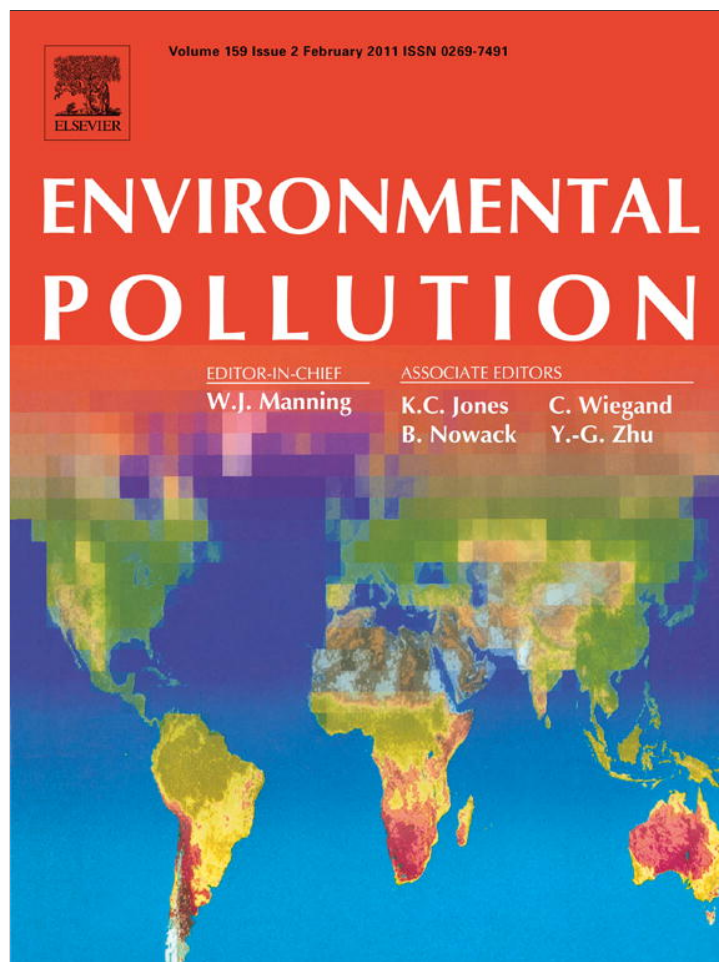


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Monitoring anthropogenic sewage pollution on mangrove creeks in southern Mozambique: A test of *Palaemon concinnus* Dana, 1852 (Palaemonidae) as a biological indicator

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The identification of the effects of the peri-urban conditions on *P. concinnus* increases the choice of possible bioindicators in East African coastal waters.

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ABSTRACT

Tropical coastal ecosystems, such as mangroves, have a great ecological and socioeconomic importance for adjacent systems and local populations, but intensive environmental impact monitoring is still lacking, mainly in East Africa. This study evaluated the potential anthropogenic disturbance on *Palaemon concinnus* population structure and fitness. *Palaemon concinnus* populations from one peri-urban (domestic sewage impacted) and two pristine mangrove creeks were studied by sampling nearly 100 shrimps per location every 15 days for 12 months. The shrimps at the peri-urban location were larger, experienced longer reproductive periods, presented higher proportion of ovigerous females and better embryo quality when compared with shrimps inhabiting pristine locations. Physiological indices (RNA/DNA ratio) were similar between shrimps at pristine and peri-urban mangroves. However, a higher level of parasitization by a Bopyridae isopod, *Pseudione elongata* indicated some degree of stress on the host at the peri-urban mangrove, with potential effects on the host population dynamics.

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1. Introduction

Over the last several decades mangrove ecosystems have been adversely affected by anthropogenic activities including deforestation and dumping activities (Duke et al., 2007; Kruitwagen et al., 2008), despite its great socioeconomic and environmental importance (Nagelkerken et al., 2008; Walters et al., 2008). Most studies done in these forests have focused on the *in situ* quantification (water, sediments, and organisms) of anthropogenic contaminants (Kruitwagen et al., 2008).

However, determining when contamination results in ecosystem pollution requires not only chemical but also biological measurements (Chapman, 2007). Studies of contaminant-induced alterations in fauna communities' abundance and diversity were

developed and are commonly used in environmental assessment (Bigot et al., 2006; Saunders et al., 2007), including mangroves (Cannicci et al., 2009), and have been considered as the most important tool for environmental impact assessment studies (Warwick, 1992). Community structure and dynamics are merely expressions of variation in the population of constituent species and the response of these species to environmental stress (Smith and Suthers, 1999). In turn, the population structure is dictated by its fitness parameters, such as growth, physiological conditions and reproductive output, that might be more or less sensitive and used as an early warning tool to determine deterioration of ecosystem health (Attrill and Depledge, 1997).

