ANESTHESIA IN OYSTERS OF THE GENUS *Crassostrea* CULTURED IN BRAZIL*

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ABSTRACT

This study evaluated: 1) the time required for anaesthesia induction and recovery of oysters *Crassostrea rhizophorae, Crassostrea gasar* and *Crassostrea giga*s using magnesium chloride ($MgCl_2$); 2) the survival after anaesthesia and gonad sampling; and 3) the D-larvae generation after anaesthesia. For each species, three groups of 10 animals were kept in 50 g L^{-1} $MgCl_2$, salinity of 36 and temperature of 22 ºC. One control group was kept in solution without $MgCl_2$. Every 1 h anaesthetised oysters were recorded, sampled to determine sex and placed in clean seawater to assess recovering every 30 min. Gonad samplings were made beside the posterior adductor muscle, using 1 mL syringes and needles (0.60 x 25 mm). Factorial crosses were generated within and between anaesthetised and non-anaesthetised oyster groups to produce D-larvae in *C. gigas*. The survival after 10 days of anaesthesia was 100% for the three studied species. For *C. rhizophorae* and *C. gigas*, 100% of the animals were anaesthetised after 360 min and were recovered after 240 and 150 min, respectively. For *C. gasar*, 87% were anesthetised after 720 min and recovered after >240 min. There were no significant differences in D-larvae numbers between factorial crosses. The salt $MgCl_2$ served as an efficient relaxant and caused no deleterious effect on the survival of the three studied species, or on the D-larvae generation in *C. gigas*.

Keywords: anaesthetics; magnesium chloride; *Crassostrea gasar*; *Crassostrea rhizophorae*; *Crassostrea gigas;* D-larvae

ANESTESIA EM OSTRAS DO GÊNERO *Crassostrea* CULTIVADAS NO BRASIL

RESUMO

Este estudo avaliou: 1) o tempo necessário para indução à anestesia e recuperação das ostras *Crassostrea rhizophorae*, *Crassostrea gasar* e *Crassostrea gigas* utilizando cloreto de magnésio ($MgCl_2$); 2) a sobrevivência após anestesia e amostragem gonadal; e 3) a geração de larvas-D após anestesia. Para cada espécie, três grupos de 10 animais foram mantidos em 50 g L^{-1} de $MgCl_2$, salinidade de 36 e temperatura de 22 ºC. Um grupo controle foi mantido em solução sem $MgCl_2$. A cada 1 h, ostras anestesiadas foram registradas, amostradas para determinar sexo e colocadas em água do mar limpa para avaliar a recuperação a cada 30 min. Amostragens das gônadas foram feitas ao lado do músculo adutor posterior, usando seringas de 1 mL e agulha de 0,60 x 25 mm. Cruzamentos fatoriais foram gerados dentro e entre grupos de ostras anestesiadas e não anestesiadas para produzir larvas-D em *C. gigas*. A sobrevivência após 10 dias de anestesia foi de 100% para as três espécies. Para *C. rhizophorae* e *C. gigas*, 100% dos animais foram anestesiados após 360 min e recuperados após 240 e 150 min, respectivamente. Para *C. gasar*, 87% foram anestesiados após 720 min e recuperados após >240 min. Não houve diferença significativa no número de larvas-D entre os cruzamentos. O sal $MgCl_2$ serviu como relaxante eficiente e não causou efeitos deletérios sobre a sobrevivência das três espécies estudadas ou na geração de larvas-D de *C. gigas*.

Palavras chave: anestésicos; cloreto de magnésio; *Crassostrea gasar*; *Crassostrea rhizophorae*; *Crassostrea gigas;* larva-D

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INTRODUCTION

Oyster culture in Brazil is a relatively minor industry compared to other regions of the world, such as China, Japan, Korea and Europe. Nevertheless, it does represent an income source for local populations that depend on marine fishery resources. The most economically important species is the Pacific oyster, *Crassostrea gigas*, which produces about 90% of all oysters cultured in Brazil (BRASIL, 2013; SANTA CATARINA, 2014). The cultivation of *C. gigas* is concentrated in the state of Santa Catarina, mostly in the North and South bays of Florianópolis (around 27º69'S; 48º57'W) (MELO *et al*., 2010) and expanded from 43 ton in 1991 to 2,932 ton in 2013 (SANTA CATARINA, 2014).

