



Environmental conditions influencing the leech infestation by *Myzobdella platensis* (Hirudinea: Piscicolidae) on the swimming crabs (*Callinectes* spp.) (Decapoda: Portunidae) in a Brazilian tropical estuary

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Abstract. There is a global lack in the literature on the studies of parasitism in tropical estuaries, particularly about the effects of estuarine conditions on the parasite population dynamics among the crustacean hosts. Here, we evaluate whether the environment conditions and biotic factors could influence the infestation by *Myzobdella platensis* in swimming crabs (genus *Callinectes*) captured in a Brazilian estuarine system. We collected a total of 1,097 crabs, and records the physical-chemical parameters monthly for two years at three sites in the estuary to analysis the effect of estuarine conditions (i.e. depth, floor temperature, dissolved oxygen, and salinity). There were cocoon and leeches feeding effectively on swimming crabs' hemolymph in the three species studied between the sexes, years, and at the three study sites. The study reveals a large infestation for *C. bocourti* with a prevalence of 91.5% by leeches and of 96.0% by cocoon, and 87.5% by leeches and of 90.2% by cocoon to *C. sapidus*, while for *C. danae* it was lower with 26.2% by leeches and 32.6% by cocoon, respectively. We did not observe neither leeches and nor cocoon in crabs smaller than 7.0 cm. Salinity and dissolved oxygen seem to have inverse correlation with infestations probably due to the higher sensitivity of the parasite to these parameters. The explanation for the lowest infestation recorded for *C. danae* is because it occurs preferentially at the site of greatest salinity and because it has the smallest size among the species studied. Infestation was higher in *C. sapidus* and *C. bocourti* and according to the collection site. The estuarine conditions were related to intensity of parasitism, once with the degree of infestation was linked to biotic and abiotic factors, such as host size, host species, and salinity at the collection points regarding both leeches fixation and deposition of cocoon.

Key words: leeches; parasitism; Portunidae; *Callinectes danae*; *Callinectes sapidus*; *Callinectes bocourti*.

Resumo: Condições ambientais influenciam parasitismo de sanguessugas *Myzobdella platensis* (Hirudinea: Piscicolidae) em siris (*Callinectes* spp.) (Decapoda: Portunidae) em um estuário tropical brasileiro. Existe uma carência global na literatura sobre estudos relacionados a intensidade de parasitismos que ocorrem em estuários tropicais, particularmente sobre os efeitos das condições estuarinas na dinâmica populacional de parasitas de crustáceos hospedeiros. No presente estudo avaliamos se as condições ambientais e os fatores bióticos podem influenciar na infestação por *Myzobdella platensis* em siris (gênero *Callinectes*) capturados em um sistema estuarino brasileiro. Amostras de 1.097 siris e registros de parâmetros físico-químicos (profundidade, temperatura do sedimento, oxigênio dissolvido e salinidade) foram realizados mensalmente por dois anos em três locais do estuário.

Constataram-se sanguessugas e casulos se alimentando efetivamente da hemolinfa das três espécies de siris estudadas, em ambos os sexos, nos dois anos e nos três locais de estudo. O presente estudo revela uma grande infestação nas espécies de siris. *C. danae* evidenciou um parasitismo de 26,2% por sanguessugas e 32,6% por casulos; *C. sapidus* demonstrou um parasitismo de 87,5% por sanguessugas e de 90,2% por casulos; e *C. bocourti* apresentou um parasitismo equivalente a 91,5% por sanguessugas e de 96,0% por casulos. Não foi observado parasitismo em siris menores que 7,0 cm. A salinidade e o oxigênio dissolvido provavelmente determinam uma correlação inversa com as infestações, possivelmente devido à maior sensibilidade do parasita a estes parâmetros. A explicação para a menor infestação registrada para a espécie *C. danae* relaciona-se com o fato de sua ocorrência mais destacada em locais contendo maiores salinidades e pelos indivíduos alcançarem menores tamanhos em relação as outras espécies estudadas. A infestação foi maior em *C. sapidus* e *C. bocourti* e de acordo com o local de amostragem. As condições estuarinas estiveram relacionadas à intensidade do parasitismo, uma vez que o grau de infestação foi associado a fatores bióticos e abióticos, como tamanho do hospedeiro, espécie hospedeira, oxigênio dissolvido e salinidade nos locais de amostragem quanto à fixação de sanguessugas e deposição de casulos.

Palavras-chave: sanguessugas; parasitismo; Portunidae; *Callinectes danae*; *Callinectes sapidus*; *Callinectes bocourti*.

Introduction

A considerable biological diversity has been already described for leeches. Such diversity includes different types of symbiosis. However, there is a global lack in the literature on the intensity of parasitism in tropical estuaries, particularly about the effects of estuarine conditions on the parasite population dynamics among the crustacean hosts (Zara et al., 2009; Ocegüera-Figueroa & León-Règagnon, 2014).

