RECORD OF *Pseudomyicola spinosus* IN CULTURED *Perna perna* FROM SOUTHERN BRAZIL*

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ABSTRACT

The copepoda *Pseudomyicola spinosus* was identified infecting culture *Perna perna* mussels from Palhoça, Florianópolis, Governador Celso Ramos and Penha, municipalities of Santa Catarina state, Brazil. Adult stage of the parasite was observed in the mantle cavity, with higher prevalence and infestation rate in mussels from Florianópolis (3.33 and 1.08%, respectively). Nauplius phases of the copepod were observed in the digestive gland, gonad and gill of mussels, with highest prevalence in mussels from Penha (17.78%) and highest infestation rate from Palhoça (1.19). The analysis showed that the highest prevalence of the adult phases of the copepods occurred in the autumn (2.22%) while nauplius phases were prevalent in summer (16.67%). Even in situations of higher prevalence and infestation, no histopathological evidence of damage to host was found.

Key words: copepod; mussel; mussel culture; pathology.

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REGISTRO DE *Pseudomyicola spinosus* EM CULTIVOS DE *Perna perna* DO SUL DO BRASIL

RESUMO

O copépode *Pseudomyicola spinosus* foi identificado infectando cultivos de mexilhões *Perna perna* nos municípios catarinenses de Palhoça, Florianópolis, Governador Celso Ramos e Penha, Brasil. O estágio adulto do parasita foi observado na cavidade do manto em maior prevalência e taxa de infestação nos mexilhões de Florianópolis (3,33 e 1,08%, respectivamente). A fase naupliar deste copépode foi observada na glândula digestiva, gônada e brânquia dos mexilhões, em maior prevalência nos mexilhões da Penha (17,78%) e maior taxa de infestação nos mexilhões da Palhoça (1,19). A análise mostrou que a maior prevalência das fases adultas dos copépode ocorreu no outono (2,22%), enquanto as fases naupliares foram prevalentes no verão (16,67%). Mesmo em situações de maior prevalência e taxa de infestação, não foram registrados danos histopatológicos ao hospedeiro.

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INTRODUCTION

Copepoda (Maxillopoda: Crustacea), comprising around 14,000 species (ITIS, 2016). Copepods are the most abundant metazoans on Earth and are the basis of the pelagic trophic chain with several reproductive strategies to maximize population in response to predation (TURNER, 2004). According to HUYS and BOXSHALL (1991) the Copepoda are classified into 10 orders, half of which contain only parasitic or symbiotic species. The family Mycolidae (Poecilostomatoida) includes the species *Pseudomyicola spinosus*. Worldwide copepod *P. spinosus* have been recorded in more than 50 bivalve hosts (KIM, 2004). In Brazil *P. spinosus* was described infecting the clams *Anadara ovalis* (FERREIRA JR *et al.*, 2015), *Anomalocardia brasiliana* (SABRY *et al.*, 2008), the oyster *Crassostrea rhizophorae* (SABRY *et al.*, 2008, 2011) and the mitilid hosts (references in Table 1).

In previous study (CARNEIRO-SCHAEFER *et al.*, 2017) registered for the first time the occurrence of the genus *Pseudomyicola* in cultured mussels *Perna perna* (LINNAEUS, 1758) from Santa Catarina coast, Brazil. The present study identified the species of genus *Pseudomyicola* using specific techniques and it relation with the cultured mussels *Perna perna*.

Table 1. Review of mytilid hosts of copepod *Pseudomyicola spinosus*. Details of origin (wild or culture) local, prevalence (%), infestation and tissue/organ parasitized.

Specie of bivalve	Origin from	Local	Tissue/organ parasitized (prevalence in %)	Authors
Mytilus edulis	Wild	France	Digestive gland	KLEETON (1964)
(Linnaeus, 1758)		Holland	-	STOCK (1965)
		France	-	HUMES (1968)
		Japan	Mantle cavity and esofagus	HO (1980)
		Japan	Mantle cavity	KAJIHARA and NAKAMURA (1985)
		Korea	Mantle cavity	KIM (2004)
		Japan		
Mytilus galloprovincialis	Wild	France	Intestine	HUMES (1968)
(Lamarck, 1819)		France	Intestine	
		Japan	Mantle cavity	KAJIHARA and NAKAMURA (1985)
	Culture	Mexico	Mantle cavity (100)	CÁCERES-MARTÍNEZ et al. (1996)
	Culture	Mexico	Gills (66), mantle cavity (90) and digestive tract (100)	OLIVAS-VALDEZ and CÁCERES-MARTÍNEZ (2002)
Mytilus californianus	Culture	Mexico	Mantle cavity (100)	CÁCERES-MARTÍNEZ et al. (1996)
(Conrad, 1837)	Wild	Mexico	Mantle cavity	CÁCERES-MARTÍNEZ and VÁSQUEZ-YEOMANS (1999)
Septifer virgatus	Wild	Mexico	Mantle cavity	CÁCERES-MARTÍNEZ and VÁSQUEZ-YEOMANS (1999)
(Wiegmann, 1837)				
Septifer virgatus	Wild	Japan	Mantle cavity	HO (1980)
(Wiegmann, 1837)		Japan		DO and KAJIHARA (1986)
Perna perna (Linnaeus, 1758)	Culture	Brazil	Gills, mantle cavity and digestive tract	Present study

METHODS

Local sampling

During the period from July 2010 to June 2011, 240 mussels were sampled monthly along four localities on the Santa Catarina coast in southern Brazil. Mussel samples (n=30) were taken in commercial farms from three municipalities, Palhoça (27º45'23"S, 48º37'07"W), Governador Celso Ramos (27º22'16"S, 48º33'43"W) and Penha (26º46'59"S, 48º36'17"W) and in the experimental culture area of the UFSC, Florianópolis (27º29'27"S, 48º32'23"W), totalling 2.880 mussel specimens. From this total, 1.440 mussels were analyzed using a stereomicroscope to observe the presence or absence of copepods. The other 1.440 mussels were submitted to a histopathology protocol. The local salinity and temperatures was recorded along the sampling period.

