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Variation in population and reproductive parameters of the amphipods, *Cymadusa filosa* Savigny, 1816 and *Sunamphitoe pelagica* (H. Milne Edwards, 1830), associated with *Sargassum* beds in an historically impacted bay

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Abstract

The aim of this study is to investigate if *Sargassum*-associated herbivorous amphipods *Cymadusa filosa* Savigny, 1816 and *Sunamphitoe pelagica* (H. Milne Edwards, 1830) present differences in their population parameters at sites located at different distances from a state marina, which is the main source of pollution (especially heavy metals) in an impacted bay. The study was conducted at four beach sites within Flamengo Bay, Ubatuba municipality, northern coast of São Paulo State, Brazil. The beaches are Lamberto and Ribeira close to the pollution source and Flamengo and Santa Rita, which are more distant. We observed the predominance of juveniles in the populations of *C. filosa* and *S. pelagica*, followed by females, with the sex ratio for both species being favored toward females, and the highest densities of individuals were observed during the summer. *Sunamphitoe pelagica* presented lower density, smaller ovigerous females and egg volumes at Lamberto beach, indicating a possible higher sensitivity to metal pollution for this species. *Cymadusa filosa* showed no clear alteration of density, number of ovigerous females and egg volumes between sites. Our results emphasize the importance of studying the life history and reproductive parameters of herbivorous amphipods, showing how these parameters can be altered in contaminated areas.

Keywords

Ampithoidae, density, life history, macroalgae, metal pollution

Introduction

Marine macrophytes (seaweeds) occur at high densities in coastal regions, presenting a wide variety of shapes that can form extensive beds that cover rocky shores and constitute an important biological substrate for a high diversity of marine invertebrates (Lewis and Stoner, 1983; Christie *et al.*, 2009, Walker *et al.*, 2013). In Brazil, seaweed beds are conspicuous in shallow waters and account for approximately 80 % of algal cover and biomass in certain coastal areas of the state of São Paulo and Rio de Janeiro (Oliveira Filho and Paula, 1980; Széchy and Paula, 2000). They are represented mostly by brown algae, macrophytes of the Phaeophyta, and the genus *Sargassum* (Tararam and Wakabara, 1981; Wakabara *et al.*, 1983).

However, with increasing urbanization and industrialization in the coastal region, macrophytes and their associated fauna are susceptible to anthropogenic changes, like the presence of contaminants of different origins in the environment (Roberts *et al.*, 2008b; Johnston and Roberts, 2009). Gorman *et al.* (2020) showed that seaweed loss is occurring on the Brazilian coast, particularly in *Sargassum* beds. This is mainly caused by ocean warming and other pressures such as high human population density and changed coastal setting — either exposed or sheltered, with greater loss in sheltered sites. Lourenço *et al.* (2019) demonstrated that *Sargassum* beds can also be affected by hydrocarbon pollution in seawater, which also affects the associated amphipods.

Among the great diversity of invertebrates associated with *Sargassum* sp. beds, Peracarida crustaceans have both a high abundance and species richness (Tararam and Wakabara, 1981; Tanaka and Leite, 2003; Leite and Turra, 2003 Machado *et al.*, 2019), with high representation from species in the herbivorous family Ampithoidae, such as *Cymadusa filosa* Savigny, 1816 and *Sunamphitoe pelagica* (H. Milne Edwards, 1830) (Jacobucci and Leite, 2006; Jacobucci and Leite, 2014). These species of herbivorous amphipods are closely associated with the algae they occupy, not only because they consume the leaflets (Duffy, 1990), but also because they use them to construct tubes as shelter for their newly hatched juveniles (Barnard and Karaman, 1991; Appadoo and Myers, 2003). However, the genus *Sargassum* is identified as an important contaminant bioaccumulator and heavy metal bio-absorber (Davis *et al.*, 2003; Miao *et al.*, 2014; Lourenço *et al.*, 2019), exposing these herbivores to the contaminants concentrated in the leaflets.

Amphipods can absorb metals either directly, resulting from the exposure to metals dissolved in the water, or indirectly, through the ingestion of contaminated algae (Perrett *et al.*, 2006; Roberts *et al.*, 2006). High rates of absorption and concentration of metals in these organisms generate energy costs associated with their excretion and detoxification, which can result in reduced growth and reproduction (Marsden and Rainbow, 2004). Many studies have been conducted showing the effects of pollutants, present in macroalgae, directly on individual amphipods resulting in higher mortality rates, reduced growth and fecundity of these organisms, including inducing changes in their secondary sexual characteristics (Besser *et al.*, 2005; Felten *et al.*, 2008; Pastorinho *et al.*, 2009; Prato *et al.*, 2013), and lowering densities (Jacobucci and Leite, 2014). These changes affect the survival, maintenance and growth of these contaminated populations. These aforementioned studies demonstrate the utility of amphipods as bioindicators of marine pollution and stress (Bellan-Santini, 1980; Guerra-Garcia and Garcia-Gomes, 2001).

This study analyzed the population and reproductive parameters of two abundant herbivorous amphipod species associated with *Sargassum* beds in an historically impacted bay, Flamengo Bay, investigating if sites at different distances from a main contamination source within the bay show differences in population parameters. The hypothesis is that populations closer to the contaminant source will have reduced population density and parameters indicating reduced reproductive potential.

