BIOLOGICAL SCIENCES

Population structure of *Callichirus major* (Say 1818) (Crustacea: Callianassidae) and conservation considerations at Southeast coast of São Paulo, Brazil

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Abstract: Globally, there is limited knowledge about management and conservation of Callianassidae family. Their catches seem to be recently increasing in several parts of the world and evidences raise concern on the need of specialized literature focused on populations monitoring. At Southeast of Brazil, the species *Callichirus major* (Say 1818), known as the ghost shrimp, is frequently captured for use as bait in sport fishing and, according to previous studies, the overexploitation could threaten the population. The aim of this study was evaluated over a year this harmful human pressure on the species *C. major* in two beaches contiguous but susceptible to different anthropogenic impacts due to the existence of legislation on one beach that prohibits the capture of this crustacean. The population of *C. major* presented where the prohibition regulation does not exist a density almost three times lower when compared to the beach that prohibits the capture, with individuals with smaller carapace oval area and a shorter reproductive period. This paper alert to some evidence of *C. major* structure population disturbance resulting from its exploitation at Southeast of Brazil. Recommendations were also addressed to conservation management considering the species' ecological importance.

Key words: Callianassidae, ghost shrimp, conservation, overexploitation, sandy beaches, crustacean.

INTRODUCTION

The burrowing crustaceans of the species *Callichirus major* (Say 1818) (Crustacea: Axiidea) belongs to the family Callianassidae (Dana 1852) which are known as ghost shrimps, occurring in galleries excavated in the sediment of the intertidal region of sandy beaches. Its presence is detected on the surface of the sand through characteristic holes of 5 mm in diameter with surrounded by fecal pellets (Weimer & Hoyt 1964, Frankenberg et al. 1967, Rodrigues & Shimizu 1997, Botter-Carvalho et al. 2007, Alves-Júnior et al. 2014a, 2018). It is distributed from North Carolina in the United States to the state of Santa Catarina in Brazil (Rodrigues 1983, Coelho & Ramos-Porto 1986, Manning & Felder 1986, Melo 1999, Dworschak 2000).

This crustacean has great ecological importance because it favors the establishment of other species in its galleries, such as bivalves, polychaetes and other crustaceans (Rodrigues & Shimizu 1997, Berkenbusch et al. 2000, Souza & Borzone 2003, Peiró & Mantellato 2011, 2016). It also performs bioturbation, revolving the sediment and increasing the cycling of organic matter and nutrients in its zones of occupation (Webb & Eyre 2004, Abrunhosa et al. 2008).

However, *C. major* individuals are frequently captured for use as bait in sport fishing along the Brazilian coast (Souza & Borzone 2003, Botter-Carvalho et al. 2007) and this excessive activity has been resulting in a decline in the species' population (Botter-Carvalho et al. 2007, Peiró & Mantelatto 2016) and even causing overexploitation (Souza & Borzone 2003, Rodrigues & Shimizu 1997). Apart from overfishing, it is also recognized that others possible factors could affect its densities, such as sediment size (Borzone & Souza 1996, Dumbald et al. 1996, Strasser & Felder 1999, Dworschak 2000, Botter-Carvalho et al. 2002), individual body size (Botter-Carvalho et al. 2007, Alves-Júnior et al. 2014b, 2018) and food availability (Suchanek & Colin 1986, Rodrigues and Shimizu 1997).

The simple observation of the intensification of capturing of the species led to the creation of law 850 in 1992, prohibiting the exploitation of these animals throughout the municipality of Santos, state of São Paulo, southeast of Brazil, even collection for scientific purposes. Notably, this law was created using no scientific support, such as the description of density and population structure of the species. However, seventeen years later, Pedrucci and Borges (2009) found higher densities of the species at the José Menino Beach (where the catching of *C. major* is prohibited) than at the Itararé Beach, located in São Vicente (where the catching of *C. major* is not prohibited), bordering the Santos city. This would be the first evidence showing the effectiveness of the law. Other studies have also shown that beaches free from anthropogenic influence have demonstrated higher density of callianassids species (Wynberg & Branch 1997, Contessa & Bird 2004, Hernaéz & Wehrtmann 2007). Based on these statements, it is believed that the prohibition of the capture of *C. major* in one of the beaches may contribute to the

conservation of the species as confirmed by these authors. Even though the capture of the species may occurs illegally in Santos, the exploitation is unquestionably less intense due to effective monitoring by cameras (24 hours per day) and municipal guards. In addition, the population structure of *C. major* in the beach where there is no specific legislation, as the Itararé Beach, may be subject to changes because of excessive catch observed in this area.

