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Evaluation of some hemato-immunological parameters in the mangrove oyster *Crassostrea rhizophorae* of different habitats of Santa Catarina Island, Brazil

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Abstract – The main purpose of this study was to establish the pattern of variability of some hemato-immunological parameters in the mangrove oyster, *Crassostrea rhizophorae,* from 3 different environmental conditions in Santa Catarina Island: two natural habitats (mangrove and rocks of the coastal bay) and one oyster culture station. The water quality was adequate in all localities. The animals were collected seasonally over a period of one year (n = 30, per locality). The oyster hemolymph comprised two basic cell populations, hyaline (HH) and granular hemocytes (GH). Both cell populations contained carbohydrates and glycogen in their cytoplasm (periodic acid Schiff reaction, PAS staining) and the occurrence of lysosomes was suggested by the detection of acid phosphatase (Gomori's method). Both hemocyte populations were able of phagocytosis of zymosan particles *in vitro* and producing cytotoxic molecules, such as the superoxide anions (nitroblue-tetrazolium, NBT reduction). The oyster hemograms significantly differed in the different habitats and also among seasons. The total circulating hemocyte counts (THC) and the percentage of GH in the mangrove and rock oysters always differed from each other, but not from those of the cultured oysters. The highest THC and the lowest percentage of GH were always found in summer. The total plasma protein concentration (PC) exhibited a similar seasonal pattern in all oyster populations, with a marked decrease in spring. The PC of the cultured oysters was almost always significantly lower than that of the other oyster populations. The plasma agglutinating titer was higher in the cultured oysters regardless of season. The histological organization of the oyster digestive gland and gills was basically similar to that of other oyster species. Their structural aspect did not show any detectable alteration, corroborating that the oysters were in good health. The results of this study will serve as a basis for further analyses on the monitoring of *C. rhizophorae* health status and environmental quality in different aquatic habitats.

Key words: Mollusks / Bivalves / *Crassostrea rhizophorae* / Hemato-immunological parameters / Hemolymph / Hemocytes / Digestive gland / Gills

Résumé – Évaluation de quelques paramètres hémato-immunologiques chez l'huître de mangrove *Crassostrea rhizophorae* de différents habitats de l'île de Santa Catarina, Brésil. Le but de cette étude est d'établir la variabilité de paramètres hémato-immunologiques de l'huître de mangrove, *Crassostrea rhizophorae*, en différentes conditions environnementales de l'île Santa Catarina : deux habitats naturels (mangrove et zone rocheuse de la baie) et une station ostréicole. La qualité de l'eau est adéquate dans les 3 sites. Les huîtres sont collectées de façon saisonnière sur une période d'un an (n = 30, par site). L'hémolymphe des huîtres comprend 2 groupes de cellules de base, des hémocytes hyalins (HH) et granuleux (GH). Les deux groupes de cellules contiennent des glucides et du glycogène dans leur cytoplasme (réactif de Schiff, PAS) et la présence de lysosomes est mis en évidence par réduction de l'acide phosphatase (méthode de Gomori). Les deux groupes d'hémocytes sont capables de phagocyter des particules de zymosane *in vitro* et produisent des molécules cytotoxiques, tels que des anions superoxides (réduction du nitrobleu de tétrazolium, NBT). Les hémogrammes diffèrent significativement entre les différents habitats et aussi entre les saisons. Le nombre total d'hémocytes circulant (THC) et le pourcentage de GH chez les huîtres de la mangrove et ceux de la zone rocheuse diffèrent toujours, mais non avec ceux des huîtres cultivées. Le THC le plus élevé et le plus faible pourcentage de GH sont toujours trouvés en été. La concentration totale des protéines du plasma (PC) montre un schéma similaire chez toutes les huîtres, avec une nette diminution au printemps. La concentration de PC des huîtres cultivées est presque

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toujours plus faible que celles des autres populations d'huîtres. Le titrage du plasma agglutiné est plus élevé chez les huîtres cultivées quelle que soit la saison. L'organisation histologique de la glande digestive et des branchies est similaire à celle des autres espèces d'huîtres. Leur aspect structural ne présente pas d'altération détectable, corroborant que ces huîtres sont en bonne santé. Ces résultats serviront pour le suivi de la santé de *C. rhizophorae* et celui de la qualité environnementale en différents habitats.