In spite of its potential for rapid growth, the cultivation of *C. gigas* in Brazil is limited to southern regions because of its low tolerance to warm temperatures (POLI, 2004). Thus, additional research on mangrove oysters, *Crassostrea rhizophorae* and *Crassostrea gasar*, is needed, especially for culture in tropical regions, in order to achieve optimum production.

Hatchery production is the most effective method for obtaining oyster seeds with minimal impact over natural populations (FERREIRA and NETO, 2007). Adults with ripe gametes are induced to spawn in hatcheries by thermal shock plus air exposure treatment (SILVEIRA *et al*., 2011) or they are opened and the gametes stripped from the gonadal tissue (FAO, 2015). In both techniques, prior to every spawning event, gonadal tissue sampling is obtained from up to 40 breeding animals for assessing gamete quality. Sample numbers of broodstock can be much greater when gonadal development conditions are not ideal and when it is difficult to equalize sex ratios for subsequent fertilization.

Oyster tissue sampling, without sedation, often relies on lethal procedures, since the shell has to be removed from the meat to obtain samples. Avoiding oyster sacrifice is particularly valuable for research and commercial aquaculture, as it enables experiments to be conducted for better design of breeding strategies (SUQUET *et al*., 2009; 2010). Anaesthesia induction techniques have been employed in aquaculture to minimise handling stress, to reduce research-related mortalities (BUTT *et al*., 2008) and to facilitate tissue biopsies and gametogenesis studies (SUQUET *et al*., 2010).

Muscle relaxants benzocaine and propylene phenoxetol have been successfully used as anaesthetics during the surgical operative process in the pearl production of *Pinctada albina*, *Pinctada maxima*, *Pinctada margaritifera* and *Pinctada fucata* (NORTON *et al*., 1996; ACOSTA-SALMÓN *et al*., 2005; MAMANGKEY *et al*., 2009). However, these chemical relaxants have proven unsuitable for use in other species, such as the queen conch *Stombus gigas* (ACOSTA-SALMÓN and DAVIS, 2007) and the Sidney rock oyster *Saccostrea glomerata* (BUTT *et al*., 2008).

The use of salt magnesium chloride ($MgCl_2$) to anaesthesia induction has also been effectively applied in oyster production, for both research and commercial purposes. Magnesium chloride has proven to be a non-toxic, easily administered and efficient relaxant (ACOSTA-SALMÓN and DAVIS, 2007) and has been successfully used to induce anaesthesia on the Sydney rock oyster *S. glomerata* (BUTT *et al*., 2008), Pacific oyster *C. gigas* (SUQUET *et al*., 2009), European flat oyster, *Ostrea edulis* (SUQUET *et al*., 2010), and Chilean flat oyster, *Ostrea chilensis* (ALIPIA *et al*., 2014). Gonadal tissue sampling of anaesthetised oysters (*C. gigas* and *O. edulis*) also caused very low subsequent mortality using biopsy with needles for sex determination (SUQUET *et al*., 2009; 2010).

A reliable anaesthesia and tissue sampling protocol has been described for Pacific oyster *C. gigas*, but adapted tools are still required for Brazilian mangrove oysters *C. rhizophorae* and *C. gasar* to improve broodstock management. Also, there is a need to investigate the effects of anaesthesia induction on the successful spawning and D-larvae generation for the three above cited species. For native species, the development of a non-destructive method would enable successive samplings of the same individuals, and therefore would allow descriptive studies such as those on gametogenesis for future selective breeding programs. Addressing these needs, this study evaluated the time required for induction and recovery in *C. rhizophorae, C. gasar*

and *C. gigas* (control species) using $MgCl_2$, the oyster survival after anaesthesia and gonad sampling, and the spawning and D-larvae generation after anaesthesia.