In Brazil, the estuary system of Santos and São Vicente on the coast of the state of São Paulo has an intense human occupation, including the country's largest industrial pole and the largest seaport. Both cause anthropogenic degradation due to water and atmospheric pollution by industries and domestic sewage (Cetesb, 2001; Silva & Gomes, 2012; Duarte et al., 2016, 2017). The proximity to the Port of Santos exposes the estuary to the introduction of exotic species (NISC, 2001, Seebens et al., 2013). Invading crustaceans, specifically, may be vectors of pathogens, parasites, and exotic commensals (Bojko, 2017). Despite the impacts, this system has maintained a capacity to renew its waters periodically under the influence of tides and freshwater discharges of adjacent rivers (Silva & Gomes, 2012), remaining a breeding ground and harboring an expressive natural biological community.

In these environments, Portunidae stand out, including some species exploited economically by artisanal fishing (Pita et al., 1985; Severino-Rodrigues et al., 2001; Scalco et al., 2004). Nine

species of swimming crabs were identified in the Bay/Estuary Complex of Santos and São Vicente. Four of them (*Callinectes bocourti*, *C. danae*, *C. exasperatus*, and *C. sapidus*) have a life cycle that involves migrations between the bay and the estuary (Pita et al., 1985).

During their stay in the estuaries, leeches of the genus *Myzobdella* (Leidy, 1851) (Christoffersen, 2008, Ringuelet, 1985) can parasite swimming crabs, some species of fish and other crustaceans (Daniels & Sawyer, 1975; Zara et al., 2009; Ocegüera-Figueroa & León-Règagnon, 2014). They suck the blood of these animals (hemolymph) through suckers located at both ends of the body (Sawyer, 1981).

Leeches of the genus *Myzobdella* are hermaphroditic hematophagous parasites (Faisal & Schulz, 2009; Schulz & Faisal, 2010; Faisal et al., 2011; Schulz et al., 2011; López-Jiménez et al., 2012) with a wide geographic distribution, from the western Atlantic in New Jersey (USA) to Uruguay (Burreson, 2009). They are associated with salinity (0-26‰) predominantly in brackish waters (Sawyer et al., 1975).

Upon reaching sexual maturity, the leeches detach themselves from their hosts and deposit cocoons on consolidated substrates, from which juveniles emerge to find their own hosts because they do not have larval stages (Becker & Katz, 1965; Daniels & Sawyer, 1975; Sloan et al., 1984; Sawyer, 1986). *Myzobdella* can deposit egg capsules directly on the shell of some crustaceans, avoiding abandonment of their primary hosts (Sawyer et al.,

1975; Zara et al., 2009; López-Jiménez et al., 2012). Juveniles when hatching from cocoons do not survive in environments with more than 15 ppm (salinity) (Sawyer et al., 1975). Daniels & Sawyer (1975) reported a parasitic relation of *Myzobdella lugubris* initially on fish, later depositing cocoons on decapods.

This parasitic relationship may initially cause an histopathologic response (Amin, 1981; Volonterio et al., 2004) and lead to the transmission of potentially harmful opportunistic pathogens such as bacteria (*Flavobacterium* sp., *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp.) (Kikuchi & Fukatsu, 2005; Noga et al., 1990; Goffredi et al., 2012; Pomposini et al., 2019), and viruses (*viral hemorrhagic septicemia* - VHSV) (Sawyer et al., 1975; Faisal & Schulz, 2009), which are responsible for the mortality of fish and crabs (Hutton & Sogandares-Bernal, 1959; Morrison et al., 2011).

Little is known about the parasitic relationship between leeches and crabs in tropical estuaries. Zara et al. (2009), for example, confirmed such parasitic relationship for *C. bocourti*, but did not show the intensity with which they occur nor the main variables that determine infestations. This study aims to evaluate the infestation by *M. platensis* on crabs of the genus *Callinectes* (Stimpson, 1860) captured in the Brazilian estuary of Santos and São Vicente, and its possible relations with factors such as crab species, sex, size, and the abiotic conditions of the catch site.

Material and Methods

Study site: Samplings were carried out monthly for two years (2015 and 2016) at three sites located in the estuarine system of Santos and São Vicente: 1) Branco River, 2) Mariana River, and 3) Queiroz River (Fig. 1).

Sample collection: The capture of crabs was performed using baited traps of the type "puçá" thrown by a motorized aluminum boat (Severino-Rodrigues et al., 2001). The captured specimens were individualized in labeled plastic bags to prevent loss of leeches, which easily detach from the host when removed from the water. At the time of collection, a multi-parameter probe was launched to measure the environmental data (four measurements per site) depth (m), floor temperature (°C), dissolved oxygen (mg/L), and salinity (ppm).

The crabs (Melo, 1996) and the leech (Ringuelet, 1985) were identified in laboratory. The sex (abdomen shape) and the development stage in juveniles and adults (abdomen shape and adhesion to



Figure 1.- Map of the Santos and São Vicente Estuary System (São Paulo, Brazil) showing the sampling sites: Mariana, Branco, and Queiroz Rivers (Source: adapted from Google Earth® satellite images).

thoracic sternites or not) were recorded macroscopically. The width of the carapace at the base of the lateral spines (LS) was measured with a steel pachymeter (mm). The specimens were recorded with or without cocoons and leeches; in parasitized animals, they were separated and counted in each individual. All parasites were quantified on three crab's hemolymph, both sexes, in both years, and at the three study sites.