1 Introduction

The mangrove oyster *Crassostrea rhizophorae* is currently one important alternative for the oyster aquaculture industry, and has been cultivated in several Brazilian regions. However, in Santa Catarina state, Southern Brazil, the exotic species *Crassostrea gigas* or Pacific oyster largely dominates oyster culture. At present, Santa Catarina is the largest Brazilian producer of cultivated bivalves, consisting primarily of oysters and mussels. The indigenous oyster *C. rhizophorae* is commonly found in mangrove and estuarine environments, adhered to rocks and aerial roots. The cultivation of *C. rhizophorae* was attempted in Santa Catarina during 1970s and 1980s, but the results were not satisfactory. New efforts commenced in the 1990s with greater success. Farming of *C. rhizophorae* may contribute to a continuous annual oyster production, specially in summer, when large-scale mortalities occur in *C. gigas*. In addition, other factors are also driving *C. rhizophorae* farming, such as the problems involved in the introduction of exotic species, the degradation of natural oyster banks by overexploitation and also its good taste and nutrient value for human consumers.

The immune system of bivalves of commercial interest is a crucial tool for understanding their resistance to infections, which currently represent a serious risk to the success of their cultivation. Moreover, the exposure of bivalves to aquatic xenobiotics can also result in a change of their immunocompetence, making the animals more susceptible to disease. The immune system of *C. rhizophorae* is largely unknown, compared to that of *C. gigas* and *C. virginica*, which have been widely studied (Olafsen et al. 1992; Lacoste et al. 2002; Romestand et al. 2002; Hégaret et al. 2004). As in other invertebrates, the immune system of bivalves is mainly associated with their blood or hemolymph. This fluid comprised circulating cells or hemocytes and a variety of humoral factors. The hemocytes are mainly concerned with the cellular immune responses, including the phagocytosis and encapsulation of invading organisms and their degradation through a variety of cytotoxic and/or lytic molecules. On the other hand, the humoral factors comprise non-self recognition molecules and immune effectors, such as lectins, opsonins, cytolytic proteins and components of the prophenoloxidase (proPO) system (see reviews by; Roch 1999; Vargas-Albores and Barracco 2001). Cellular and humoral reactions act synergistically in bivalves to ensure their survival against a variety of opportunistic and pathogenic organisms.

Several hemato-immunological parameters have been proposed to serve as tools in the monitoring of bivalve health status. The most common are hemograms, phagocytic index, production of reactive oxygen species (ROS), plasma protein concentration and agglutinating activity (Oubella et al. 1993; Coles et al. 1995; Cajaraville et al. 1996; Fisher et al. 1996; Romestand et al. 2002). Changes in the histological pattern of some tissues can also reflect the presence of pathogens or xenobiotics (Smith et al. 1985; Cajaraville et al. 1996).

The aim of the present study was to characterize the variability of some hemato-immunological parameters in the mangrove oyster *Crassostrea rhizophorae,* from three different habitats in Santa Catarina Island. These results will serve as a basis to compare the health status of the oysters when exposed to xenobiotics or infections.

2 Materials and methods

2.1 Animals

The mangrove oyster *Crassostrea rhizophorae* (Guilding 1828) was collected from three different habitats in the Northwest region of Santa Catarina Island (27°28′30″ S; 48°33′40″ W): mangrove area (Ecological Station of Carijós in the region of Ratones – Environmentally protected area), rocks of the coastal bay of Sambaqui beach, and Mollusk Culture Station close to Sambaqui beach Figure 1. The mangrove and rock oysters were exposed to natural tidal variations, whilst those from the culture station remained constantly submersed. Oysters were collected seasonally, 30 animals from each location, during one year. Shell length ranged from 60 to 120 mm for the cultured oysters, 30 to 75 mm for the mangrove oysters and 30 to 65 mm for the rock oysters. The larger size of the cultivated oysters did not represent a different developmental stage, since they usually grow much faster than the oysters from the other habitats. Immediately after collection, the oysters were transported to the Laboratory of Immunology Applied to Aquaculture, and maintained in aquaria (20 L) containing filtered seawater (cultured and rock oysters) and constant aeration, for a minimum period of 24 h and a maximum of 48 h. The mangrove oysters were maintained under the same conditions, except for salinity, which was adjusted to the values similar to those at the collection site. The environmental parameters, temperature and salinity, were monitored throughout the study period.