Based on that, the aim of this study was analyzing the influence (even as first evidence) of exploitation of *C. major* in terms of their densities at Itararé and José Menino, two contiguous beaches but susceptible to different anthropogenic impacts due to local specific legislation. In addition, at the the Itararé Beach (where capture is not prohibited), a secondary objective was to describe the biological aspects of the species particularly based on sex ratio, population structure in terms of length and gender and reproductive periods, in order to compare them with other studies and identify possible changes in the population, due to the intense exploitation of these animals at the Itararé Beach.

MATERIALS AND METHODS

Study area

Itararé Beach in São Vicente (361803.06 m E and 7348449.56 m S, UTM, Datum WGS 84), where there is no specific legislation prohibiting the capture of *C. major,* and José Menino Beach in Santos (362859.12 m E and 7348518.91 m S, UTM, Datum WGS 84), where law 850/1992 prohibits the capture of the species, are located in Santos Bay, state of São Paulo, southeastern Brazil (Figure 1). The beaches present a dissipative morphodynamic state and they are very environmentally similar to each other, with homogeneous profiles composed of fine to
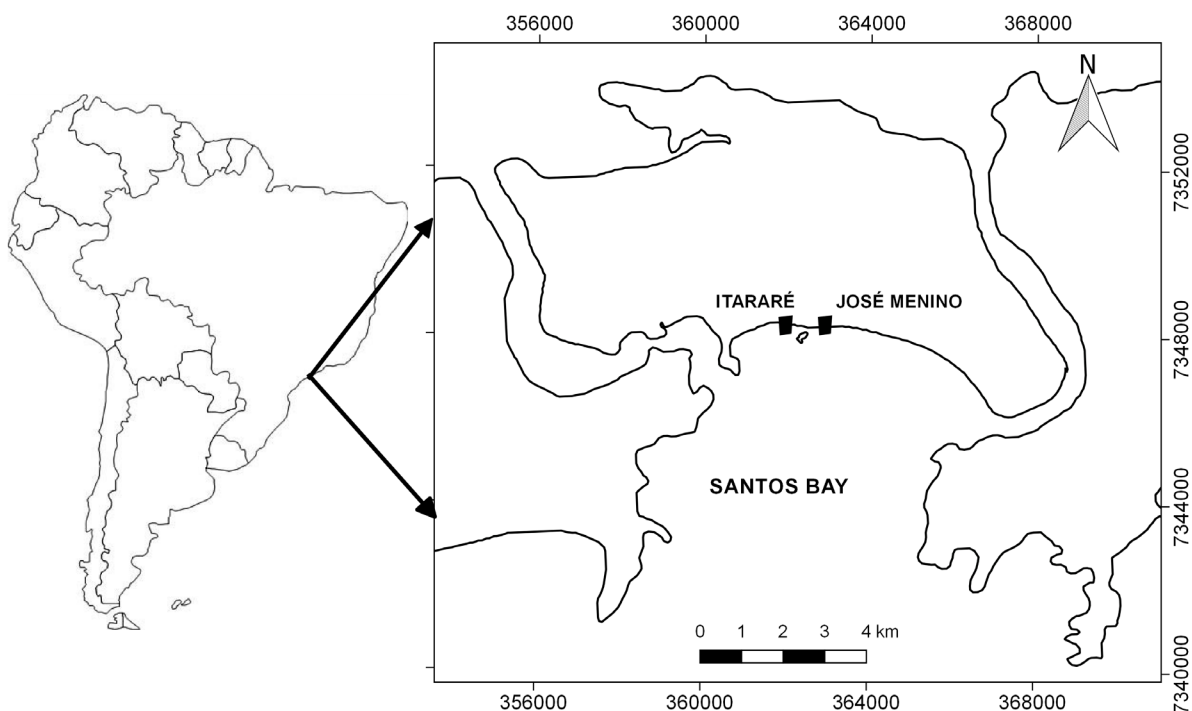
Figure 1. Location of the study beaches in Santos Bay, state of São Paulo, Brazil Google Earth (2018). UTM, Datum WGS 84.

very fine sands (Farinaccio et al. 2009). Both are very urbanized, however, only Santos beaches counted with intensive monitoring in order to enforce the law regarding the capture of *C. major*.

