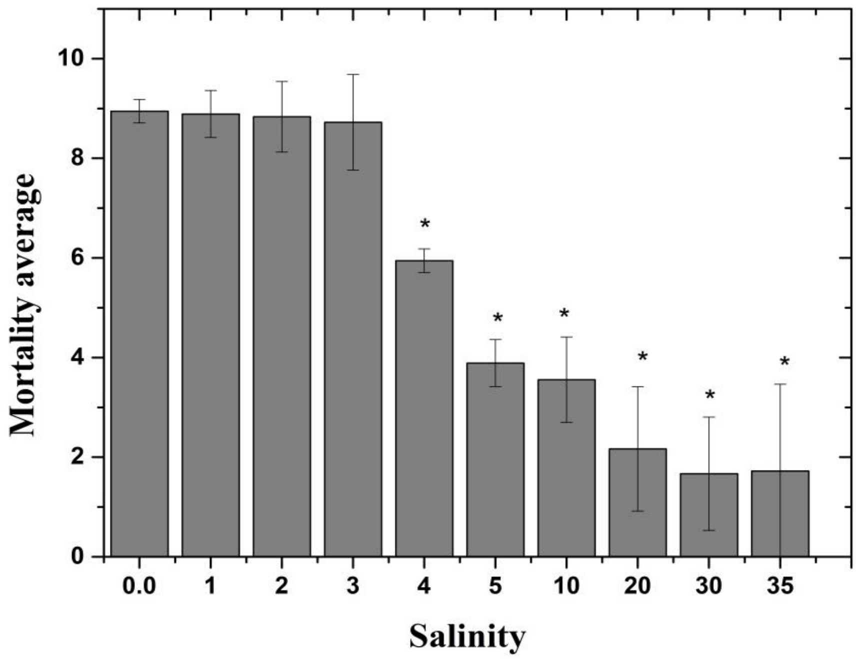

Figure 2. Mean survival for oysters exposed to different salinities (g L^{-1}), in 30 days of experiments. Bars indicate standard deviations (n=90). *Significant differences ($p<0.05$).

Specific oxygen consumption

Oysters exposed to salinity gradients showed a reduction in specific oxygen consumption from 10 g L^{-1}. There was a decrease in the mean specific oxygen consumption of 55.4% in relation to salinities of 1 to 4 g L^{-1} (Figure 3). No statistical difference was observed in the metabolic rate between salinities from 0 to 5 g L^{-1} ($p<0.05$).

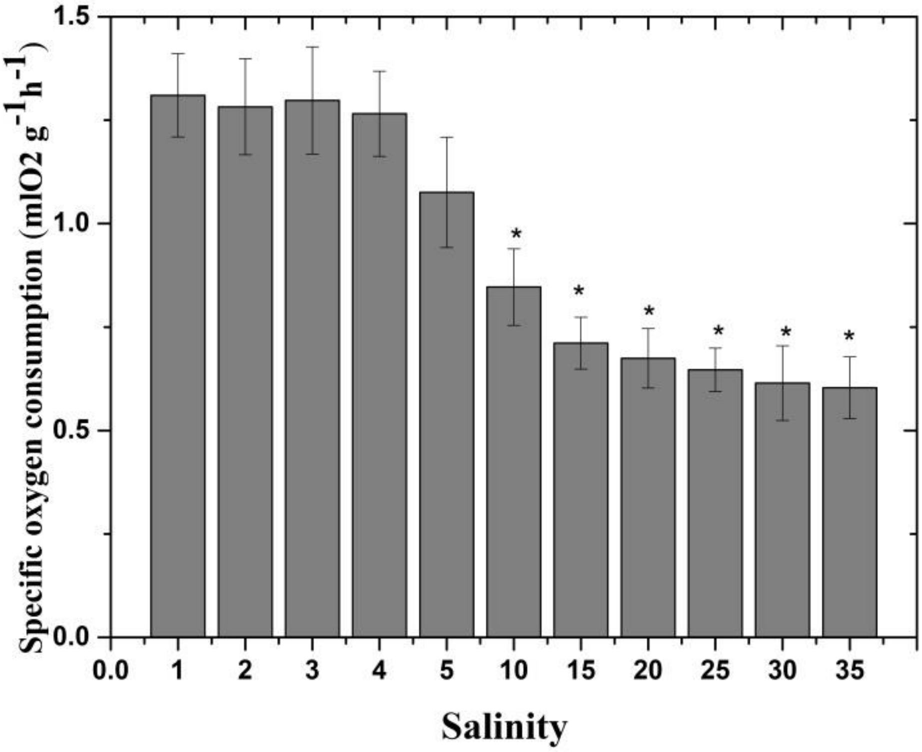

Figure 3. Means of specific oxygen consumption of oysters exposed to different salinity concentrations (g L^{-1}), at 20°C. Bars indicate standard deviations (n=9). *Significant differences ($p<0.05$).

