

# Growth, mortality and susceptibility of oyster *Crassostrea* spp. to *Perkinsus* spp. infection during on growing in northeast Brazil

Crescimento, mortalidade e susceptibilidade de ostras *Crassostrea* spp. à infecção por *Perkinsus* spp. em cultivo no Nordeste do Brasil

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## Abstract

*Crassostrea rhizophorae* and *C. gasar* oysters are cultivated in the northeast region. *Perkinsus* parasites infect bivalves, and their effects on oysters from tropical regions are poorly understood. This study evaluated the impact of *Perkinsus* infection on the productive traits of native oysters. Oysters were sampled bimonthly during 7 months, from July 2010 to February 2011, to evaluate growth rate, mortality and shell color patterns (white and dark-gray) (n = 500), and to determine the prevalence and intensity of *Perkinsus* (n = 152). *Perkinsus* and *Crassostrea* species were determined using molecular tools. Results showed that most dark-gray (90%, n = 20) and white (67%, n = 18) oysters were *C. gasar* and *C. rhizophorae*, respectively. Oysters showed a high growth rate and moderate cumulative mortality (44%). *C. gasar* oysters grew better and showed lower mortality and lower incidence of *Perkinsus* compared to *C. rhizophorae*. The mean prevalence of *Perkinsus* was moderate (48%), but the infection intensity was light (2.2). Perkinsiosis affected very small oysters (19.4 mm). In conclusion, native oysters, especially *C. gasar*, have a great potential for culture, mortality is not associated with perkinsiosis, and the shell color of oysters can be used to improve selection for spats with better performance.

**Keywords:** Oyster productive traits, *Perkinsus marinus*, *Perkinsus beihaiensis*, PCR-RFLP, ITS rDNA, 16S mtDNA.

## Resumo

*Crassostrea rhizophorae* e *C. gasar* são cultivadas na região Nordeste. Parasitas *Perkinsus* infectam bivalves e seus efeitos em ostras de regiões tropicais são pouco compreendidos. Este estudo avaliou o impacto da infecção por *Perkinsus* em parâmetros de produção de ostras nativas. Ostras foram coletadas bimestralmente durante 7 meses, de julho de 2010 a fevereiro de 2011, para avaliar crescimento, mortalidade e padrão de coloração da concha (branca e cinza-escura) (n = 500); além da presença e intensidade de *Perkinsus* (n = 152). *Perkinsus* e *Crassostrea* foram identificados por abordagem molecular. Os resultados mostraram que as ostras cinza-escuras (90%, n = 20) e brancas (67%, n = 18) eram *C. gasar* e *C. rhizophorae*, respectivamente. As ostras mostraram uma boa taxa de crescimento e mortalidade acumulada moderada (44%). *C. gasar* cresceu melhor com menor mortalidade e menor incidência de *Perkinsus* que *C. rhizophorae*. A prevalência média de *Perkinsus* foi moderada (48%), mas a intensidade de infecção foi leve (2,2). A perkinsiose afetou ostras pequenas (19,4 mm). Em conclusão, ostras nativas, especialmente *C. gasar*, têm grande potencial de produção; sem mortalidade associada à perkinsiose; e, a cor da concha pode ser usada para melhorar a seleção de sementes com melhor desempenho.

**Palavras-chave:** Parâmetros de produção de ostras, *Perkinsus marinus*, *Perkinsus beihaiensis*, PCR-RFLP, ITS rDNA, 16S mtDNA.

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## Introduction

Two mangrove oyster species, *Crassostrea rhizophorae* and *C. gasar*, inhabit the estuaries of the northeast region of Brazil. Additionally, they have wide geographical distribution, with *C. rhizophorae* inhabiting from the southern Caribbean to Uruguay and *C. gasar* from South America to Africa (LAPÈGUE et al., 2002).

Mangrove oysters are produced in the northeast region of Brazil using artisanal techniques, which include the sampling of naturally settled spats at different places in an estuary (bottom substrate, roots of mangrove tree, and culture structures) and hand-managing oysters for all culture stages. Estimates of the oyster production in northeastern Brazil are scarce and underestimated. In contrast, the state of Santa Catarina (SC, South Brazil) is the biggest national producer of bivalves (17,000 tons of *Perna perna* mussels and 3,000 tons of *C. gigas*) (SANTOS & COSTA, 2015). The production of the exotic Japanese oyster *C. gigas* (POLI, 2004) depends on hatchery-reared spats and has a somewhat mechanized system. Since 1990, spats are annually produced by the Laboratory of Marine Mollusks at the Federal University of Santa Catarina and are sold to local farmers. In contrast, recently (2014), a private enterprise implemented a hatchery facility in the northeast region to produce spats of *C. gasar* (DA SILVA et al., 2016). However, the supply of continuous spats is not certain because of the high costs of running these facilities and the need for financial support.

