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Relationship between reproductive cycle of *Anomalocardia brasiliana* (Mollusca: Veneridae) and the suspended particulate matter in the Paranaguá Estuarine Complex, Brazil

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Abstract The aim of the present work was to analyze the reproductive cycle of *Anomalocardia brasiliana* and its relationships with the concentrations of suspended particulate matter (SPM) in the euhaline portion of Paranaguá Estuarine Complex (PEC). Sampling was performed in spring low tide, between September 2008 and August 2009, in the intertidal plain. Twenty seven individuals were manually collected monthly at three levels. In laboratory individuals were cleaned and measured. Sex identification, gonadal maturation stage (GMS), condition index (CI), meat yield (MY) and the daily food index (FCI) were determined. Water samples were collected from August 2008 to July 2009 to characterize the concentration of SPM. Fluorescence (Clorophyll-*a)* was recorded in the same period. The indices showed magnitudes of 3.49 ± 1.04 % for CI; 23.60 ± 5.64 % for MY and 0.009 ± 0.004 mg L^{-1} for FCI. The SPM ranged from 0.03 to 0.41 mg.L^{-1} in the adjacent water and fluorescence from 0.09 to 17.05 µg.L^{-1} . The annual continuous reproduction of the species in the area can be confirmed, with difference in relation to levels of occurrence. There was an increase of SPM and reproductive cycle of *A. brasiliana* during spring, summer and autumn.

Keywords: bivalve, infauna, feeding, reproduction, South Atlantic.

Resumo. **Relação entre o período reprodutivo de *Anomalocardia brasiliana* (Mollusca: Veneridae) e a matéria particulada em suspensão no Complexo Estuarino de Paranaguá, Brasil**. O objetivo do presente trabalho foi analisar o período reprodutivo de *Anomalocardia brasiliana*, relacionando com as concentrações de material particulado em suspensão (MPS) na porção euhalina do Complexo Estuarino de Paranaguá (CEP). Amostragens foram realizadas em três níveis intermareais, entre setembro de 2008 a agosto de 2009. Vinte e sete indivíduos foram manualmente coletados mensalmente em cada nível. Em laboratório, os indivíduos foram limpos e medidos. Determinou-se a identificação do sexo, estágio de maturação gonadal (EMG), índice de condição (IC), índice de rendimento da carne (IR) e índice diário de alimento (IDA). Para caracterizar a concentração de MPS foram coletadas amostras de água de agosto de 2008 a julho de 2009. Dados de fluorescência (clorofila-a) foram obtidos durante o mesmo periodo. Os índices variaram de 3,49 ± 1,04 para IC; 23,60 ± 5,64 para IR e 0,009 ± 0,004

mg L^{-1} para a IDA. O MPS variou de 0,03 a 0,41 $mg.L^{-1}$ na água adjacente e a fluorescência variou de 0,09 a 17,05 $\mu g.L^{-1}$. Os índices reprodutivos indicaram contínuos estágios reprodutivos da espécie no CEP, com diferença entre os níveis de ocorrência. Houve aumento de MPS e ciclo reprodutivo de *A. brasiliana* durante a primavera, verão e outono.

Palavras chaves: bivalve, infaunal, alimentação, reprodução, Atlântico sul

Introduction

The infaunal clam *Anomalocardia brasiliana* lives at the intertidal region of bays and inlets, buried in sandy or sandy-muddy in calm waters (predominantly down to 10 cm depth). Reproductive cycle is continuous throughout the year in most of the Brazilian coast (Grotta & Lunetta 1980, Boehs 2000, Barreira & Araújo 2005). However Araújo (2001) in a study at "Reserva Extrativista Marinha de Pirajubaé", Santa Catarina, found a period of reproductive rest in winter. The commercial exploitation of these clams by coastal populations is an important economic source in this region. Intensive exploitation of the natural banks, based mainly on extraction, threatens the natural stocks due to overfishing. The possibility of increasing production requires protective measures of natural stocks in conjunction with cultivation activities.

In order to achieve the appropriate cultivation of this species it is necessary to study the concentration and distribution of the suspended particulate matter in the water, since this species does not have great filtration ability to select particulate matter (Rasgalla-Jr & Piovezan 2009, Navarro *et al.* 2010). This kind of studies would indicate the appropriate locations for the installation of marine farms, without affecting the sustainability of natural banks, thus aiming at sustainable aquaculture. Another important point of study is the characterization of the appropriate period for the collection of breeders used in laboratory maturation for optimization of the use of microalgae in feeding of those individuals since the cultivation of microalgal biomass represents 40% of the total cost of seed production (Laing & Helm 1981).

The present study has the following objectives: 1. Analyze possible differences in the reproductive period of *A. brasiliana* in relation to three levels of the intertidal plain of Rasa da Cotinga Island on Paraná coast; 2. Relate the concentrations of suspended particulate matter with the reproductive cycle of *A. brasiliana* in the euhaline portion of Paranaguá Estuarine Complex.