Oysters exposed to temperature gradients showed an increase in specific oxygen consumption starting from 25°C. The mean specific oxygen consumption at a temperature of 30°C increased by 82.3% in relation to a temperature of 15°C (Figure 4). No statistical difference was observed in the metabolic rate between temperatures of 15 and 20°C and between 25 and 30°C.

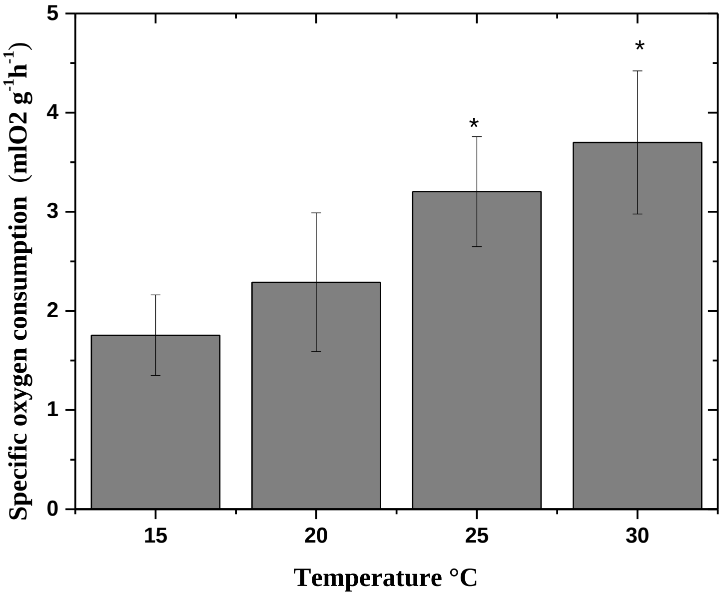

Figure 4. Means of oxygen consumption for oysters exposed to different temperatures at a salinity of 25 g L^{-1}. Bars indicate standard deviations (n=9). *Significant differences ($p<0.05$).

Specific ammonia excretion

The means of specific ammonia excretion of oysters exposed to salinity gradients used in the experiments showed a statistical difference starting from 15 g L^{-1}, in relation to salinities from 1 to 5 g L^{-1} (Figure 5). At 30 g L^{-1} there was an increase of 47.5% in ammonia excretion in relation to salinity of 5 g L^{-1}. Between 1 to 10 g L^{-1} there was no significant difference. The means of specific ammonia excretion of oysters exposed to temperature gradients used in this study showed a statistical difference from 25ºC onwards, in relation to temperatures of 15 and 20°C (Figure 6). At the temperature of 30ºC, there was an increase of 52.4% in ammonia excretion in relation to the temperature of 15ºC. Between temperatures of 15 and 20ºC there was no significant difference.