MATERIAL AND METHODS

This study was performed at Laboratory of Marine Molluscs of Federal University of Santa Catarina (LMM-UFSC), located at Barra da Lagoa, Florianópolis, Brazil. Adult animals of *C. rhizophorae* (48.6 ± 7.1 mm height; 20.0 ± 5.0 g weight) *C. gasar* (70.7 ± 22.9 mm height; 46.3 ± 5.0 g weight) and *C. gigas* (114.0 ± 16.4 mm height; 167.7 ± 41.0 g weight) were collected in the experimental culture area of LMM, located at Sambaqui beach (27º35'S and 48º32'W), Florianópolis, and transported to the hatchery of the LMM. The animals of each species were placed separately in conditioning tanks (500 L), and maintained in a continuous flow water system, at constant aeration, temperature (23 ºC) and salinity (36) for subsequent anaesthesia experiments. During the maintenance period, the animals were fed with *Chaetoceros muelleri* microalgae at final concentration of 20 to 40 x 10^4 cell mL^{-1}, in continuous system.

Two anaesthesia experiments were performed for this study. Experiment I, developed with *C. rhizophorae*, *C. gasar* and *C. gigas*, evaluated the time for anaesthesia induction and recovery, and the survival of anaesthetised and sampled (gonadic tissue) animals. Experiment II was developed only with *C. gigas*, and assessed the effects of anaesthesia on spawning activity (D-larvae number). This experiment was not performed with *C. gasar* and *C. rhizophorae* because there were no females and males available for these species, respectively, after anaesthesia and sex determination.

Experiment I

The oysters of each species (n = 40) were randomly taken from the 500 L tanks and placed in 30 L tanks under fasted condition for 12 h (overnight) in filtered seawater with constant aeration, salinity (36) and temperature (22 °C) before the experimental induction. The height and fresh weight of each oyster were registered.

After this period, three replicate groups of 10 animals of each species were anaesthetised in 10 L containers, using 50 g L^{-1} (525 mM) magnesium chloride ($MgCl_2$) and water, according to the protocol established for *C. gigas* in SUQUET *et al*. (2009). Dilution medium was 6 L of freshwater and 4 L of seawater in order to avoid salinity increase by $MgCl_2$ concentration. A control group of 10 animals by species were placed separately in 10 L containers without $MgCl_2$. Aeration remained constant during the experimental period.

This induction experiment (I) was completed in 6 h with counting every 1 h (60, 120, 180, 240, 300, 360 min) to evaluate the state of anaesthesia. Salinity and temperature were also monitored during the anaesthesia induction. Oysters that were not anaesthetised 100% after 360 min were kept more 360 min in the induction tank for final evaluation. Oysters were considered anaesthetised when the shell valves were opened and no closure was observed after successive pressures.

The anaesthetised oysters were separated in two groups. One group (R) was transferred directly to the recovery tanks, separated by species [*C. rhizophorae* (Rrh; n = 15); *C. gasar* (Rga; n = 16); and *C. gigas* (Rgi; n = 10)]. The other group (S) was sampled for sex determination by species [*C. rhizophorae* (ASrh; n = 15); *C. gasar* (ASga; n = 14); and *C. gigas* (ASgi; n = 20)] and then transferred to the recovery tanks.

Gonadal tissue sampling was performed on the left valve of the anaesthetised oysters, above the adductor muscle, using a 1 mL syringe with a needle of 0.60 x 25 mm, 23 gauge (SUQUET *et al*., 2009). Tissue samples (approximately 0.05 mL) were placed in glass laminas and the sex was determined using a binocular light microscope (with 40x and 100x of amplification).