2.2 Hemolymph extraction and plasma preparation

Hemolymph was obtained individually by the insertion of a 21 G needle coupled to a syringe (1 ml) in the posterior adductor muscle of each oyster, after making a small opening in the edge of the shell. For plasma preparation, the hemolymph was collected as 3 pools (10 animals per pool) for each oyster habitat and each season. The samples were immediately centrifuged at $800 \times g$ for 5 min at 8 °C. The cell pellet was discarded and the supernatant or plasma removed, aliquoted and frozen (−20 °C) for a maximum of 20 days.

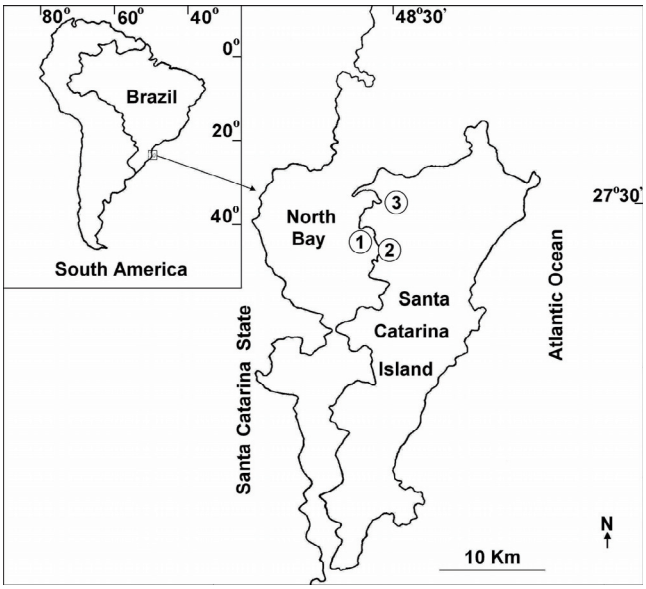

Fig. 1. Sites where the oysters were extracted in the Northwest region of Santa Catarina Island. 1– Mollusk Culture Station of Sambaqui, 2 – rocks of the Sambaqui beach, 3 – Ecological Station of Carijós (Ratones).

2.3 Cell identification in wet smear preparations

Hemolymph samples were collected (v/v) in 4% formaldehyde-anticoagulant (27 mM sodium citrate, 336 mM NaCl, 115 mM glucose, 9 mM EDTA, pH 7.0), slightly spread on a microscope slide and immediately observed under the differential interference microscope.

2.4 Giemsa staining

Hemolymph fresh smears (n = 10) were fixed in Baker's formol-calcium (4% formaldehyde, 3.2% NaCl, 1% calcium acetate) for 20 min, washed thoroughly with running water and stained with a Giemsa solution (1:6 v/v, in distilled water). The smears were then washed with distilled water, mounted with coverslips and observed under the microscope.

2.5 Detection of carbohydrates and glycogen – PAS Reaction (Periodic Acid-Schiff)

Hemolymph fresh smears (n = 10) were fixed as above and after washing thoroughly with running water, the smears were incubated with 1% periodic acid, washed again and exposed to Schiff's reagent for 30 min (Bancroft and Stevens 1982). The slides were rinsed for 3 min in each of 3 consecutive sulphurous baths (10 ml of 1 N HCl in 210 ml of 0.05% $Na_2S_2O_5$) and then in running water, before being counterstained with Harris' hematoxylin. Histological sections of cat large intestine served as positive controls. For detection of glycogen, hemolymph smears were pre-treated with human salivary amylase for 1 h at 37 °C before the incubation with the periodic acid. In controls, the salivary amylase was replaced by 150 mM NaCl.

2.6 Detection of acid phosphatase (lysosomes) – Gomori method

Hemolymph smears, fixed as described above, were incubated in the Gomori's medium (Bancroft and Stevens 1982) for 1 h at 37 °C. They were then washed with 0.1 M Tris-maleate buffer, pH 5.0, treated with a 1% ammonium sulphide solution for 1 min and counterstained with 2% methyl green. After washing in running water, the smears were mounted and observed under the microscope. In control experiments, the smears were incubated in Gomori's medium devoid of the enzymatic substrate sodium β-glycerophosphate.