The mussels were collected from the rope ends and from the center of five strings on each commercial farm, stored in thermic boxes and then transported to the laboratory at the Center for Aquatic Pathology Studies/UFSC.

Analysis at stereoscope

The shell was opened by adductor muscle dissection and subsequent exposition of mantle and the remaining part of the animal was divided in two transversely, attached at the valve. The presence of copepods in the gill, mantle and the external part of the gonadic tissue was registered. Tissue anomaly were observed and registered.

Collected copepods were submitted to a protocol for differential phase contrast microscopy (DIC) and scanning electron microscopy (SEM).

Differential phase contrast microscopy (DIC) photography

For DIC, live copepods were fixed in 70% ethanol, clarified using lactic acid for a few minutes, and conditioned over a slide for analysis (HUMES and GOODING, 1964).

Scanning Electron Microscopy (SEM)

For SEM, live copepods were fixed in glutaraldehyde, washed with sodium cacodylate, dehydrated in an ascendant ethanol series, covered with gold and photographed under a scanning electron microscope (JEOL JSM-6390LV), (DAWES, 1971; HAYAT, 1972; DYKSTRA, 1993), at the Electron Microscopy Center Laboratory (LCME) of UFSC.

Histopathology

Mussel tissues were sectioned into 2 mm slices (HOWARD and SMITH 1983; HOWARD *et al.* 2004), fixed in Davidson solution (BELL and LIGHTNER, 1988), dehydrated in an ascendant ethanol series and embedded in paraffin (PAULETE-VANRELL, 1967). Sections of 5 µm thickness were cut in a microtome (LUPE, MRP-03) using disposable razors, mounted on slides, and stained with hematoxylin-eosin (HOWARD and SMITH, 1983). Coverslips were added, mounted with Erv-Mount and the sections were analyzed and photographed using an optical microscope.

The sex of copepod, number and stage of development for each mussel sample were recorded, as well as possible tissue damage to the bivalve.

Statistical analysis

The prevalence and infestation rate of copepod in the mussels was analysed by site and season. The prevalence was calculated by the number of pathogens/the number of hosts ratio (BUSH *et al.* 1997). Considering that the data of prevalence and infestation were nonparametric (non normal distribution), datas were analyzed using a t-test with permutation using proc multitest in SAS® (WESTFALL *et al.*, 1999).

RESULTS

This study registered the presence of the copepod *Pseudomyicola spinosus* in the gills and mantle of cultured mussel *Perna perna*. Copepod moving quickly in the mussels tissue was observed.

Throughout the experimental period, the temperature and salinity in all sampled sites were similar (Table 2) and none parameter showed statistical differences between the study sites.

Mussels length from Governador Celso Ramos

was significantly ($p<0.05$) lower than Florianópolis, Palhoça and Penha. Also, mussels length in the summer was significantly higher ($p<0.05$) autumn and winter (Table 2).

Table 2. Mean (± standard deviation) of length of cultured mussels *Perna perna* sampled, prevalence (%) and infestation rate of nauplius (N) phase (stereoscope) and adult (A) phase (histopathology) of copepod *Pseudomyicola spinosus* in the mussels and mean (± standard deviation) of temperature and salinity by study site and season. Where GCR = Governador Celso Ramos.

Study site/ Season	Mussels lengths (mm)	Prevalence (%)		Infestation		Temperature (ºC)	Salinity (‰)
		N	A	N	A		
Palhoça	85.69 ± 11.74	7.50	0.83	1.19	1.00	20.62 ± 3.39	32.25 ± 2.52
Florianópolis	88.88 ± 13.96	5.56	3.33	1.15	1.08	20.45 ± 3.44	32.66 ± 2.70
GCR	76.29 ± 18.54	7.78	0.56	1.00	1.00	20.62 ± 4.21	33.08 ± 4.44
Penha	80.06 ± 49.68	17.78	0.28	1.00	1.00	21.25 ± 3.78	32.66 ± 2.87
Winter	76.55 ± 11.63	6.94	0.56	1.40	1.00	17.33 ± 2.23	34.00 ± 1.34
Spring	84.37 ± 11.71	9.44	0.83	1.56	1.00	20.95 ± 2.09	32.50 ± 1.67
Summer	91.98 ± 51.50	16.67	1.39	1.20	1.00	24.75 ± 1.95	29.75 ± 4.20
Autumn	78.48 ± 13.20	5.56	2.22	1.20	1.13	19.91 ± 3.42	34.41 ± 2.27

