

**EFFECT OF THE SALINITY VARIATION ON THE OSMOTIC RESPONSES OF
CRASSOSTREA RHIZOPHORAE (GUILDING, 1828) (MOLLUSCA-BIVALVIA-
OSTREIDAE): PHYSIOLOGICAL SUBSIDIES TO CULTIVATION**

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RESUMO

Efeito da variação da salinidade sobre as respostas osmóticas de Crassostrea rhizophorae (Guilding, 1828) (Mollusca - Bivalvia - Ostreidae): subsídios fisiológicos para cultivo. A sobrevivência de adultos (4,5 cm de comprimento médio) e de sementes (2,0 cm de comprimento médio), a capacidade de isolamento e a osmolalidade da hemolinfa da ostra *Crassostrea rhizophorae*, foram analisadas em indivíduos aclimados a 30 psu, durante 48 h e submetidos a meios hiper e hiposmóticos. Após 20 dias, a tolerância de adultos foi maior que a das sementes, em todas salinidades testadas (entre 10 e 35 psu). Sem atingir o LT_{50} , os maiores níveis de sobrevivência (acima de 90%) foram atingidos pelos adultos e sementes, nas faixas de salinidade entre 15 e 35 psu e entre 20 e 35 psu, respectivamente. Nos meios mais diluídos, a lenta diluição do fluido perivisceral de ostras adultas, evidenciou a existência de estratégia de proteção temporária contra uma rápida diminuição da concentração dos fluidos corpóreos. A concentração da hemolinfa variou linearmente com a concentração do meio ($R^2= 0,9687$), confirmando em *C. rhizophorae* a característica de animal osmoconformador. Embora *C. rhizophorae* apresente alto grau de eurialinidade, as salinidades iguais ou inferiores a 10 psu representam meios de alta letalidade, sendo inadequados para o cultivo da espécie. Na seleção de áreas para cultivo de *C. rhizophorae*, recomenda-se que a salinidade do meio esteja dentro de uma faixa de variação entre 15 e 35 psu.

Palavras-chave: Ostra, sobrevivência de adultos e sementes, capacidade de isolamento, osmolalidade da hemolinfa.

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ABSTRACT

Effect of the salinity variation on the osmotic responses of *Crassostrea rhizophorae* (Guilding, 1828) (Mollusca-Bivalvia- Ostreidae): physiological subsidies to cultivation. The survival of adults (4.5 cm mean length) and of seeds (2.0 cm mean length), the isolation capacity and the osmolality of the haemolymph of the oyster *Crassostrea rhizophorae* were analysed in individuals acclimated to 30 psu during 48 h and submitted to hyper- and hyposmotic media. After 20 days, the tolerance of adults to various salinities was higher than of seeds, with the highest survival levels (above 90%) occurring between 15 and 35 psu for adults, and between 20 and 35 psu for seeds. In all tested salinity range, from 10 to 35 psu, the LT_{50} was not reached. In adult oysters, the perivisceral fluid presented a slow dilution, an evidence of the existence of a strategy for temporary protection against a sudden decrease of the body fluids concentration, mainly in more diluted media. The concentration of the haemolymph varied linearly with the osmotic concentration of the medium ($R^2 = 0.9687$). Although the data have shown that *C. rhizophorae* is an osmoconformer, with a high degree of euryhalinity, media with salinities equal to or lower than 10 psu are highly lethal, making them unsuitable for cultivation. Salinities between 15 and 35 psu are recommended for selecting cultivation areas.

Key words: Oyster, survival of adults and seeds, isolation capacity, haemolymph osmolality.

INTRODUCTION

Crassostrea rhizophorae is an oyster distributed from the southern Caribbean to Uruguay (RIOS, 1994). Though mostly occurring in estuaries, this species is also found in bays, coves, and along rocky coastlines (GUZENSKI, 1996). Exploited as an important natural resource for human consumption, *C. rhizophorae* has been cultivated in some Brazilian estuaries, where salinity fluctuation may represent one of the biggest difficulties to a successful enterprise.