2.7 Phagocytosis and production of superoxide anion

The hemolymph of 5 oysters was collected (v/v) in 50 mM Tris, 2% NaCl, 0.03% $CaCl_2$, 0.1% nitroblue-tetrazolium (NBT), 1% bovine serum albumin (BSA), 0.15% zymosan, pH 8.4 (Pipe et al. 1995) and incubated in the dark under gentle shaking for 40 min at 20 °C. Hemocyte monolayers were then prepared on pyrogen free glass coverslips (22 × 22 mm, baked for 4h at 180 °C), maintained in 6-well microplates. An aliquot of 120 μl of this suspension was added to each coverslip, and the cells were allowed to adhere for 20–30 min. The coverslips were then washed (3×) with 50 mM Tris, 2% NaCl and 0.03% $CaCl_2$, pH 8.4, fixed in Baker's formol-calcium, washed again with 2% NaCl and mounted on slides to detect the formation of the blue precipitate (formazan). In controls, the incubation solution did not contain zymosan. A control for specific superoxide anion production was done by adding the enzyme superoxide dismutase (SOD – 600 U ml^{-1} in 2% NaCl, Sigma) in the incubating solution. Inactivated SOD (boiled for 30 min) was also used.

2.8 Hemograms – total (THC) and differential (DHC) hemocyte counts

Hemolymph was collected in 4% formaldehyde-anticoagulant (1:2 v/v) as 3 pools (10 animals per pool) for each location and each season. The blood of each animal was thus, collected twice: a first withdrawal, for plasma preparation and a second withdrawal carried out immediately after the first, for hemogram determination. The THC was estimated using a Neubauer chamber. The relative percentage of the different hemocyte populations (DHC) was calculated by counting 200 cells in each hemolymph pool, under the phase contrast microscope.

2.9 Total protein concentration (PC) in the plasma

Oyster plasma PC was determined according to the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

2.10 Agglutinating titer

Samples of plasma (50 μl) were serially diluted with TBS (10 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, pH 7.4) in 96-microwell plates (U-shaped bottom) and incubated with an equal volume of human A erythrocytes (2% in TBS) for 2–3 h at 20 °C. In controls, the plasma was replaced by TBS. The titer of the natural agglutinating activity of the plasma of the oysters from the different habitats was expressed as the reciprocal of the highest dilution showing a positive pattern of agglutination. The values of the agglutinating titers were converted to $\log_2$. The analyses were carried out in duplicate.

2.11 Histological analysis of the digestive gland and gills

Histological observations were made on 5 animals from each habitat in the summer and winter seasons only. The gills and the digestive gland were removed and transversely sectioned at three equidistant points (3 animals from each location) or longitudinally at the mid-point (2 animals from each location). The tissue sections were immediately fixed in Davidson's solution (Howard and Smith 1983). After fixation, the tissues were transferred to 70% alcohol and prepared according to conventional histological techniques for light microscopy, involving embedding in paraffin, sectioning (6 μm), and staining with hematoxylin-eosin (HE).

2.12 Statistical analysis

Data from THC, DHC, PC and agglutinating titer were first submitted to Bartlett's test for homogeneity of variance. A two-way analysis of variance (ANOVA) was used to evaluate the season and habitat effects, and the possible interaction between them. When a significant interaction was detected, one-way ANOVA was applied, followed by Tukey's test for the comparison of means. The percentage values of DHC were arcsin transformed. Results were considered significant at $p < 0.05$.

3 Results

3.1 Environmental parameters

The average water temperatures at the different oyster sites, Sambaqui (culture station and rocks) and Ratones (mangrove) were, respectively: 27 and 26 °C in summer, 19 °C at both locations in autumn, 20 and 18 °C in winter and 22 and 24 °C in spring. The average salinity at Sambaqui and Ratones was, respectively: 31 and 22‰ in summer, 32 and 24‰ in autumn, 32 and 26‰ in winter, and 31 and 23‰ in spring.