Statistical analysis: The data were organized and grouped in two-month groups aiming to have a

better fit of the data. The statistical and graphical analyses were performed using the software R 2.13.0 (Ihaka and Gentleman, 1996). The variables obtained for the physical-chemical parameters and the number of leeches by species were submitted to Levene's tests of homogeneity of variances (L) and to Shapiro-Wilk normality test (W). Data that presented a normal distribution ($p > 0.05$) were submitted to analysis of variance (ANOVA) with comparison of means "*a posteriori*" by Tukey test (Zar, 1999; Morrison, 2002).

A Spearman correlation analysis was performed involving the means of physical-chemical parameters and the mean number of leeches recorded per species. Spearman coefficients were compared to the values recorded with $n-2$ degrees of freedom at 5% of statistical significance (Zar, 1999). Relations with significant correlation coefficients were represented by scatter plots. A multifactorial analysis (principal component analysis, PCA) was performed according to Kaufman and Rousseeuw (1990) in order to evaluate correlations between leech infestation on the swimming crabs' species and physical-chemical parameters of the sites. Calculations were performed using the FactoMineR package using R environment (Le et al., 2008; Husson et al., 2012).

Results

A total of 1,097 crabs (559 in 2015 and 538 in 2016) of the genus *Callinectes* (497 *C. danae* [CW: 6.60 ± 1.72], 376 *C. sapidus* [CW: 10.83 ± 1.65], and 224 *C. bocourti* [CW: 10.44 ± 1.24]) were collected in Mariana River (314 specimens), Branco River (372 specimens) and Queiroz River (411 specimens). There were leeches and cocoon feeding effectively (*personal observation done during each specimen's collection by both authors*) on crabs' hemolymph in the three species studied, both sexes, in both years, and at the three study sites. Noticeably the occurrence of parasitized females of the three species was lower than that of males, namely: 12 specimens of *C. danae*, 40 specimens of *C. sapidus* and 36 specimens of *C. bocourti*. It was documented cocoons deposited on different sites of the carapace and locomotor appendages (Fig. 2).

Table I shows the prevalence for the three species of *Callinectes*. The results indicate a large infestation on *C. bocourti* and *C. sapidus* rather than *C. danae* in a tropical estuary. *C. danae* showed a parasitism of 26.2% by leeches and 32.6% by cocoon. *C. sapidus* showed a parasitism of 87.5% by leeches and 90.2% by cocoon. *C. bocourti* showed a

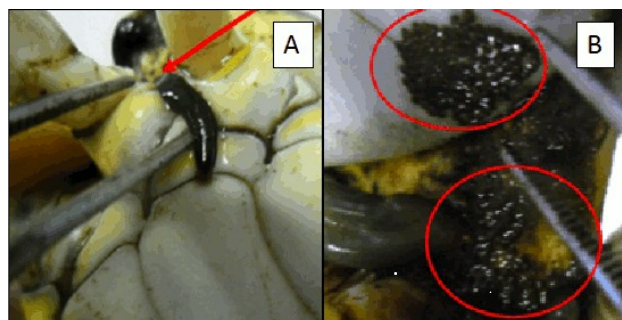


Figure 2.- Parasitism observed in *Callinectes bocourti* by the leech *M. platensis* in Santos and São Vicente Estuary System (São Paulo, Brazil): (A) adult animal attached to the host's joints; (B) capsules of eggs (cocoons).

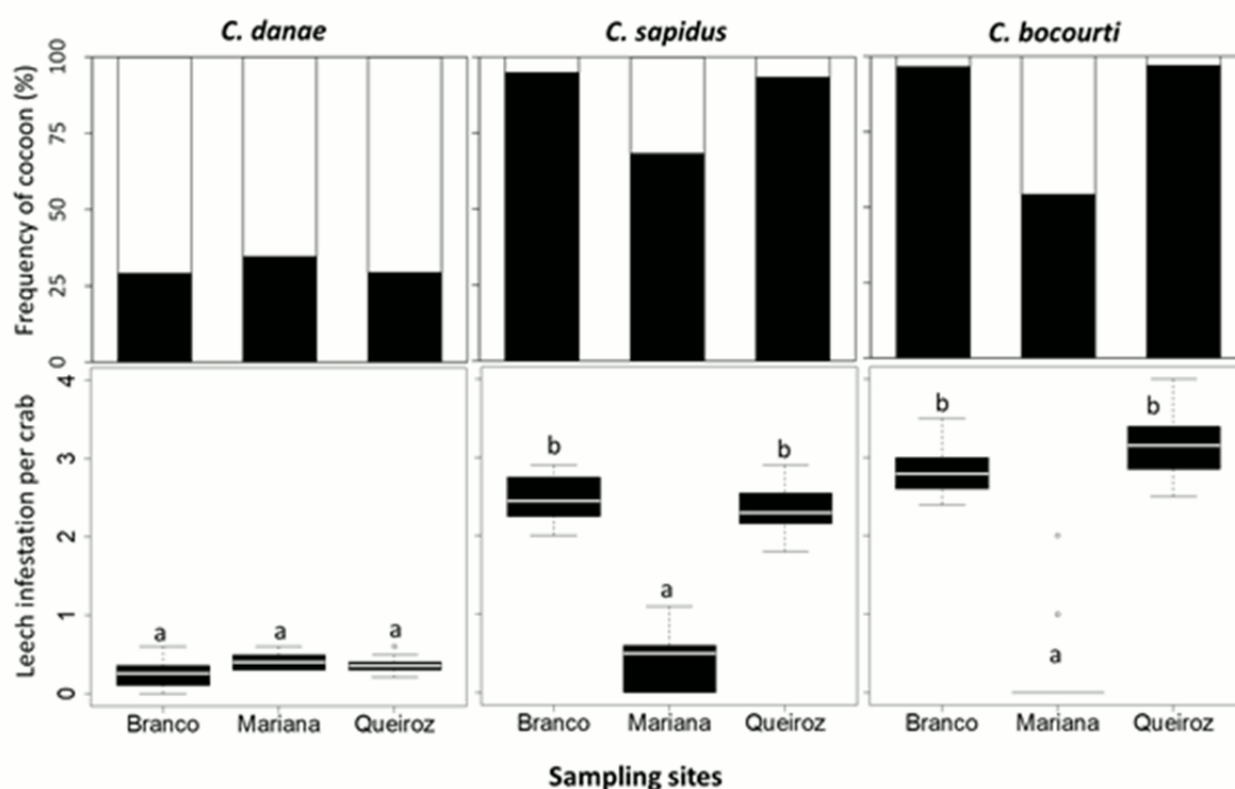
parasitism of 91.5% by leeches and 96.0% by cocoon. Besides this proportion of parasitized crabs, the intensity of parasitism per animal was also recorded. *C. danae* showed a lowest range of infestation considering it presented 1-3 leeches/individual, while other two species presented 1-7 leeches/individual.