Pseudomiycola spinosus sampled under the microscope stereoscope

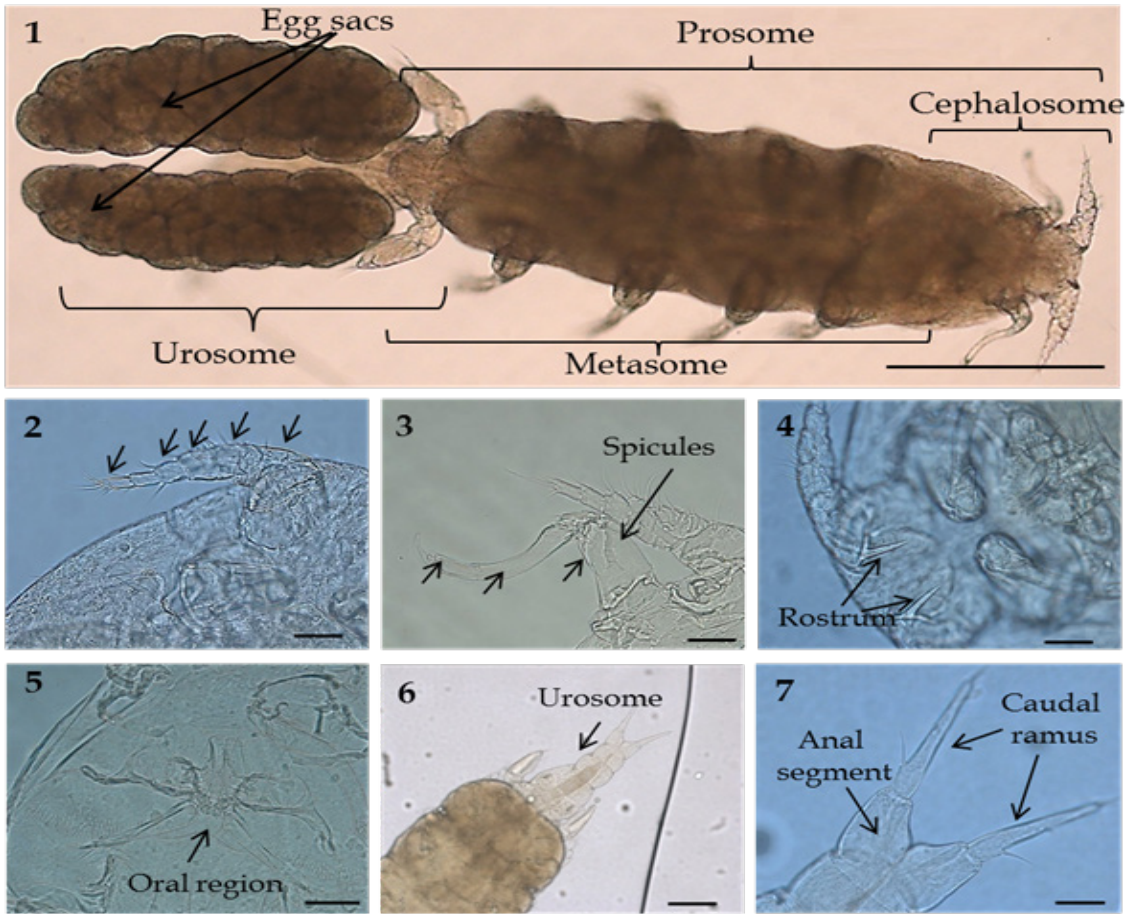
Under the microscope stereoscope, a total of 19 free-living adults copepod were found in the mantle cavity of *P. perna*. From this founded copepods, only females specimens that presented egg sacs (about 20 eggs; Figure 1) were studied (n=10) to characterize the species. These females showed six segments in the first antenna (Figure 2); the second antenna (Figure 3) has three segments; the third segment has variable number of spicules (12 to 20 spicules). The rostrum and oral region are shown in Figures 4 and 5, respectively. The final part of the urosome (Figure 6) has two caudal ramus (Figure 7) corresponding to the copepods Poecilostomatoida order and family Myicolidae. Photographs from DIC permitted the observation of the first and second pairs of antennae; the first, second, third, fourth and fifth pairs of legs; and the caudal ramus (Table 3 and Figure 8).

Copepods specimens founded showed total length 870 ± 70 µm (maximum 980 µm and minimum 740 µm) and width 260 ± 30 µm (maximum 300 µm and minimum 220 µm).

Carapace of 9 female adults specimens of *P. spinosus* photographed at the SEM (Figure 9), showed presence of the 1st and 2nd pairs of antennas with their targets characteristics, the oral region and the 5th pairs of legs. In the urosome, the presence of the anal segment and the caudal ramus were observed.

Table 3. Pairs of legs and ornamentation of endo and exopoditos. "Roman numerals" indicate the number of thorns and the "Arabic number" of bristles.

Pair of legs	Exopod	Endopod
1	IV-4 I-1 I-0	II-4 0-1 0-1
2	IV-5 I-1 I-0	III-3 II-0 I-0
3	V-5 I-1 I-0	IV-2 0-2 0-1
4	IV-5 I-1 I-0	IV-1 0-2 0-1



Figures 1 to 7. Specimens of *Pseudomyicola spinosus*, collected in cultured mussels *Perna perna* from Santa Catarina, Brazil, using lactic acid technical and photograph in DIC. Fig. 1 – Dorsal view of female copepod (arrow: egg sacs) (bar: 200 µm); Fig. 2 – Detail of the first antenna (arrows indicating the 5 segments) (bar= 32 µm); Fig. 3 – Detail of the second antenna (arrows indicating the 3 bands) (bar= 32 µm); Fig. 4 – rostrum detail (bar= 20µm); Fig. 5 – Detail of the oral region (bar= 20 µm); Fig. 6 – Urosome detail (bar= 130 µm); Fig. 7 – Detail of the caudal ramus and anal segment (bar= 32 µm).

Histopathology

Nauplius phases of *P. spinosus*, with an average length of 107.70 ± 28.14 µm were recorded through the analysis of histological sections in the gonads, gills, digestive glands and lumen of the stomach in the mussels (Figures 10 to 13).

Prevalence and infestation

The largest number of copepodites (nauplius), 175 (95.63%) was observed in the digestive gland of mussels, and 5 in the gill filaments (2.73%) and in the gonads (3 copepodites; 1.64%). Histopathology showed pre-emergent stage of these parasites in the connective tissue of the digestive gland of two mussels.

The prevalence of adult *P. spinosus* in mussels from Florianópolis was significantly higher ($p<0.05$) compared to that in Governador Celso Ramos and Penha. Autumn showed a higher prevalence, significantly different ($p<0.05$) to the other seasons (Table 2). Infestation rates were not significantly different between sites or seasons (Table 2).

The prevalence of nauplius stage *P. spinosus* was significantly ($p<0.05$) higher in Penha compared to Governador Celso Ramos, Palhoça and Florianópolis. Analysing season, summer showed higher ($p<0.05$) prevalence compared to the other seasons (Table 2). Infestation rates were not significantly different between sites or seasons.