Material and methods

Study area: Paraná coast, located in southern Brazil, extends from the Ararapira village in the north (25°12'44"S, 48º01'15"W) to Saí-Guaçu river mouth in the south (25º58'38"S, 48º35'26"W), comprise two main estuaries: Paranaguá Estuarine Complex and Guaratuba Bay (Fig. 1), Paranaguá Estuarine Complex (PEC) is the largest estuarine system in the state of Paraná. To east is influenced by the Atlantic Ocean through North and Southeast mouth and is surrounded by hydrographic basins with about 3,361 Km^2 (Maack 1981), comprise four bays: Laranjeiras and Pinheiros at the north-south axis and Antonina and Paranaguá in the east-west axis (Noernberg *et al.* 2006). Rasa da Cotinga Island (25 º32'S, 48°24'W) is located in the euhaline portion, has elongated shape oriented northwest-southeast, displays a slope of 0.28 $cm.m^{-1}$ and a sandy-muddy sediment, with a predominance of very fine sand (Boehs 2000). Due to these characteristics and to a lower anthropogenic influence Rasa da Cotinga Island was the site chosen for the implementation of this study (Fig. 1).

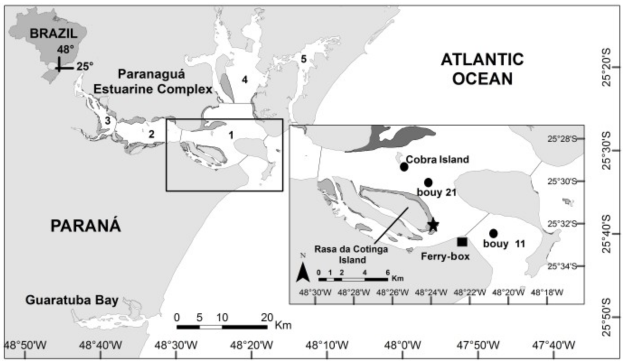

Figure 1 - Location of the sampling sites: sampling of *Anomalocardia brasiliana* in intertidal plain (★); sampling of suspended particulate matter and Clorophyll-*a* (●) and Ferry-box station (■). 1-Mixture Bay; 2-Paranaguá Bay; 3- Antonina Bay; 4- Laranjeiras Bay; 5- Pinheiros Bay (Noernberg *et al.* 2006).

Sampling methodology: Samples were collected between August 2008 and July 2009. Three transects were established in the mesolittoral at a distance of 500 m, from the interface of the salt marsh to the lower limit of the plain (400m in length) in low spring tide. In each transect three levels (upper-U, middle-M and lower-L) were defined based on the gradual increase of desiccation period which causes a gradual decrease in the period of filtering by the organisms (Fig. 2).

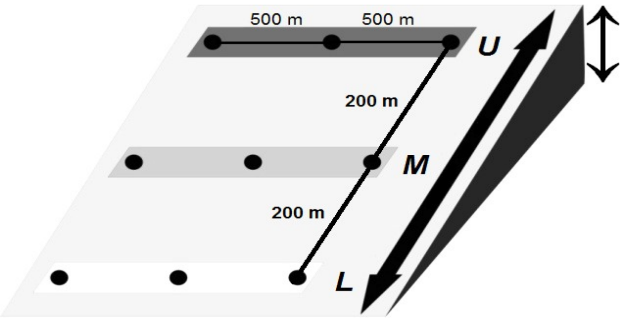

Figure 2 - Schematic drawing of sampling: sites: (●) three transects of 500 m and three levels (upper-U, middle-M and lower-L), in low spring tide (⟷); ↕- slope of the intertidal plain of 0.28 cm.m^{-1} (Boehs, 2000).

At each level of each transect 27 individuals (≥ 20 mm) were manually collected on a monthly basis. For sediment characterization samples were taken in three different months (September 2008, April 2009 and June 2009) at each level. All samples were transported in insulated box to the Laboratory of Marine Mollusks/CEM and immediately frozen until processing. Samples of water for analysis of suspended particulate matter (*SPM*) were taken in surface water of the region immediately below the lower end of the transect, and in front of them in the same day of biological sampling, and stored in cooler with ice until arrival at the laboratory. These samplings started one month after the biological collections (September 2008) and ended a month after (July 2009). Data from Microbiology Laboratory/CEM (unpublished) were also used to characterize the variation of the *SPM* at two points located near Rasa da Cotinga Island (Cobras Island and Channel buoy 21) and a third point, which lies at the south mouth of PEC (Channel buoy 11) during the years 1999 to 2006 (Fig. 1). A bibliographical research was also made of *SPM* variation in euhaline portion of PEC. Fluorescence, temperature and salinity of water, between August 2008 and July 2009, were performed by a Scufa sensor (Turner Designs) and provided by a monitoring database from a stationary Ferry-box, located at the south outlet of PEC (Fig. 1).