3.2 Hemocyte identification and characterization

Two basic cell populations were identified in the hemolymph of *C. rhizophorae*, hyaline (HH) and granular hemocytes (GH) (Fig. 2). The HH were round to oval cells of variable size (6–13 μm), with a spherical nucleus (3.5–6.5 μm) and whose cytoplasm was generally agranular (Figs. 2A, D, E). The smaller HH resembled undifferentiated cells with a high nucleocytoplasmic ratio and a basophilic cytoplasm in Giemsa staining. The larger HH did not always exhibit a basophilic cytoplasm. The GH were also round to oval cells of variable size (5–16 μm). They contained abundant cytoplasmic granules of different size (0.4–1.5 μm) (Figs. 2B-C, F-H). The nucleus (3–6 μm) was generally spherical and eccentrically located (Figs. 2F-H). According to the size of the granules, the GH could be further subdivided in large (1.0–1.5 μm) (Figs. 2C, F, G) and small granule (0.4–0.8 μm) cells (Figs. 2B, H). The granules were generally basophilic in Giemsa staining.

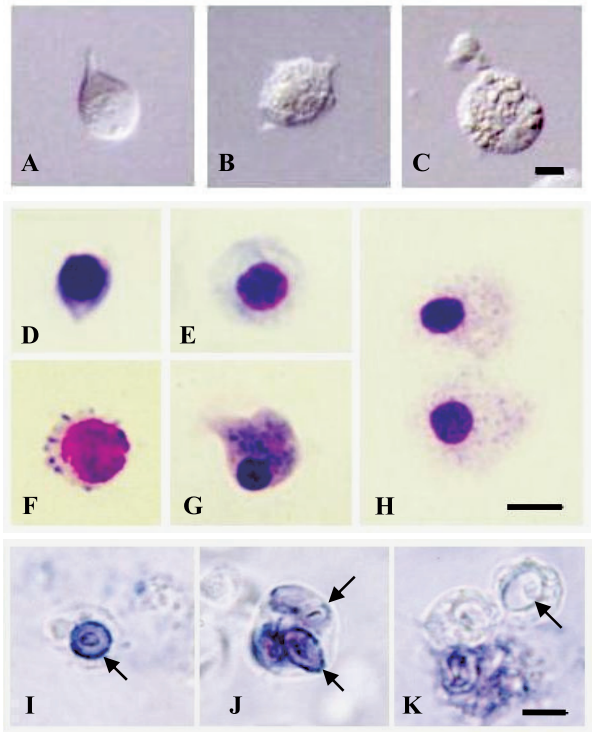

Fig. 2. Photomicrographs of the hemocytes of *C. rhizophorae*. **A-C**: hemocytes observed by interference microscopy (Nomarski), hyaline hemocyte (A), small granule hemocyte (B) and large granule hemocyte (C). **D-H**: Hemocytes stained by Giemsa, small (D) and large (E) hyaline hemocytes, small granule (H) and large granule hemocytes (F, G). **I-K**: phagocytosis of zymosan particles by the oyster hemocytes (arrows) and NBT reduction by the production of the superoxide anion. In K the arrow shows a phagocytic vacuole where NBT was not reduced. Bar = 5 μm.

3.3 Histochemical characterization, phagocytic activity and production of superoxide anion

The hemocytes of *C. rhizophorae* were all positive to the Schiff' (PAS), which confirmed the presence of polysaccharides. The majority of the cells (61%) exhibited a weak reddish cytoplasmic staining. Their pre-incubation with amylase reduced, but did not completely eliminate the Schiff staining, suggesting the presence of glycogen (Table 1).

Table 1. Histochemical staining or reaction in *C. rhizophorae* hemocytes.

Reaction/Staining	Intensity[1]	% hemocytes[2]
	0	0
Periodic acid - Schiff (PAS)	1	37.5
(polysaccharides)	2	61.0
	3	1.5
Amylase-PAS (glycogen)	0–1	100
Gomori (acid phosphatase)	0	30.5
	1–2	69.5

[1]The numbers indicate the reaction intensity (0 – negative; 1 – weak; 2– moderate; 3 – strong).
[2]The hemocytes were taken altogether, since it was not possible to differentiate them.

Acid phosphatase was detected by the Gomori method in the majority of the hemocytes (69.5%).These results suggested the occurrence of lysosomes and the role of these cells in phagocytosis. The phagocytic capacity and the production of the superoxide anion (O_2^-) by the oyster hemocytes were effectively confirmed after their incubation with zymosan particles (Figs. 2I–K). The majority of the phagocytic vacuoles exhibited a strong positive blue color as a consequence of the reduction of NBT to formazan by the O_2^- (Figs. 2I, J). Only very few vacuoles were left unstained (Fig. 2K). The presence of SOD in the incubation medium reduced considerably the formation of the blue precipitate, confirming the specific production of O_2^-. On the other hand, heat-inactivated SOD did not cause any evident change in formazan production.