Positive indicators, also named opportunistic species, from polychaete worms to fishes (Tsai, 1975 in Méndez et al., 1997; Smith and Suthers, 1999), tend to increase their abundance in polluted areas by out competing with local species. Negative indicators are usually key fauna species in the environment that tend to decrease biomass, show reduced growth rates (Crouau and Moia, 2006; Amara et al., 2009), as well as low reproduction output and quality (Smith and Suthers, 1999; Crouau and Moia, 2006). They are efficient indicators of contamination due to a general reallocation

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of resources favouring tolerance to stress, by combating contaminants, low oxygen levels or repairing genetic or physiological damage caused by pollutants (Diaz and Rosenberg, 1995; Wu, 2002). Morphological structures and physiological processes (such as RNA/DNA ratios and cytological modifications) have also been an alternative indication of an organism's (including foraminifera, bivalves and crustaceans) condition when facing stress or pollution (Norkko et al., 2006; Amaral et al., 2009a).

Parasite infection has also been recently considered as a good indicator of anthropogenic pollution due to a decrease in the host defence mechanisms and fitness, thereby affecting the susceptibility (Sures, 2004), abundance, tolerance to pollution, behaviour and mortality of the latter (Lafferty, 2008).

While these population fitness indicators have proven to be reliable for different anthropogenic contaminants in many different ecosystems, they are scarce for mangrove habitats. A range of ecological responses in few key mangrove species have been attributed to sewage pollution including: increased or decreased abundance, increased mortality and reduced growth, changes in reproduction parameters, behaviour and ecosystem functioning (Bartolini et al., 2009; Penha-Lopes et al., 2009a,b, 2010, in press).

Recently, a multi-disciplinary approach to investigate the impacts of sewage pollution on single species, mainly polychaetes and fishes, has been used as indicators of anthropogenic impacts (e.g., Smith and Suthers, 1999; Amara et al., 2009), but once again nonexistent for mangrove habitats. The shrimp *Palaemon concinnus* Dana, 1852 was selected as a potential bioindicator of organic pollution for Eastern African mangroves due their high abundance and fidelity in mangrove creeks, and low importance for local or commercial fisheries. Nevertheless it is one of the least studied species in mangrove habitats. Only a few studies have focused on this species' geographic distribution, taxonomic or morphological variations (Dutt and Ravindra, 1974; De Grave, 1999) although it is probably a food source for commercial fish species that migrate into the mangrove during high tides (Nagelkerken et al., 2008).

The purpose of this work was to examine whether or not marked differences in anthropogenic disturbance altered *Palaemon concinnus* population structure and fitness, and therefore if these can be used as a reliable biomonitor of pollution in mangrove habitats. We used several different indices: population structure and average size, reproductive parameters (such as maturation curves, fecundity, potential fertility and embryo quality assessed through fatty acid analysis), parasite infection, and RNA/DNA ratio to test for habitat quality differences between a contaminated peri-urban and two pristine mangrove creeks in southern Mozambique.

2. Materials and methods

2.1. Study area and sampling design

We followed a Beyond BACI unbalanced design, comparing the ecological features recorded in one peri-urban mangrove swamp with those characteristic of two nearby pristine mangroves of similar ecological traits in Mozambique. Mangrove areas, sampling design and location selection were already extensively described in previous studies (Cannicci et al., 2009; Penha-Lopes et al., 2009b), however, a brief description will be done here.

The sampling was conducted in three different mangrove ecosystems: a peri-urban mangrove, Costa do Sol (CS – 25°55'S, 32°35'E), and two pristine mangroves located at Inhaca Island (26°S, 32°55'E), Saco (S) and Ponta Rasa (PR) (Fig. 1). The Saco mangrove covers an area of 2.1 km² and is located in a small, enclosed and shallow bay in the south of the island (Fig. 1). *Avicennia marina* and *Rhizophora mucronata* dominate its vegetation (Kalk, 1995). Ponta Rasa is the smallest mangrove system on the island, covering about 0.2 km², and is located on the southwest coast facing Maputo Bay (Fig. 1). The creek is dominated by *Rhizophora mucronata*, with sparser patches of *Ceriops tagal*, *Bruguiera gymnorrhiza* and *Avicennia marina* (Paula et al., 2003). Ponta Rasa has unique characteristics such as closure during neap tide due to the presence of a sill at the entrance of the creek, very sandy soils and a lower sediment fauna diversity and abundance than the Saco mangroves, but the same diversity in terms of the macrofaunal species (Kalk, 1995). Costa do Sol mangrove is