Genetic variability among native mangrove oyster populations is high (LAZOSKI et al., 2011), which is reflected by the wide variation in shell morphology and shell color. Additionally, due to their phenotypic plasticity, distinguishing between the two sympatric oyster species by morphological characteristics becomes very difficult. Therefore, Rios (2009) considered mangrove oysters from Brazil to be the same species (*C. rhizophorae*). By contrast, Absher (1989) observed some differences in growth rates and larval morphology among sympatric lineages of *Crassostrea*, which supports the existence of two species, *C. rhizophorae* and *C. gasar*. Notwithstanding, observations by oyster farmers indicate two macroscopically distinguishable shell color patterns, white and dark-gray. They noticed potentially different performances of these oysters in culture as well. White oysters seemed to take a longer time to reach commercial size (pers. comm.). Since the shell color patterns of these oysters could represent different oyster species, choosing the correct species for production is desirable, especially considering the lack of hatchery-reared spats. Nevertheless, the performances of these two oyster species in culture have never been directly compared.

In Brazil, *C. gigas* production is already consolidated, while native oyster production is still in the exploratory phase. Recently, some studies were conducted in order to evaluate oyster maturation (RAMOS et al., 2014), larval settlement (SILVEIRA et al., 2011) and performance in culture (LOPES et al., 2013).

Perkinsosis is a disease caused by protozoan parasites from the *Perkinsus* genus, which includes two species, *Perkinsus marinus* and *P. olseni*, which are responsible for mortality in their hosts, consisting of *C. virginica* and *Ruditapes* spp. (VILLALBA et al., 2011). *Perkinsus* spp. were recently reported in Brazil, infecting

*C. rhizophorae* and *C. gasar* oysters and *Anomalocardia brasiliensis* clams from the northeastern region (DA SILVA et al., 2013; FERREIRA et al., 2015; LUZ & BOEHS, 2016; QUEIROGA et al., 2013, 2015; SABRY et al., 2009, 2013). *P. marinus* and *P. olseni* are both notifiable to the World Organisation for Animal Health (OIE) and were reported to infect *C. gasar* oysters from the estuary of the São Francisco River, Sergipe State (DA SILVA et al., 2014).

Little is known about the dynamics of *Perkinsus* spp. infection in tropical bivalve species under cultured conditions (DA SILVA et al., 2014, 2016; LUZ & BOEHS, 2016). No differences in *P. beihaiensis* prevalence was observed among cultured (61%) and wild (77%) *C. rhizophorae* oysters from Bahia state (LUZ & BOEHS, 2016), while prevalence varied in oysters (cultured 80% and wild 57%) from the Sergipe state, probably because in the latter region, there are two species of *Perkinsus* and *Crassostrea* oysters (DA SILVA et al., 2014).

For the oyster industry, it is imperative to know the impact of *Perkinsus* spp. on the productive traits of oysters, such as growth and mortality, during the complete cultured period. For the first time, Da Silva et al. (2016) evaluated the incidence of *Perkinsus* sp. in *C. gasar* oysters from spats reared in a hatchery facility to adult (commercial size) during growth in shrimp ponds. Mortality was not estimated. However, the effects of *Perkinsus* spp. on oysters cultured in Brazilian estuaries and marine environments were never analyzed.

The aim of the present study was to investigate oyster growth and mortality, identify the oyster species according to the shell color patterns and assess the impact of *Perkinsus* spp. on oysters during the entire outgrowth period.

## Materials and Methods

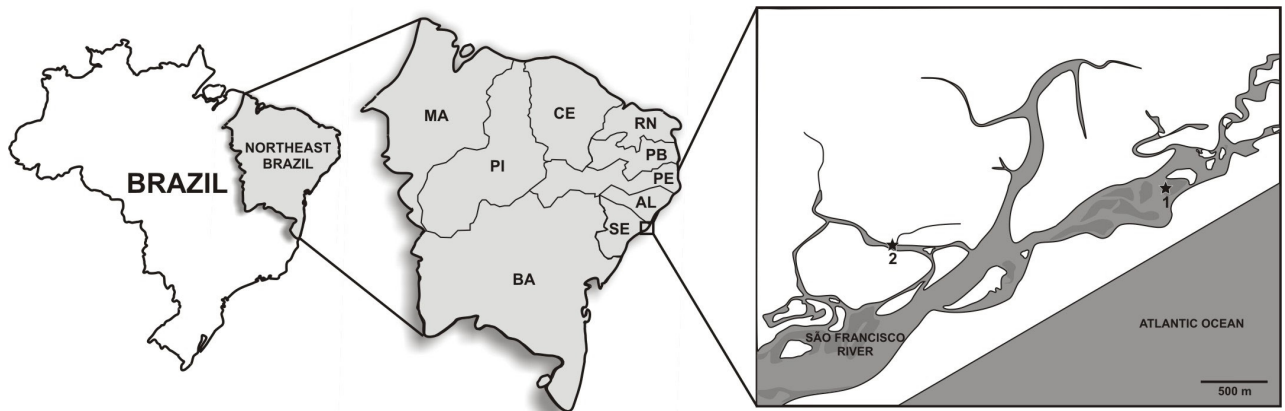
### Experimental design for oyster on growing

Oysters were cultured in a suspended - fixed system in the estuary of the River São Francisco (10° 32' 0.60" S, 36° 29' 36.24" W) in the Sergipe state of northeastern Brazil (Figure 1).

In July 2010, spats (n = 1,000) were obtained from a natural settlement on culture structures (ropes, bags, and stakes) and distributed into two nurseries bags (1 m<sup>2</sup>) with 500 oysters on each. Oyster spats (n = 50 per bag) were sampled for their initial biometry (shell height and whole weight), determination of their shell color (white or dark-gray) and *Perkinsus* spp. diagnosis by PCR (n = 12; Table 1).