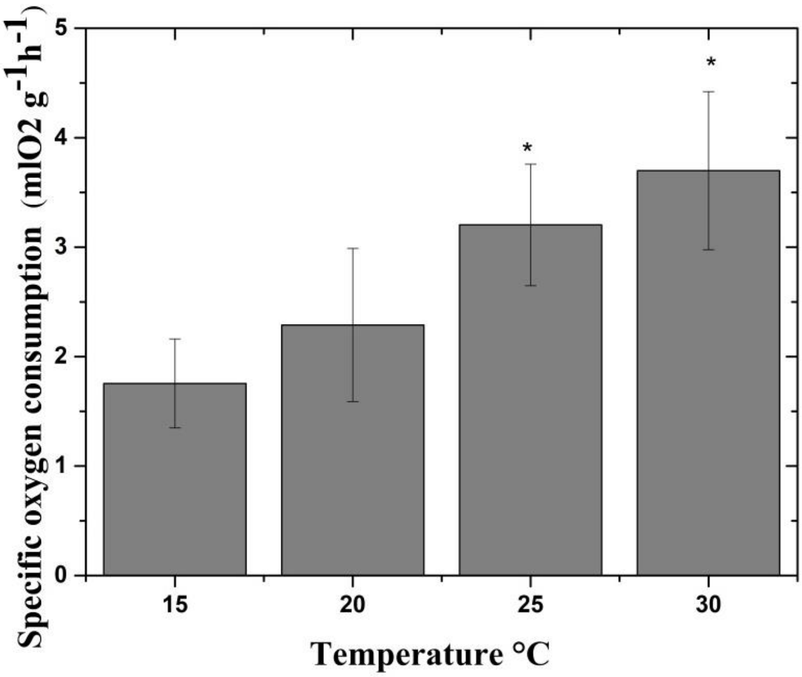

Figure 5. Means of specific ammonia excretion for oysters exposed to different salinity concentrations (g L^{-1}), at 25°C. Bars indicate standard deviations (n=9). *Significant differences ($p<0.05$).

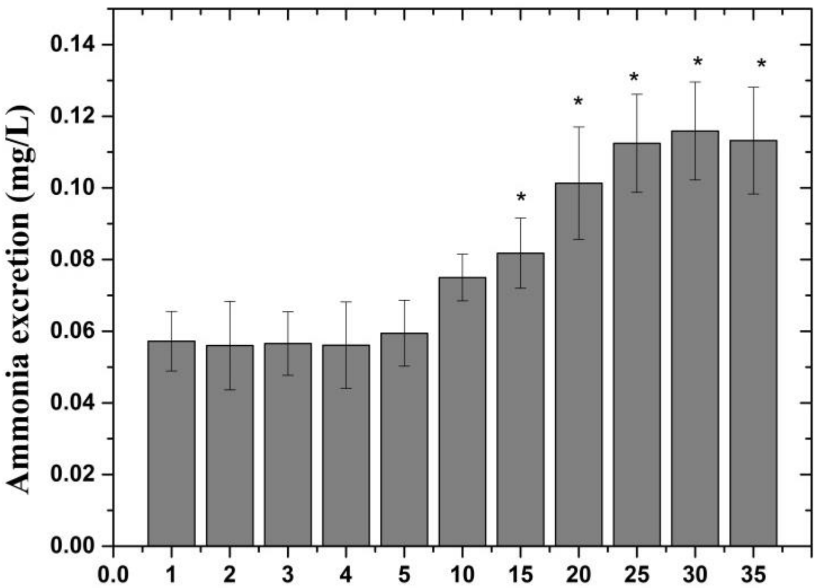

Figure 6. Means specific ammonia excretion for oysters exposed to different temperatures at salinity of 25 g L^{-1}. Bars indicate standard deviations (n=9). *Significant differences ($p<0.05$).

Investment

The investment required for implementing oyster cultivation amounted to US$ 77,534.43 (Table 2). Items with a useful life of less than 10 years were included as expenses in their respective replacement years. The items that most burdened the investment were land lease and construction, representing approximately 75% of the total value.

Table 2. Investment required for implementation of mangrove oyster *Crassostrea gasar* cultivation, in 0.1 ha, in the Cananeia region, southeastern Brazil, May 2024.

Item	Qty.	Total Price[1]	Lifespan (replacement)[2]	Annual depreciation[3]	Interest	Total
Land lease	1	36000.00	-	-	3780.00	3780.00
Warehouse Office (8x5 m) - 40 m^2	1	24000.00	20	1200.00	1260.00	2460.00
Trays (supports + screens)	20	4800.00	5(1)	960.00	252.00	1212.00
Labor for construction and assembly (man-day)	120	4272.00	-	-	448.56	448.56
Aluminum vessel (6 m) + Outboard motor (15 HP)	1	6400.00	5(1)	1280.00	336.00	1616.00
Tool kit	1	500.00	2(5)	250.00	26.25	276.25
Pressure washer (1,500 pounds)	1	400.00	5(1)	80.00	21,00	101.00
Plastic box	25	700.00	10	70.00	36.75	106.75
Documentation and Project Preparation	3%	462.43	-	-	48.56	48.56
Total		**77534.43**				**10049.12**

[1] Amounts expressed in US dollars (US$ = R$ 5.20); [2] Lifespan and replacement ( ) in years; [3] Estimated depreciations, based linear methodology.
Source: Research data.