Since they do not have extracellular anisosmotic regulation, marine and estuarine bivalves use only cell volume regulation (GILLES, 1972; GILLES and JEUNIAUX, 1979), showing variation in their body fluids concentration in changing environments (PIERCE, 1970; 1971). To escape or to diminish temporarily the amplitude of an osmotic shock, bivalves use strategies like adduction of the valves, siphon retraction, production of mucus, and deepening in the substrate (FREEMAN and RIGLER, 1957; PIERCE, 1971; CASTAGNA and CHANLEY, 1973; SALOMÃO *et al.*, 1980; GILLES and JEUNIAUX, 1979; LEONEL and SILVA, 1988). Besides osmotic problems, salinity may affect reproductive rate, development, and growth of embryos and larvae of molluscs (GILLES and JEUNIAUX, 1979).

Individuals of *C. rhizophorae* living attached to mangrove roots in estuarine areas, may be affected by large salinity variations. In Brazil, particularly

in the Northeast region, a pronounced and prolonged decrease of salinity occurs throughout the rainy season (from March to August), when the volume of fresh water increases in the estuaries.

In order to improve cultivation of this species, the present study aims to elucidate some physiological aspects of *C. rhizophorae*, including their isolation capacity, tolerance levels of seeds and adults, and their osmotic behaviour in various salinities.

MATERIAL AND METHODS

The experiments were performed with seeds (2.0 cm mean length) acquired from the company Conatura of the State of Sergipe, where they were maintained at a mean salinity of 28 psu, and with adult oysters (4.5 cm mean length) collected in the estuary of the Catuama river, with a mean salinity of 32 psu, in the State of Pernambuco, in Northeast Brazil. Before starting the experiments, the oysters were previously acclimated during 48 h at 30 psu (control salinity) in continuously aerated water.

The experiments were carried out by using potentially mature males and females, because specimens of *C. rhizophorae* from 1.9 cm length on, present functional gonads (NASCIMENTO, 1978).

The hyposmotic media were prepared with sea-water at 35 psu, paper filtered, and diluted with dechlorinated tap water, using the formula $V_1C_1 = V_2C_2$ (V_1 = volume of the water to be used; C_1 = concentration of the water to be used; C_2 = final concentration of the water, and V_2 = the volume to be obtained).

The salinity values were obtained by using a Portable Refractometer Model SZJ-S10 ATC. The water temperature was measured with a mercury thermometer.

The tolerance of *C. rhizophorae* to salinity variation was analysed separately in sets (N=60) of adults and seeds transferred from the control medium to aquaria containing 20 L of sea-water of one of the following salinities: 10, 15, 20, 25, 30, and 35 psu, at ambient temperature. The animals were observed for 20 days, at 24 h intervals, and they were considered to have died when they failed to react to mechanical stimulation at the mantle edge, as suggested by LEONEL and SILVA (1988). The water in the aquaria was changed at 48 h intervals.

In order to analyse the interference of adduction of the valves on the maintenance of the concentration of the perivisceral fluid (PVF), the salinity of the PVF was tested in adult oysters (4.5 cm mean length) by using the same range of salinity as used when evaluating their survival. The initial measurement (time zero) of the PVF salinity was performed in animals that had just been removed from the control salinity. After transferring the oysters to a given salinity concentration, the observations were carried out after 1, 3, 6, and 12 h. After

this time, they were monitored at 24 h intervals up to a maximum period of 96 h. At each observation time, the salinity of the PVF was determined in five specimens for each salinity concentration, with the specimens discarded afterwards. Samples of PVF, from 1.5 to 2.0 mL, were withdrawn from the pallial cavity of each specimen using a 5-mL hypodermic syringe, with the PVF salinity immediately measured in the refractometer. The mean and standard deviation were determined for each pool of samples.