Oysters were sampled bimonthly (September, October, and December 2010 and February 2011) in duplicates (n = 50) or quadruplicates (n = 25), totalizing 100 oysters per month to estimate the shell height, whole weight as well as the oyster shell color. For *Perkinsus* spp. diagnosis by Ray's fluid thioglycollate medium (RFTM) oysters were sampled in duplicates (n = 15) or quadruplicates (n = 10), totalizing 30 or 40 oysters per month.

The mortality rate was estimated as the proportion of dead oysters at each bimonthly sample, in duplicates (n = 50) or quadruplicates (n = 25), totalizing 100 oysters per month and the cumulative mortality was the sum of the mortality rates. The shell color of dead oysters was also recorded.



**Figure 1.** Map of Brazil showing the Northeastern region, the estuary of the São Francisco River and the sampling site (\*1) where oysters were cultivated.

**Table 1.** Number of oysters used for *Perkinsus* diagnosis and the identification of parasite and host species. Sequenced samples were the same used for PCR-RFLP.

Diagnosis	<i>Perkinsus</i>	<i>Crassostrea</i>
RFTM	138	-
PCR	12	-
PCR-RFLP	25	38
Sequencing	12	12

RFTM - Ray's fluid thioglycollate medium

### *Perkinsus* spp. diagnosis

Gills were excised from each oyster and divided into two fragments, which were (i) incubated in RFTM (RAY, 1966) and (ii) preserved in 96% ethanol for molecular diagnosis. After 7 days of incubation in RFTM at room temperature in darkness, the gills were chopped, stained with lugol and analyzed for the presence and intensity of *Perkinsus* hypnospores. Oysters were classified using the Mackin (1962) intensity scale adapted by Da Silva et al. (2013), including uninfected (level 0) or infected oysters (1- very lightly, 2- lightly, 3- moderately and 4-heavily).

The mean of the infection was estimated as the sum of the infection intensity levels (1-4) divided by the number of infected oysters at each sampling time (BUSH et al., 1997). The prevalence of *Perkinsus* sp. infections was estimated as the proportion of infected oysters at each sampling time.

### PCR assays

The oyster gill samples (50 - 80 mg) were submitted for DNA extraction with a DNAzol (Invitrogen, Brazil) reagent, according to the manufacturer's instructions (Table 1).

To identify the species of *Perkinsus*, the ITS regions of the *Perkinsus* spp. gene complexes were amplified from the isolated DNAs using the genus-specific primers PerkITS-85 and PerkITS-750 (CASAS et al., 2002). These primer pairs were used to amplify a specific region of the internal transcriber spacer (ITS) of the small subunit of ribosomal RNA (SSU rRNA) in the genomic DNA. DNA

samples from *in vitro* cell cultures of *P. olseni*, from *P. marinus*-infected oysters (GenBank JX144335) (DA SILVA et al., 2013) and from *P. beihaiensis*-infected oysters (GenBank FJ472346) (SABRY et al., 2009) were used as controls for the PCR and PCR-RFLP analyses.

To identify the species of *Crassostrea*, PCR reactions were performed as previously described (DA SILVA et al., 2013). The primer pairs (16SAR - 16SBR) were used to amplify a region of the mitochondrial DNA of the large subunit of ribosomal RNA (16S). DNA samples of *C. gasar*, *C. rhizophorae* and *C. gigas* oysters were used as controls for the PCR and PCR-RFLP analyses.

For both PCRs, the reactions were done in a total volume of 25  $\mu$ l, containing 1  $\mu$ l of genomic DNA (50 ng), buffer at 1x concentration, MgCl<sub>2</sub> at 1.5 mM, 0.2 mM nucleotides, 0.4  $\mu$ M of primers, and 0.04 U/ $\mu$ l of Taq DNA polymerase (Invitrogen, Brazil). PCR products were electrophoresed on 1.5% agarose gels, stained with ethidium bromide, and visualized by UV illumination.

### Restriction Fragment Length Polymorphism (RFLP) analysis

The freshly amplified ITS region of *Perkinsus* rDNA from DNA samples of 25 *Perkinsus*-infected oysters (Table 1) and control DNA samples (*P. olseni*, *P. marinus*, *P. beihaiensis*) were digested with the enzyme *Hinf*I (ABOLLO et al., 2006; QUEIROGA et al., 2015) following the manufacturer's protocols (Fermentas, Brazil). Similarly, PCR-RFLP analysis was performed to identify oyster species among 25 *Perkinsus* sp.-infected oysters and 13 uninfected individuals from all sampling dates and color patterns, including white (18) and dark-gray (20) (Table 1). The freshly amplified 16S mtDNA products were digested with the enzyme *Alu*I (Fermentas, Brazil) (DA SILVA et al., 2013). Restriction patterns were compared with those obtained from the control species (*Perkinsus* spp. and *Crassostrea* spp.). Ten microliters of digestion products were resolved on an 8% polyacrylamide gel and then stained with ethidium bromide.