Operational and Production Costs

The estimated annual total production cost (TCP) for *C. gasar* cultivation corresponded to 24.7% of the invested amount (Tables 2 and 3), indicating the high operational expenses required to sustain the activity.

In Table 3, it is observed that the total annual operational cost (TOC) was US$ 10,797.92. This value was used in the cash flow calculations [13].

Labor was the most burdensome item, costing US$ 2,550.07 per production cycle, representing approximately 27% of the TCP (Table 3).

Under the infrastructure expenses item, costs for water, electricity, and telephone were considered, with the latter intended for commercial contacts, totaling US$ 600.00 for a production cycle of six months. An additional item was included for fuel and maintenance of the vessel, necessary for conducting the activity (Table 3).

The Brazilian Union Property Secretariat (SPU), a governmental body responsible for the jurisdiction of marine areas in the country, stipulates a fee for the use of the area, amounting to US$ 360.00 ha^{-1} annually (Table 3).

Table 3. Operating cost per cycle and annually for the culture of mangrove oyster *Crassostrea gasar*, in 0.1 ha, in the Cananeia region, southeastern coast of Brazil, May 2024.

Item	EOC[2]	Social Burdens[3]	Financial Burdens[4]	TOC[5]	Fixed Costs	TCP
Permanent labor force (minimum wage)	1694.40	677.76	177.91	2550.07	-	2550.07
Vessel fuel and maintenance of the vessel	1200.00	-	126.00	1326.00	-	1326.00
Infrastructure expenses	600.00	-	63.00	663.00	-	663.00
Occupancy rate (SPU)	18.00	-	1.89	19.89	-	19.89
Civil Construction Depreciation[6]	-	-	-	-	1080.00	1080.00
Boat, Tools and Equipment Depreciation[6]	-	-	-	840.00	-	840.00
Annual interest on investment capital	-	-	-	-	3104.56	3104.56
Total cicle^{-1}	**3512.40**			**5398.96**		**9583.52**
Total year^{-1}	**7024.80**			**10797.92**		**19167.04**

[1] Amounts expressed in US dollars (US$ = R$ 5.20); [2] Effective Operating Cost; [3] 40% of the EOC; [4] 10.5% per annum, of EOC added to social burdens; [5] Total Operating Cost; [6] Depreciation estimated according to lifespan.
Source: Research data.

To determine the production costs per dozen of *C. gasar*, three different survival conditions were considered, which served as the basis for the production system calculation (Table 1). The estimated final survival rates were 63% (6,300 dozen year^{-1}), 72% (7,200 dozen year^{-1}), and 81% (8,100 dozen year^{-1}) for conditions A, B, and C, respectively. This produced biomass resulted in different EOC, TOC, and TCP, ranging from US$ 0.87 dozen^{-1} for EOC in condition C to US$ 3.04 dozen^{-1} for TCP in condition A (Table 4)

Table 4. Effective operating cost (EOC), total operating cost (TOC) and total production cost (TPC) of mangrove oyster *Crasssotrea gasar* farmed in 0.1 ha, on the Cananeia region, southeastern coast of Brazil, May 2024.

Production cost (US$ dz^{-1})	**Condition A**	**Condition B**	**Condition C**
Effective Operating Cost (EOC)	1.12	0.98	0.87
Total Operating Cost (TOC)	1.71	1.50	1.33
Total Production Cost (TPC)	3.04	2.66	2.37

Source: Research data.

The prices practiced for the sale of a dozen oysters (US$ 3.00 and US$ 4.00) were used to obtain financial and profitability indicators, reflecting the variation in market prices in the region. The financial indicators Operating Profit (OP), and the profitability indicators Gross Margin (GM) and Profitability Index (PI), showed positive values in all three survival conditions evaluated (Table 5). The Internal Rate of Return (IRR) ranged from 0.81% for condition A at the lowest selling price to 24.83% for condition C at the highest selling price (Table 5). Only for conditions A and B, when sold at US$ 3.00, there was no economic viability, as the obtained IRRs were below the Minimum Attractive Rate (MAR), set at 10.5%. The same occurred for the Net Present Value (NPV), where all evaluated conditions showed positive values, except for conditions A and B when sold at US$ 3.00 (Table 5), demonstrating that the higher the selling price, the greater the profitability. The Payback Period (PP) in the worst evaluated condition was 9.52 years, considered high risk. Only in conditions B and C, with a selling price of US$ 4.00, does the PP become attractive, providing a greater chance of success and a quick return on invested capital, exposing the activity to lower risk (Table 5).