Sample and data processing: In laboratory each *A. brasiliana* was cleaned and weighed (*Wg* - gross weight = weight of the shell and meat), using a scale with a precision of 0.001g, opened by sectioning the adductor muscles and then eviscerated. After removing the shell, soft tissues were weighed to obtain the wet weight (*Wmw* - wet weight of meat). Wet weight of shell (*Wsw*) was obtained by subtracting meat wet weight from the gross weight. The gonadal maturation stage (GMS) was determined using a stereoscopic microscope and classified in five levels: empty (E: absence of gonad covering the digestive gland); partially empty (PE: with gonad covering 1/3 of the digestive gland); partially full (PF: with gonad covering 2/3 of the digestive gland); full (F: with gonad covering the whole digestive gland) and undetermined (U) (Christo & Absher 2006). After this process a small portion of the gonadal product was removed by puncture, placed on a slide and examined under optical microscope for sex identification.

Thereafter, the soft parts were oven-dried at 60°C until constant weight (24 hours) to obtain meat dry weight (*Wmd* - dry weight of the meat). These weights (*Wg, Wsw, Wmw* and *Wmd*) were used to calculate condition index (*CI*) and meat yield (*MY*) of individuals by the following equations (Absher & Christo 1993):

$$CI = \left(\frac{Wmd}{Wsw}\right) * 100$$

$$MY = \left(\frac{Wmw}{Wg}\right) * 100$$

To determine the size of individuals in reproductive activity length (L), height (H) and width (W) of shell were measured using a caliper (precision of 0.5 mm).

The index of calculation of food by individuals per day (*FCI*) proposed here was adapted from the cells volume of Helm *et al.* (2006), for the weight of 1 million cells of microalgae *Chaetoceros muelleri* and *Isochrysis galbana* at 50:50 ratio.

For *SPM*, 1 liter of water of each sample was analyzed by filtration with Whatman GF/C (47 mm) filters according to the methodology of Niencheski *et al.* (2006). To determine the possible density of adults (*D*) feeding in the available particulate matter, a ratio between the particulate suspended matter (*SPM*) and the index of calculation of food per day (*FCI*) was calculated. These two variables (*FCI* and *D*) are described according to the following equations:

$$FCI = \frac{a * Wmd}{100}$$

$$D = \left(\frac{SPM}{FCI} * b * c\right) * 100$$

Where *FCI* is the breeders food calculation index (mg.l^{-1}); *a* - coefficient of the amount of daily ration that an individual would need, feeding with a ration of 3% of the biomass of organism, *a* = 3; *Wmd* - dry weight of meat of the organisms; *D* - density of adult organisms.m^{-2}; *SPM* - suspended particulate matter (mg.l^{-1}); *b* - coefficient of the natural density of *Anomalocardia brasiliana* in intertidal plain of Rasa da Continga Island, *b* = 0.3 (Boehs *et al.* 2004); *c* - coefficient of the percentage of particulate organic matter in *SPM*, *c* = 0.25 (Table I).

Granulometric analysis followed methodology described by Suguio (1973), organic matter and carbonate content followed methodology of Gross (1971). Granulometric values were analyzed using SYSGRAN 3.0 program and ranked on Φ (phi) scale.

Relative frequency (Rf) was used to characterize sex and GMS. A bi-factorial ANOVA for repeated measures (sample and level) was used to show potential differences in characteristics (*CI*, *MY* and GMS) of the levels in intertidal plain and post-hoc Student-Newman-Keuls (SNK) was performed to identify possible differences. Principal Component Analysis was performed to identify relationships among the environmental parameters (fluorescence, suspended particulate matter, grain size, percentage gravel and organic matter of the sediment), indexes (*CI* and *FCI*), levels of the intertidal plain (upper, middle and lower) and months (September 2008; April 2009 and July 2009).

Results

Throughout the study period an average of 11.12 ± 3.63 g was verified for weight "*in natura*", 29.05 ± 3.71 mm for length, 24.30 ± 3.00 mm for height and 20.51 ± 2.98 mm for width. A sexual relationship of 1: 1.65 (972 females-Fe, 1,606 males-M and 95 indeterminate, 2,673 individuals analyzed) was found.

Reproductive differences: The condition index (*CI*) was 3.49 ± 1.04 %, and meat yield (*MY*) was 23.60 ± 5.64 %. A pattern of stratification for this two indexes was identified differences between levels only occurred in November 2008, January and June 2009 for *CI* ($F = 17.06$, $p < 0.001$) and September and November 2008, January, May and June of 2009 for *MY* ($F = 40.92$, $p < 0.001$) (Figs. 3 - 4).

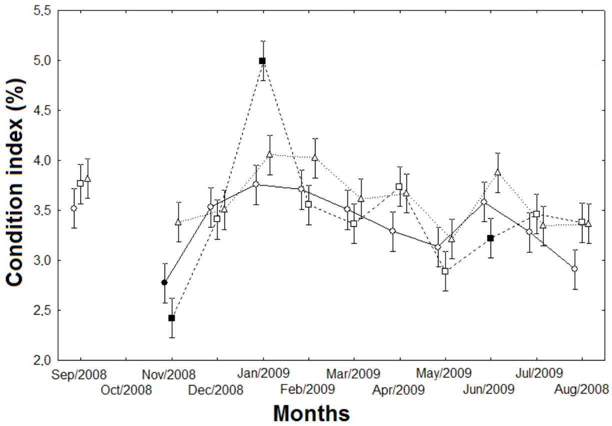

Figure 3 - Variation of average Condition Index (*CI*) of *Anomalocardia brasiliana* at three levels in the intertidal plain of Rasa da Cotinga Island Paranaguá Estuarine Complex - Paraná, Brazil. - mean and standard deviation of the upper level; - mean and standard deviation of the middle level; - mean and standard deviation of the lower level; - significant difference (α = 0.05).