During the research period no histopathological evidence of damage to the host, macroscopic alteration or mussel mortality was observed.

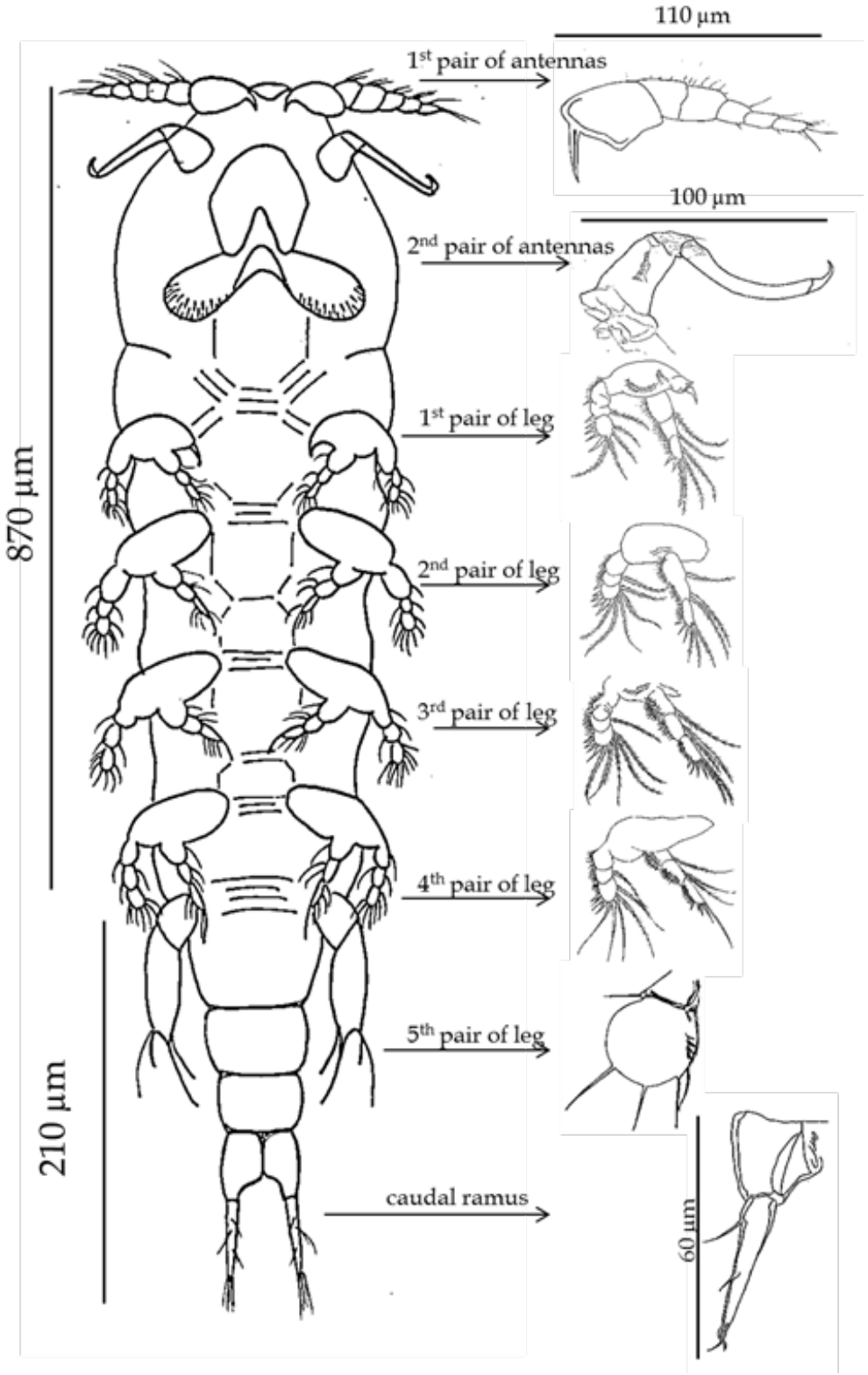


Figure 8. Schematic representation of female *Pseudomyicola spinosus* in ventral view, with details of the antennas (two pairs), legs (five pairs) and caudal ramus that characterize this copepod species.