Materials and Methods

Study area

This study was conducted in Flamengo Bay, Ubatuba, on the north coast of the state of São Paulo, Brazil (23°29′42″–23°31′30″S 45°05′–45°07′30″W). The bay has low wave intensity with moderate hydrodynamics and bottom dynamics, which allows for high rates of sediment deposition and the accumulation of organic matter (Fig. 1) (Lançone *et al.*, 2005). The inner portions of Flamengo Bay are divided into two main areas: Saco do Perequê-Mirim and Saco da Ribeira. In the Saco da Ribeira region, there is a large marina that offers nautical garage services, offshore loading and unloading, fishing, transportation for tourist activities and floating fuel stations for boats (CETESB, 2013). Due to these services, Saco da Ribeira is used as a monitoring and sampling point in the State's surface water quality reports by the São Paulo State Environmental Company (CETESB); and it is considered the main source of metal pollution to the bay (CETESB 2013; 2014; 2015). Data from CETESB (2013; 2014; 2015) indicate contamination by heavy metals (copper and zinc), along with elevated total nitrogen and phosphorus, present in sediments in Saco da Ribeira, with concentrations above reference values in the three years that this study was conducted (Tab. 1). Also, in a previous study conducted by P.A.S. Longo *et al.* (2021) in Flamengo Bay, it was shown that *Sargassum* fronds located in sites within Saco da Ribeira had high concentrations of heavy metals (Cu, Zn, and Fe) in their tissues.

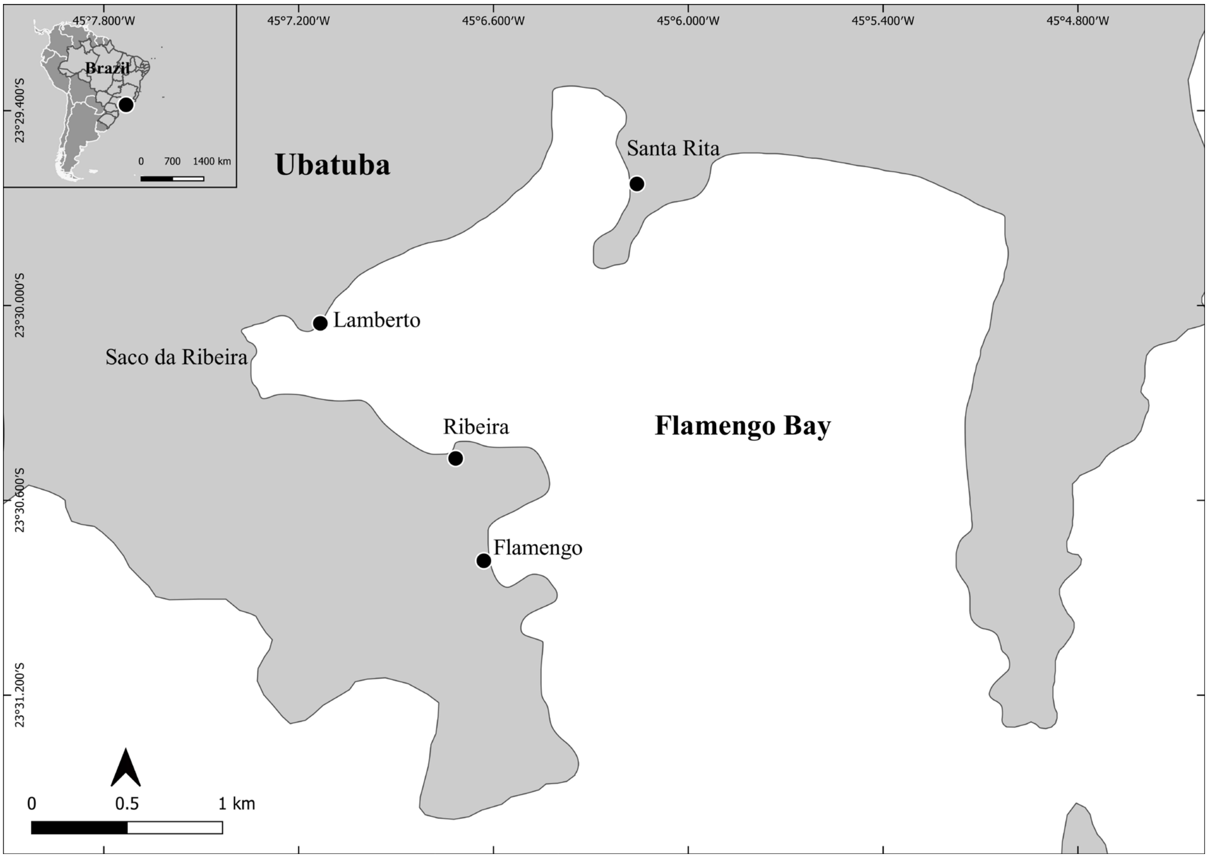

Figure 1. Map of the study area, Flamengo Bay, Ubatuba, on the north coast of São Paulo State, with the locations of the four beaches where the *Sargassum* fronds were collected. Inset (upper left) shows the position of the bay on the Brazilian coast, indicated by a point.

Table 1. Concentrations (mean ± standard error) (mg/kg) of heavy metals, total nitrogen, and phosphorus present in the sediment in Saco da Ribeira. Reference values are in the CETESB Reports. (CETESB 2013, 2014 and 2015). "–" = Absence of reference values.