Table 5. Financial and profitability indicators for the cultivation of mangrove oyster *Crasssotrea gasar*, in 0.1 ha, on the Cananeia region, southeastern coast of Brazil, May 2024.

Cash Flow	**Condition A**		**Condition B**		**Condition C**	
Selling Price (US$ dz^{-1})	3.00	4.00	3.00	4.00	3.00	4.00
Gross Revenue (US$)	18900.00	25200.00	21600.00	28800.00	24300.00	32400.00
Operating Profit (US$)	8102.08	14402.08	10802.08	18002.08	13502.08	21602.08
Gross Margin (%)	75.03	133.38	100.04	166.72	125.04	200.06
Profitability Index (PI) (%)	42.87	57.15	50.01	62.51	55.56	66.67
Internal Rate of Return (IRR) (%)	0.81	13.20	6.53	19.21	11.61	24.83
Net Present Value (NPV) 10.5%	-28802.29	8179.26	-12562.40	30743.96	3677.49	52397.15
Leveling Point (LP) oyster (dz)	3599.31	2699.48	3599.31	2699.48	3599.31	2699.48
Payback Period (PP) (year)	9.52	5.46	7.17	4.22	5.75	3.61

Source: Research data.

DISCUSSION

Physiological variations in farmed mangrove oysters (*Crassostrea gasar*)

Intrinsic factors such as body size and life stage [16,17] and extrinsic factors such as temperature, dissolved oxygen, and salinity [18] play important roles in the biology of many species of mollusks. In aquatic organisms, respiration generally increases with increasing temperature [17,18]. The responses of mollusks to changes in salinity are, however, more complex [7]. Occurrences of high mortality resulting from prolonged rainfall, related to low salinities, have already been observed in oyster farming areas by Pereira and coauthors [1] for *C. brasiliana*, Ellard and coauthors [19] for *C. gigas*, and La Peyre and coauthors [20] for *C. virginica*.

In these studies, almost 100% mortality was observed when oysters were exposed to salinities below 5 g L^{-1}, when this condition persisted for more than 10 days.

The observed trend of decreasing mortality with increasing salinity suggests that *C. gasar* exhibits a higher survival rate in environments with higher salinity levels. This finding aligns with previous studies that have demonstrated the salinity tolerance of *C. gasar* and its ability to thrive in habitats with elevated salinity. The decrease in mortality with increasing salinity may be attributed to the physiological adaptations of *C. gasar* to osmotic stress [21]. As salinity rises, the osmoregulatory mechanisms of the oysters may become more efficient, allowing them to maintain internal balance and cope with the challenges posed by high. According to Funo and coauthors [21], salinity had a significant impact on the survival and growth of the oysters. The study revealed a high survival rate for oysters within the salinity range of 10 to 45 g L^{-1}. Particularly, salinities of 20 and 25 g L^{-1} resulted in significantly higher survival rates, while a lower survival rate was observed at a salinity of 5 g L^{-1}. These results suggest that the *C. gasar* oyster demonstrates resilience across various salinity levels, rendering it suitable for cultivation in marine regions or estuary environments, as long as the salinity does not drop to or below 5 g L^{-1}. Oliveira and coauthors [22] support this information, observing better growth performance and survival in areas near the ocean, while more distant areas, influenced mainly by river discharges, are less favorable for the production of the oyster *C. gasar*, which showed greater growth during the transition period from rainy to dry season and in the dry season, with salinity between 25 and 35 g L^{-1}.