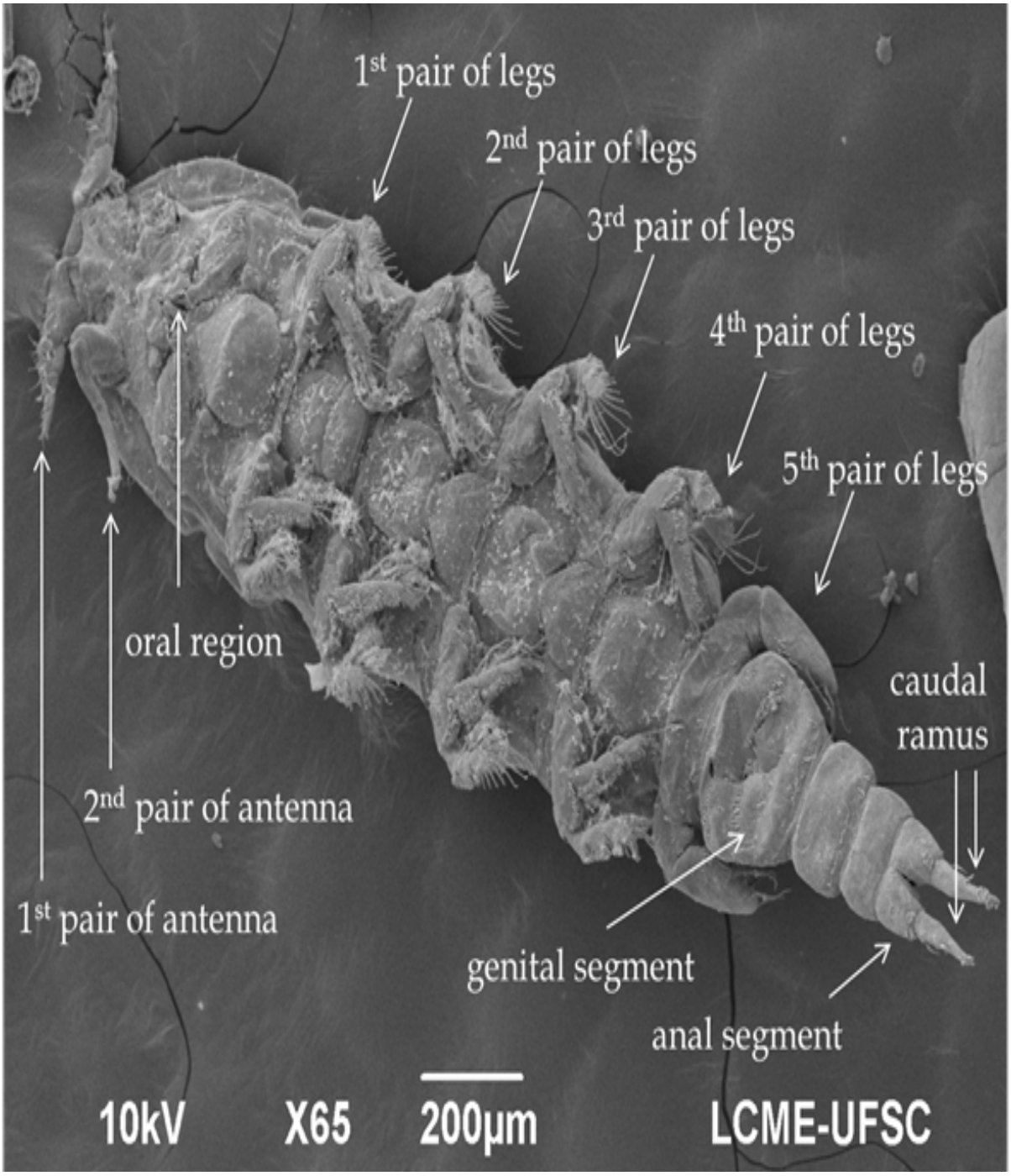
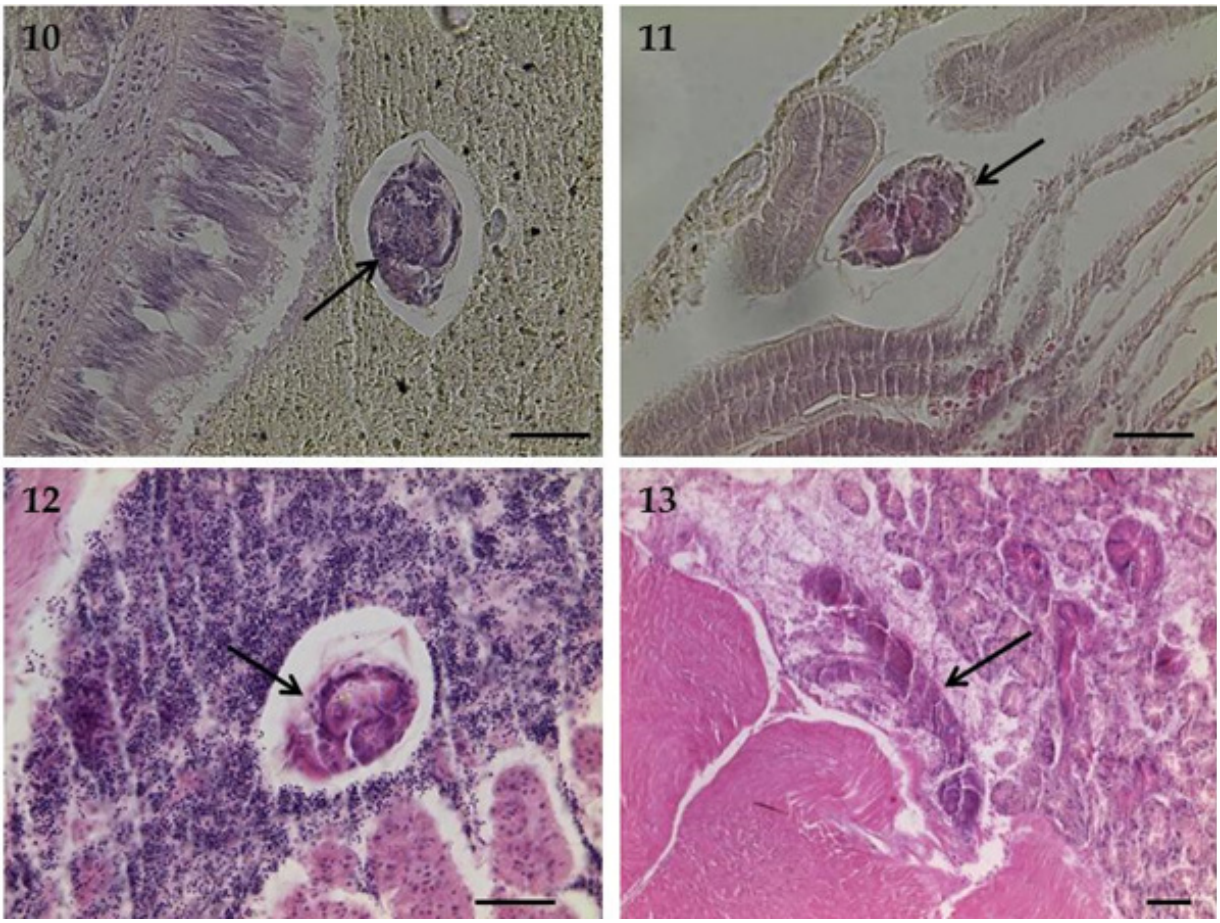


Figure 9. SEM photograph of *Pseudomyicola spinosus* female without egg sacs collected in cultured mussel *Perna perna* from Santa Catarina, Brazil. Ventral view with details of oral region, antennas (two pairs), legs (five pairs), caudal ramus and genital and anal segment that characterize this copepod species.