C. major: assessment of density and population structure

The estimation of species density was based on the method used by Souza & Borzone (2003) and Botter-Carvalho et al. (2002). Four transects were arranged randomly in the intertidal region on the both beaches, perpendicular to the water line, and each transect was composed of contiguous sample units of 1m². The densities were calculated monthly from September 2013 to September 2014 during low tide periods based on the number of burrow holes in each sample unit. As corroborated by Rodrigues

(1983), each burrow hole was considered one unique individual, except in reproductive period. In addition, it is important to mention that only this species of callianassids occurs on beach tidal region (band analyzed in this study) (Rodrigues 1983). For statistical data treatment, the densities per m² obtained in an each transect were compared between beaches and between months using the non-parametric Kruskal-Wallis test (Zar 1999).

The population structure was evaluated only at the Itararé Beach from October 2013 to September 2014. The sampling occurred monthly in each four transects in burrows located right on the boundary of the water line, due to the greater probability of sampling of the species in this section as described by Souza et al. (1998). A single researcher performed all the samplings

collection using a suction pump, with a sampling effort of 30 minutes in each transect.

Specimens captured monthly were placed in containers containing alcohol (70%) and transported to the laboratory, where they were brushed to remove sand and measured with 0.05-mm precision calipers (carapace oval area length). According to Rodrigues & Shimizu (1997), the oval area corresponds to an isometric structure to the total length of the animal's body.

The sex was also determined by inspection of the first pair of pleopods and sexual dimorphism of the chelipeds (Botter-Carvalho et al. 2007), and the sex ratio was analyzed by the Chi-Square test (Zar 1999). The reproductive period was determined through the months of occurrence of ovigerous females in the population, as proposed by Butler et al. (2009).

Environmental characteristics of the beaches: organic matter and granulometry

In order to assure that some possible differences of densities in the two beaches were accounted for, the two main natural causes that determine the population size were investigated: organic matter and grain size (Borzone & Souza 1996, Rodrigues & Shimizu 1997, Dworschak 2000, Botter-Carvalho et al. 2007). Analysis of the organic matter present in the intertidal zone was performed monthly by burning the dry sediment at 550 °C for two hours and the organic content calculated as the difference between the initial and final weights divided by the initial sample weight times 100% (Suguio 1973). The mean grain size of the sediments present in the intertidal zone was determined also monthly using the method proposed by Folk & Ward (1957) which still used in other studies (Botter-Carvalho et al. 2002, Souza & Borzone 2003).

These results of the environmental characteristics were correlated with the densities of *C. major* through the Spearman correlation coefficient. Posteriorly, the similarity between the beaches based on their environmental aspects was analyzed using the Kruskal-Wallis test.

RESULTS

Density Analysis

The density of *C. major* ranged from 0 to 18 ind. m² at the Itararé Beach (mean density of 1.33 ind.m² to 4.50 ind.m²) and from 0 to 20 ind.m² at the José Menino Beach (mean density from 5.07 ind.m² to 8.47 ind.m²) (Figure 2).

José Menino Beach presented significantly higher densities than those observed in Itararé Beach in all months studied (p<0.0001). There was also a significant difference of density between months in both beaches (p<0.0001), evidencing a population variation in the temporal distribution of the species. However, there were no peaks of densities indicating a defined seasonal period for the abundance of the species in both beaches.

The sediments of the intertidal regions were composed of fine to very fine sand (average diameter of 0.11 to 0.25 mm) and presented varying concentrations of organic matter (0.30 to 4.27% of the dry weight of the samples).

The beaches are similar as regards their mean grain size (p=0.246) and the organic content (p=0.375). However, these environmental factors did not correlate with the densities in the studied beaches (r<0.04, p<0.05).

A total of 382 individuals of *C. major* (156 males and 226 females) were collected in the Itararé Beach, with predominance of females (1:1.44, X²=12.82, p=0.0005).