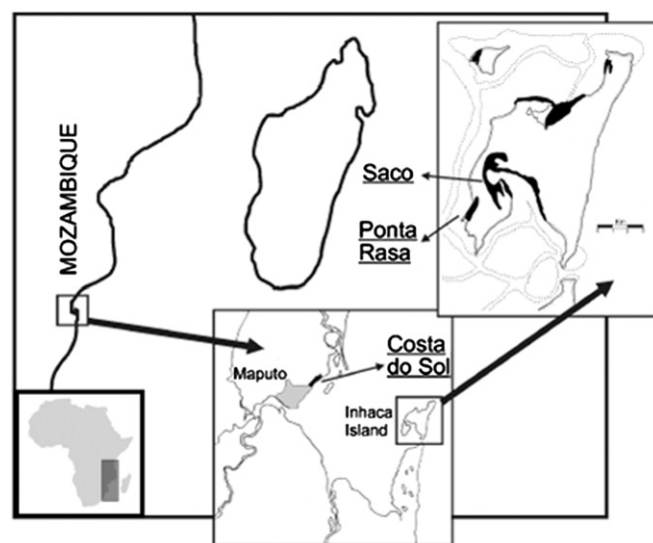


Fig. 1. Location of study sites in southern Mozambique.

located at Maputo Bay, ~7 km north of Maputo city and is nearly 2 km². The seasonal river Quinhenganine discharges in the swamp after passing through the city. The mangrove creek is bordered by a residential area and has been receiving domestic sewage, aquaculture residuals and solid dumps from various sources during the last several decades (PUMPSEA, 2008). Higher sediment organic content and pathogens present in the porewater was observed at Costa do Sol (Salvador, 2007; PUMPSEA, 2008). Also, nutrient concentration in sediment porewater, especially nitrites and nitrates, characterized Costa do Sol (0.49 ± 0.52 μM and 3.90 ± 7.69 μM, respectively) in relation to Saco (0.17 ± 0.09 μM and 0.08 ± 0.09 μM, respectively) and Ponta Rasa (0.18 ± 0.06 μM and 0.70 ± 0.06 μM, respectively) mangroves (PUMPSEA, 2008). A more detailed characterization of the mangroves can be found at Cannicci et al. (2009).

Sampling was done every low spring tide (± every 15 days) from August 2005 to August 2006 in all 3 mangroves. On each sampling occasion, nearly 100 *Palaemon concinnus* specimens were collected with the help of a 1 mm mesh net. Water temperature and salinity were also measured. Based on Kalk (1995) and data obtained during this work (see Fig. 2) the wet season was considered from December to May and dry season from June to November. While in the wet season temperatures are higher, and heavy rains common, promoting an increase of nutrient concentration in the water channels (Paula et al., 1998) and mangrove sediments, the dry season tends to be cooler with sporadic short rains.

2.2. Population structure, parasitism and maturation

Shrimp samples were preserved in buffered 4% formaldehyde diluted with seawater, and taken to the laboratory for examination. Specimens were identified, counted and their sex determined (by investigating the presence (male) or absence (female) of an appendix masculina on the second pair pleopod). Standard length (SL), from the posterior margin of the orbit to the tip of the telson, were taken under a binocular microscope with a calibrated micrometer eyepiece, to the nearest 0.02 mm. Presence of the parasite, *Pseudione elongata africana* Kensley, 1968, in the cephalothorax was also registered.

According to the criteria used by Kensler (1967), female maturation size was determined by calculating the size which corresponded to 50% of ovigerous females. The proportion of mature females by size was fitted to a logistic equation:

$$P = \frac{1}{1 - e^{(a+b \times SL)}} \quad (1)$$

With the logarithmic transformation given by:

$$\ln\left(\frac{1-P}{P}\right) = a + b \times SL \quad (2)$$

where "P" is the predicted mature proportion, "a" and "b" the estimated coefficients of the logistic equation ("a" as the y-intercept value and "b" as the slope value), and SL the standard length. The estimation of parameters in this equation was made by correlation analysis of variables of P and SL after linearization. Size at sexual maturity (SL₅₀), corresponding to a proportion of 50% sexually mature females, was estimated as the negative of the ratio of the coefficient (SL₅₀ = -(a/b)) by substituting P = 0.5 in Eq. (1).