The anaesthesia recovery was performed in 10 L tanks containing clean seawater with constant aeration, salinity (36) and temperature (22 °C). The recovering status of the animals was monitored every 30 min. Oysters were considered recovered when they regained the ability to close their valves autonomously.

After recovering, the induced oysters and the control groups returned to the conditioning tanks and were monitored for survival during 10 days. Survival was compared within species,

between three different groups: 1) anaesthetised and sampled oysters (group S); 2) anaesthetised but non-sampled oysters (group R); and 3) non-anaesthetised and non-sampled oysters (control group).

Experiment II

To evaluate the effect of anaesthesia on the spawning activity of oyster *C. gigas*, 55 specimens were randomly taken from 500 L conditioning tanks and pooled into two different groups: a control group of non-anaesthetised oysters (n = 25) and another group exposed to the anaesthetic treatment (n = 30). Anaesthesia and gonad sampling procedures were the same as described above. The anaesthesia time was fixed at 180 min.

After sex determination, two sets of four mature females and two sets of four mature males were selected, each from anaesthetised oysters (A) and control group (C). Females were evaluated under light microscopy (40x), based on the number, shape and size of oocytes in the evaluation sample. Selected females presented higher numbers of bigger round shaped oocytes. Males were evaluated under light microscopy (100x), based on the number and motility of spermatozoids. Selected males presented higher numbers of mobile spermatozoids.

The spawning was performed through animal sacrificing and stripping of gonadic tissues. After stripping of each female set (A and C), oocytes were hydrated in 10 L containers. After 1 h of hydration, three 0.2 mL samples from each oocyte solution were diluted in three 5 mL beakers containing seawater and then three 0.2 mL samples were taken and placed in Sedgewick-Rafter chambers for oocyte quantification. Each oocyte solution was divided into six equal parts, containing 150 oocytes mL^{-1}. The fertilization occurred every 20 min for 1 h by adding 30 mL of sperm (A or C). Thus, 2 x 2 factorial crosses were created within and between anaesthetised (A) and non-anaesthetised oysters (C), producing four groups in triplicates: females A x males A; females C x males C; females C x males A; and females A x males C.

After 24 h of fertilization, three samples of 0.5 mL from each replicate within each cross were taken and placed in Sedgewick-Rafter chambers for D-larvae quantification.

Statistical analysis

The induction and recovery time, oyster survival after 10 days of anaesthesia, oocytes number and D-larvae number were analysed using one way ANOVA and Tukey test in SAS program. The effects of weight and sex on the anaesthesia and recovery time were analysed using Pearson Correlation and student *t*-test, respectively.

RESULTS

Experiment I

After 120 min of exposure to $MgCl_2$, 96.7 ± 4.7% oysters *C. rhizophorae*, 93.3 ± 4.7% *C. gigas* and 83.3 ± 17.0% *C. gasar* were anaesthetised. After 360 min, 100% oysters *C. rhizophorae* and *C. gigas* were anaesthetised, but only 86.7 ± 21.7% *C. gasar* (Figure 1). Even after more 360 min (total induction time: 720 min of induction), *C. gasar* did not respond, as expected and 13.3 ± 12.5% oysters remained non-anaesthetised. Salinity (36) and temperature (22 °C) remained constant during the experimental induction period.

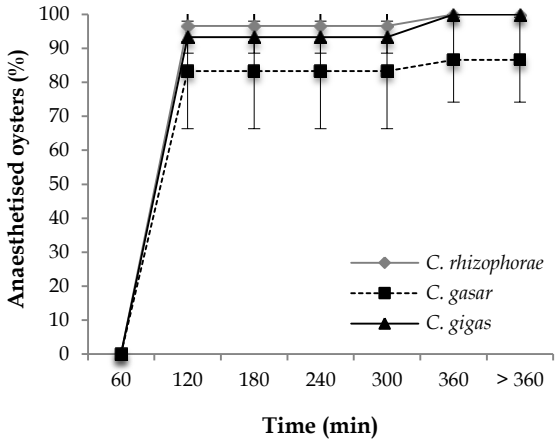

Figure 1. Cumulative percentages of anaesthetised oysters *Crassostrea rhizophorae, Crassostrea gasar* and *Crassostrea gigas* after exposure to $MgCl_2$. Times shown indicated the duration of exposure to $MgCl_2$ before induction of relaxation. Percentages were calculated in 60 min increments up to 6 h after initial exposure. Bars show standard deviation.