Determination of the osmotic concentration of the haemolymph was carried out in adult oysters (4.5 cm mean length). In order to render ineffective the isolation caused by adduction of the valves, the oysters were carefully opened with a wedge, maintaining them open throughout the experiment, as suggested by SALOMÃO *et al.* (1980). Then, sets of 20 animals were transferred to the salinities of 15, 20, 25, 30, and 35 psu. In order to speed up the osmotic change with the medium, about 5 ml of water from the respective aquarium was injected into the pallial cavity of each specimen. After 24 h, samples of haemolymph were individually collected by withdrawing the fluid from the adductor muscle of five animals per each salinity investigated, using a 1-ml hypodermic syringe. Three samples of the aquarium water were collected from each salinity. The samples of haemolymph were placed into eppendorf tubes and stored in the freezer until measurements were performed. All the samples were centrifuged (JOUAN MR Model 1822) at 10,000 rpm for 10 min at a temperature of 10 °C according to LEONEL (1981). The supernatant fluid was removed and its molality measured in an osmometer WESCOR 5520 with water vapour under pressure. The relationship between the osmotic concentrations of the haemolymph and of the medium were determined through regression analysis and analysis of covariance, using the software Statistica for Windows (STATSOFT, 1998).

RESULTS

Along the 20 days of the experiment, the tolerance to salinity variation of the adult specimens was higher than of the seeds, with the highest survival levels for adults between 15 and 35 psu (95.0 to 100%) and for seeds between 20 and 35 psu (90.0 to 96.7%) (Fig.1). The lowest survival levels of adults and seeds were observed at 10 psu with 20% specimen mortality. The mean temperature of the water for both groups was 25.8 ± 0.40 °C.

Although the salinity concentration of the PVF showed a tendency to move towards equilibrium at all tested salinities (Fig. 2, A-F), this effect was only reached by animals maintained at 35 and 25 psu, over 24 h and 48 h periods, respectively. In the oysters maintained at salinities of 20, 15, and 10 psu, the salinity concentration of the PVF showed differences between 5 and 15 psu, proportionally to the water dilution, up to the limit of the 96 h period. In

the control salinity there was no variation of the PVF salinity.

The haemolymph osmolality varied linearly with the medium osmolality ($R^2 = 0.9687$) (Fig.3). The F-ratios obtained from the analysis of covariance indicate that the haemolymph was significantly ($p < 0.01$) more concentrated than the sea-water, in all the salinities tested (Tab.1).

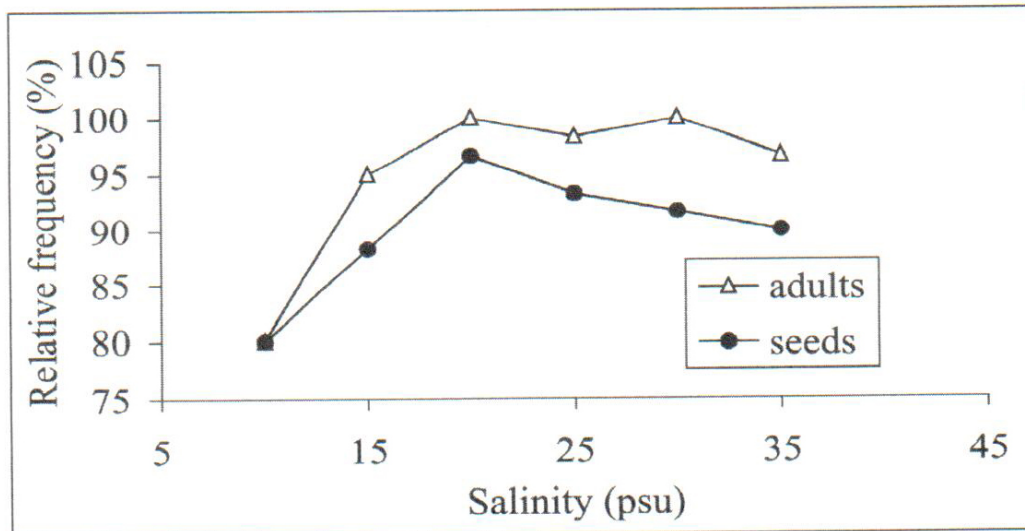


Figure 1- Relative frequency of survival of *C. rhizophorae* adults and seeds (N = 60) submitted to various salinities over a twenty-day period.