### DNA sequencing and phylogenetic analyses

The freshly amplified ITS region of *Perkinsus* rDNA and *Crassostrea* 16S mtDNA from 12 DNA samples each (Table 1) were sequenced by ACTGene *Análises Moleculares Ltda.*

(Centro de Biotecnologia, UFRGS, Porto Alegre, RS, Brazil) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems). Samples were prepared following the protocol recommended by the manufacturer.

Sequences of rDNA-ITS of *Perkinsus* and 16S mtDNA of *Crassostrea* were aligned using ClustalW (THOMPSON et al., 1994) in BioEdit 5.0.9 (HALL, 1999). Parsimony bootstrap analyses were done with MEGA 7.0 (KUMAR et al., 2016) with 100 random additions of 1,000 replicates. Bootstrap support values above 50% (*Perkinsus*) and 70% (*Crassostrea*) were calculated. The ITS tree was rooted with the *P. qugwadi* ITS-region sequence (GenBank no. AF151528), and the 16S mtDNA tree was rooted with *Saccostrea glomerata* (GenBank no. AF458911) and *Ostrea edulis* (GenBank no. JF808187). The nucleotide sequences reported in this paper are available in GenBank under the accession numbers MF595808 – MF595812 (*P. beihaiensis*); MF595813 – MF595819 (*P. marinus*); MF663010 – MF663016 (*C. gasar*); MF663017 – MF663018 (*C. gigas*) and MF663019 – MF663025 (*C. rhizophorae*).

## Results

### Oyster growth and mortality

The oysters were cultivated for 7 months. The initial mean length and weight of the spats were  $28.7 \pm 1.06$  mm and  $4.1 \pm 0.44$  g, respectively. Oysters grew from July to October; although they continued to grow in length, their weight decreased from October ( $73.8 \pm 4.92$  g) to December ( $34.8 \pm 1.55$  g). At the end of the growing period, the oysters reached a mean length of  $69.0 \pm 1.07$  mm and weight of  $50.0 \pm 1.68$  g (Figure 2). This growth corresponded to an average monthly growth of 5.8 mm and 6.6 g. Regarding the oysters with different color patterns, the dark-gray oysters grew significantly ( $P < 0.05$ ) larger ( $70.0 \pm 1.17$  mm and  $51.9 \pm 1.76$  g) than the white oysters ( $64.2 \pm 2.30$  mm and  $43.1 \pm 4.16$  g).

The highest monthly mortality rate was in September, two months following deployment. The cumulative mortality at the end of the study was 43.6%. The white oysters showed a higher cumulative mortality (52.4%) than the dark-gray oysters (34.8%) (Figure 3).

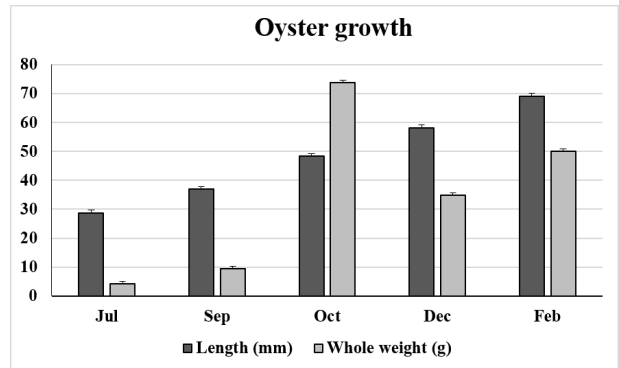
### Incidence of *Perkinsus* spp. during on growing

The mean prevalence of *Perkinsus* spp. was moderate (47.8%) during the growing period, except in October when it increased to 72.4% (Figure 4). The mean intensity of the *Perkinsus* sp. was 2.2, which corresponded to a light intensity over a 4-point scale. The prevalence of *Perkinsus* spp. infecting oysters (spats), in July, which was estimated by PCR, was 50% (Figure 4). The smallest infected oyster was 19.4 mm.

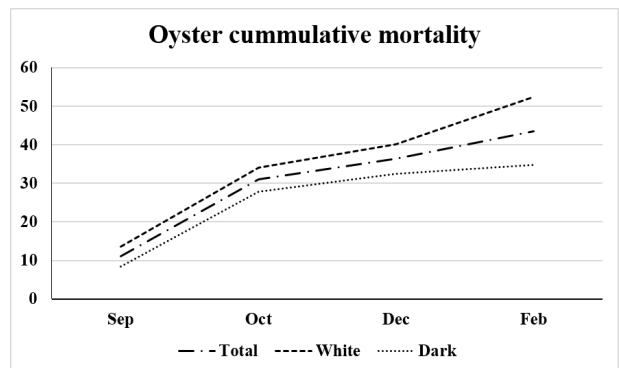
The incidence of *Perkinsus* spp. in oysters according to shell color was determined. The white oysters were more affected by perkinsosis (19 from 34, 55.9%) than dark-gray oysters (50 from 116, 43.1%).