The time required for the anaesthetised animals to recover (Figure 2) was higher in *C. rhizophorae* (240 min) and in *C. gasar* (>240 min), and statistically different (P<0.0001) compared to *C. gigas* (150 min). After 60 min, 96.7 ± 4.7% oysters *C. gigas* spontaneously closed their valves in comparison to only 7.0 ± 5.0% *C. gasar* and 0% *C. rhizophorae*. After 150 min, when 100 % oysters *C. gigas* had recovered, 38% *C. gasar* and nearly 13% *C. rhizophorae* were still anaesthetised.

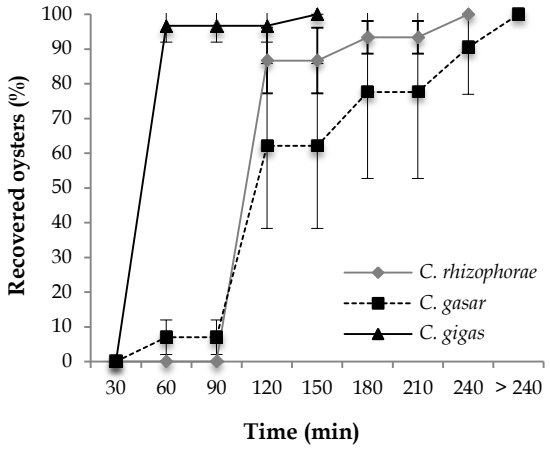

Figure 2. Cumulative percentages of recovered oysters *Crassostrea rhizophorae, Crassostrea gasar* and *Crassostrea gigas* after exposure to $MgCl_2$. Percentages were calculated in 30 min increments up to 4 h after initial recovery. Bars show standard deviation.

The shell height and fresh weight had no influence on the anaesthesia efficiency (induction and recovering) for the three studied species. Minimum and maximum values of fresh weight and height for *C. rhizophorae*, *C. gasar* and *C. gigas* are presented in Table 1. Both smaller and bigger animals required the same amount of time to anaesthetise and recover.

After 10 days of induction, no mortality was observed for the sampled group (S), non-sampled group (R) and control group in the three studied species.

Procedures for gonad sampling used in this study were able to identify female, male and indeterminate animals in the three studied oyster species (Table 2). No statistical difference was observed in relation to the sex ratio and anaesthesia induction time for the sampled animals (group S) of each species. Only one male of *C. gigas* was not anaesthetised after 120 min of induction. In relation to the recovery time after anaesthesia induction and gonad sampling, females of *C. gigas* (Figure 3C) and of *C. rhizophorae* (Figure 3B) required more time to recover than males. However, sample sizes of female *C. gigas* and male *C. rhizophorae* were too small to show statistical differences at significant level. For *C. gasar*, no sex differentiation pattern was shown during recovery time (Figure 3A).

Table 1. Maximum and minimum values of height and fresh weight of anaesthetised oysters for the three studied species (*Crassostrea rhizophorae, Crassostrea gasar* and *Crassostrea gigas)*.

Species	Height (mm)		Fresh weight (g)	
	Minimum	Maximum	Minimum	Maximum
Crassostrea rhizophorae	33.00	66.00	10.36	33.32
Crassostrea gasar	45.00	132.00	35.36	55.05
Crassostrea gigas	93.00	155.00	106.58	299.92

Table 2. Males (M), females (F) and indeterminate (I) animals identified from the gonad sampled anaesthetised oysters in the experiment I.