Pollutants	2013	2014	2015	Reference Values (ISQG)
Copper	52.31 (±8.90)	53.83 (±11.43)	82.58 (±35.61)	18.7
Zinc	116.31 (±10.14)	127.25 (±10.91)	136.55 (±18.90)	124
Lead	29.23 (±3.79)	28.51 (±2.02)	41.81 (±2.69)	30.2
Cadmium	<0.5	<0.5	<0.5	0.7
Chrome	43.05 (±0.98)	43.80 (±1.81)	40.91 (±3.09)	52.3
Iron	43672.67 (±1046.32)	41910 (±1579.33)	38406.67 (±3026.98)	–
Total Nitrogen	1848.66 (±160.62)	1453.33 (±257.47)	1454.16 (±226.42)	1000
Phosphorus	1282.66 (±315.57)	746.16 (±35.53)	781 (±40.27)	700

Sampling procedure

Guided by previous results, samples were collected at four sites within Flamengo Bay: two located inside Saco da Ribeira (Lamberto and Ribeira beaches, closer to the main pollution source) and two outside that area (Flamengo and Santa Rita beaches). Collections were made on four sampling campaigns during the southern hemisphere winter (August 2013 and 2014) and summer (January 2014 and 2015).

Sampling was conducted in the infralittoral zone of rocky shores at each site, where five fronds of *Sargassum* sp. were collected at random through free diving along a horizontal transect, parallel to the rocky shore, at depths between one and two meters. The fronds were detached from the substrate and placed in a bag with 0.2 mm mesh size for faunal retention.

In the laboratory, the associated fauna was carefully removed by successive immersion of each *Sargassum* frond in fresh water. The detached animals were fixed in 70 % ethanol for counting under a stereomicroscope and identification. After separating the total number of animals associated with the algae, the ampithoidae were identified to species level according to the literature (LeCroy, 2002; Peart, 2004). After fauna removal, the samples of *Sargassum* were oven dried for 48 hours at 60 °C to obtain their dry weight and to estimate the density of individuals.

The sex differentiation of *C. filosa* and *S. pelagica* individuals was conducted according to secondary sexual characteristics: males present a second pair of gnathopods larger than the first pair; females have two pairs of similarly sized gnathopods and additionally have oostegites. Females carrying eggs or embryos were considered ovigerous (Jacobucci and Leite, 2002). It was possible to identify all the eggs in terms of their stage of development. When all the eggs are homogeneous and the egg membranes are intact with no changes in shape, this corresponds to stage B of egg development according to Leite and Wakabara (1989). Stage B eggs are strongly oval shaped, without oil globules, with head, body segments and appendages visible (Leite and Wakabara, 1989). Individuals were considered juveniles for those smaller than the smallest ovigerous female identified.

The dorsal length (size) of the individuals — the distance between the insertion of the first pair of antennae and the telson — and the egg diameter were measured from photographs taken under the microscope with the aid of AxioVision Rel. 4.8.

Data analysis

The total density of *C. filosa* and *S. pelagica* was obtained by the ratio of the number of individuals per *Sargassum* frond dry weight (individuals/gram = ind./g). To verify if the total density of *C. filosa* and *S. pelagica*, and density of individuals in each sexual category (females, ovigerous females, males, and juveniles) for each species, varied between seasons and sites, an analysis of variance (ANOVA) (Zar, 1999) was performed for each response variable, considering two fixed factors: Season (two levels: Summer and Winter) and Site (four levels: Flamengo, Lamberto, Ribeira and Santa Rita).

Fecundity was measured as the number of eggs per ovigerous female. Egg volume was measured using the formula $V = (\pi / 6 * d^3)$ where *d* is the largest diameter of each egg (De Paula *et al.*, 2016). For *C. filosa*, ovigerous females were found at all sampling sites, but in low numbers, for that reason, the fecundity and ovigerous female size were not statistically compared between sites. For *S. pelagica*, ovigerous females were found at only two of the four sites, but with a number of specimens per site that allowed the comparison of ovigerous female size and fecundity. For each measure, a Student T-test was performed, with one fixed factor: Site (two levels: Flamengo and Lamberto). Two linear regression analyses were used to determine the relationship between the size of *S. pelagica* ovigerous females and their fecundity at each site (Flamengo and Lamberto), with fecundity as the dependent variable. Male body length for both species was analyzed by ANOVA with one fixed factor: Site (four levels: Flamengo, Lamberto, Ribeira, and Santa Rita).

The sex ratios for both species, expressed as the proportion of the number of males to the number of males plus total females, were determined. Statistically significant deviations from the 1:1 proportion between males and females at each site were evaluated by binomial tests considering all individuals present in all samples.

All statistical analyses were performed in R v3.2.3 software (R Core Team, 2019). The level of significance was set at 5 %, and a P-value of less than 0.05 indicates a significant difference. The homogeneity of variances and normality of data were checked by visual inspection of residuals. Where necessary, data were transformed by log (X + 1).

Results

Density of individuals and population structure

A total of 849 individual amphipods were identified, of which 364 were *C. filosa* and 485 were *S. pelagica*.