3.4 Total (THC) and differential (DHC) hemocytes counts

The THC of *C. rhizophorae* varied from 1100 to 4000 cells mm^{-3} (Fig. 3). In summer, the values were always significantly higher ($p < 0.05$) than in autumn and winter, independently of the oyster habitat. On the other hand, the THC of the rock oysters was significantly lower ($p < 0.05$) than that of the mangrove oysters throughout the year.

The DHC of the oysters of the three different habitats gives only the GH percentage, since the HH represent the complementary percentage (Fig. 3). The proportion of GH was always higher in winter/spring regardless the oyster habitat. In summer, the percentage of GH reached the lowest values in all oyster groups, except in the rock oysters where this low percentage (46%) continued also in autumn. The number of GH was always significantly different ($p < 0.05$) between the rock and mangrove oysters, whilst the cultured oysters exhibited a significantly lower percentage of GH in summer and winter.

3.5 Total protein concentration in plasma (PC)

The plasma PC had the same seasonal pattern in the oysters from the three different habitats, increasing significantly

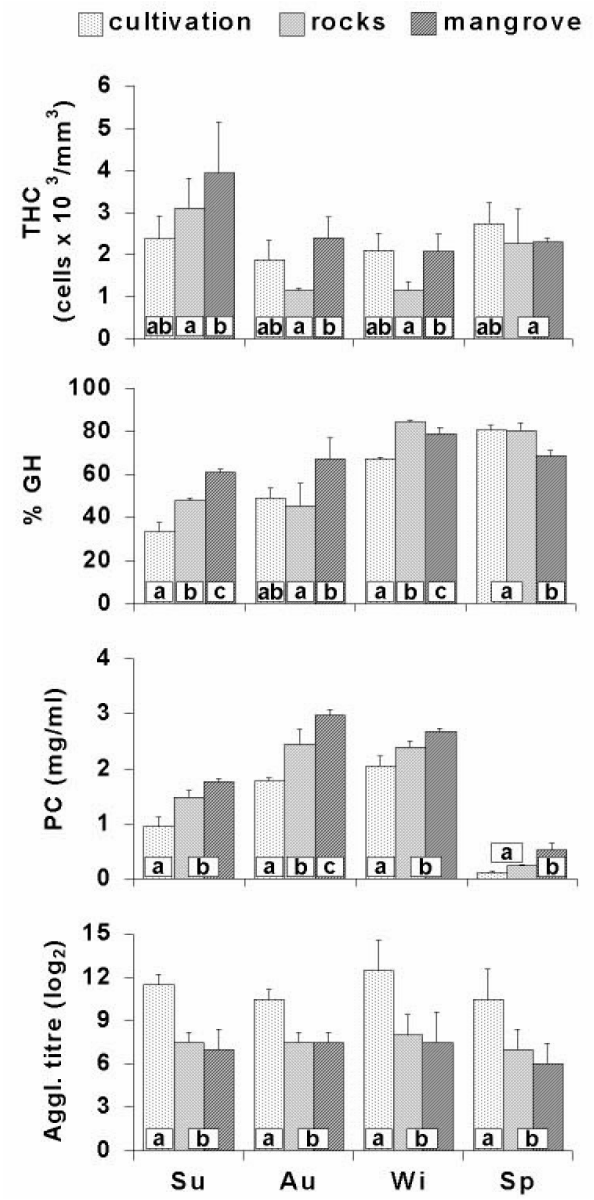

Fig. 3. Total hemocytes count (THC), percentage of granular hemocytes (GH), total concentration of plasma proteins (PC) and plasma agglutinating titer against human A erythrocytes in *C rhizophorae* from three different localities throughout the 4 seasons of the year. The bars represent the means ± standard deviations. Different letters indicate significant differences ($p < 0.05$) between the different sites of oyster extraction, within each season of the year.

($p < 0.05$) from summer to autumn and then decreasing markedly in spring. In cultured oysters, PC varied from 0.11 to 2.05 mg ml^{-1} and was always significantly lower ($p < 0.05$) than in oysters from the other localities, except in spring, when it did not differ from the rock oysters. On the other hand, the PC of the mangrove and rock oysters was similar in summer and winter, but differed significantly ($p < 0.05$) during the other seasons (Fig. 3).