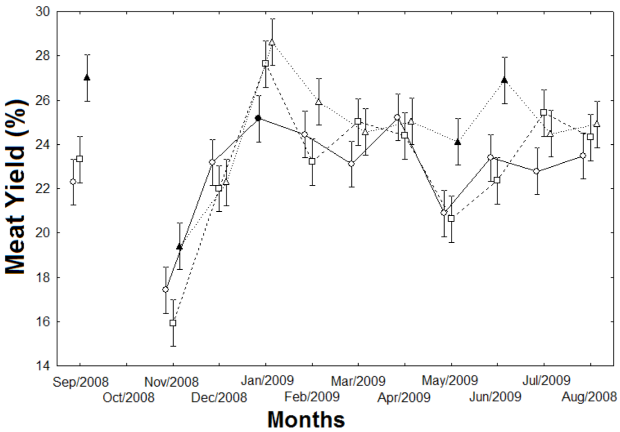

Figure 4 - Variation of average meat yield (*MY*) of *Anomalocardia brasiliana* at three levels in the intertidal plain of Rasa da Cotinga Island Paranaguá Estuarine Complex - Paraná, Brazil. - mean and standard deviation of the upper level; - mean and standard deviation of the middle level; - mean and standard deviation of the lower level; - significant difference (α = 0.05).

The GMS demonstrated the occurrence of gonads in stages Full (23.87%), P. Full (42.01%), P. Empty (30.86%), Empty (2.99%) and Undeterminate (0.26%). Spatial variation was similar at all three levels, results that indicate a continuous reproduction with three most significant periods in September 2008, January and April 2009 ($F = 4.783$, $p < 0.001$). However, when comparing the levels, the upper level tended to have lower values of gonadal repletion, even though with no significant difference among them ($F = 0.661$, $p = 0.52$) (Fig. 5).

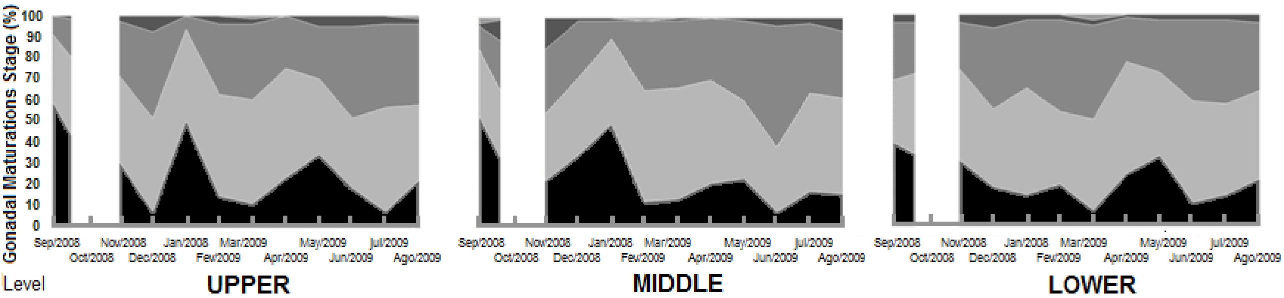

Figure 5 - Frequency of Gonadal Maturation Stages (GMS) of *Anomalocardia brasiliana* at three levels in the intertidal plain of Rasa da Cotinga Island - Paranaguá Estuarine Complex - Paraná, Brazil. ■ F: full; PF: partially full; PE: partially empty; E: empty; U: undetermined.

Environmental and biological interactions: Regarding the *FCI* of organisms, there was an average of 0.009 ± 0.004 mg.L^{-1}, with a predominance of organisms requiring higher concentration of food in the upper level of the plain (Fig. 6).

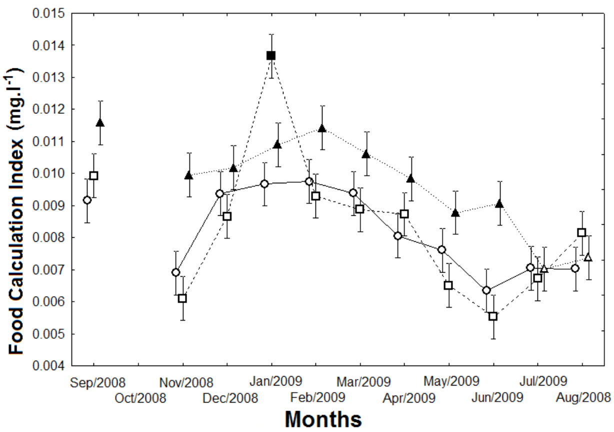

Figure 6 - Variation of average Food Calculation Index (*FCI*) of *Anomalocardia brasiliana* at three levels in the intertidal plain of Rasa da Cotinga Island- Paranaguá Estuarine Complex - Paraná, Brazil. - mean and standard deviation of the upper level; - mean and standard deviation of the middle level; - mean and standard deviation of the lower level; ●;■;▲ - significant difference (α = 0.05).