### Identification of *Crassostrea* oysters by PCR-RFLP

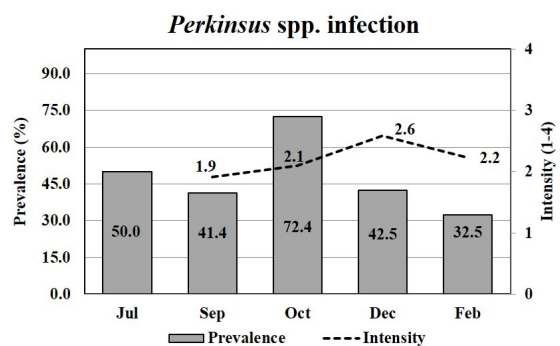
Thirty-eight oysters with distinct shell color patterns (18 white and 20 dark-gray) were collected throughout the study period and analyzed by PCR-RFLP in order to identify the oyster species (Table 1).



**Figure 2.** Mean length (mm) and whole weight (g) of oysters. Oysters were sampled in duplicates (n = 50) or quadruplicates (n = 25), totaling 100 oysters per month.



**Figure 3.** Total cumulative mortality of the oysters (%) and by oyster shell color (white and dark-gray). Mortality was estimated in duplicates (n = 50) or quadruplicates (n = 25), totaling 100 oysters per month.



**Figure 4.** The prevalence of *Perkinsus* spp. (bar) and mean infection intensities (line) in *Crassostrea* spp. oysters through oyster growth. Oysters were analyzed by RFTM in duplicates (15) or quadruplicates (10), totaling 30 or 40 oysters per month; except in July, when oysters (n = 12) were analyzed by PCRs.



Two distinct fragmentation patterns of the 16S mtDNA using the endonuclease *AluI* were obtained (Table 2). In 24 oysters, the digestion produced one large fragment of ~250 bp and a group of unresolved fragments smaller than 100 bp, corresponding to *C. gasar*. Of these, 18 were dark-gray oysters (90%) (Table 2). In 14 oysters, the restrictive pattern showed one large fragment with slightly more than 400 bp and a group of unresolved fragments of less than 100 bp, corresponding to *C. rhizophorae*. Of these, 12 were white oysters (66.7%) (Table 2).

### Susceptibility of *Crassostrea* spp. to *Perkinsus* spp.

Twenty-five oysters collected throughout the study period were analyzed by PCR-RFLP in order to identify the parasite and the host species in the same sample (Table 1).

Two distinct rDNA ITS fragmentation patterns were obtained with *HinfI*, which corresponded to *P. marinus* and *P. beihaiensis* (Table 3). Eleven samples showed the *P. marinus* pattern, which was four fragments of approximately 365, 150, 98 and 56 bp. Seven samples showed the *P. beihaiensis* pattern, which was a group of four fragments with the sizes ranging from 100 to 200 bp (quadruplet) and one smaller than ~10 bp. Seven oysters shared both patterns, indicating dual-infection (Table 3). *P. marinus* infected more *C. rhizophorae*, while *P. beihaiensis* infected more *C. gasar* oysters (Table 3).

The intensity of infection according to the *Perkinsus* spp. was also determined. The results indicated that *P. marinus* seems to

have a greater capacity to proliferate and reach heavier infection intensities (56%) in the host than *P. beihaiensis* (13.9%) (Table 4).

The smallest white and dark-gray infected oysters had shell heights of 19.4 mm and 25.6 mm, respectively.

### Phylogenetic analysis

The results of the 16S mtDNA phylogenetic analyses of the 12 *Crassostrea* spp. and the ITS sequences of the 12 *Perkinsus* spp. obtained during this study were 100% coincident with the species identifications from the PCR-RFLP analyses, since the sequences were grouped into monophyletic clades. *C. rhizophorae* and *C. gasar* had bootstrap support values of 96% and 99%, respectively (Figure 5) and *P. marinus* and *P. beihaiensis* had bootstrap support values of 99% and 90%, respectively (Figure 6).

## Discussion

The present study evaluated, for the first time, the impact of perkinsosis on the productive traits of *Crassostrea* spp. oysters cultured in Brazil as well as the susceptibility of two native oyster species, *C. rhizophorae* and *C. gasar* to *P. beihaiensis* and *P. marinus* infection.

Comparison of the productive traits among the native oyster population cultivated in Brazil is a challenge because of the wide differences in experimental conditions employed, including the spat origins (mangrove or hatchery), starting spat size, time of culture, culture methods, and geographical location. Oysters from the present study showed a similar growth rate (5.8 mm and 6.6 g per month) compared to *C. gasar* oysters grown in shrimp ponds in the neighboring state of Rio Grande do Norte (5.2 mm per month) (DA SILVA et al., 2016). Moreover, the growth rate was better than oysters growing in shrimp ponds in the Sergipe state (2.37 mm per month) (ROSA, 2014) as well as in the marine and estuarine environments of South Brazil using spat produced in a hatchery facility (LOPES et al., 2013; PEREIRA et al., 2001). The latter authors reported oyster growth rates of 2.6 mm, 3.1 mm and 3.9 g per month.

A decrease of the mean whole weight observed in December and February compared with October could be related to spawning events, which caused an important decrease in body weight, as already described in *C. rhizophorae* from Brazil (NASCIMENTO & PEREIRA, 1980; REBELO et al., 2005). Cultured *C. gasar* oysters became mature and present several spawning peaks during most parts of the year (PAIXÃO et al., 2013; RAMOS et al., 2014). It is important to consider that the oyster growth data presented here represents the mean of two different oyster species, which were likely pulled down by the species with the worst performance. Indeed, white oysters grew worse than dark-gray oysters.