Figures 10 to 13. Nauplius of *Pseudomyicola spinosus* registered through the analysis of histology in cultured mussels *Perna perna* from Santa Catarina, Brazil. Fig. 10 – Nauplius in the stomach lumen; Fig. 11 – Nauplius between the gill filaments; Fig. 12 – Nauplius in gonadal tissue of male mussel; Fig. 13 – Copepod pre-emergent stage in the tissue of the digestive gland. Colour: HHE. Bars= 40 µm (Fig. 10 to 12) and 100 µm (Fig. 13).

DISCUSSION

This is the first report of *Pseudomyicola spinosus* infecting the mussel *Perna perna*. Copepod *P. spinosus* species identification was conducted through the analysis of histological sections, differential phase contrast microscopy (DIC) and scanning electron microscopy.

Specimens of *P. spinosus* observed in the mussel *P. perna* showed body divided into the prosome (cephalosome and metasome) and the urosome, as described by HUMES (1968) for *P. spinosus* species. According to HUMES (1968) and observation of specimens of *P. spinosus* in the present study, the first pair of antennae or antennules, with six segments, as well as the second pair is located in the cephalosome. The second pair of antennae has three segments, where the first segment has from 12 to 20 spikes. In this part of the body they are also located the jaw, first and second maxilla and maxilliped. The five pairs of legs are located in the metasome and consist of endopods and exopods, with adornments of differing numbers of thorns and bristles (Table 3), except for the fifth pair of legs that has a sub-circular distal segment. The urosome consists of the genital segment, the first and second abdominal segments, the anal segment and the caudal ramus. The female is distinguished by carrying two bags of dorsolateral eggs.

The main hosts group of copepod parasites are invertebrates (HUMES, 1994; KIM, 2004; HUYS *et al.*, 2006). Most copepods belong to the Poecilostomatoida order, which includes *P. spinosus* from the Myicolidae family.

Histopatholgy in the present study showed adults stage *P. spinosus* in the mantle cavity of the mussels *P. perna* and nauplius stage in the digestive gland, gonad and gills.

Adult copepods at high infestation rates have also been observed in the oyster *Saccrostrea glomerata* from New Zealand (DINAMANI and GORDON, 1974); in the clams *Anadara obesa* from Panama (HUMES, 1984) and *Austroventus stutchburyi* from New Zealand (LEUNG and POULIN, 2007); in the mussels *Mytilus galloprovincialis* from Mexico (CÁCERES-MARTÍNEZ *et al.*, 1996; OLIVAS-VALDEZ and CÁCERES-MARTÍNEZ, 2002) and *Mytilus californianus* (CÁCERES-MARTÍNEZ *et al.*, 1996); and in the scallop *Argopecten ventricosus* from Mexico (CÁCERES-MARTÍNEZ *et al.*, 2005). In Brazil, SABRY *et al.* (2011) observed a copepod possibly belonging to the *Pseudomyicola* genus in the stomach of cultured *Crassostrea rhizophorae* oysters.

The higher incidence of adult females of *P. spinosus* in the current research coincides with the results of DINAMANI and GORDON (1974) in *S. glomerata*, New Zealand; DO and KAJIHARA (1986) in *Mytilus edulis*, Japan; and CÁCERES-MARTÍNEZ and VÁSQUEZ-YEOMANS (1997) in *M. galloprovincialis* and *M. californianus*, in Mexico.

Relation between host size and copepod infestation was not observed in the present study. However, relationship between larger host size and higher infestation rate of adults and nauplius copepod stages was reported by CÁCERES-MARTÍNEZ *et al.* (1996) in *M. galloprovincialis* and *M. californianus* and OLIVAS-VALDEZ and CÁCERES-MARTÍNEZ (2002) in *M. galloprovincialis*. GOATER and WEBER (1996) recorded a positive association between abundance of copepods *Mytilicola orientalis* and size, age, filtration rate and tolerance of the host *Mytilus trossulus*. This relationship was also noted by GEE and DAVEY (1986) for *Mytilicola intestinalis* infecting *M. edulis* on European coasts.

Even in situations of higher prevalence and infestation observed in the present study, no histopathological evidence of damage to host was found. Externally, the copepod can cause erosions and hemolymph spill. Inside the tissue the copepod *M. intestinalis* can cause hemocyte infiltration (FIGUERAS *et al.*, 1991) and obstruction of the intestine (ROBLEDO *et al.*, 1994) in *M. galloprovincialis*. The copepod *P. spinosus* was also found attached to the walls of the gut, stomach and digestive diverticulum causing obstruction in *M. galloprovincialis* (OLIVAS-VALDEZ and CÁCERES-MARTÍNEZ, 2002).

Highly infected *P. perna* mussels with adult and nauplius copepods did not show evidence of increased mortality, indicating balance in the copepod-host relationship. According to CHENG (1966), high infestation of *P. spinosus* in the gills can cause extensive weight loss and mortality of the mussels. However, other authors suggest that massive mortality in mussels parasitized by *P. spinosus* is uncertain for *M. edulis* in the North Sea (DAVEY, 1989) and in *M. galloprovincialis* and *M. californianus* in Mexico (CÁCERES-MARTÍNEZ and VÁSQUEZ-YEOMANS, 1997). Mortality in cultured *M. galloprovincialis* has been attributed to the parasite *M. intestinalis* in the Netherlands (KORRINGA, 1950), Canada (LI and CLYBURNE, 1979) and Italy (MUNFORD *et al.*, 1981).

According to DOS SANTOS and COIMBRA (1995), bivalve molluscs can have a symbiotic relationship with some predators, competitors or parasites that can cause gonadal damage and significant decrease in production when grown in high concentrations.