The results of this study reinforce previous findings that prolonged exposure to low salinity is a critical factor influencing oyster mortality, with prior research indicating near-total mortality occurs when oysters are exposed to salinities below 5 g L^{-1} for more than 10 days [19, 20]. However, the present study extends this exposure time to 30 days, providing a more comprehensive evaluation of the long-term effects of low salinity on oyster survival and physiological responses. While initial mortality trends align with prior reports, where oysters experience severe mortality under low salinity, the extended exposure may have influenced additional physiological stress responses, potentially accelerating irreversible damage, suggesting a cumulative effect over time where prolonged osmotic stress exceeds the oysters' metabolic compensation capacity. Additionally, prolonged exposure to low salinity likely impacted the oysters' metabolic rates, as previous research has shown that osmoregulatory mechanisms to cope with osmotic stress are energetically costly [23], and the sustained metabolic demand could deplete energy reserves, further compromising survival. Assessing metabolic markers, such as oxygen consumption, ammonia excretion, and enzymatic activity, under extended exposure conditions could offer valuable insights into how the oysters' physiological state deteriorates. Moreover, environmental factors like temperature and nutrient availability may modulate the impact of prolonged low salinity exposure [24], and if oysters face additional stressors, such as temperature fluctuations or food scarcity, their ability to withstand osmotic stress may be further reduced. Future studies should incorporate multi-factorial analyses to explore the interactive effects of salinity duration, temperature, and nutritional status on oyster resilience.

Responses to changes in external salinity involve the closing of bivalve shell valves, adjustment of intracellular ion concentrations [25], and decreased functional activity [26], interfering with their growth. The lack of statistical differences in mortality means between salinities ranging from 10 to 35 g L^{-1} suggests that *C. gasar* may exhibit a relatively stable mortality rate within this salinity range. This indicates that, once the salinity surpasses a certain threshold, the mortality rate of *C. gasar* may not be significantly affected by further increases in salinity. However, it is essential to note that local adaptation, genetic variations, and other ecological factors may influence the response of *C. gasar* populations to different salinity levels.

The oyster is an osmoconformer, it can tolerate considerable dilution, to some extent it can resist periodic dilution of estuary water as it keeps its shells closed [27,28,29] and require energy to maintain tissue hydration levels [16], an energy that can be reallocated from growth or another physiological function. When salinity drops too much, they close their shells, however, when salinity increases, they open the shells and release ammonia. La Peyre and coauthors [30] report that the high mortality rate of *Crassostrea* oysters exposed to low salinity is related to the inability to control plasma osmolality for prolonged periods due to valve closure in these extreme conditions. This fact was observed in this study (Figure 5) where starting from a salinity of 15 g L^{-1} there was a significant increase in the excretion of ammonia.

The increase in the respiratory rate of *C. gasar* with the decrease in salinity observed during this study corresponds to the results obtained by Fuhrmann and coauthors [31] for *C. gigas* and Horodesky and coauthors [29] for *C. gasar*. Oysters’ metabolic responses to changes in salinity are highly variable across species [27]. In this study, an increase in salinity was associated with a decrease in the metabolic rate of *C. gasar*. However, the absence of any significant difference in the respiration rate and specific ammonia excretion of *C. gasar* above the salinity range of 15 g L^{-1} suggests that this oyster species is well adapted to inhabit environments characterized by variations in salinity between 15 and 35 g L^{-1}. Such conditions occur in the middle course of permanently open estuaries with substantial freshwater inflow and saltwater inflow during high tide. Within these systems, salinity fluctuates a lot due to tidal influences, especially in spring tides [32]. On the other hand, when *C. gasar* was subjected to salinities below 5 g L^{-1} for a longer period, mortality was high.

This condition of adaptation to more saline environments led to the choice of *C. gasar* cultivation in the sea, in southern Brazil, in suspended systems with lanterns, as an alternative to *C. gigas*, which are the most cultivated oyster species in the region; however, there is a high mortality in crops during the summer months [33]. The increase in the respiration rate of oysters at salinities lower than 5 g L^{-1} suggests that this bivalve may undergo osmotic stress in fresh water. This fact can occur mainly in the summer when rainfall is higher in the Cananeia estuary, and some areas of the estuary may have hyposaline conditions for several days [32]. At salinities less than 5 g L^{-1} the increase in oyster metabolic rate may be due to rapid loss of salts [34]. As there was an increase in mortality and metabolic rate during the experiments at salinities below 5 g L^{-1}, the data suggest that these bivalves are not able to tolerate hyposaline conditions for a long time. This can be a limiting factor especially in the summer in some areas of the estuary, which can experience high fluctuations of salinity over 24-hour periods [33] and remain at a low salinity for days.