The mean cumulative mortality observed was moderately high (43.6%) for native *Crassostrea* spp. oysters produced in Brazil. Studies developed in the south of Brazil (Santa Catarina State) showed low mortality rates (7.5%) when using culture hatchery-reared *Crassostrea* spp. spat (MACCACCHERO et al., 2007) and cumulative mortalities ranged from 10 to 36% when using 50 mm mangrove-obtained spat (PEREIRA et al., 2001).

**Table 2.** Identification of oyster species by PCR-RFLP in relation to shell color patterns.

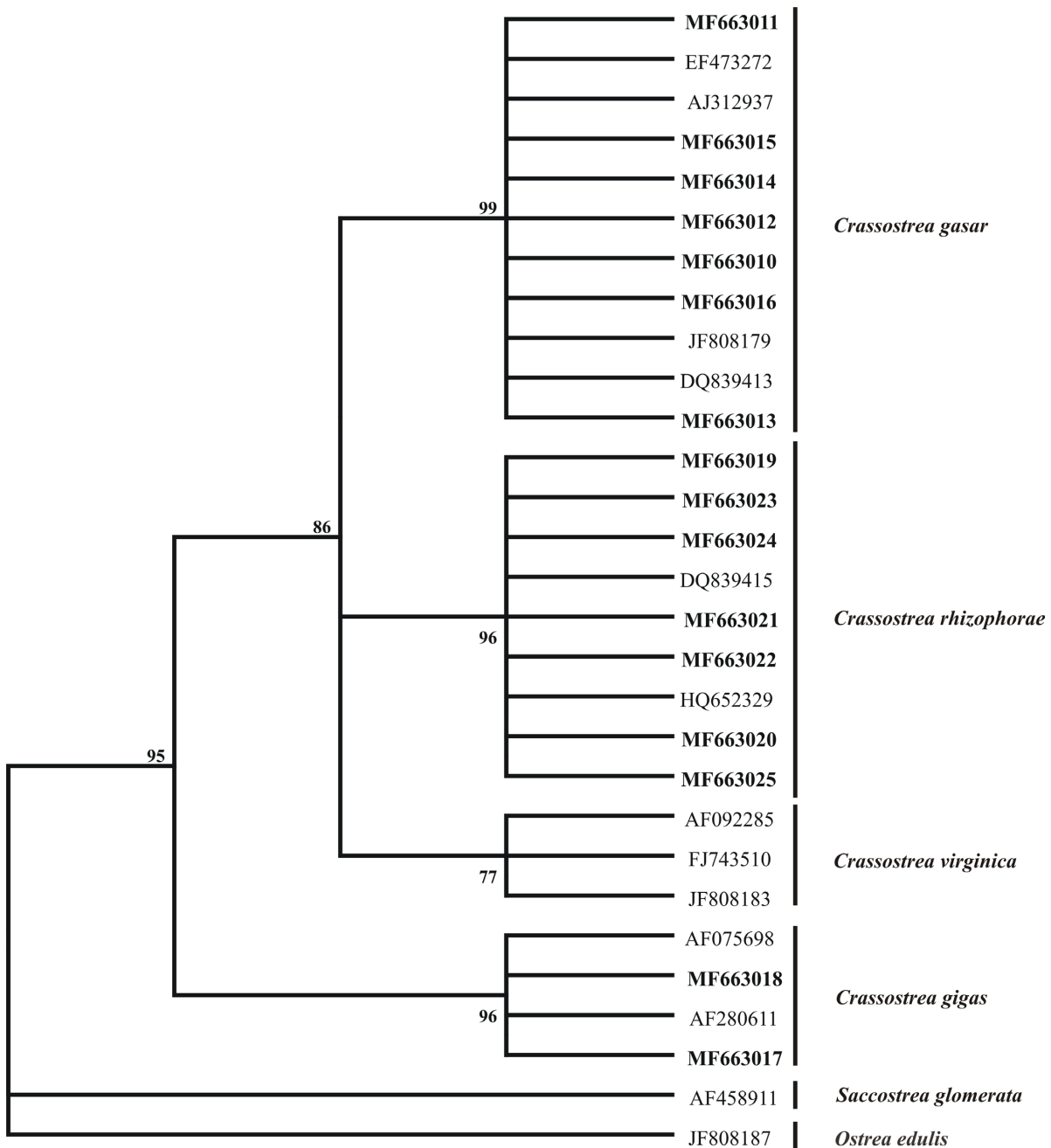
Shell color pattern	Oyster species		N
	<i>C. rhizophorae</i>	<i>C. gasar</i>	
White	12 (66.7%)	6 (33.3%)	18
Dark-gray	2 (10%)	18 (90%)	20
Total	14	24	38

**Table 3.** Identification of parasite species by PCR-RFLP in relation to the oyster species. Percentages were calculated considering the rows (*Perkinsus* spp.) or (*I*) columns (*Crassostrea* spp.).