The length of the oval area ranged from 2.63 mm (smaller male collected in October 2013) to 11.88 mm (largest male collected in July 2014), with males and females presenting a modal length in the class of 8-9 mm.

The ovigerous females were recorded in the Itararé Beach from December 2013 to March 2014 (7.07% of the total females sampled) and their relative record monthly were 15.38% in December, 27.27% in January, 36.84% in February and 5.55% in March. These individuals presented an oval length ranging from 8.09 mm (December 2013) to 10.88 mm (March 2014) (mean of 9.19±0.73). Table I shows the monthly means obtained for this structure, incluing the number of males, females and ovigerous females sampled during the study months.

Considering all of the individuals sampled, significant differences in the oval length were found among the months (p<0.0001), suggesting oscillations in the body sizes throughout the study.

DISCUSSION

The densities of individuals of *C. major* at José Menino were higher than those recorded at Itararé and superior than the results obtained at José Menino by Rodrigues (1983) (1.5 to 7.9 ind.
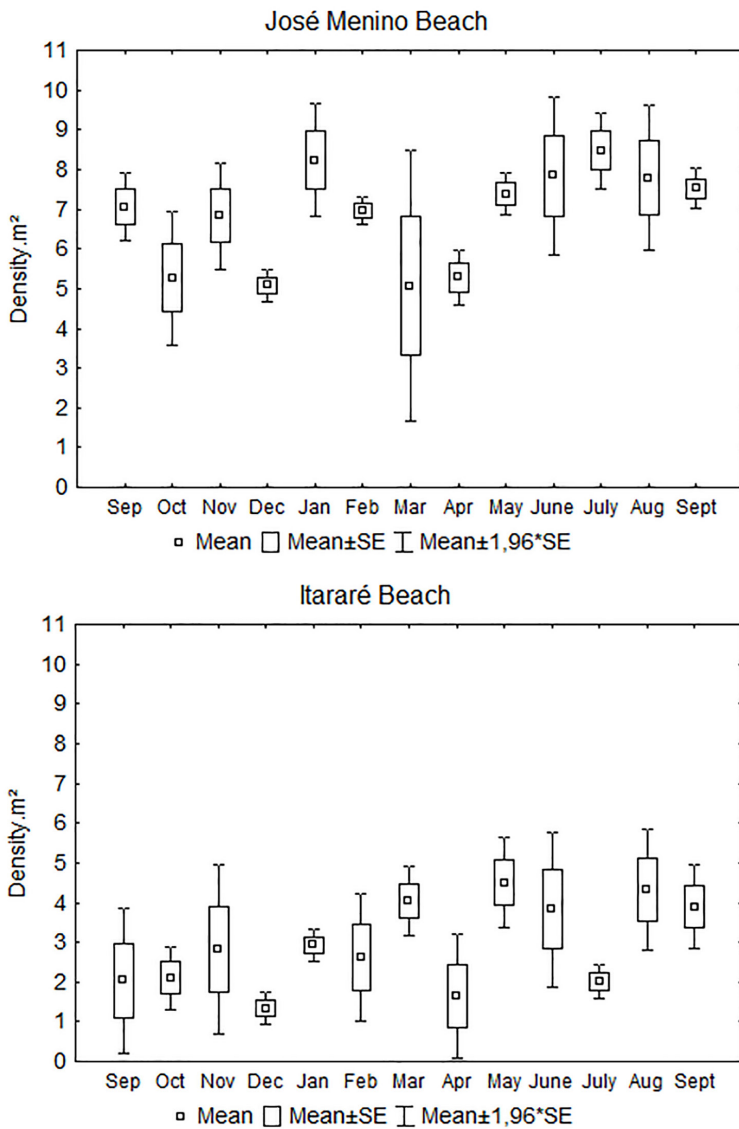

Figure 2. Densities of *C. major* at José Menino and Itararé monthly from September 2013 to September 2014.

Table I. Monthly mean oval lengths (mm) of *C. major* specimens sampled at the Itararé Beach, São Vicente, state of São Paulo.