DISCUSSION

The data we obtained show clearly that *C. rhizophorae* is an osmoconformer animal, since the osmotic concentration of its haemolymph varied linearly with the concentration of the medium. As is the case for other marine and estuarine bivalves submitted to hyposmotic media (PIERCE, 1970; SHUMWAY, 1977; LEONEL, 1981; LEONEL and SILVA, 1988; SALOMÃO and LUNETTA, 1989), the haemolymph of *C. rhizophorae* is hyperosmotic to the media over the salinity range tested. Such difference probably is due to the Gibbs-Donnan equilibrium, as suggested by PIERCE (1970) for three species of *Modiolus*.

The gradual variation of salinity of the PVF of *C. rhizophorae* submitted to hyper- and hyposmotic media, indicates a slow process of ionic change between the PVF and the medium, helping the animal to survive to direct osmotic shock, since it prevents a sudden change of concentration of the extra- and intracellular fluids. As a behavioural strategy of bivalves under stress in unfavourable media, this isolation is carried out by adduction of the valves, as observed in *Modiolus demissus granosissimus* (PIERCE, 1971), *Perna perna* (SALOMÃO *et al.*, 1980), and *Mytella guyanensis* (LEONEL and SILVA,

1988). Besides this behavioural strategy, it is known that exposure of bivalves to diluted media induces a temporary reduction of the ciliary activity (THEEDE, 1978), decreasing the speed of the internal flux of water. In this way, these behaviours induce a slower dilution of the PVF, permitting it to act as a buffer fluid between the external medium and the haemolymph and increasing the probability of survival to salinity variations.