<i>Perkinsus</i> spp.	Oyster species		N
	<i>C. rhizophorae</i>	<i>C. gasar</i>	
<i>P. marinus</i>	7 (64/58%)	4 (36/31%)	11
<i>P. beihaiensis</i>	2 (29/17%)	5 (71/38%)	7
Dual infection	3 (43/25%)	4 (57/31%)	7
Total	12	13	25

**Table 4.** Identification of parasite species by PCR-RFLP in relation with the parasite burden on the host.

<i>Perkinsus</i> spp.	Intensity of <i>Perkinsus</i> spp. infection				N
	Very light	Light	Moderate	Heavy	
<i>P. marinus</i>	2 (22%)	0	2 (22%)	5 (56%)	9
<i>P. beihaiensis</i>	2 (28.7%)	2 (28.7%)	2 (28.7%)	1 (13.9%)	7
Dual-infection	0	2 (67%)	0	1 (33%)	3
Total					19

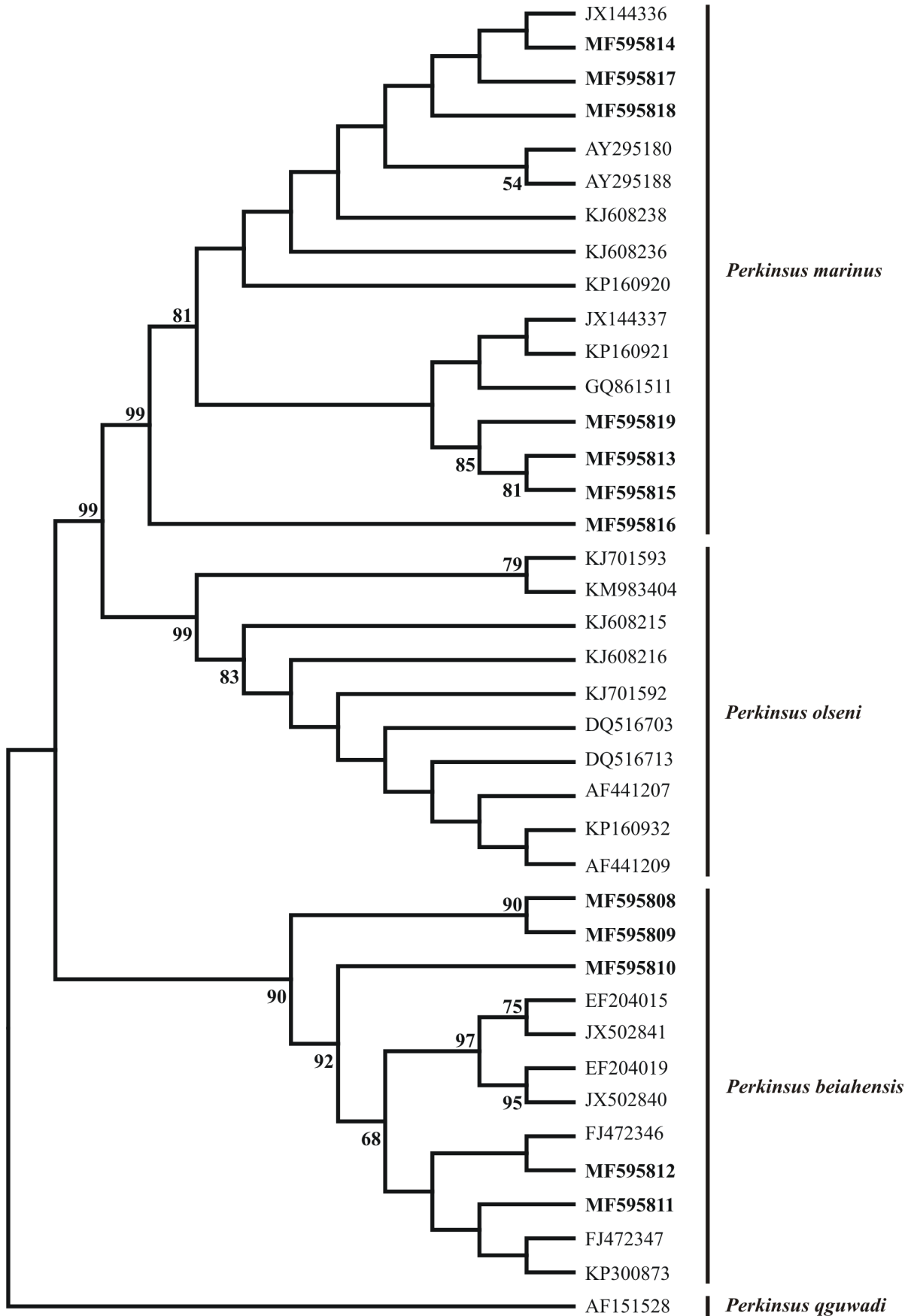


**Figure 5.** Phylogram showing the results of the maximum parsimony analyses on *Crassostrea* spp. 16S mtDNA sequences. Bootstrap support values are indicated at the nodes. Sequences from this study are indicated by GenBank accession number (in bold). Sequences MF663016 (*C. gasar*), MF663025 (*C. rhizophorae*) and MF663017 - MF663018 (*C. gigas* from Santa Catarina State) were the controls used for PCR-RFLP.

Notably, in the present study, white oysters showed higher cumulative mortality (52.4%) than dark-gray oysters (34.8%) under the same culture conditions.