Figure 3 shows information on parasitism on the species of crabs (sexes grouped) according to sampling site. In general, *C. danae* was less infected by leeches and cocoon at the three study sites. This species presented general averages below two leeches/crab at all sampling sites, with no significant differences according to Tukey test ($p > 0.05$). More than 60% of *C. sapidus* specimens contained cocoon at the three sampling sites, with lower values for leeches on the Mariana River (Tukey test: $p < 0.05$). Similarly, *C. bocourti* presented cocoon and leeches with equivalent mean values at the three study sites. Table II presents the results for infestations by leeches and oothecae on the three species of crabs every two months. There were no seasonal peaks of parasitism related to floor temperature in a tropical estuary.

It is also interesting to analyze to which size crabs begin to be parasitized in a tropical estuary. Regardless of species and sex, crabs begin to be parasitized from 7 cm of carapace width, not been observed in 245 animals smaller than this (28.7%). The smallest individual of *C. danae* parasitized (including those that only carried oothecae) measured 7.12 cm (male). In *C. sapidus*, the smallest measured 7.05 cm (female) and *C. bocourti* presented a female with 7.13 cm as the smallest specimens recorded. The size range of parasitized animals in a tropical estuary is also important to describe. Figure 4 shows the prevalence (%) of leeches in the three species of crabs per length class

Table I – Prevalence of *M. platensis* leeches and presence of oothecae in the three species of *Callinectes* sampled in the estuarine system of Santos and São Vicente during 2015 and 2016.

Species	n	2015				2016				Total			
		Leeches		Cocoon		Leeches		Cocoon		Leeches		Cocoon	
		n	%	n	%	n	%	n	%	n	%	n	%
<i>C. danae</i>	497	68	52.3	83	51.2	62	47.7	79	48.8	130	26.2	162	32.6
<i>C. sapidus</i>	376	175	53.2	182	53.7	154	46.8	157	46.3	329	87.5	339	90.2
<i>C. bocourti</i>	224	205	51.2	112	52.1	100	48.8	103	47.9	305	91.5	215	96
Total	1,097	448	58.6	377	52.6	316	41.4	339	47.3	764	69.6	716	65.3

**Figure 3.-** Infestations by oothecae and leeches of the species *M. platensis* on three species of crabs: *C. danae*, left; *C. sapidus*, center; and *C. bocourti*, right, at the study sites (2015 and 2016). Above: average of relative frequency (%) of crabs infected by cocoon (black box = individuals with oothecae, white box = individuals without oothecae). Below: Box graphs (medians, quartiles, and range of variation) of mean adult leech infestation per sampled individual. The means followed by the same letter did not show a statistical difference by Tukey test at 5% ($p > 0.05$).