The total density of *C. filosa* showed variation between locations, higher at Flamengo beach (0.33 ind./g) compared to Santa Rita beach (0.09 ind./g), and between seasons, with higher density of individuals in summer (summer: 0.33 ind./g; winter: 0.10 ind./g) (Fig. 2a, b; Tab. 2a). The density of *C. filosa* females and ovigerous females did not vary between seasons and sites (Fig. 2c; Tab. 2b, c). The density of *C. filosa* males varied only between seasons, with higher density of individuals in summer (summer: 0.09 ind./g; winter: 0.013 ind./g) (Fig. 2c; Tab. 2d). The populations of *C. filosa* showed a predominance of juveniles (individuals with < 7.54 mm length, size of the smallest ovigerous female) at all sites, with higher values during the summer (summer: 1.07 ind./g; winter: 0.23 ind./g) (Fig. 2c) and between sites, higher at Flamengo (1.02 ind./g) compared to Santa Rita beach (0.29 ind./g) (Tab. 2e). The sex ratio for *C. filosa*, considering all sites, differed from 1:1; it skewed towards females at Flamengo and Santa Rita beaches, but had no difference in the male:female ratio between other beaches (Tab. 3a).

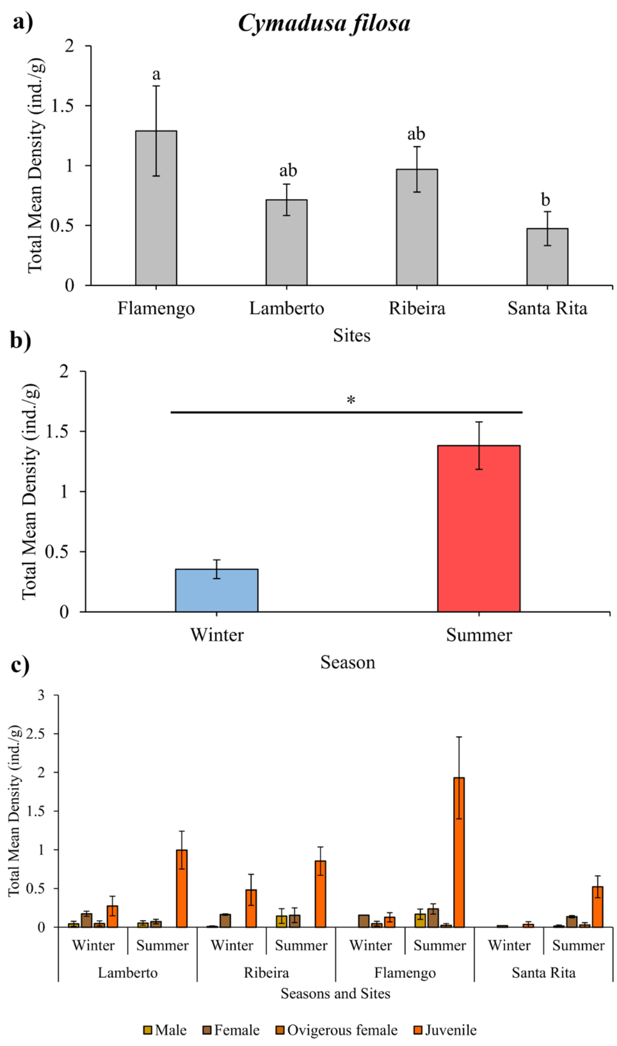

Figure 2. *Cymadusa filosa*. (**a**) Total mean density (ind./g) of individuals in different sites (± standard error); (**b**) Total mean density (ind./g) between seasons (± standard error); (**c**) Total mean density (ind./g) of males, females, ovigerous females and juveniles at each beach in winter and summer (± standard error). Asterisk represents significant differences and bars with the same letter indicate no significant difference at each beach.

Table 2. ANOVA results for *Cymadusa filosa* for comparisons of amphipod densities between sites and seasons. (**a**) Total density; (**b**) density of females; (**c**) density of ovigerous females; (**d**) density of males and (**e**) density of juveniles. Bold font indicates significant *P* values.

	Df	Mean Sq	*F*	*P*
a) Total density				
Season	1	5.097	37.931	**<0.001**
Sites	3	0.40	2.978	**0.037**
Season: Sites	3	0.114	0.851	0.470
Residuals	70	0.134		
b) Density of females				
Season	1	0.003	0.091	0.764
Sites	3	0.02	0.631	0.597
Season: Sites	3	0.028	0.747	0.528
Residuals	70	0.038		
c) Density of ovigerous females				
Season	1	0.041	0.96	0.33
Sites	3	0.046	1.095	0.357
Season: Sites	3	0.053	1.246	0.3
Residuals	70	0.042		
d) Density of males				
Season	1	0.089	7.56	**<0.01**
Sites	3	0.02	1.321	0.274
Season: Sites	3	0.02	1.747	0.165
Residuals	70	0.011		
e) Density of juveniles				
Season	1	3.942	32.473	**<0.001**
Sites	3	0.333	2.742	**0.049**
Season: Sites	3	0.285	2.344	0.08
Residuals	70	0.121		

Table 3. Sex ratio and Binomial Test results (p-values) for deviations from a 1:1 ratio between sex categories (male:female) of amphipod populations at each site. (**a**) *Cymadusa filosa*; (**b**) *Sunamphitoe pelagica.* Bold font indicates significant *P* values.