Variation of SPM was 0.03 to 0.41 mg.L^{-1} in adjacent waters to the intertidal plain of Rasa da Cotinga Island, 1.02 to 131.74 mg.L^{-1} in the other points (Cobras Island, Channel buoy 11 and 21) and from 0.09 to 17.05 µg.L^{-1} of fluorescence in water at Ferry-box station (Table I).

Bibliographical research indicated concentration ranged from 1.18 to 221 mg.L^{-1} for *SPM* and 0.38 to 68 µg.L^{-1} for Clorophyll-*a* (Cl-*a*) in various portions of the system and environments of PEC between 1981 and 2006 (Table I).

The estimated densities of adult organisms varied from 16 to 371 specimens.m^{-2}. This estimate indicates densities with values above 250 individuals in December 2008, January, March, April and July 2009 (Fig. 7).

Salinity ranged from 11.85 to 34.12, with higher values in winter months (August 2008, June and July 2009) and water and air temperatures ranged from 19.37 to 28.77°C and 20.28 to 30.75°C, respectively (Fig. 8).

Results of Principal Component Analysis demonstrated that the individuals are feeding (*FCI*) more in April 2009 when temperature, suspended particulate matter and fluorescence values are higher for all levels of the intertidal plain. Characteristics of the sediment (grain size and organic matter percentage), of the condition indexes (*CI*) and of the organisms demonstrated higher relationships during September 2008 in the middle and lower levels. The salinity showed higher values in June 2009 (Fig. 9).

Discussion

Studies of the reproductive cycle of *A. brasiliana* in various regions of Brazil show a continuous reproduction of the species throughout the year. In low latitudes, as in Ceará (Brazil), Barreira & Araújo (2005) verified this type of reproduction with no more significant reproductive periods. Similar results were also observed on the coast of Paraiba. However, in São Paulo coast there are two most significant periods of reproduction - in the spring (February to April) and autumn (September to November) (Grotta & Lunetta, 1982). In this study, using the *CI* and *MY* there is indication of a likely ongoing cycle with periods of increased activity in the spring, summer and fall. These results coincide with those of Boehs (2000), except for higher reproductive data observed in this study in the summer period (January/2008). However the lowest values of *CI* were observed at the upper level. This may be related to the quality and/or quantity of phytoplankton and microphytobenthos at and a higher period of exposure to desiccation (Fonseca *et al.* 2008), which in long term decrease filtration time and hence reduce the intake of energy for the gametogenic processes (Nascimento & Perreira 2004).

Table I - Concentration of Chl-*a,* tendency of water fluorescence (Flu), concentration of suspended particulate matter (SPM) and concentration of particulate organic matter (POM) in the Paranaguá Estuarine Complex - Paraná, Brazil. * Laranjeiras Bay; ** < Suspended matter< 300 µm (N = 10), corresponding to 85.9% (SD: ± 8.8) SPM; *** units of values transformed from $mg.m^{-3}$ to $1000µg.1000L^{-1}=µg.L^{-1}$; **** concentration of particulate organic carbon; & maximum value of work; && daily value.

Reference	Location	N	Chl-*a* ($µg.l^{-1}$)	Flu ($µg.l^{-1}$)	SPM ($mg.l^{-1}$)	POM ($mg.l^{-1}$)
Knopers & Optiz 1984	G. Guananditubа *	14	5.43-15.05		13.5–45.00	0.6–2.0 ****
Brandini *et al.*1988	Axis W-E of PEC	14	0.75-24.6		3.84–51.96**	
Rebelo & Brandini 1990	Two points in PEC	7	0.38-6.75 ***		2.76–34.32	0.57–1.77 ****
Brandini & Thamm 1994 ***	Two points in PEC	22	13.23 & (1.0-7.2) &&		3.85–51.96	
Machado *et al.* 1997	Axis W-E of PEC	13	0.4–49.0		1.7–221	0.2–4.0 ****
Fernandes *et al.*1999	Outlet of the south (OS)	12	0.06–3.27 $mg.cm^{-2}$			
Brandini *et al.*2001	Outlet of the south (OS)	12	1.4-11			
Kolm *et al.* 2002	Axis W-E of PEC	6			13.32–30.78	1.37–5.65 ****
Siqueira & Kolm 2005	Gamboa of Maciel	13			3.78–125.95	2.04–55.86
Mafra Junior *et al.*2006	Axis W-E of PEC	7	0.6–68.0			
Kolm & Absher 2008	Five points in PEC	28			1.18–65.13	
Kolm & Nowicki 2011	Gamboa of Maciel	2	0.42–1.83		7.14 – 86.23	1.83–81.45
THIS STUDY	Cobras Island	4	4.20–16.70		25.58–127.06	
	Channel buoy 11	13	1.90–10.80		1.04–118.56	
	Channel buoy 21	12			1.02–131.74	
	Ferry-box (OS)	28		0.09–10.95		
	Rasa da Cotinga Island	10			0.03–0.41	

Table II - Sedimentological characterization of substrate of the intertidal plain of Rasa da Cotinga Island - Paranaguá Estuarine Complex - Paraná, Brazil. $CaCO_3$: calcium carbonate content; OM: organic matter content, Ca: gravel content; Fi: fines content; GS: grain size; SG: selection of grain, SD: standard deviation.