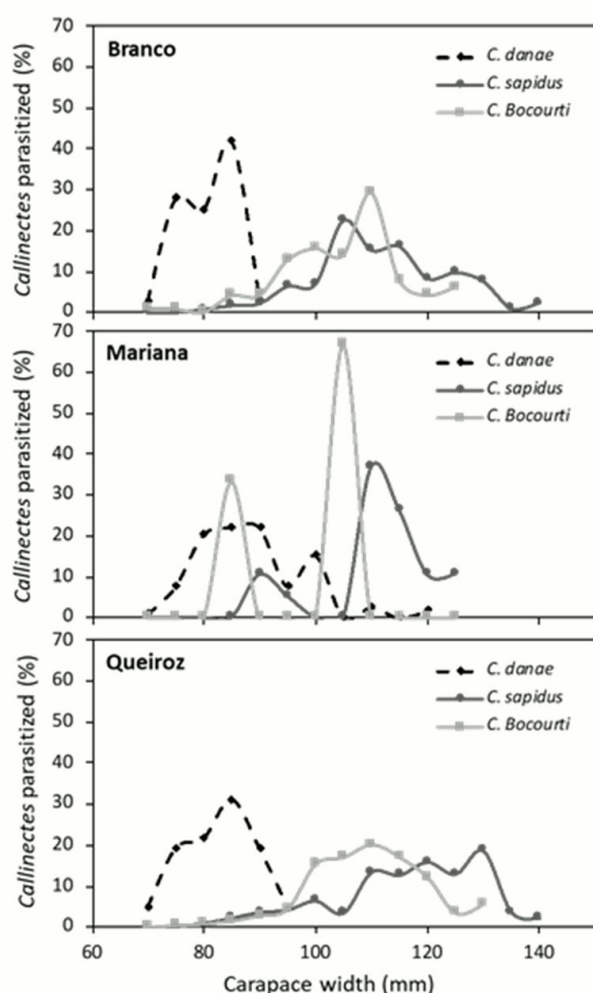
(CW). The highest prevalence occurred in **1) *C. danae***: in CW classes between 8.0 and 9.0 cm; **2) *C. bocourti***: between 10.0 and 11.5 cm; **3) *C. sapidus***: in CW classes between 10.5 and 13.0 cm. Therefore, *C. danae* have presented the smallest infested specimens in relation to the other two species.

Table III shows the results of the physical-chemical parameters at the three sampling points. The mean floor temperature in the rivers was not different in 2015 nor in 2016 (Tukey test, $p > 0.05$),

ranging from 23°C to 25.3°C. The Mariana River presented a higher relative salinity in both years (Tukey test, $p < 0.05$). The results for the Branco and Queiroz Rivers were significantly lower than half this value in both years. The three sampling points presented significant differences in oxygen dissolved in both years (Tukey test, $p < 0.05$). The depths were different among rivers, ranging from 2.1 m (Queiroz River) to 3.1 m (Mariana River) (Tukey test, $p < 0.05$).

Table II - Percent distribution of leeches and oothecae of the species *Myzobdella platensis* every two months, and average floor temperature in both years analyzed.

2015/16		<i>C. danae</i> (%)		<i>C. sapidus</i> (%)		<i>C. bocourti</i> (%)		Total	
Two-month	Temp.	Leeches	Cocoon	Leeches	Cocoon	Leeches	Cocoon	Leeches	Cocoon
1 st	28.3	32.7	37.6	88.9	88.9	92.4	84.1	93.2	62.5
2 nd	25.6	24.2	28.3	82.4	82.4	73.5	92.1	100	56.1
3 rd	22.1	26.5	33.7	91.2	91.2	94.7	92.9	92.9	59.5
4 th	20.8	23.9	31.3	83	83	88.7	92.7	95.1	60.9
5 th	23.2	21.9	32.9	95.3	95.3	100	92.3	97.4	60.7
6 th	24.9	25.7	31.1	90.5	90.5	93.7	94.1	97.1	63.2

**Figure 4.-** Prevalence (%) per class of length (CW) of crabs of the genus *Callinectes* parasitized by the leech *M. platensis* at the three study sites (2015 and 2016).

It is crucial, however, to identify how leech infestations is relating with ecology of tropical estuaries. Figure 5 shows the results of principal components analysis (PCA) of physical-chemical parameters (depth [m], floor temperature [°C], dissolved oxygen [mg/L], and salinity [ppm]) and of

the means related to the prevalence (leeches/crab) recorded for the three species of crabs of the genus *Callinectes* at the three study sites (2015 and 2016). The first two dimensions explained 78.98% of the variances (DIM 1: 60.63% and DIM 2: 18.35%). The first was responsible for the correlation (positive and negative) of the variables: salinity (0.97), O₂ (0.87), leeches in *C. sapidus* (-0.88), and in *C. bocourti* (-0.96).

Complementary to these results, these same relationships were also significant according to Spearman's correlation coefficients ($p < 0.05$). Figure 6 shows the resulting logarithmic and linear regressions. According to them, when salinity increases, there is a decrease in leech infestation on the species *C. sapidus* and *C. bocourti*. The same pattern was also observed with increases in dissolved oxygen.

Discussion

There is a global lack in the literature on the intensity of parasitism in estuaries (Pomposini et al., 2019), especially in tropical ones (Zara et al., 2009; Ocegüera-Figueroa & León-Règagnon, 2014). In addition, studies about leech infestations related to the ecology of estuaries are scarce. The presence of leeches in the joints of species of crabs is a feature of sucking Hirudinea that use their rigid mandibles to cut soft tissues and feed on host juices (Ruppert et al., 2004; Abdualkader et al., 2013). Hutton & Sogandares-Bernal (1959) recorded perforations on the back, the ventral surfaces of the carapace, and near the base of the locomotor appendages of *C. sapidus*.

Leeches parasite aquatic organisms for the deposition of cocoons on gills of fish (Pomposini et al. 2019), shells and appendages of crabs (Zara et al., 2009). This is a strategy of *Myzobdella* spp. for dispersion of offspring (Daniels & Sawyer, 1975;

Table III - Physical and chemical parameters (depth [m], floor temperature [°C], dissolved oxygen [mg/L], and salinity [ppm]) at the study sites (2015 and 2016). The means of each parameter per sampling site followed by the same letter did not show statistical differences by Tukey test at 5% ($p > 0.05$).