	Lamberto	Ribeira	Flamengo	Santa Rita
a) *Cymadusa filosa*				
P	0.052	0.359	**0.016**	**0.006**
Sex ratio	1:0.3	1:0.37	1:0.27	1:0.083
b) *Sunamphitoe pelagica*				
P	**0.001**	**<0.001**	**<0.001**	**<0.001**
Sex ratio	1:0.14	1:0.13	1:0.2	1:0.17

The total density of *S. pelagica* varied between locations and seasons, with higher densities in summer (1.40 ind./g) than in winter (0.14 ind./g) at Flamengo beach; and lower densities at Lamberto (0.07 ind./g) and Ribeira (0.15 ind./g) during summer compared to Flamengo (Fig. 3a; Tab. 4a). The populations of *S. pelagica* showed a higher density of females (2.45 ind./g), ovigerous females (0.40 ind./g), males (0.52 ind./g) and juvenile individuals (2.67 ind./g) (<3.09 mm, size of the smallest ovigerous female) at Flamengo beach during the summer, while the populations at Lamberto (females: 0.15; ovigerous females: 0.01; males: 0.04 and juveniles: 0.07 ind./g) and Ribeira beaches (females: 0.325; ovigerous females: 0.01; males: 0.07 and juveniles: 0.21 ind./g) showed low densities of individuals and equivalent values between sexual categories throughout the study period (Fig. 3b; Tab. 4b, c, d, e). The sex ratio for *S. pelagica*, considering all sites, differed from 1:1, skewed towards females at Lamberto, Ribeira, Flamengo and Santa Rita beaches (Tab. 3b).

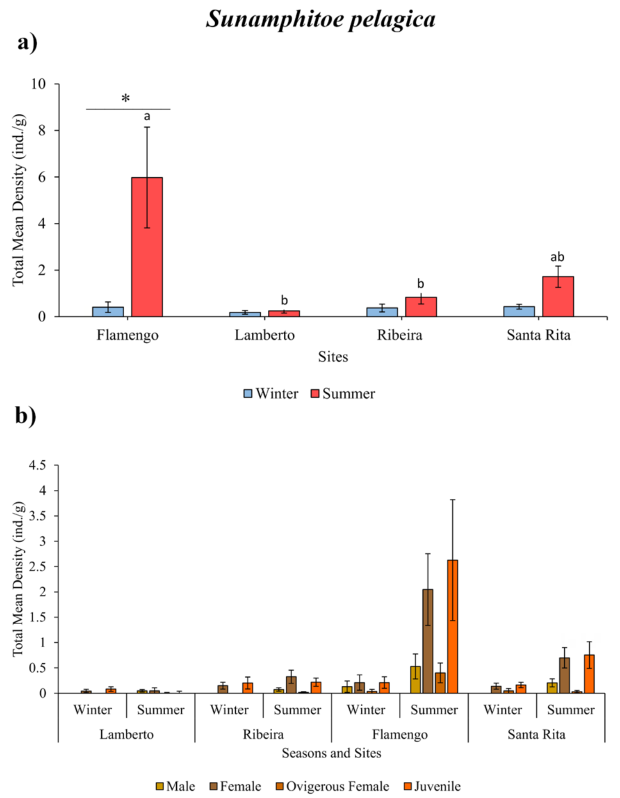

Figure 3. (**a**) Total mean density (ind./g) of *Sunamphitoe pelagica* at each beach in winter and summer; (**b**) total mean density (ind./g) of males, females, ovigerous females and juveniles at each beach in winter and summer (± standard error). Bars with the same letter indicate no significant differences. Asterisk represents significant difference

Table 4. ANOVA results for *Sunamphitoe pelagica* for comparisons of amphipod densities between sites and seasons. (**a**) Total density; (**b**) density of females; (**c**) density of ovigerous females; (**d**) density of males and (**e**) density of juveniles. Bold font indicates significant *P* values.

	Df	Mean Sq	F	P
a) Total density				
Season	1	5.823	23.549	**<0.001**
Sites	3	1.504	6.081	**<0.001**
Season: Sites	3	1.134	4.587	**0.005**
Residuals	71	0.247		
b) Density of females				
Season	1	2.162	18.818	**<0.001**
Sites	3	0.62	5.408	**<0.01**
Season : Sites	3	0.42	3.662	**0.016**
Residuals	70	0.114		
c) Density of ovigerous females				
Season	1	0.808	5.585	**0.02**
Sites	3	0.602	4.163	**<0.01**
Season : Sites	3	0.331	2.288	0.086
Residuals	70	0.144		
d) Density of males				
Season	1	0.35	10.109	**<0.01**
Sites	3	0.15	4.402	**<0.01**
Season : Sites	3	0.044	1.273	0.290
Residuals	70	0.034		
e) Density of juveniles				
Season	1	1.491	9.413	**<0.01**
Sites	3	0.722	4.561	**<0.01**
Season : Sites	3	0.546	3.447	**0.021**
Residuals	70	0.158		

Reproductive parameters

Ovigerous females of *C. filosa* were found at Lamberto, Santa Rita and Flamengo, with only seven ovigerous females at these three locations (Lamberto: N = 3; Santa Rita: N = 2; Flamengo: N = 2) with 145 eggs in total. The number of ovigerous females was too small for comparisons and fecundity calculations. The average egg volumes were higher at Santa Rita beach (0.054 ± 0.0015 mm^3) followed by Lamberto beach (0.037 ± 0.0010 mm^3) and Flamengo beach (0.022 ± 0.0011 mm^3). Males of *C. filosa* were found at Lamberto, Ribeira and Flamengo, comprising 23 males at these three locations (Lamberto: N = 9; Ribeira: N = 6; Flamengo: N = 8).