Months	LEVEL	$CaCO_3$ (SD)	OM (SD)	Ca (SD)	Fi (SD)	GS (SD)	SG (SD)
Sep-2008	Upper	4.58 (±3.99)	3.38 (±1.91)	0.40 (±0.47)	9.06 (±0.65)	3.14 (±0.10)	1.07 (±0.01)
	Middle	5.27 (±3.85)	3.45 (±1.62)	1.16 (±1.51)	9.76 (±1.71)	3.15 (±0.02)	1.09 (±0.03)
	Lower	4.97 (±2.71)	1.05 (±0.56)	1.43 (±1.83)	7.44 (±2.62)	3.06 (±0.08)	0.87 (±0.42)
Apr-2009	Upper	6.19 (±1.70)	4.79 (±6.15)	0.66 (±0.67)	10.68 (±4.69)	3.24 (±0.15)	0.87 (±0.29)
	Middle	2.75 (±1.16)	2.63 (±2.03)	0.83 (±0.38)	9.63 (±1.67)	3.13 (±0.10)	1.08 (±0.03)
	Lower	4.64 (±2.47)	2.32 (±0.53)	0.28 (±0.11)	8.54 (±1.34)	3.06 (±0.00)	1.06 (±0.01)
Jun-2009	Upper	3.28 (±0.28)	3.21 (±1.12)	4.38 (±6.97)	8.94 (±6.02)	3.15 (±0.23)	1.04 (±0.12)
	Middle	4.75 (±1.58)	2.67 (±0.94)	0.87 (±0.93)	9.16 (±3.48)	3.09 (±0.08)	1.09 (±0.04)
	Lower	7.02 (±4.57)	3.18 (±1.64)	0.43 (±0.53)	10.33 (±0.94)	3.10 (±0.04)	1.09 (±0.01)

In relation of GMS there was a predominance of full and developing gonads. Regarding the annual variation was possible to identify the same features for all three levels, indicating a likely continuous cycle with peak periods in spring (September 2008), summer (January 2009) and autumn (April 2009). This predominance of partial stages of GMS may be related to the absence in *A. brasiliana* of an interval of rest in reproductive activity, this is, gonadal restructuring resume soon after spawning (Grotta & Lunetta 1980).

Larger individuals were observed in the upper level (mode exceeding 30 mm in length). This may be related to the type of distribution that each level has, being a uniform distribution at the upper level and middle and lower level with aggregated distributions (Boehs 2000).

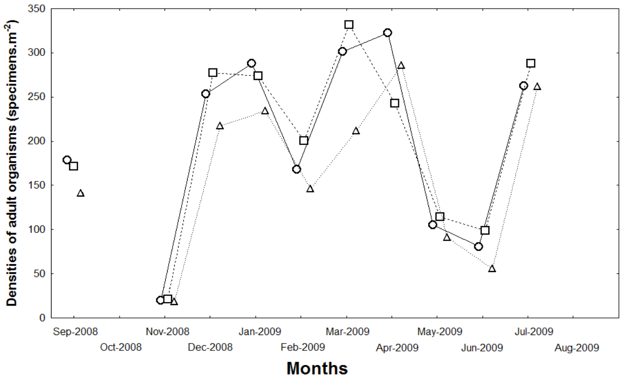

Figure 7 - Variation of estimated densities of adult organisms.m^{-2} (*D*) of *Anomalocardia brasiliana* at three levels in the intertidal plain of Rasa da Cotinga Island-Paranaguá Estuarine Complex - Paraná, Brazil. Δ- mean of the upper level; □- mean of the middle level; ○- mean of the lower level.

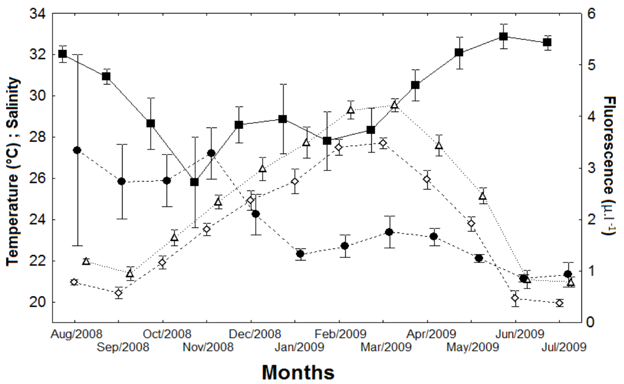

Figure 8 - Variation of environmental parameters of the Ferry-box at the outlet of the south Paranaguá Estuarine Complex - Paraná, Brazil. ○ - average of water temperature; Δ - Mean of air temperature; ■ - Average of salinity; ● - Mean water fluorescence. ⌶ - Variations of the parameters.