Oysters exposed to temperature gradients exhibited an increase in specific oxygen consumption, with the trend becoming evident at a temperature of 25°C (Figure 4). Specifically, at a temperature of 30°C, the mean specific oxygen consumption showed a substantial increase of 82.3% compared to a temperature of 15°C. However, no statistically significant difference in metabolic rate was observed between temperatures of 15 and 20°C or between 25 and 30°C.

The observed increase in specific oxygen consumption with rising temperature suggests an elevated metabolic demand of the oysters as they experienced warmer conditions. Higher temperatures can lead to increased metabolic activity in ectothermic organisms, such as oysters, due to the temperature dependence of enzymatic reactions involved in energy production and utilization [24]. The significant rise in specific oxygen consumption at 30°C indicates a notable acceleration of the metabolic rate, likely reflecting the enhanced physiological activities necessary for the oysters to maintain vital functions under elevated temperature stress.

The increase in specific oxygen consumption and specific ammonia excretion for *C. gasar* in response to a temperature gradient observed in this study corresponds to results of metabolic variations with another oyster species like *C. corteziensis* [35], and for other invertebrates in temperature gradients [36]. The rate of oxygen consumption often increases progressively and steadily with increasing temperature [17], a fact also observed in this study for *C. gasar*.

Interestingly, the absence of statistical differences in metabolic rate between temperatures of 15 and 20°C and between 25 and 30°C suggests a potential threshold effect or an optimal temperature range for the metabolic response of the oysters. It is possible that within these temperature ranges, the oysters were able to regulate their metabolic rate to cope with the environmental conditions effectively. This regulation could involve physiological adjustments to optimize energy utilization and maintain homeostasis.

The means of specific ammonia excretion showed a statistical difference starting from 15 g L^{-1}, in relation to salinities from 1 to 5 g L^{-1} (Figure 5). At 30 g L^{-1} there was an increase of 47.5% in ammonia excretion in relation to salinity of 5 g L^{-1}. Between 15 to 35 g L^{-1} there was no significant difference.

The observed increase in specific ammonia excretion with increasing salinity suggests that *C. gasar* exhibits an elevated excretory response to higher salinity levels. Ammonia excretion is an essential process for marine organisms to eliminate nitrogenous waste products, and its rate is influenced by various factors, including salinity. The increased ammonia excretion at higher salinities may be attributed to the osmoregulatory adjustments made by *C. gasar* to maintain osmotic balance under hyperosmotic conditions [17]. The excretion of ammonia helps regulate internal osmotic pressure and maintain proper physiological functioning. The absence of significant differences in ammonia excretion between salinities ranging from 15 to 35 g L^{-1} suggests that *C. gasar* may have a threshold or optimal range of salinity tolerance for ammonia excretion. Within this range, the oysters may be able to maintain a relatively stable ammonia excretion rate, indicating a capacity for efficient osmoregulation [36]. This adaptability may be crucial for oysters to cope with varying salinity conditions and maintain their physiological equilibrium.

It is important to consider that ammonia excretion is influenced by multiple factors, including temperature, food availability, and metabolic rate. Therefore, further studies investigating the interactions between these factors and their combined effects on ammonia excretion in *C. gasar* would provide a more comprehensive understanding of the oysters' excretory responses.

The observed increase in ammonia excretion with increasing temperature suggests an increase in metabolic activity and energy demand of the oysters as they experienced higher temperature levels (Figure 6). Elevated temperature can have physiological impacts on marine organisms, including oysters, by increase in metabolic activity processes and altering the functioning of various metabolic pathways [7]. Exploring the underlying mechanisms and molecular pathways involved in the temperature regulation of ammonia excretion in *C. gasar* would contribute to our knowledge of osmoregulation and nitrogenous waste management in marine organisms. Understanding the physiological adaptations and regulatory mechanisms of oysters in response to different salinity and temperature conditions can have implications for the sustainable management of oyster populations and aquaculture practices.

Economic feasibility in farmed mangrove oysters (*Crassostrea gasar*)

Even using a reduced area (0.1 ha) when compared to other marine farms in Brazil [37] and worldwide [38], the results obtained in this study demonstrated the economic viability of *C. gasar* cultivation on a small scale, which can be attributed to the good commercial value of the product and the short production cycle adopted in the fattening system, optimizing time and use of cultivation structures (trays).