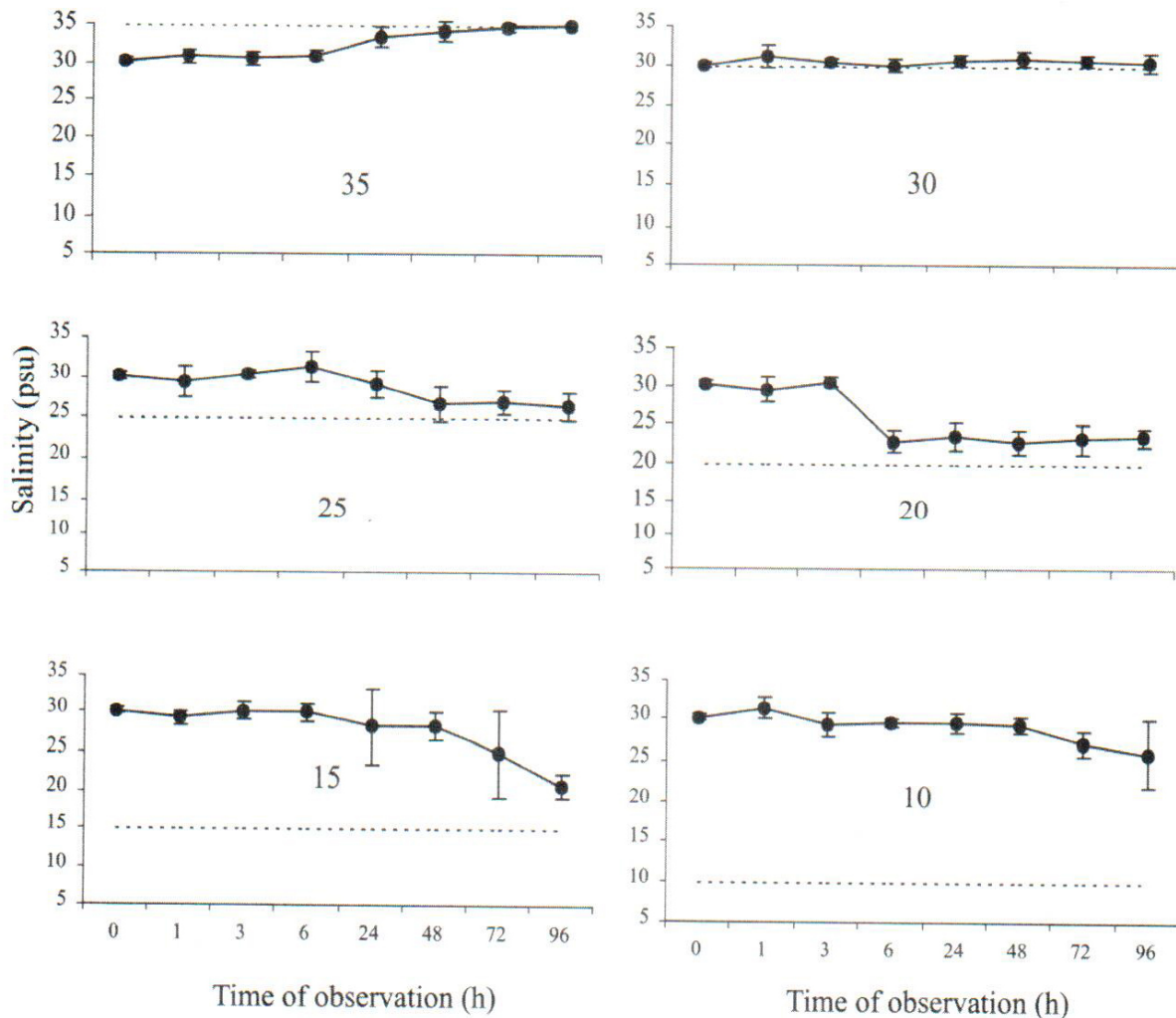


Figure 2 - Salinities of the perivisceral fluid (PVF) of *C. rhizophorae* cultivated in various salinities: 35, 30, 25, 20, 15 and 10 psu at the times indicated. The dashed line indicates the salinity-test. Error bars represent standard deviations. (N = 4 to 5 specimens).

Considering that (i) selection and intake of food depend on the ciliary activity (BAYNE *et al.*, 1976), (ii) hyposmotic media depresses the ciliary activity throughout the acclimation period (THEEDE, 1978; GILLES and JEUNIAUX, 1979), and (iii) the regulation of the cell volume involves energy expenditure (GILLES, 1975), we hypothesize that prolonged exposure periods to adverse

salinities would negatively affect the oyster development.