Species	M (%)	F (%)	I (%)
Crassostrea rhizophorae	13	67	20
Crassostrea gasar	57	07	36
Crassostrea gigas	35	10	55

Experiment II

After 180 min of exposure to $MgCl_2$, 100% oysters *C. gigas* were anaesthetised. The evaluation of gonad samples identified 28% males, 28% females and 44% indeterminate individuals within the control group (C). In the anaesthetised group (A), 33% males, 40% females and 27% indeterminate animals were identified.

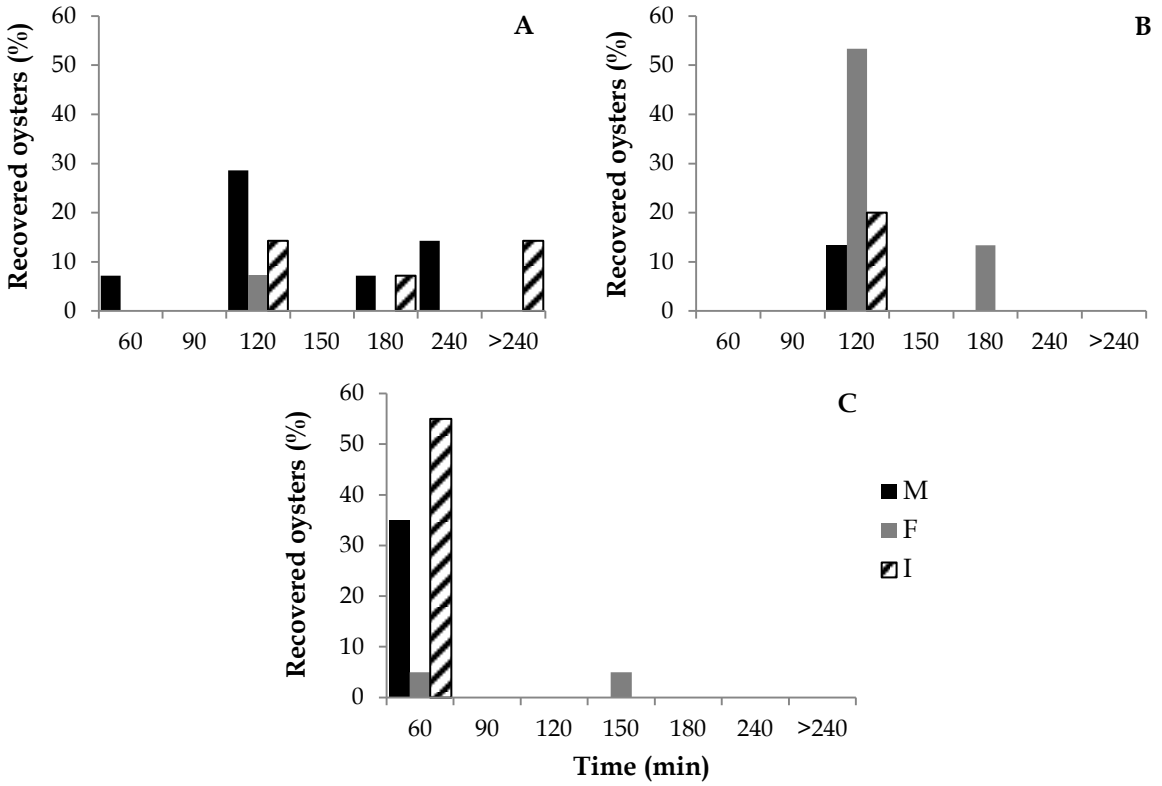

Figure 3. Percentages of females (F), males (M) and indeterminate (I) oysters recovered after exposure to $MgCl_2$ and gonad sampling in the three studied species: *Crassostrea gasar* (A); *Crassostrea rhizophorae* (B); *Crassostrea gigas* (C). Percentages were calculated in 30 min increments up to 4 h after initial recovery.