The distribution observed may be related to the natural self-limiting ability of the species with intra-specific competition, thereby causing a decrease in the size of the individuals in regions with higher densities (Monti *et al.* 1991). The uniform distribution observed at the top level indicates that this place would be more appropriate for recruitment due to the smallest density of adults and to the decrease in competition for food among adults and juveniles, favoring the youths' feeding, because smaller organisms have less capacity of filtration (Monti *et al.* 1991, Pezzuto & Echternacht 1999, Boehs *et al.* 2004, 2008). This difference in the feeding rate may represent a decrease in the intra-specific competition of the species, thus confirming the type of distribution found by Boehs (2000). Individuals with size range from 20 to 30 mm in length are the main contributors of gametes for the population and individuals larger than 30 mm have a lower fertility rate (Boehs 2000). Thus it can be said that the lower and middle levels have the greatest contribution of gamete in the non vegetated intertidal plain and the upper level with less reproductive contribution because this region has older individuals and juveniles.

The estimated densities (*D*) ranged from 16 to 371 specimens.m^{-2}, and this oscillation is associated with the primary production variations during the year. A review of works by Monti *et al.* (1991), Pezzuto & Echternacht (1999), Boehs *et al.* (2004, 2008) of population densities of *A. brasiliana* in natural environment values similar to those estimated in this study were observed.

Among the factors affecting the reproductive period of bivalve molluscs in the natural environment we can highlight the amount and composition of SPM (Montes-Hugo & Alvarez-Borrego 2007). The concentration of *SPM* in the water adjacent to the intertidal plain was higher in January, February and April 2009. Studies conducted during the last fourty years in the CEP found higher values of the concentration of SPM in autumn, spring and summer (Knoppers & Opitz 1984, Brandini *et al.* 1988, Rebelo & Brandini 1990, Kolm & Absher 2008, Kolm & Nowicki 2011). Siqueira and Kolm (2005) found maximum values of SPM in deep waters in the winter (55.86 mg.L^{-1}) at the mouth of the Gamboa of Maciel, near the Cotinga Island. Studies described in the CEP demonstrated that approximately 25% of the composition of *SPM* is particulate organic matter (POM), which directly reflects the ability of maturation of *A. brasiliana* (Rasgalla-Jr & Piovezan 2009). The *SPM* can still be influenced mainly by the oscillation of the annual phytoplankton productivity and concentration of particulate inorganic matter (PIM) due to anthropogenic influences (dredging of the bed of the port channel of PEC), which can affect the sustainability of filtering communities of the region.

For chlorophyll fluorescence higher values were observed in October and December 2008, January and April 2009. Other studies in the CEP have found higher values of Chl-a in the winter, spring and summer (Knoppers *et al*. 1984, Rebelo & Brandini 1990,

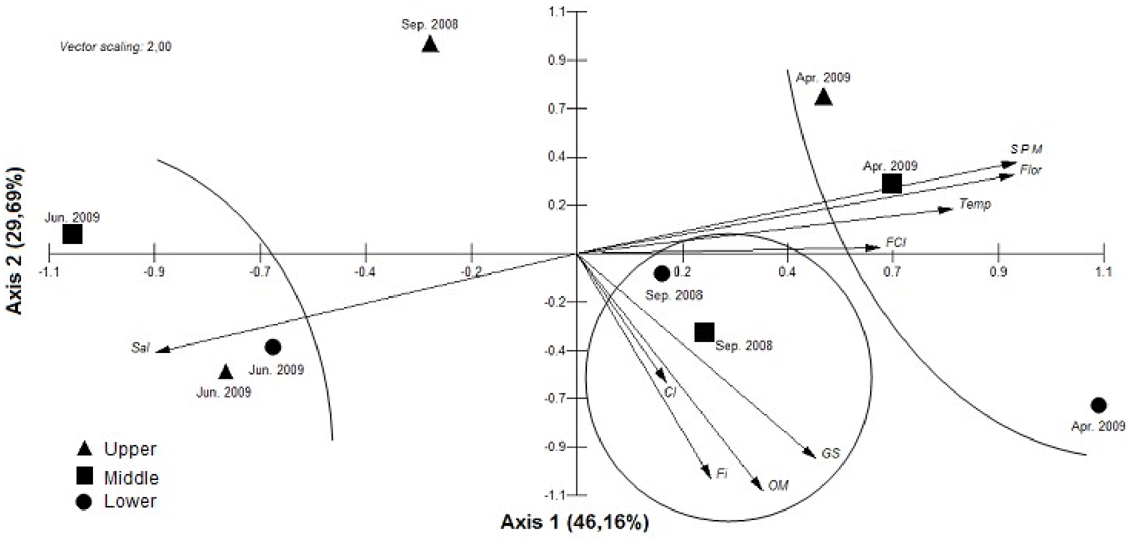

Figure 9 – Principal Component Analysis of environmental parameters (Temp – water temperature, Flor – fluorescence, SPM - suspended particulate matter, GS – grain size, Ca- gravel percentage and OM - organic matter of the sediment), indexes (CI and FCI), intertidal levels of the plain (Lower, Middle and Upper) and months (September 2008; April 2009 and June 2009).