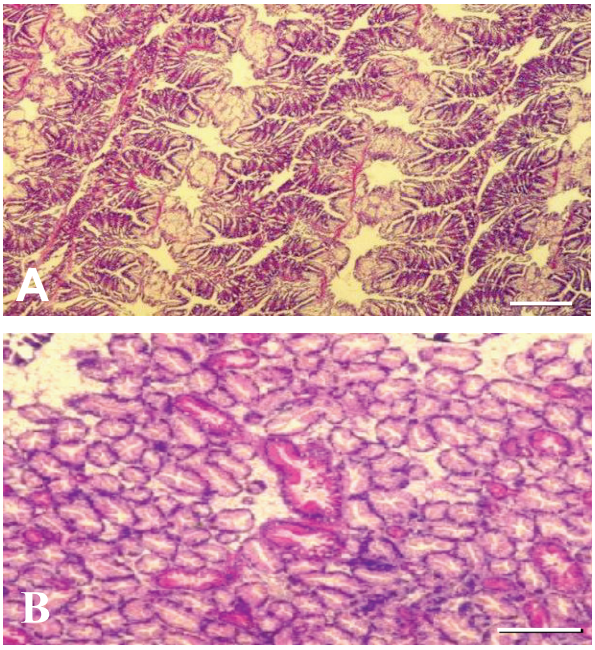

Fig. 4. General view of the histological organization of the gills (A) and digestive gland (B) of *C. rhizophorae* (HE staining). Both tissues did not show any evident structural alteration in oysters from the three habitats, suggesting that the animals were in good health. Bar = 200 μm.

3.6 Agglutinating activity of the plasma

The plasma agglutinating titer of the cultured oysters varied from 512 to 16 384 and was always significantly higher ($p < 0.05$) than that of the oysters from the other locations (Fig. 3). On the other hand, the agglutinating activity of the rock and mangrove oysters was similar to each other and did not vary throughout the seasons.

3.7 Histological analysis of the digestive gland and gills

The histological organization of the digestive gland and gills of the oysters from the three different localities did not show any apparent tissue alteration (Fig. 4). Parasites and inflammatory reactions were not observed within the limits of detection of the light microscope.

4 Discussion

The main purpose of this study was to characterize variability of several hemato-immunological parameters in the healthy mangrove oysters *Crassostrea rhizophorae* in three different habitats of Santa Catarina Island. In addition, the oyster gills and digestive gland were also histologically examined, since these tissues can be severely affected by pathogens and xenobiotics from the aquatic environment. *C. rhizophorae* has a considerable potential for aquaculture and characterizing normal variability of hemato-immunological parameters could be of relevance for assessment of oyster health status and environmental quality.

The immune system of *C. rhizophorae* is practically unknown. According to our observations, the hemocytes of this oyster species had similar characteristics to the cells of other bivalves (see review of Hine 1999). The small undifferentiated and the large basophilic HH resembled those of *C. gigas* (Bachère et al. 1988). On the other hand, the small and large granule hemocytes, whose granule content was generally basophilic, contrasted with those of *C. gigas* (Auffret 1989) and *Ostrea edulis* (Brereton and Alderman 1979). In these oysters, the GH are differentiated in eosinophilic and basophilic cells. Conversely, in the mussel *Perna perna,* only one GH population with eosinophilic granules was described (Barracco et al. 1999).

The cytoplasm of all *C. rhizophorae* hemocytes contained polysaccharides and glycogen (PAS staining), although in variable quantities. Glycogen was also reported in the cytoplasm of *C. gigas* GH (Bachère et al. 1988), whereas in *C. virginica* (Hawkins and Howse 1982) and *Mya arenaria* (Seiler and Morse 1988), glycogen was found mainly in the HH.