The restriction patterns and the analyzed 16S mtDNA sequences indicated that most white oysters belong to *C. rhizophorae* species and dark-gray oysters belong to *C. gasar* species. Therefore, *C. gasar* showed better performance in culture than *C. rhizophorae*, reaching larger sizes and showing higher survival. These results corroborate the assumption of producers and suggest that *C. rhizophorae* is less suitable for culture, demanding, for example, some genetic improvement.

The prevalence of *Perkinsus* spp. was moderate (45.7%). Although, in October, after three months of culture, when oysters were 48.3 mm long, the prevalence reached the maximum value (72.4%). This high prevalence was not associated with mortality this month (17.8%) because the highest monthly mortality rate (22.5%) was observed in the previous month. In addition, the light infection (level 2) in oyster tissues during the whole culture period did not support the hypothesis that *Perkinsus* infection caused oyster mortality. A period of high *Perkinsus* infection intensity commonly precedes or is concomitant with a mortality event (VILLALBA et al., 2011).



**Figure 6.** Phylogram showing the results of the maximum parsimony analyses on *Perkinsus* spp. ITS region sequences. Bootstrap support values are indicated at the nodes. Sequences from this study are indicated by GenBank accession number (in bold).

The present study also highlights that very young oysters were already infected by *Perkinsus* spp. and that the infection persisted in the population until harvest. These facts suggest that the parasite is well established in the population and that it is a result of the proximity of older, infected oysters that allows parasite transmission (BEN-HORIN et al., 2015). Spats (3 - 15 mm) of clams *Ruditapes philippinarum* also acquired infection with *Perkinsus* spp. (*P. olseni* dominant) after immersion challenge with zoospores and suffered a high mortality (SHIMOKAWA et al., 2010; WAKI et al., 2012). Concerning *P. marinus*, infection in *C. virginica* oysters smaller than 30 mm is rare (BURRESON, 1991). The same result was found in *Crassostrea corteziensis* infected by *P. marinus* of the Pacific coast of Mexico, whose prevalence was almost insignificant in oysters of 20 - 49 mm (CÁCERES-MARTÍNEZ et al., 2016). Our results are in contrast with the latter results because 50% of oysters in our study were already infected with 29 mm (July spats). Moreover, the present result does not support the hypothesis that larger and older oysters are more infected because of their higher filtration rate and consequently higher parasite acquisition (BURRESON & RAGONE-CALVO, 1996; CÁCERES-MARTÍNEZ et al., 2016; VILLALBA et al., 2005).

The restriction patterns and analyzed ITS sequences identified two *Perkinsus* species (*P. marinus* and *P. beihaiensis*). This result allowed study of the differential susceptibility of Brazilian sympatric oysters to *Perkinsus* spp. infection. Interestingly, *C. rhizophorae* seemed to be more susceptible (55.9%) to *Perkinsus* spp. infection than *C. gasar* (43.1%). Herein, we put in evidence that *C. rhizophorae* is more affected by *P. marinus*, whose proliferation seemed to be more favored because more cases of heavy intensities were found. In turn, *C. gasar* is more likely to be infected by *P. beihaiensis*, whose proliferation seems to be more limited because fewer cases reached heavy intensities. Queiroga et al. (2015) found the opposite, that *P. beihaiensis* reached moderate and heavy intensities in *C. gasar*, while *P. marinus* seemed to be more limited. These results suggest that, when two susceptible host species are present, the progression of the two *Perkinsus* species can be modified in order to favor parasite progression in the most susceptible oyster species. Despite the knowledge that *P. marinus* causes outbreaks in *C. virginica* oysters from the East Coast of the USA (BURRESON & RAGONE-CALVO, 1996), *P. marinus* was not associated with mortality in other susceptible oyster species (*Crassostrea* and *Saccostrea*) (CÁCERES-MARTÍNEZ et al., 2010, 2012, 2016; MOSS et al., 2008).

In contrast, the pathogenicity of *P. beihaiensis* is poorly studied. Moss et al. (2008) noted some histopathological effects of *P. beihaiensis* in the digestive system (stomach, intestine and digestive gland) of *Crassostrea hongkongensis* from China, including cases of moribund oysters with heavy infections. Similar symptoms were observed in *C. rhizophorae* (SABRY et al., 2009) and *C. gasar* (QUEIROGA et al., 2015) from Brazil. In contrast, Sanil et al. (2012) reported some digestive tubule lesions in *Crassostrea madrasensis*, but these lesions were not directly associated with *P. beihaiensis*.

In Brazil, a natural population of *C. rhizophorae* oysters (State of Paraíba, NE Brazil) is dramatically affected (prevalence up 100% and moderate intensities, level 3) by *P. marinus* (DA SILVA et al., 2013). This oyster species is also susceptible to *P. beihaiensis*, with

very low prevalence (not exceeding 10%) and mostly light intensities (DANTAS-NETO et al., 2015; SABRY et al., 2009, 2013).

In conclusion, the occurrence of some variability in productive traits and *Perkinsus* spp. susceptibility between native oyster species was demonstrated. One oyster species, i.e., *C. gasar*, on average, showed a significantly better performance through growth. Perkinsosis did not cause mortality in any stage of oyster culture. The results obtained with the best-performing oyster species suggest that even under perkinsosis pressure and premature infection, farming in northeast Brazil is feasible and can be profitable using appropriate oyster spats and species.

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