Although no changes were observed in the tissues of *P. perna* mussels parasitized by the copepod, CÁCERES-MARTÍNEZ *et al.* (1996, 2005), consider *P. spinosus* a facultative parasite that can be harmful to mussel farms.

CONCLUSIONS

Copepods adults in the mantle cavity and nauplius in the digestive gland, gonad and gill of the species *Pseudomyicola spinosus*, were observed parasitizing *Perna perna* mussels cultured at southern Brazil, without causing harm to the host.

Nauplius stage in *P. perna* showed high prevalence in Penha and high infestation in Palhoça. Aduld stage showed high prevalence and infestation in Florianópolis.

Summer showed high prevalence of nauplius and autumn of adults of *P. spinosus* in *P. perna*.

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REFERENCES

BELL, T.A.; LIGHTNER, D.V. 1988 *A handbook of normal penaeid shrimp histology*. Baton Rouge, World Aquaculture Society. 114p.

BUSH, A.O.; LAFFERTY, A.D.; LOTZ, J.M.; SHOSTAK, A.W. 1997 Parasitology meets ecology on its own terms: Margolis *et al.*, revisited. *Journal of Parasitology, 83*(4): 575-583.

CÁCERES-MARTINEZ, J.; VÁSQUEZ-YEOMANS, R. 1997 Presence and histopathological effects of the copepod *Pseudomyicola spinosus* in *Mytilus galloprovincialis* and *Mytilus californianus*. *Journal Invertebrate Pathology, 70*: 150-155.

CÁCERES-MARTINEZ, J.; VÁSQUEZ-YEOMANS, R. 1999 Metazoan parasites and pearls in coexisting mussel species: *Mytilus californicus, Mytilus galloprovincialis* and *Septifer bifurcatus*, from an exposed rocky shore in Baja California, NW México. *Veliger, 42*(1): 10-16.

CÁCERES-MARTÍNEZ, C.; CHÁVEZ-VILLALBA, J.; GARDUÑO-MÉNDEZ, L. 2005 First record of *Pseudomyicola spinosus* in *Argopecten ventricosus* in Baja California, Mexico. *Journal of Invertebrate Pathology, 89*(2): 95-10.

CÁCERES-MARTINEZ, J.; VÁSQUEZ-YEOMANS, R.; SUÁREZ-MORALES, E. 1996 Two parasitic copepods *Pseudomyicola spinosus* and *Modiolicola gracilis*, associated with the edible mussels, *Mytilus galloprovincialis* and *Mytilus californianus*, from Baja California, NW. México. *Journal Shellfish Research, 15*(3): 667-672.

CARNEIRO-SCHAEFER, A.L.; SÜHNEL, S.; MELO, C.M.R., MAGALHÃES, A.R.M. 2017 Estudo patológico em mexilhões cultivados em Santa Catarina, Brasil. *Boletim do Instituto de Pesca, 43*(1): 124-134.

CHENG, T.C. 1966 The coracidium of the cestode Tylocephalum and the migration and fate of this parasite in the american oyster, *Crassostrea virginica*. *Transactions of the American Microscopical Society, 85*(2): 246-255.

DAVEY, J.T. 1989 *Mytilicola intestinalis* (Copepoda: Cyclopoida): A ten year survey of infested mussels in a Cornish estuary, 1979–1988. *Journal of the Marine Biological Association of the United Kingdom, 69*: 823-826.

DAWES, C.J. *Biological techniques in electron microscopy*. New York: Barnes & Noble, 1971. 193p.

DINAMANI, P.; GORDON, D.B. 1974 On the habits and nature of association of the copepod *Pseudomyicola spinosus* with the rock oyster *Crassostrea glomerata* in New Zealand. *Journal of Invertebrate Pathology, 24*: 305-310.

DO, T.T.; KAJIHARA, T. 1986 Studies on parasitic copepod fauna and biology of *Pseudomyicola spinosus*, associated with the blue mussel *Mytilus edulis galloprovincialis* in Japan. *Bulletin of Institute of Oceanic Research and Development Tokai University, 23*: 1-63.

DOS SANTOS, A.M.T.; COIMBRA, J. 1995 Growth and production of raft-cultured *Mytilus edulis* L., in Ria de Aveiro:gonad symbiotic infestation. *Aquaculture, 132*: 195-211.

DYKSTRA, M.J. 1993 *A manual of applied techniques for biological electron microscopy*. New York: Plenum Press. 258p.

FERREIRA JR, A.L.; CHRISTO, S.W.; ABSHER, T.M. 2015 First occurrence of *Pseudomyicola spinosus* in *Anadara ovalis* in the Paranaguá Estuarine Complex – Brazil. *Boletim do Instituto de Pesca, 41*(2): 449-456.

FIGUERAS, A.J.; JARDON, C.F.; CALDAS, J.R. 1991 Diseases and parasites of rafted mussels (*Mytilus galloprovincialis* LMK): preliminary results. *Aquaculture, 99*(1-2): 17-33.

GEE, F.M.; DAVEY, J.T. 1986 Experimental studies on the infestation of *Mytilus edulis* (L.) by Mytilicola intestinalis (Copepoda, Cyclopoidea). *Journal du Conseil Permanent International pour l'Exploration de la Mer, 42*(3): 265-271.

GOATER, C.P.; WEBER, A.E. 1996 Factors affecting the distribution and abundance of *Mytilicola orientalis* (Copepoda) in the mussel, *Mytilus trossulus*, in Barkley Sound, B.C. *Journal of Shellfish Research, 15*: 681-684.

HAYAT, M.A. 1972 *Basic electron microscopy techniques*.

New York: UNR. 119p.

HO, J.S. 1980 Origin and dispersal of *Mytilus edulis* in Japan deduced from its present status of copepod parasitism. *Publications of the Seto Marine Biological Laboratory, 5*(6): 293-313.