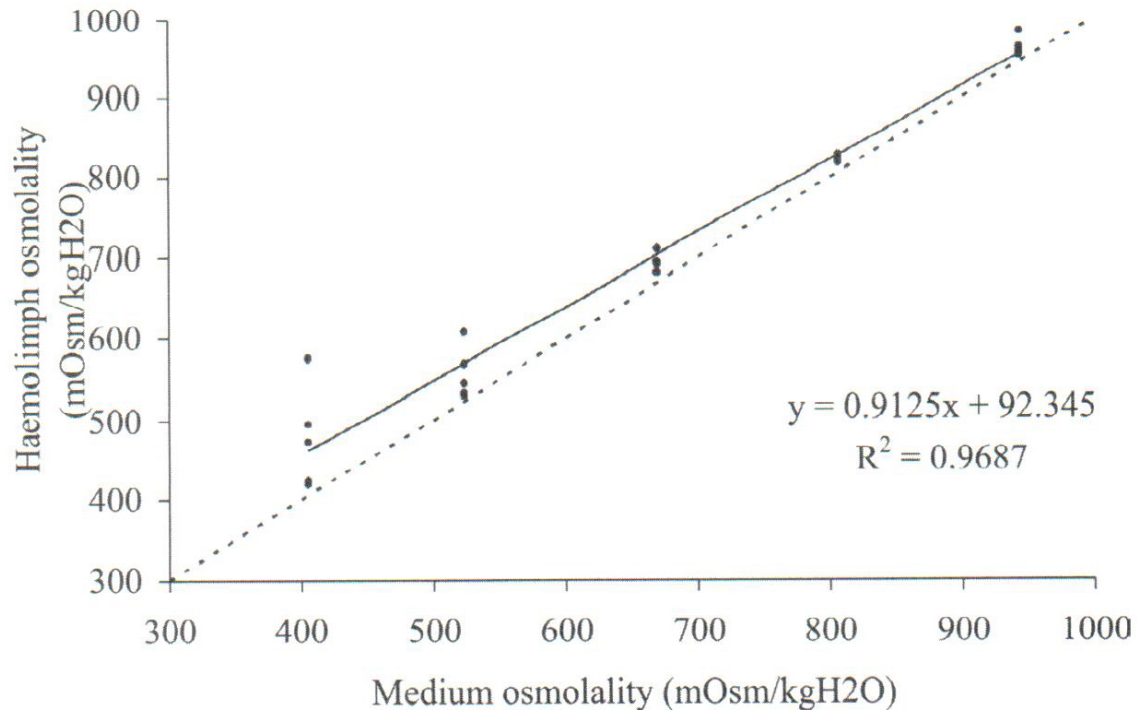


Figure 3 - Variation of the osmotic concentration of the haemolymph of *C. rhizophorae* submitted to various salinities (N = 5). The dashed line represents the isosmotic concentration.

Table 1 - Covariance analysis and difference of osmotic concentration (mOsm/kgH₂O) between mean values of the haemolymph (N = 5) of *C. rhizophorae* and of the sea water (N = 3) in different salinities.

	Salinity (psu)				
	15	20	25	30	35
Haemolymph	477.4±64.14	557.8±32.29	692.6±11.72	824.6±4.62	963.8±12.52
Medium	405.0±1.73	523.0±1.73	669.3±2.08	806.3±1.15	943.6±2.08
Difference of concentration (mOsm/kgH ₂ O)	72.4	34.8	23.3	18.3	20.2
Covariance	F (37.1)= 12.24		p<0.01		

The isolation caused by valve adduction of *C. rhizophorae* was effective in the salinity range of 20 to 10 psu over a 96 h period when the PVF did not reach a complete equilibrium with the medium, as it did in the salinities

between 35 and 25 psu. This indicates that *C. rhizophorae* is able to identify the dilution grade of the medium, showing a longer time of adduction of the valves as larger is the medium dilution. According to DAVENPORT (1981) the opening of the valves is controlled by osmoreceptors situated on the mantle edge and in the inhalant siphon. Depending on the medium dilution, the movement of the valves may return to its normal rhythm within 5 to 10 days, as observed in *Modiolus demissus granosissimus* (PIERCE, 1971). We believe that the response observed at 20, 15 and at 10 psu was probably affected by the short observation period selected in our experiment, which was not sufficient to observe the recovery of the opening rhythm of the valves.