Study months		N	Males Mean length	Sd		N	Females Mean length	Sd		N	Ovigerous Mean length	Sd
2013	October	12	5.72	1.26		27	7.22	0.91		-	-	-
	November	10	7.34	0.64		28	7.51	0.97		-	-	-
	December	21	8.30	0.95		13	8.60	0.57		2	8.45	0.36
2014	January	14	8.29	1,15		22	8.93	0.72		6	9.01	0.50
	February	15	9.18	1.12		19	9.10	1.04		7	9.32	0,54
	March	10	8.52	1.30		18	8.87	1.17		1	-	-
	April	7	9.10	0.92		11	6.96	1.61		-	-	-
	May	7	8.34	1.87		20	7.37	1.56		-	-	-
	June	8	8.52	1.56		9	8.15	1.74		-	-	-
	July	14	8.06	1.48		14	8.51	1.05		-	-	-
	August	25	7.34	1.09		23	8.51	1.30		-	-	-
	September	13	7.95	0.89		22	8.19	1.45		-	-	-

m²) and by Rodrigues & Shimizu (1990) (1.5 to 7.9 ind.m²). Pedrucci & Borges (2009) found higher values than those obtained in this study for *C. major* at José Menino (mean density of 14.39 ind. m²) and lower density (2.23 ind.m²) at Itararé.

The increase in the number of individuals of *C. major* at José Menino over the years is possibly associated with the protective law. In the present study, the density was almost three times higher than that observed at the Itararé beach. Hypothetically, this population difference could be the consequence of the creation of the municipal law 850/1992. It is believed that even if the capture of *C. major* may occurs illegally at José Menino, the municipal law implies in lowest intensity of this activity.

Table II shows that the densities of the species of callianassids are variable in several localities, even in sandy beaches with morphodynamics characteristics similar to beaches evaluated in this study. Most of the authors reported the occurrence of capture activity of the callianassids in these localities, except by Felder and Griffs (1994).

Other factors (abiotic and biotic) could be determining the size of the local populations, while the differences found to the beaches studied could be an effect from the exploitation prohibition, since they are connected geographically.

No defined seasonal periods were identified for the densities in the both beaches, different from the study developed by Souza et al. (1998) and Souza & Borzone (2003), who found the highest densities of the species in the summer (rainy season) in the southern region of the country. Botter-Carvalho et al. (2002) reported a decline in *C. major* densities from the month of April (beginning of rainy season) in a beach at the northeast of Brazil. Perhaps the temperature is more deterministic in influencing the density of the species than the rains.

It is important to mention the impossibility of the occurrence of more than one animal per gallery for the *C. major* according to Rodrigues (1983). The occasional meeting between *C. major* individuals in the galleries leads immediately to competition and reproduction (Rodrigues &

Table II. Densities of callianassids in various localities along the American Coast (Atlantic and Pacific Ocean).

Species	Locality	Density (ind.m²)	References
Callichirus islagrande (Schmitt ,1935)	Louisiana, EUA	mean of 150	Felder & Griffs (1994)
Lepidophthalmus louisianensis (Felder and Rodrigues, 1993)	Mississipi, EUA	mean of 400	Felder & Griffs (1994)
Callichirus seilacheri (Bott, 1955)	Las Machas Beach, Chile	1.4 to 20.2	Hernaéz & Wehrtmann (2007)
Callichirus major	Coast of Paraná, south coast of Brazil	0.12 to 11.42	Borzone & Souza (1996)
Callichirus major	Atami Beach, Paraná, south coast of Brazil	mean of 10.1	Souza et al. (1998)
Callichirus major	Leste Beach, Paraná, south coast of Brazil	8.5 to 9.3	Souza & Borzone (2003)
Callichirus major	Piedade Beach, Pernambuco, northeast coast of of Brazil	mean of 6.07	Botter-Carvalho et al. (2002)
Callichirus major	Itararé Beach, Southeast coast of São Paulo, Brazil	mean of 2.63	Present study
Callichirus major	José Menino Beach, Southeast coast of São Paulo, Brazil	mean of 6.41	Present study

Shimizu 1997), a behavior attributed by Felder & Lovett (1989) to territorial defense or mating. The densities of the *Sergio mirim* (Rodrigues 1971) was not considered too, since their occurrence and distribution in deeper areas (Rodrigues 1983), a fact that attributed greater accuracy to the density of *C. major* estimated.