The presence of lysosomes, through the detection of acid phosphatase, was observed in a large fraction (70%) of *C. rhizophorae* hemocytes, thus suggesting the role of these cells in phagocytosis. The phagocytic capacity of *C. rhizophorae* hemocytes was effectively confirmed by the incubation of these cells with zymosan particles. Moreover, the reduction of NBT to formazan during the phagocytic reaction demonstrated the capacity of the hemocytes to produce cytotoxic molecules, such as the superoxide anion, as described in other oyster species (Bachère et al. 1991; Anderson 1994; Austin and Paynter 1995; Fisher et al. 1996; Lacoste et al. 2002; Hégaret et al. 2004). The generation of reactive oxygen species (ROS) in common with the lysossomal enzymes is the major lytic and degrading mechanism capable to ensure the bivalve survival during infections. In further studies, it will be examined whether ROS production in *C. rhizophorae* is modulated by infections or aquatic contaminants, as already reported in *C. virginica* (Volety and Chu 1995; Bramble and Anderson 1999; Fisher et al. 2000).

In addition to this initial hemocyte characterization, a seasonal evaluation of several hemato-immunological parameters was also performed, comparing oyster populations from three different habitats, all presenting good water quality, apparently free of high-impact contaminants.

Amongst the examined hemato-immunological parameters, the agglutinating activity of the plasma of cultured oysters was always higher than that of oysters from the other sites, independently of the season. The reason for this difference remains unclear. In *C. gigas*, Olafsen et al. (1992) reported that the agglutinating activity was increased in oysters infected by *Vibrio anguillarum.* However, no record of infection was found at the culture station throughout the period of our study.

The total concentration of plasma proteins (PC) in the cultured oysters was surprisingly, consistently lower than in the oysters from the other habitats, corroborating that the agglutinin concentration in the cultured oysters was effectively increased. On the other hand, the PC of the mangrove oysters was always higher than oysters from the other habitats. In

mangroves, the salinity is normally lower than in marine environments and it fluctuates daily according to the tidal cycles and to freshwater run-off (rivers and rain). Therefore, to adjust their osmolarity, the mangrove oysters might activate a variety of enzymes and/or proteins, (Shumway 1977; Nirchio and Pérez 1997), that can result in a variation of their plasma PC. It is noteworthy, that the PC of *C. rhizophorae* followed the same seasonal profile in the three different oyster populations, showing a marked fall in spring and a progressive increase towards the colder seasons. Also in *C. virginica*, a seasonal variation of PC was described, with the highest values in winter months and the lowest in summer months (Fisher et al. 1996). The authors suggested that the highest protein concentrations were related to the final stage of gamete maturation. Considering that in Santa Catarina the spawning peak of *C. rhizophorae* is in autumn (March to June) (Ostini and Poli 1990), a correlation between PC and gamete maturation.

Concerning the hemograms (THC and DHC), the THC of rock oysters was always different from that of the mangrove oysters, whilst neither of them differed from the cultured oysters. On the other hand, the THC values of all three-oyster populations were usually greater in the warmer (spring and summer) than in the colder periods of the year (autumn and winter). These results suggested that the oysters produce or release more hemocytes during the hot seasons.

The percentage of *C. rhizophorae* GH, which is considered to be the most immunocompetent cell type (Ford et al. 1989; Anderson 1994; Oubella et al. 1996; Paillard et al. 1996), was always higher in winter and spring regardless the oyster habitat. These results apparently indicate that during the hot seasons, where THC is maximal, there is a greater production/release of hyaline cells over granular hemocytes. By contrast, in *C. virginica*, the number of GH is greater in the months of higher temperatures (Chu and La Peyre 1993). It would be interesting to verify if the lower proportion of GH in *C. rhizophorae* during the summer results in an increased susceptibility to infections.

The histological organization of *C. rhizophorae* digestive gland and gills was similar to that described in other oyster species (Galtsoff 1964; Eble 1996). No structural and cellular alterations were observed in the oysters, suggesting that the animals from the three different habitats were in good health conditions.

In conclusion, in the present study we established a basic hemato-immunological profile of the oyster *C. rhizophorae* from 3 habitats of different environmental conditions, during the four seasons of the year. Some of the examined hemato-immunological parameters showed an apparent correlation with the oyster habitat or with the season of the year. Briefly, (1) the plasma agglutinating activity was always higher and the total protein concentration lower in cultured oysters; (2) the hemograms (THC and DHC) of the mangrove and rock oysters always differed from each other, although both were similar to those of the cultured oysters; (3) the THC of the three oyster populations was always higher, and the percentage of GH lower, during the summer; (4) the PC of all oyster populations markedly decreased in spring. The results of this study will serve as a basis to future analyses on the monitoring of *C. rhizophorae* health status and environmental quality at different marine habitats.

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