According to Henriques and coauthors [3], the major capital investment in marine aquaculture projects is related to the acquisition of equipment and the construction of cultivation structures. This data was confirmed for the present study, in which the initial investment of US$ 77,534.43 can be considered high for a small producer or artisanal fisherman, restricting the activity to entrepreneurs with greater financial resources. However, an alternative for individuals with lower financial capacity to enter this activity would be through associations or cooperatives of producers, a fact that already occurs incipiently in the studied region.

The data used in the proposed production system include the costs associated with the infrastructure necessary to implement the activity on a small-scale family system, as well as its operational and labor costs. Larger cultures tend to be economically viable in their operation due to the possibility of better dilution of fixed costs and the use of contracted or temporary labor. Theodoridis and coauthors [38] point out that family farming, occupying smaller areas, is an interesting alternative, due to the potential flexibility advantage, but that is threatened, however, by high fixed costs.

Several financial indicators were used to assess the feasibility of investing in such an enterprise. In the present study, the IRRs, for all proposed conditions, ranged from 0.81% to 24.83%, influenced by the survival rates tested and by the market prices of the product. Fagundes and coauthors [4] obtained up to 54.82% IRR, with mangrove oyster farming in the Cananeia region, but worked with areas 10 times larger (1 ha), and this factor diluted fixed costs, improving the return on investment at that time. In the present study, we chose to work in smaller areas because it currently reflects the reality of the region, where large marine farms have not been observed for a long time.

The value allocated to labor was the item that accumulated the most operational costs for the activity, about 27%. It should be emphasized that the basic calculation of this item is due to the remuneration of a worker, in this case, the aquaculturist himself, on a full-time basis (8 hours/day), sufficient to maintain the necessary productive routine for the smooth running of cultivation. This is much more expensive when compared to aquaculture in the Philippines, where labor is not remunerated and represents only 10% to 14% of the cost, a value calculated by the opportunity cost, which represents the remuneration that individuals would obtain in other activities if there were no option to practice aquaculture [39]. It is important to emphasize that in some Asian countries, this workforce consists mainly of rural workers seeking to overcome the poverty line, receiving extremely low wages [40]. In the present study, although uncommon, it was opted to regularize the remuneration of the labor used in the productive process, including all labor rights. Underestimating this cost directly leads to underestimating the real production cost of the entire aquaculture activity [14].

The Payback period in the best evaluated condition (81% survival) was 3.61 years, a value close to the 4.10 years obtained by Henriques and coauthors [3] for fattening mangrove oyster in the same region. However, they considered a survival rate of 64% and used 80 trays on 0.5 ha, an area of production five times larger. This fact when compared to our condition A with 63% survival makes the PP rise to 5.46 years. On the other hand, our results were better than the six years of PP obtained by Taylor and coauthors [41] for the cultivation of *Nodipecten subnodosus* in Magdalena Bay, Baja California Peninsula, Mexico, under the same survival conditions, but with different production technology, in that location trays are not used, the cultivation is also bottom-based, but with the use of pillows, without contact with the substrate.

Brazilian aquaculture is still in its infancy compared to other countries, especially in Asia, mainly due to the lack of public policies prioritizing alternatives with greater socioeconomic impact, aiming to harness the potential and regional vocation [42, 43]. This activity contributes to social development, generating income through the creation of direct and indirect jobs with significant gains for the local economy, resulting in an improvement in the quality of life of the involved aquaculturists [44, 45]. Finally, the job creation promoted by the activity also contributes to reducing the unemployment rate, one of the most serious social problems in Brazil currently, as well as anchoring producers in their place of origin, maintaining their tradition.

CONCLUSION

The decrease in mean mortality with increasing salinity suggests a higher survival rate of *Crassostrea gasar* in environments with higher salinity levels. The findings of this study shed light on the metabolic responses of oysters to salinity gradients. The observed reduction in specific oxygen consumption with increasing salinity highlights the physiological adjustments made by oysters to cope with osmotic stress. The present study demonstrated that the production and commercialization of *C. gasar* can be economically viable according to the particularities described here, confirming the hypothesis of profitability change according to the proposed survival conditions. Research focused on zootechnical improvement and the feasibility of new integrated farming systems could support the success of new projects and, consequently, generate jobs and income, tax revenue, local development, and also discourage harmful practices such as oyster extraction in mangroves, a condition that still occurs indiscriminately in the region

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