Although previous studies indicate that sediments composed of fine to very fine sand support the occurrence of high densities of *C. major* (Borzone & Souza 1996, Rodrigues & Shimizu 1997, Dworschack 2000, Souza & Borzone 2003, Botter-Carvalho et al. 2007), the results recorded in both beaches did not indicate the influence of this factor. Pinheiro et al. (2011) analyzed the density of *C. major* at the beaches in Santos close and distant from the drainage channels (constructed mainly to drain rainwater from the urban region), but these authors recorded a higher density of *C. major* associated with this human intervention as a consequence of higher organic material

local content. However, the content of organic matter in this study was not correlated with the densities.

The higher density of *C. major* at the José Menino Beach when compared to the density at the Itararé Beach, similar as observed by Pedrucci & Borges (2009), could be a consequence of the lower capture effort by fishermen over time in the area due to law 850/1992, which prohibits the catching of this crustacean, considering that the beaches are similar in environmental aspects (organic content and sediment size), connected geographically, and that these facts did not influence the densities obtained.

The capture of individuals can cause fluctuations in the density and temporal distribution of the species. Overexploitation does not require the removal of large quantities of specimens from the beaches due to distinct biological potential that species have for their repopulating that also depend of environmental quality.

Table III. Reproductive periods of *C. major* and other callianassids in different localities.

Species	Locality	Reproductive period	References
Sergio mirim (Rodrigues, 1971)	Cassino Beach, Rio Grande do Sul, south coast of Brazil	September-January	Pezzuto (1998)
Callichirus seilacheri (Bott ,1955)	Las Machas Beach, Chile	March-September	Hernaéz and Wehrtmann (2007)
Callichirus major (Say, 1818)	Atami Beach, Paraná, south coast of Brazil	November-January	Souza et al. (1998)
Callichirus major (Say, 1818)	Piedade Beach, northeast coast of Brazil	December - May	Botter-Carvalho et al. (2007)
Trypaea australiensis (Dana, 1852)	Western Port, Victoria, Australia	September - December	Butler et al. (2009)
Biffarius arenosus (Poor, 1975)	Western Port, Victoria, Australia	September – February	Butler et al. (2009)
Callichirus major (Say, 1818)	Itararé Beach, Southeast coast of São Paulo, Brazil	December - March	Present study

Some vulnerable species can present significant declines in population even with relatively minimal fishing effort levels (Tamaki & Ingole 1993, Kura et al. 2004, Duarte et al. 2010, 2014, FAO 2018). Wynberg and Branch (1997) confirmed through experiments that the catching of the callianassid *Callianassa kraussi* (Stebbing 1990) in South Africa from the use of suction pumps caused a decline in the population density of this species, as well as a destruction of its galleries. In Australia, a study by Contessa and Bird (2004) also showed the capture of the callianassid *Trypaea australiensis* (Dana 1952) using a suction pump caused a reduction in the densities due to changes in sediment properties, such as porosity and oxidation, creating conditions unfavorable for the occupation of the species. Hernaéz and Wehrtmann (2007) affirmed that the catching of *Callichirus seilacheri* (Bott 1955) for use as bait in fishing can affect density considerably over time.

As regards the population structure, females predominated in the sampled population, consistent with other studies (Rodrigues et al.

1994, Rodrigues & Shimizu 1997, Souza et al. 1998, Botter-Carvalho et al. 2007). This proportion can be explained because most of the male callianassids construct deeper galleries, making them harder to capture with a suction pump (Rodrigues & Shimizu 1997).

As for the months of occurrence of ovigerous females, the reproductive period was seasonal (December to March), diverging from the results of Rodrigues (1983) and Rodrigues et al. (1986), who obtained ovigerous females over the course of one year at José Menino, and Peiró et al. (2014), who collected ovigerous females over one year at the northern coast of the state of São Paulo. Different reproductive periods reported for *C. major* and other callianassids can be seen on Table III.

Proportionally, low numbers of ovigerous females were sampled at Itararé in comparison to the studies carried out at José Menino by Rodrigues et al. (1994) (41.3%) and Rodrigues & Shimizu (1997) (45%), by Peiró et al. (2014) at the northern coast of the state of São Paulo (41%), and by Botter-Carvalho et al. (2007) (21.40%) at the northeast of Brazil. Botter-Carvalho et al.