HOWARD, A.W.; SMITH, C.S. 1983 *Histological techniques for marine bivalve mollusks*. NOAA Technical Memorandum NMFS-F/NEC-25. Woods Hole, MA: Oxford Lab., National Marine Fisheries Service. 97p.

HOWARD, D.W., LEWIS, E.J., KELLER, B.J., SMITH, C.S. 2004 *Histological techniques for marine bivalve mollusks and crustaceans*. NOAA Technical Memorandum NOS NCCOS5. Oxford, MD: Oxford Lab. National Marine Fisheries Service. 218p.

HUMES, A.G. 1968 The cyclopoid copepod *Pseudomyicola spinosus* (Raffaele and Monticelli) from marine pelecypods, chiefly in Bermuda and the West Indies. *Beaufortia, 14* (178): 203-226.

HUMES, A.G. 1984 Ostrincola and Pseudomyicola (Crustacea, Copepoda, Poecilostomatoida) associated with marine bivalve mollusks on the Pacific coast of Panama. *Proceedings of The Biological Society of Washington, 97*(3): 589-600.

HUMES, A.G. 1994 How many copepods? Ecology and Morphology of Copepods. In: FERRARI, F.D.; BRADLEY, B.P. *Hydrobiologia*. Kluwer Academic Publishers. 292/293. p. 1-7.

HUMES, A.G.; GOODING, R.U. 1964 A method for studying the external anatomy of copepods. *Crustaceana, 6*: 238-240.

HUYS, R.; BOXSHALL, G.A. 1991 *Copepod evolution*. London: The Ray Society. 1.468p.

HUYS, R.; LLEWELLYN-HUGHES, J.; OLSON, P.D.; NAGASAWA, K. 2006 Small subunit rDNA and Bayesian inference reveal *Pectenophilus ornatus* (Copepoda incertae sedis) as highly transformed Mytilicolidae, and support assignment of Chondracanthidae and Xarifiidae to Lichomolgoidea (Cyclopoida). *Biological Journal of the Linnean Society, 87*: 403-425.

ITIS – Integrad Taxonomic Information System. Disponível em: < http://www.itis.gov > Acesso em 14 mar. 2016.

KAJIHARA, T.; NAKAMURA, K. 1985 Lifespan and ovoposition of the parasitic copepod *Pseudomyicola spinosus* under rearing conditions. *Marine Biology, 87*: 55-60.

KIM, I.H. 2004 Poecilostomatoid Copepods Associated with Bivalves in Korea and their Distribution. *Zoological Studies, 43*(2): 187-192.

KLEETON, G. 1964 Sur la présence de *Pseudomyicola spinosus* (Raff. & Mont.) (Crustacea, Copepoda) dans l'Atlantique, avec une note sur la synonymie de *P. spinosus* et P. glaber Pearse. *Beaufortia, 11*(45): 171-177.

KORRINGA, P. 1950 De aanval van de parasiet *Mytilicola intestinalis* op de Zeeuwse mosselcultuur. *Visserij-Nieuws, 3*(7): 1-7.

LEUNG, T.L.F.; POULIN, R. 2007 Interactions between parasites of the cockle *Austrovenus stutchburyi*: hitch-hikers, resident-cleaners, and habitat-facilitators. *Parasitology, 134*: 247-255.

LI, M.F.; CLYBURNE, S. 1979 Mortalities of blue mussel (*Mytilus edulis*) in Prince Edward Island. *Journal Invertebrate Pathology, 33*: 108-110.

MUNFORD, J.G.; DA ROS, L.; STRADA, R. 1981 A study on the mass mortality of mussels in the Laguna Veneta. *Journal of the World Mariculture Society, 12*(2): 186-l 99.

OLIVAS-VALDEZ, J.; CÁCERES-MARTÍNEZ, J. 2002 Infestation of the blue mussel *Mytilus galloprovincialis* by the copepod *Pseudomyicola spinosus* and its relations with size, density and condition index of the host. *Journal Invertebrate Pathology, 79*: 65-71.

PAULETE-VANRELL, J. 1967 *Guia de Técnica Microscópica*. Faculdade de Filosofia, Ciências e Letras de São Leopoldo, São Leopoldo. não paginado.

ROBLEDO, J.A.F.; SANTARÉM, M.M.; FIGUERAS, A. 1994 Parasites loads of rafted blue mussels (*Mytilus galloprovincialis*) in Spain with special reference to the copepod, *Mytilicola intestinalis*. *Aquaculture, 127*: 287-302.

SABRY, R.; DA SILVA, P.M.; HO J, BARRACCO, M.A.; MAGALHÃES, A.R.M. 2008 Ocorrência do copépode *Pseudomyicola spinosus* em espécies

de bivalves (*Carssostrea rhizophorae, Crassostrea gigas* e *Anomalocardia brasiliana*) da Ilha de Santa Catarina. In: ENCONTRO BRASILEIRO DE PATOLOGISTAS DE ORGANISMOS AQUÁTICOS, 10., Búzios, 17-20 nov./2008. *Anais...* Búzios: Associação Brasileira de Patologistas de Organismos Aquáticos. 1 CD-ROM.

SABRY, R.; DA SILVA, P.M.; GESTEIRA, T.C.V.; PONTINHA, V.A.; MAGALHÃES, A.R.M. 2011 Pathological study of oysters *Crassostrea gigas* from culture and *C. rhizophorae* from natural stock of Santa Catarina Island, SC, Brazil. *Aquaculture, 60*: 43-50.

STOCK, J.H. 1965 On Copepoda associated with Dutch molluscs. *Basteria, 1*(4): 65-71.

TURNER, J.T. 2004 The importance of small planktonic copepods and their roles in pelagic marine food webs. *Zoological Studies, 43*(2): 255-266.

WESTFALL, P.H.; TOBIAS, R.D.; ROM, D.; WOLFINGER, R.D.; HOCHBERG, Y. 1999 *Multiple Comparisons and Multiple Tests using the SAS® System*. SAS Institute, Cary. 397p.