Although periods of gradual acclimation to salinity may alter survival levels (SHUMWAY, 1977), in our experiments *C. rhizophorae* showed a wide tolerance to salinity variations. Since this species is tolerant to a variation of 25 psu, it can be considered a highly euryhaline animal, according to criteria suggested by KINNE (1971). A salinity of 10 psu can be considered the limit of tolerance, representing a loss of 20% of the cultivated specimens. FERNANDES and SANCHEZ (1980), analysing the survival of adults of *C. rhizophorae* (5.0 cm length) acclimated to 25 psu and transferred to salinities between 15 and 1 psu, reported a high mortality of specimens in the 7 to 1 psu range, where the LT_{50} was reached within eight days. As a complement to our results, the mortality rates determined by FERNANDES and SANCHEZ (1980) confirm observations in an estuary of Northeast Brazil, where a high mortality of *C. rhizophorae* was noticed at the upper Mamanguape river (State of Paraíba) after a period of intense rains, when the salinity decreased to levels similar to fresh water (R.M.V. Leonel, unpublished).

Despite the tolerance of several invertebrates to salinity variation have been associated to age, sex and reproductive cycle (KINNE, 1964; VERNBERG and VERNBERG, 1978), this relationship in molluscs have been associated to the ontogenetic phases of their development (BAYNE, 1976). The differing survival to salinity observed for adults and seeds indicate that in the experimental conditions we used, the tolerance level of *C. rhizophorae* was influenced by the animal age. Our results confirm the data of tolerance to salinity variation obtained by CASTAGNA and CHANLEY (1973) in 36 species of bivalves which showed that (i) small bivalves frequently succumbed more rapidly to lethal salinities than did larger individuals and that (ii) the tolerance range of each species does not depend on the specimen size.

The 100% survival of seeds (obtained from the same sampling set of our experiment), cultivated throughout 30 days in natural feeding condition and salinity (28/29 psu) similar to nursery conditions (PEDRESCHI, 2004), was an evidence that under laboratory conditions, the seeds tolerance may have been influenced by both salinity and food deprivation. However, these influences are not sufficient to explain neither the differences of survival detected between adults and seeds nor the specific tolerance variations of each group.

Using the measurements of length, width, and height of seed (2.0 cm length) and of adult shells (4.5 cm length) obtained by PEDRESCHI (2004), we observed that the surface: volume ratio of seeds was 2 times larger than that of adults. Considering that the surface: volume ratio affects the metabolic activity of animals (SCHMIDT-NIELSEN, 1995), we believe that the largest surface: volume ratio of seeds would induce higher metabolic expenditure in this group of animals than in adults.

Glycogen being the main storage of energy in bivalves, its content may vary as a function of available food level (GABBOTT and WALKER, 1971; GABBOTT and STEPHENSON, 1974) and of sexual cycle (GIESE, 1969). Being able to reproduce from 1.9 cm length on, *C. rhizophorae* population is made up of individuals in different stages of gonadal repletion (NASCIMENTO, 1978), with different glycogen contents. As it is known that during starvation oysters loses glycogen (GABBOTT and WALKER, 1971) and that under osmotic stress the regulation of cell volume implies in energy expenditure (GILLES, 1975), we consider that these processes have contributed to increase the energy deficit of animals. Thus, considering that physiological and biochemical variations have occurred in both experimental groups, the seeds size represented the differential factor that would explain the higher sensitivity of this group to osmotic stress. The individuals differences in the reserve contents and in the capacity for regulating the cell volume, would also explain the variations of tolerance observed in each group.

The high levels of survival detected for adults and seeds of *C. rhizophorae* indicate strongly that isolation by adduction of the valves represents a favourable mechanism for gradual acclimation of oysters to stressful conditions of the media. Since *C. rhizophorae* is an osmoconformer, this strategy only favours its survival in a specific salinity range.

The experiments carried out under controlled conditions in the laboratory may not reflect all the abiotic interactions of the organisms, nevertheless, the data we obtained indicates the need for careful cultivation management of *C. rhizophorae* with respect to salinity variations in estuarine waters. Since salinity fluctuations may affect the survival of adults and seeds, we recommend the selection of cultivation areas that have a mean variation of salinity in the 15 to 35 psu range. In geographical regions with an accentuated seasonal variation of the pluviometric index, it is recommended that oyster cultivation from seeds, start at periods of higher salinity.

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