The sperm motility analysis showed 80 and 100% mobile spermatozoids on the anaesthetised and non-anaesthetised males, respectively. From the total number of females, 43% of the group C and 33% of the group A presented higher numbers of round shaped oocytes and were used in the spawning event.

Total numbers of oocytes from anaesthetised (A) and non-anaesthetised (C) females were 37.5 x 10^6 and 40.0 x 10^6, respectively. There were no significant differences in D-larvae numbers between crosses (Table 3). Thus, exposure to $MgCL_2$ had no deleterious effect on the spawning activity and on the D-larvae generation of *C. gigas*.

Table 3. Average number and survival (%) of D-larvae from factorial crosses within and between anaesthetised (A) and non-anesthetised (C) oysters *Crassostrea gigas*. Oocyte number per cross was fixed in 150 mL^{-1}.

Cross		Average number of D-larvae (mL^{-1})	Survival of D-larvae (%)
Females	**Males**		
A	A	47 ± 3	32 ± 2
A	C	42 ± 7	28 ± 4
C	A	55 ± 3	37 ± 2
C	C	53 ± 6	36 ± 4

DISCUSSION

The salt $MgCl_2$ proved to be an effective muscle relaxant for the three studied species of *Crassostrea* genus. After the anaesthesia period, 100% recovery and 100% survival were observed for each oyster species. Our results were similar to

the very low mortalities observed using this compound in other species of gastropods (ACOSTA-SALMÓN and DAVIS, 2007) and bivalves (BUTT *et al*., 2008; SUQUET *et al*., 2009; 2010; ALIPIA *et al*., 2014).

Among authors, there is considerable variation between mollusc species in their reaction to particular relaxants and varying concentrations (for review, see LEWBART and MOSLEY, 2012). In the present study, 87% oysters *C. gasar* were anaesthetized after 12 h of exposure to 50 g L^{-1} $MgCl_2$ and 100% *C. gigas* and *C. rhizophorae* after 6 h of induction. Using the same relaxant and the same concentration, BUTT *et al*. (2008) showed similar results of induction time (6 h) for Sidney rock oysters (*S. glomerata*), but SUQUET *et al.* (2009) took 16 h to achieve 100% anaesthesia in *C. gigas*. For flat oysters *O. edulis* (SUQUET *et al*., 2010) and *O. chilensis* (ALIPIA *et al*., 2014), complete anaesthesia was obtained after 3 h using 50-72 g L^{-1} and 30 g L^{-1} $MgCl_2$, respectively. No visible effect of relaxation was shown in the pearl oyster *P. albina* (NORTON *et al*., 1996) after 1 h of induction using 30 g L^{-1} $MgCl_2$, or in the American oyster *Crassostrea virginica* (YANG *et al.,* 2013) after 36 h of treatment with 5% Dead Sea salt (containing 33.3% $MgCl_2$).

It is important to highlight that prolonged exposure to the anaesthetic can be lethal (WOODALL *et al*., 2003) for molluscs. These authors recommend short-term anaesthetic treatment, to ensure restoration of synaptic connections between injured neurons. In the pearl production, after rapid relaxation (for 10 min or more) and recovery (less than 30 min), using benzocaine and propylene phenoxetol, no mortality was observed for *P. albina*, *P. margaritifera*, *P. fucata* and *P. maxima* (NORTON *et al*., 1996; ACOSTA-SALMÓN *et al*., 2005; MAMANGKEY *et al*., 2009). Nevertheless, in this study, the increase in exposure time with $MgCl_2$ (12 h) did not caused subsequent oyster mortality for *C. gasar*. Longer induction times and repeated monthly anaesthesia of the same individuals also did not affect subsequent mortality for *C. gigas* (SUQUET *et al*., 2009). BUTT *et al*. (2008) concluded that, although immunological perturbations were evident during first 48 h, the physiological stresses associated with exposure to this relaxant did not have long-term (after 96 h) impacts on immunological parameters of Sidney rock oysters (*S. glomerata*).