Year	Site	Temperature	Salinity	Dissolved oxygen	Depth
2015	Mariana	25.3 ± 2.3 a	26.1 ± 1.2 b	30.0 ± 1.2 c	3.1 ± 0.3 b
	Branco	23.9 ± 2.3 a	10.5 ± 1 a	28.1 ± 0.82 b	2.8 ± 0.3 ab
	Queiroz	24.0 ± 2.3 a	8.13 ± 0.7 a	25.2 ± 0.6 a	2.4 ± 0.2 a
2016	Mariana	24.0 ± 3.4 a	23.5 ± 2.4 b	29.9 ± 0.82 c	2.8 ± 0.1 b
	Branco	23.0 ± 2.2 a	9.92 ± 1.8 a	27.9 ± 0.81 b	2.5 ± 0.3 ab
	Queiroz	23.2 ± 1.4 a	8.54 ± 0.5 a	25.4 ± 0.54 a	2.1 ± 0.1 a

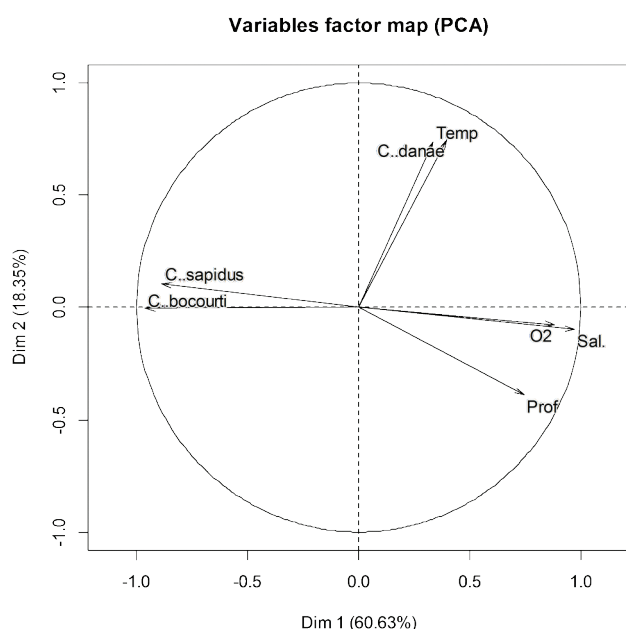


Figure 5.- Principal components analysis (PCA) of the physical-chemical parameters (depth [m], floor temperature [°C], dissolved oxygen [mg/L], and salinity [ppm]) and of means related to the frequencies of infestation (leeches/crab) recorded for the three species of crabs of the genus *Callinectes* at the three study sites (2015 and 2016).

Sloan et al., 1984; López-Jiménez et al., 2012; Saglam et al., 2018; Volonterio et al., 2004; Pomposini et al., 2019). Zara et al. (2009) described *M. platensis* as a parasite of crabs effectively feeding on their hemolymph.

In the present study, *C. danae* was the most abundant species (higher than 50 and 60% in 2015 and 2016, respectively), corroborating the results of Pita et al. (1985) and Severino-Rodrigues et al. (2001), who considered it the numerically dominant species in the region. However, this species presented the lowest infestation by leeches and the lowest occurrence of cocoons in both years

analyzed, preferentially occupying the Mariana River. This very low infestation is probably due to territorial characteristics, since this collection site presented the highest salinity levels. This fact emphasizes that, according to Sawyer et al. (1975), the distribution of *Myzobdella* spp. is limited by salinity.

C. sapidus was the second species in abundance, supporting greater infestations (with higher prevalences than 88.0%) by leeches and by cocoons (above 89%), predominantly occupying the Branco and Queiroz Rivers. *C. bocourti* was the least abundant and caused the greatest infestations (with percentages higher than 93%) by leeches and occurrence of cocoons with a greater intensity in the Branco and mainly Queiroz Rivers. Pita et al. (1985) and Severino-Rodrigues et al. (2001) described the last two species as those that occupy the innermost part of the estuary, therefore with a lower salinity.

C. sapidus and *C. bocourti* showed a number of leeches/individual higher than *C. danae*, with a mean of 2.27 to 2.58 leeches/individual on *C. sapidus* and 2.86 to 3.19 leeches/individual on *C. bocourti*, corroborating Zara et al. (2009), who, in the same estuarine region, also identified *C. bocourti* as the most infected species. This leech/individual ratio may be underestimated since, during the collection process, leeches quickly leave the hosts when removed from the water (*personal observation*).

The results of the multivariate analysis, as well as Spearman's correlations, show that the incidence of *M. platensis* on the three species of swimming crabs is related more to the environment than to the host species itself. Salinity is the factor that seems to influence infestation in swimming crabs in estuarine systems directly. Sawyer et al. (1975) also reported the relation of *Myzobdella* to this variable.