Sunamphitoe pelagica ovigerous females were found only at Lamberto and Flamengo, totalling 27 ovigerous females for all periods analyzed (Lamberto: N = 12; Flamengo: N = 15) with 212 eggs in total. The size of ovigerous females and fertility showed a significant and positive relationship only at Flamengo beach (Flamengo: $F = 4{,}942$, $p = 0.044$, Adjusted $R^2 = 0.219$; Lamberto: $F = 4{,}347$, $p = 0.063$, Adjusted $R^2 = 0.233$) (Fig.4 a, b). The size of these females was larger at Flamengo beach than at Lamberto (Fig. 4c; Tab. 5a). However, fecundity (number of eggs per female) did not vary between sites (Fig. 4d; Tab. 5b). The average egg volumes were higher at Flamengo beach (0.031 ± 0.0012 mm^3) than at Lamberto beach (0.022 ± 0.0006 mm^3). Males of *S. pelagica* were found at all sites, totalling 91 males in all periods analyzed (Lamberto: N = 39; Ribeira: N = 4; Santa Rita: N= 9; Flamengo: N = 39). Male body length did not vary between sites for either *C. filosa* or *S. pelagica* (Tab. 6).

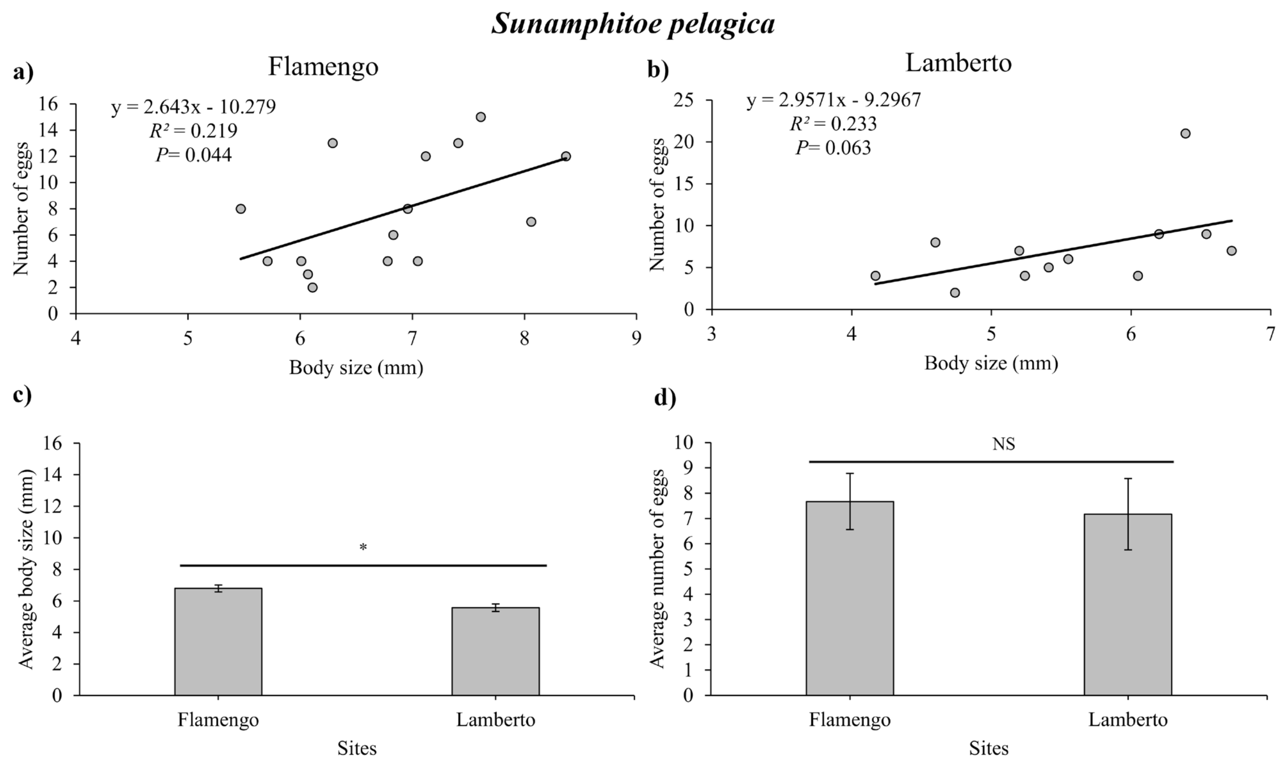

Figure 4. Linear regression between average body size and number of eggs of *Sunamphitoe pelagica* species at two locations, (**a**) Flamengo and (**b**) Lamberto; (**c**) Female body size (mm) at two locations and (**d**) Fecundity of *S. pelagica* at two locations, Flamengo and Lamberto (± standard error). NS represents no significant difference.

Table 5. (**a**) Student T-test results for comparison of size of ovigerous females of *Sunamphitoe pelagica* and (**b**) Student T-test results for comparison of fecundity of *S. pelagica*.

	Df	*T*	*P*
a) Ovigerous female size			
Sites	21.729	3.723	**0.001**
b) Fecundity			
Sites	24.271	0.294	0.77

Table 6. ANOVA results for comparisons of the following parameters between sites: (**a**) male body length of *Cymadusa filosa*; (**b**) male body length of *Sunamphitoe pelagica*.