(2007) stated that ovigerous females tend to move to the top of the galleries in order to expose their eggs to ventilation and can therefore be captured more easily. However, contrary to this statement, Peiró et al. (2014) mentioned that ovigerous females tend to inhabit the bottom of the galleries, making the capture of these individuals difficult with the suction pump. According to Hernáez et al. (2008), the males of *C. major* fertilize low numbers of females during mating events, resulting in low ovigerous abundance. There is still the possibility of the capture of individuals of larger body sizes for use as bait, and this would include the removal of adult or ovigerous females from the populations (McPhee & Skilleter 2002).

The size of ovigerous females recorded (8.09-10.88 mm) was lower than those observed in other studies (11.91-16.2 mm by Sendim et al. 2007 and 10.3-15 mm by Peiró et al. 2014). Similar smaller sizes those obtained in the present study were found by Souza et al. (1998) (minimum of 8.5) and Botter-Carvalho et al. (2007) (7.2-12.6 mm) precisely in beaches where an overexploitation of the population was documented. According to Sparre & Venema (1998) and Kura et al. (2004) a population could reproduce earlier under fishing pressure.

In terms of body sizes of the all individuals, the mode of the oval length registered at Itararé for both sexes (8.08±1.41 mm) was inferior to results obtained at José Menino by Rodrigues et al. (1994) (12 mm for this structure in both sexes). The results are also lower than those found by Rodrigues & Shimizu (1997) at the same beach, where the authors related the displacement of the mode to the lower classes (from 15 to 12 mm) and associated this fact with the increase in the number of juveniles, as a result from overexploitation of *C. major.* In the present study was observed for all months a mean of oval length smaller than 9 mm, which

refute the supposed high frequency of juveniles and could be related to the recruitment period of the species.

In addition, a temporal body size decrease is usually an indicator for identifying overexploited populations (Sparre & Venema 1998, Kura et al. 2004).

The body oscillations evidenced throughout this study differentiate from the data obtained by Rodrigues et al. (1986), who found no variation over time in the oval length at José Menino and affirmed that the growth of the individuals was slow. Thus, the importance of a new sampling of *C. major* individuals at José Menino is evident in order to evaluate their population structure and update information about the population structure and reproductive biology. At the northeastern region of Brazil, Botter-Carvalho et al. (2007) detected significant variations over the months in the oval length of *C. major,* linking this event to the excessive capturing of the animals.

Similar to what was observed by Rodrigues and Shimizu (1997), the population structure of *C. major* may have been affected by excessive species capture in this study region. Furthermore, the capture method may also alter the density and the population structure of the species, as reported in other studies developed with calianassids (Wynberg & Branch 1994).

Even with the existence of law 850/1992 in Santos, there are no current conservation proposals for the species in Brazil, and as such, the establishment of periods closed to capture or a limit on the number of individuals captured may be necessary (Peiró & Mantelatto 2016).

The information provided in this study is relevant to understand the current population in order to address strategies about the exploitation management. It will serve to present a general view of the sustainability of the capture of this crustacean as bait to fish at the southern São Paulo State. These findings

represent a necessary first step toward the maintenance of healthy and sustainable stocks of C. major and other callianassids.

CONCLUSIONS

Considering that the beaches are contiguous, have similar morphodynamics characteristics and the mean grain size and the organic content present in the sand did not influence the results, it can be assumed that the exploitation (Itararé) and non-exploitation (José Menino) of the *C. major* is leading the significant differences of the densities between the beaches.

In the same sense, the presence of individuals with smaller body sizes in Itararé beach when compared with other studies and a shorter reproductive period, can also inferred that the capture of *C. major* is causing changes in their population structure. Decreasing specimens mean size observed in this paper may result from the fishing mortality, meaning that the individuals of the population cannot reach their maximum body size, and thus, possibly affects negatively the reproductive success and density afterward. Given the ecological importance of *C. major*, the data presented suggests that the adoption of conservation management, such as the prohibition or control of the collect of specimens by specific law at the Itararé beach, should be necessary.

Other studies about calianassids should be intensified, especially in urban beaches where the population of this group is susceptible to capture for use in fishing activities.

Acknowledgments

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Moschetto FA developed this study, processed the data, performed the analysis, interpreting the results and worked on the manuscript. Duarte LFA verified the statistical analysis and worked on the manuscript. Borges RP conceived of the presented idea, contributed to sample preparation, interpreting the results and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript and revisions.