The mean temperature in the rivers was not different in both years. Although differences in depth

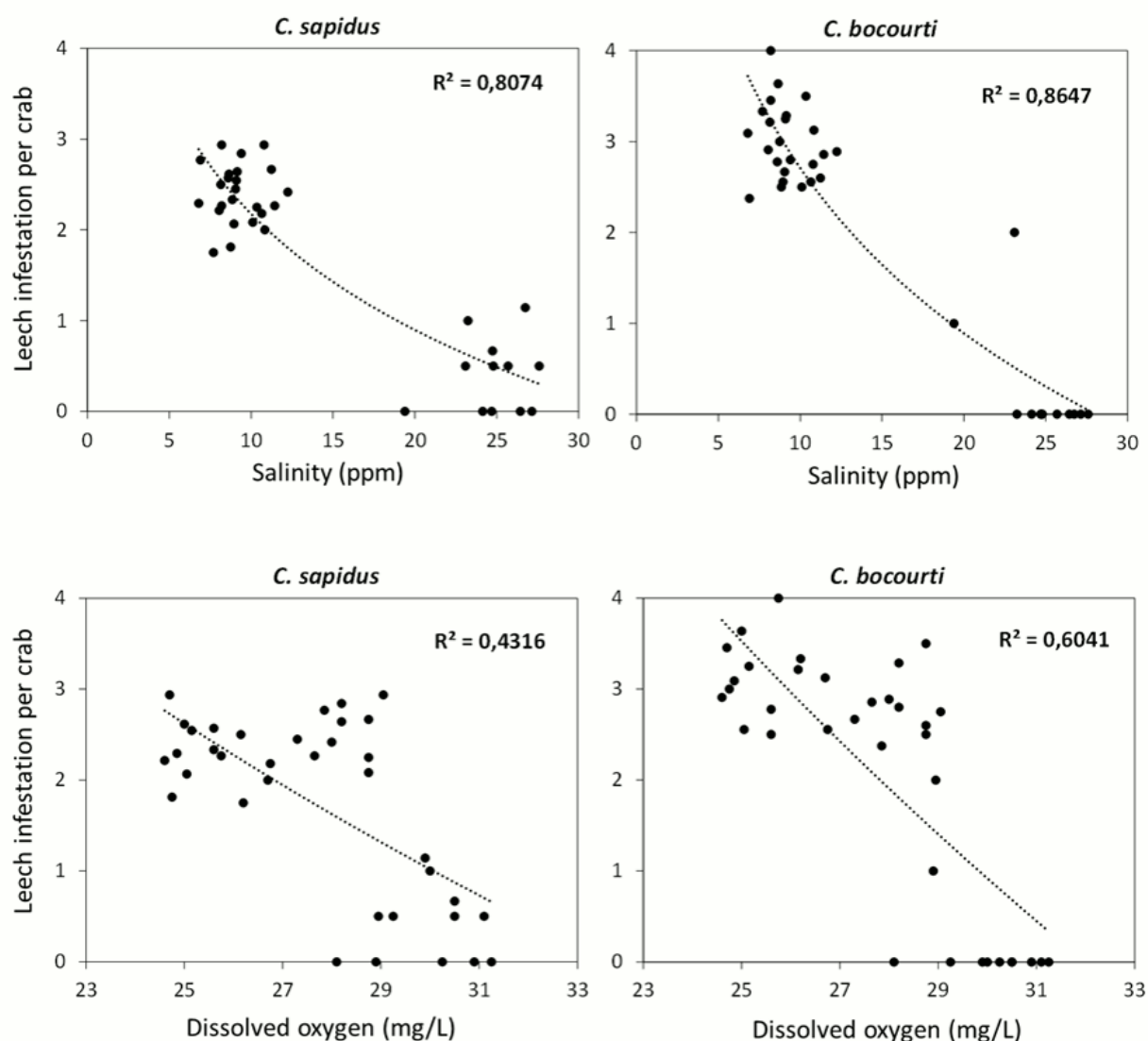


Figure 6.- Regressions (logarithmic [above] and linear [below]) of Spearman's correlations were significant ($p < 0.05$) between physical-chemical parameters (depth [m], floor temperature [$^{\circ}\text{C}$], dissolved oxygen [mg/L], and salinity [ppm]) and frequency of occurrence of infestation (leeches/crab) recorded for the three species of crabs.

were recorded, this variable was also not explanatory for the observed infestations.

Besides salinity, the dissolved oxygen also had a relation to the parasitism. Data indicate a probably higher sensitivity of the parasite *M. platensis* to both parameters. The relative salinity in the Mariana River was the double than that of Branco and Queiroz Rivers. Mariana River was also the collection point where there was less incidence of leeches and cocoons. As for dissolved oxygen, the three sampling points presented significant differences, with lower values in Queiroz River and higher values in Mariana River. However, this variable is dependent on the salinity itself, that is,

the higher the amount of dissolved salt in the water, the lower the oxygen (Truesdale & Downing, 1954).

The results of principal component analysis (PCA) and regressions also indicated a negative correlation between these variables and infestation by leeches on the species *C. sapidus* and *C. bocourti*. The greater the salinity and the dissolved oxygen, the lower the tendency to occur parasitism on these species. This explains the higher occurrences of leeches and cocoon in Branco and Queiroz Rivers, whose values for these parameters were lower than those recorded in the Mariana River. However, Rezende et al. (2015) observed, in laboratory conditions, that *Myzobdella* sp. fixed on *C. bocourti* and *C. sapidus*, with a lower incidence

on *C. danae*, although the environmental factors temperature and salinity were constant in the experiment. In short, environmental variables determine parasitism intensely, although there is also a secondary selection of species by leeches.