	Df	Mean Sq	*F*	*P*
a) Body length of *Cymadusa filosa*				
Sites	2	0.012	0.457	0.640
Residuals	20	0.026		
b) Body length of *Sunamphitoe pelagica*				
Sites	3	0.036	1.403	0.247
Residuals	87	0.026		

The main biological characteristics (body size, number of ovigerous females, number of eggs, and egg volume) for *C. filosa* and *S. pelagica* are summarized and compared to other studies in Tab. 7.

Discussion

This study emphasizes that even on a small spatial scale, differences in population parameters of the two amphipod species could be observed. We observed the predominance of juveniles, a sex ratio favorable to females, and the highest densities of individuals during the summer for both species. *Sunamphitoe pelagica* presented lower density, smaller ovigerous females and egg volume at Lamberto beach. *Cymadusa filosa* showed no clear alteration of density, ovigerous females or egg volume between sites.

Sunamphitoe pelagica had the lowest density and a reduction in ovigerous females and egg volume at

Table 7. Summary of biological characteristics for both species, *Cymadusa filosa* and *Sunamphitoe pelagica*, at each site in this study (Lamberto, Ribeira, Flamengo and Santa Rita) and in other studies elsewhere (Appadoo and Myers, 2004; Jacobucci and Leite, 2006). "–" = Absence of reference values.

	Lamberto	Ribeira	Flamengo	Santa Rita	Appadoo and Myers (2003)	Jacobucci and Leite (2014)
a) *Cymadusa filosa*						
Female body size (mm)	11.09 ±0.59	10.06±0.88	10.09±0.56	8.64±0.36	9.71±0.21	-
Male body size (mm)	10.34±1.07	10.92±0.87	9.68±0.46	13.89	8.825±0.23	-
Number of ovigerous females	3	0	2	2	393	16
Number of eggs in brood	16-24	0	10-27	10-41	8-56	15-69
Mean number of eggs	20.33±2.33	0	18.5±8.5	29.33±9.73	20.6 ± 8.0	29.63 ± 17.04
Mean egg volume (mm^3)	0.04±0.002	0	0.021±0.001	0.038±0.001	-	0.034 ± 0.008
b) *Sunamphitoe pelagica*						
Female body size (mm)	4.57±0.27	3.86±0.13	4.74±0.11	4.24±0.15	-	-
Male body size (mm)	5.69±0.32	5.95±0.51	5.87±0.17	5.31±0.43	-	-
Number of ovigerous females	15	0	12	0	-	13
Number of eggs in brood	2-21	0	2-15	0	-	10-22
Mean number of eggs	7.16±1.40	0	7.66±1.11	0	-	14.92 ± 3.84
Mean egg volume (mm^3)	0.02±0.0006	0	0.03±0.001	0	-	0.016 ± 0.005

Lamberto beach, the site closest to the main source of pollution in Flamengo Bay, the Saco da Ribeira marina. Local contaminants, such as heavy metals, can accumulate both in the sediment and in macroalgae tissues (Roberts *et al.*, 2006), ultimately becoming available to herbivorous species that use the algae as shelter and a food source (Scheffer *et al.*, 1984; Jacobucci and Leite, 2002; Roberts *et al.*, 2008a). The ingestion of contaminated algal tissues demands more energy for their excretion and consequently can negatively affect individuals, influencing aspects of amphipod population biology (Rainbow, 2002; Perrett *et al.*, 2006; Roberts *et al.*, 2006). For example, Jelassi *et al.* (2019), studying the talitrid amphipod, *Orchestia montagui* Audouin, 1826, demonstrated an increase in mortality and reduced body growth when exposed to heavy metal contamination. Vanucci-Silva *et al.* (2019) showed behavioral changes in an amphipod species exposed to silver and iron nanoparticles. Löf *et al.* (2016) showed that higher metal and hydrocarbon concentrations may be related to changes in reproductive parameters of an amphipod species, with an increase in the rate of embryo malformation. In this study, the lower population density of *S. pelagica* found at Lamberto could be related to a higher mortality rate of individuals, and higher energy expenditure for detoxification. This would reduce energy investment for reproduction, which is reflected in the lower egg volumes when compared to populations at Flamengo beach, farther away from the source of contamination.

However, *C. filosa* exhibited a different pattern of variation, with higher density at Flamengo than Santa Rita, but without statistically significant differences when compared to the sites closest to Saco da Ribeira (Lamberto and Ribeira beaches). Populations of *C. filosa* showed an exceedingly small number of ovigerous females at each site, but with a high number of eggs per female, the largest of which were found in the Lamberto population. Jacobucci and Leite (2006) also found only 16 ovigerous females of *C. filosa* in their study after one year of monthly collections in Fortaleza Bay, also located in the municipality of Ubatuba. The same females showed greater fecundity and egg volume than the ovigerous females of *S. pelagica* and *Ampithoe ramondi* Audouin, 1826. One possible explanation for the low number of ovigerous females found could be the greater mortality risk that the adults suffer due to predation by selective visual predators like fishes (Sainte-Marie, 1991; Jacobucci and Leite, 2006). The amphipod *C. filosa* is larger than *S. pelagica*, and mainly the ovigerous females. Because the ovigerous females are larger, they can be more easily located by visually-oriented predators and then more heavily predated (Ryer, 1988; Edgar and Aoki, 1993). In addition, in another study carried out with *C. filosa*, it was observed that the preference for refuge of this species is in the alga *Padina gymnospora* compared to *Sargassum filipendula* (see Machado *et al.*, 2019). Therefore, the ovigerous females may have been found in smaller numbers in the current study because they were preferentially sheltering in *P. gymnospora,* which is also very common in Flamengo Bay (personal observation).