Machado *et al*. 1997, Brandini *et al*. 2001, Mafra Junior *et al*. 2006, Kolm & Nowicki 2011) Brandini & Thamm (1994) identified maximum values of microalgae in water during the rainy summer with 467,160 cells.L^{-1} and dominance of centric diatoms (90%), mainly the species *Skeletonema costatum*. Besides these dominant species benthic species were found of the genus *Navicula*, *Cocconeis* and *Diploneis*, whose irregular occurrence is due to the suspension of bottom sediment in the flood tide (Brandini & Thamm 1994). Studies made in the Paranaguá Bay with benthic diatom identified its dominance in the microphytobenthos, with peaks of density and concentration of Chl-a observed during spring and summer, having numerically dominated by the species *Cylindrotheca closterium, Navicula cf. pargemina*, *N. phyllepta* and *N. platyventris* (Fernandes *et al*. 1999). The suspension of microphytobenthos can be considered component of the diet of the organisms from the intertidal region and in the food web of these ecosystems, because in the Paranaguá Estuarine Complex microphytobenthic primary production is 20tC.day^{-1} (Fonseca *et al*. 2008). Moreover, studies of stomach contents of oyster from this region identified the presence of specimens of the genus Navicula and Diploneis (Christo *et al*. *in press*).

The predominance of organisms that require more concentration of food at the upper level of the plain, created a variation in the values of *FCI* (0.0014 to 0.0416 mg.L^{-1}). This may show that these organisms require a greater amount of food for the maturation when using only microalgae as food. This difference in the upper level compared to the other levels (middle and lower) may also be related to a longer period of exposure to air at this level (Fonseca *et al.* 2008) and therefore causing more desiccation of larger organisms.

Therefore these specimens of *A. brasiliana* reduce its ability to feed itself, since bivalves tend to close the valves in extreme conditions, decreasing food filtration so essential for the gametogenic processes (Nascimento & Perreira 2004).

The pattern of variation in water temperature in this study was similar to that found by Boehs *et al.* (2008), with the temperature rising from September, with maximum in summer, and decreasing from March, with lower values in winter. The exposure of organisms to air did not contribute to thermal stress, since the difference between the water temperature and the air did not have a great variation (from 0.90 to 1.92°C). The salinity recorded by the station Ferry-box has a pattern with higher values in winter and lower in summer, which is related to increased rainfall this time of year. Boehs *et al.* (2008) also found this pattern in a study conducted with the salinity of pore water and water from adjacent intertidal plain of Rasa da Cotinga Island. Sedimentological values were similar to those found by Boehs *et al.* (2008). Brustolin *et al.* (2013) identified in an intertidal plain of Papagaios′ Island, located in the same section of the PEC, similar sedimentological characteristics found in this work. These authors reported that *A. brasiliana* could affect the vertical distribution of sedimentological characteristics as: organic matter, phaepigments and microphytobenthic through bioturbation. This influence of *A. brasiliana* in the phaepigments and microphytobenthic vertical distribution in Papagaios´ Island can demonstrate the influence of the feeding of this species in the surface sediment. The relationships observed in PCA demonstrated an association of the indexes (*CI* and *FCI*), percentage of organic matter (OM) and grain size (GS) of the sediment, thus showing that the conditions and the need of food of *A. brasiliana* are directly related to the OM percentages, besides the seasonal variation in the phytoplankton and microphytobenthic primary productivity. This association of infaunal bivalves in feeding of OM was also reported for other species of the Brazilian coast and of the world (Arruda *et al.* 2003, Braeckman *et al.* 2011a, b).

Conclusions

The annual continuous reproduction of the species in the area can be confirmed. We also recognized the difference in reproductive size in relation to the three levels of the non-vegetated intertidal plain, with adults and juveniles of larger sizes in the upper level of the plain, thus showing that individuals with lower reproductive capacity were at higher level, since the rate of reproduction of the species decreases with lengths exceeding 30 mm. Individuals with size greater than or equal to 30 mm could be the appropriate organisms for commercial extraction, since these would not affect the sustainability of the population and its withdrawal would reduce intraspecific competition for space and food for smaller classes. Our results also indicated that the studied region perhaps is a potentially suitable for cultivation. However, to corroborate this information, further studies are needed relating the density of the species to its reproductive cycle in the levels of the sandbank. In relation to SPM and the reproductive cycle of *A. brasiliana* studies of filtration capacity of the organisms, composition and nutritional value of SPM, phytoplankton and microphytobenthic in the region. are needed to supplement the information described here.

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