The number of leeches parasitizing crabs varied according to size, species, and site of sampling. To *C. sapidus* and *C. bocourti* it is again emphasized that leech concentrations occurred in regions with a lower salinity and in larger specimens, since for *C. sapidus* a maximum CW of 16.8 cm is cited in the literature, and for *C. bocourti*, 13.2 cm (measured at the base of the lateral spines) (Scalco et al., 2014). For this last species, it was observed in the present study, an adult male was detected in the Queiroz River measuring 13.9 cm and one female in the Branco River measuring 13.0 cm, being therefore the maximum CW reported in the literature for both sexes. These characteristics reinforce the idea that, in addition to salinity, larger specimens offer a larger carapace area for the fixation of leeches and deposition of the cocoon.

The presence of leeches or cocoon in individuals smaller than 7.0 cm was not observed, as was also observed by Rezende et al. (2015) in laboratory tests with the same species. Such behavior is likely to be associated with the fact that smaller individuals offer a reduced area for deposition of egg capsules, in addition to undergoing ecdyses in a shorter period, discarding them along with the exuvia.

Few females were parasitized since the occurrence of adult females, mainly of *C. danae*, was low in the interior of the estuary because they moved to more salty water for spawning (Pita et al., 1985; Severino-Rodrigues et al., 2001, 2009).

The slightly higher presence of adult females of *C. sapidus* and *C. bocourti* is due to the characteristic that, although they have a life cycle similar to *C. danae*, they occupy regions in the interior of the estuary, thus requiring a longer migratory process, causing the presence of some specimens still in the estuary (Severino-Rodrigues et al., 2001, 2009).

The number of crabs with egg capsules also varied according to the size and the site of collection, reaffirming that also in this case, the factor salinity seems to be the most limiting. *C. danae* was the species and the Mariana River was the site with the lowest incidence of cocoons (higher incidence on individuals between 7.5 and 9.0 cm, predominantly in the Mariana River). The other species had a small occurrence of cocoons in

Mariana River and a higher incidence in Branco and Queiroz Rivers, concentrating on *C. sapidus* with a CW between 9.0 and 11.5 cm and in *C. bocourti* with a CW between 8.5 and 11.5 cm, a species with a higher incidence of cocoon.

The importance of the environment (low salinity) related to the survival of juveniles once again is emphasized, since Sawyer et al. (1975) pointed out that they do not survive in salinity levels higher than 15 ppm.

Daniels and Sawyer (1975) reported an inverse relationship between seasonal infestation and water temperature in *M. lugubris* parasitizing *Ictalurus catus* in the South Carolina estuaries (USA). In the present study, there was a high rate of infestation throughout the analyzed period without observing seasonal concentrations related to temperature probably because in tropical regions, temperature variations are lower (Harrison, 2004; Freitas et al., 2007).

It is also important to emphasize the negatively affect to the parasitized organisms. There is little available literature regarding its health impacts on hosts (Pomposini et al., 2019). Volonterio et al. (2004), for example, studied the infestation dynamics and histopathology of *M. platensis* on teleost fish from eastern Uruguay, *Hoplias malabaricus* (Characiformes, Erythrinidae) and *Rhamdia quelen* (Siluriformes, Pimelodidae). The authors described an intense aggregated distribution on the first 2 gill pairs of the fish species and call attention for the following histopathology injuries: hemorrhage with clot formation and fibrin deposition; gill filaments with inflammatory reaction, edemas and infiltration of mononuclear leukocytes; and gills lamellae presenting telangiectatic changes. Depending on the intensity of these damages, could lead to more severe complications and culminating in individual death (Pomposini et al. 2019).

The present study reveals worrying results on the infestation of *M. platensis* in species of the genus *Callinectes* sp. in a tropical estuary and also related with its ecology. In view of the above, further studies are recommended seeking to clarify the severity of biological damages caused by the parasitism to which the swimming crabs are strongly submitted.

Conclusions

The occurrence of infestation of leeches on swimming crabs was observed in the three species of the genus *Callinectes* from three sampling points in

the Santos and São Vicente estuarine system. However, the degree of infestation was related to biotic and abiotic factors, such as host size, host species, and salinity at the collection points regarding both leeches fixation and deposition of cocoon.

Acknowledgments

The authors thank to artisanal fishermen from the “Vila dos Pescadores” for assistance in the determination of samplings sites. To the trainees: Silmara Pereira Matos, Mirela Caleffi Ancona, Victor Inacio Rezende, Raphael Gomes Hoelz and to the master Pedro Mestre Ferreira Alves (*in memory*) for the collaboration in obtaining and processing the samples. To the Researcher Roberto da Graça Lopes for the useful contributions and suggestions in the finalization of the article. To all the staff of the Fisheries Institute, who somehow contributed to the drafting of this article. LFAD thanks to the São Paulo State Research Foundation (FAPESP) for his post-doctoral scholarships (Cases #2014/13448-2 and 2016/05414-6).

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Received: January 2020

Accepted: May 2020

Published: June 2020