Both species had an increase in density in summer, which could be a consequence of greater availability of food sources during this period, due to an increase in temperature that can favor the growth of epiphytes, for example, which provide food as well as greater protection against predators for associated fauna (Martin-Smith, 1993; Tanaka and Leite, 2003).

In general, the sex ratio for both species tended to skew towards females, except for *C. filosa* in Lamberto and Ribeira, which maintained a 1:1 proportion. Those deviations might be related to differences in life cycles, mortality rate, longevity and behavior patterns between males and females (Jacobucci and Leite, 2006). One possible explanation for the deviation from the 1:1 ratio may be the differences in habitat use between the sexes in these Ampithoidae species. For example, males leave their tubes more frequently to search for females for reproduction and thus potentially suffer from higher predation pressure (Leite *et al.*, 2003; Appadoo and Myers, 2004).

The differences in life history parameters of closely related species allow them to occur in sympatry, which is commonly reported for macroalgae-associated amphitoid species (Gilat, 1962; Appadoo and Myers, 2004; Jacobucci and Leite, 2006). *Sunamphitoe pelagica* and *C. filosa* have similar niches in that they are both tubicolous and herbivorous species (Appadoo and Mayers, 2003; Bueno *et al.*, 2017). *C. filosa* has a high capacity to build tubes, constructing them in a few hours from debris, faeces and algal fronds (Appadoo and Myers, 2003). Although both are meso-herbivores, their preferences and dietary restrictions are distinct, with *C. filosa* being a more generalist species that can be associated with different host species such as red, green and brown macroalgae (Ruffo, 1982; LeCroy, 2002; Machado *et al.*, 2019; Peres *et al.*, 2019), and with a dispersion capacity higher than expected for a species with direct development (Peres *et al.*, 2019). In addition to the host algae, *Sargassum*, they are also able to feed on any epiphytic algae, such as *Hypnea musciformis* and *Canistrocarpus cervicornis*, especially in periods when *Sargassum* densities are low. *Sunamphitoe pelagica*, in contrast, is a more specialist species and has diet and habits restricted to *Sargassum* beds (Tararam *et al.*, 1986; Dubiaski-Silva and Masunari, 2008; Machado *et al.*, 2017).

Therefore, we hypothesize that differences in the patterns of variation in population parameters of *S. pelagica* and *C. filosa* are related to differences in contaminant concentrations between sites, associated with the ecological niche of each species. Flamengo Bay is considered a polluted area, however, with intermediate values of metal concentration compared to other sites (Longo *et al.*, 2021). Therefore, metal concentrations found in the areas closer to the pollution source allow amphipod survival. However, this may have a greater effect on specialist species, which are dependent on a particular resource that is affected by contamination, while more generalist species can easily change resources when needed (Rohr *et al.*, 2006). Thus, the more specialist *S. pelagica*, which is highly dependent on *Sargassum* fronds for food and shelter, is potentially more exposed to indirect contamination through their diet (Roberts *et al.*, 2006) due to the high capacity for absorption of contaminants in *Sargassum* (see Davis *et al.*, 2003; Seepersaud *et al.*, 2018). This fact might explain the lower densities of this species found at Lamberto. *Cymadusa filosa*, a generalist, is possibly more tolerant, and expresses less density differences and higher egg volumes at Lamberto beach.

Alternatively, other factors such as environmental conditions (*e.g.*, physicochemical water parameters, food quality and food availability) and ecological interactions (*e.g.*, predation, interspecific competition with other associated species and impact of invasive species) might also be relevant to explain the differences in amphipod population parameters (McLusky, 1967; Coull and Wells, 1983; Conlan, 1994; Duffy and Hay, 1991; Rainbow, 2002; Angelini *et al.*, 2011; Ros *et al.*, 2013; Machado *et al.*, 2019). All these above factors can positively or negatively modulate amphipod growth, as they change the number and frequency of molting (Leite and Güth, 2003), which is partially dependent on age and maturity, consequently affecting the size of amphipod individuals (Hartnoll, 1992). These factors should be investigated in future studies.

In this study, we observed the predominance of juveniles in the populations of *C. filosa* and *S. pelagica*, followed by females, with the sex ratio for both species favoring females. The highest densities of individuals were observed during the summer, which is also presented in other studies, and could be related to an increase in the quantity of host algae and epiphytes that serve as food and shelter for juveniles during the warmer months, thus increasing their survivorship (Appadoo and Myers, 2004; Jacobucci and Leite, 2006).

Even though *S. pelagica* and *C. filosa* are phylogenetically close species, with similar niches (Tararam and Wakabara, 1981; Tanaka and Leite, 2004; Jacobucci and Leite 2006; Peart and Ahyong, 2016; Sotka *et al*., 2017), they exhibit local differences in population parameters within a semi-enclosed, anthropogenically-impacted bay. These differences should be further investigated in future experimental studies and toxicological trials with these two species, isolating factors and verifying the lethal and sublethal effects of different concentrations of contaminants on their physiology and population biology. This would assist in a better understanding of the susceptibility of these two important coastal amphipods to anthropogenic impact, as well as their potential to act as tropical bioindicator species.

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