The inhibitory effect of the Mg^{2+} from the magnesium chloride on the Ca^{2+} synaptic transmission (KANDEL *et al*., 2000) may not be so efficient in *C. gasar*, as observed for *C. rhizophorae* and *C. gigas*. This difference between species can be related to the physical (e.g. temperature and salinity) and chemical (e.g. pH) parameters of seawater during exposure to $MgCl_2$. SUQUET *et al*. (2009) demonstrated that reducing water temperature from 19.5 °C to 15.3 °C resulted in a significant decrease in anaesthesia efficacy in *C. gigas*. In the present study, relaxation in *C. gigas* was more effective at 22 °C temperature compared to the above results. In the flat oyster *O. edulis*, also increasing water temperature from 14.9 °C to 18.8 °C significantly increased the number of oyster anaesthetised after 3 h (SUQUET *et al*., 2010). ACOSTA-SALMÓN and DAVIS (2007) observed that, although $MgCl_2$ increased the seawater salinity (from 32 to 47) during anaesthetic induction, the short duration of exposure (20 min) was not enough to cause adverse effects in the gastropod *S. gigas* at 27.8 °C. ALIPIA *et al.* (2014) suggested that variations in anaesthesia time over different relaxant concentrations in the flat oyster *O. chilensis* may result more from osmotic shock than any neuromuscular action of $MgCl_2$. Considering that salinity was maintained constant during experimental induction in this study, the lower percentage of anaesthetised oyster *C. gasar* can be related to its ability to maintain valves closed for long periods. This species behaviour was commonly observed under handling in label routine at LMM-UFSC.

For the studied species (*C. rhizophorae*, *C. gasar* and *C. gigas*), oyster height (45 to 132 mm) and fresh weight (35.36 to 55.05 g) had no effect on the anaesthesia induction and recovery times. These results agree with NORTON *et al*. (1996) that observed no association of the anaesthetic and the animal size for *P. albina* (dorsal-ventral size: 70 to 100 mm) and *P. margaritifera* (dorsal-ventral size: 90 to 170 mm). On the other side, SUQUET *et al.* (2009) showed that lower weight significantly increased the number of anaesthetized oysters *C. gigas*. ZAHL *et al*. (2010) also observed that the size of fish *Hippoglossus hippoglossus* affected the anaesthesia induction and recovery times. In

animals with body weights of 33 to 1243 g, larger fish had longer induction times and shorter recovery times.

Gonad sampling of anaesthetised oysters did not affect survival after 10 days of exposure to $MgCl_2$ for the three studied species (*C. rhizophorae*, *C. gasar* and *C. gigas*). Similar results on the survival of sampled anaesthetised oysters were observed for *C. gigas* (SUQUET *et al*., 2009) and *O. edulis* (SUQUET *et al*., 2010). In the present study, tissue sampling also allowed sex determination in mature individuals within each species and could be used for future research on gonad maturation and spawning induction of mangrove oyster species.

The native oyster culture is expanding in Brazil, and consequently the demand for seed supply is increasing. For seed production in hatcheries, anaesthesia can be a useful technique to sex determination without sacrificing animals. These aspects are important for equalizing numbers of mature females and males used in mass spawning, and for future selective breeding programs. The anaesthesia protocol developed for *C. gigas* by SUQUET *et al*. (2009) also proved to be efficient in the present study, for *C. rhizophorae*. However, other relaxants, concentrations, salinities, and temperatures need to be studied further to determine optimum conditions for *C. gasar*.

CONCLUSION

The salt $MgCl_2$ (50 g L^{-1}) anaesthetised 100% *C. rhizophorae* and *C. gigas* after 360 min, and 87% *C. gasar* after 720 min. Oysters *C. rhizophorae* and *C. gasar* take more time (≥ 240 min) to recovery compared to *C. gigas* (150 min). Anaesthesia and gonad sampling had no deleterious effect on oyster survival. The use of $MgCl_2$ did not affect fertilization and development of D-larvae *C. gigas.*

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