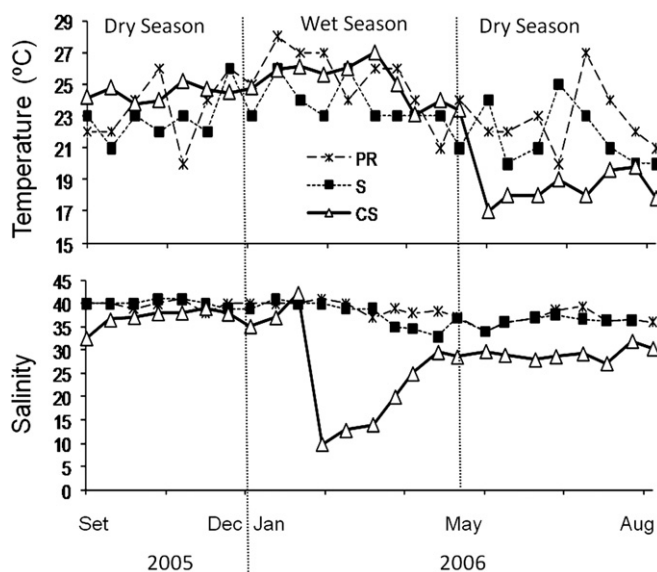


Fig. 2. Temperature and salinity dynamics measured at the low spring tide in all three mangrove creeks during a period of 12 months. Values are given as average (\pm SE).

2.3. Condition indices (RNA/DNA)

During the January–February 2006 campaign at all sampling locations, 7 haphazardly selected ovigerous female shrimps in intermoult stage, as evaluated *in situ* by the hardness of the carapace, were measured and not preserved in formalin. The individuals were immediately transported to the laboratory in cool boxes. In the laboratory, body muscle was removed, promptly freeze dried, and stored at -80°C until nucleic acid quantification. Nucleic acids were quantified using the Schmidt–Thannhauser method as described by Amaral et al. (2009a,b). Analyses were performed using ~ 15 mg (freeze-dried weight) white claw muscle. RNA and DNA absorbances were measured in a NanoDrop[®] ND-1000 full spectrum spectrophotometer (NanoDrop, USA). The full spectrum feature allows inspection of nucleic acid contaminations by calculation of 260 nm/280 nm and 260 nm/230 nm ratios, concurrently with sample analysis.

2.4. Fecundity and embryo development

In this study we used the same fecundity and potential fertility concepts employed in previous studies (Penha-Lopes et al., 2007; Torres et al., 2007) since the goal was to evaluate the initial egg number as well as the changes observed throughout the embryonic development, mainly regarding brood loss and embryo fatty acid composition. Thus, fecundity is considered as the number of newly extruded embryos (stage I), while potential fertility, on the other hand, is calculated based on the number of embryos just before hatching (stage IV).

Fecundity and potential fertility were obtained for both wet season, known to account for the highest reproductive activity in crustaceans in these subtropical mangroves (Penha-Lopes et al., 2009b; Torres et al., 2009), and dry season (except for Saco where no ovigerous females were found). Two ratios were then developed: “Fecundity per SL ratio” and “Potential Fertility per SL ratio”. Fecundity per SL is calculated dividing the number of embryos at stage I divided by the female SL, while Potential Fertility per SL uses the egg number at stage IV.

2.5. Fatty acids analysis

Fatty acid (FA) profiles of *P. concinnus* embryos were determined during the highest reproductive season (wet). Three replicates per treatment were used, each one comprising 4–7 embryo batches (from different females). FA processment and analysis were conducted following the methodology described by Penha-Lopes et al. (2009b).

2.6. Statistical analysis

PERMANOVA test, permutational multivariate analysis of variance (Anderson and Robinson, 2001), with asymmetrical design were used to test the null hypothesis of no difference in 1) total, males, females and ovigerous females size at all 3 sampling locations and between seasons; 2) RNA/DNA ratio, RNA content and DNA content at all 3 location during the wet season, and 3) in both the Fecundity per SL and Potential Fertility per SL among peri-urban and non-urban locations. Similarity matrices were computed using Euclidean distance on untransformed data. All analyses were based on 9999 permutations of residuals within a reduced model and Type III sums of squares to cope with the unbalanced design (Anderson et al., 2008). Three and two-way univariate PERMANOVA tests were applied and factors used are described in each respective table. All analyses were performed using PRIMER v. 6.1 (Clarke and Gorley, 2006) and the PERMANOVA+ for PRIMER routines (Anderson et al., 2008).

Possible differences in the relative frequencies of males, females and ovigerous females as well as differences in the relative frequencies of parasites in males and females at impacted and control locations were determined using chi-square test. To compare fatty acids composition between populations and embryo stages a two-way ANOVA test was used (followed by Tukey test in case of significant difference). All results were considered statistically significant at $p < 0.05$ levels.

3. Results

3.1. Environmental conditions

Water column temperatures in all three mangrove creeks increased slowly from August 2005 to March 2006, decreasing afterwards in all mangroves but dramatically at CS during February to April 2006 (Fig. 2). Salinity ranged between 35 and 40 in pristine mangroves, while at CS a decrease to values near 10 occur at the start of the rainy season (January 2006), increasing afterwards although remaining with slightly lower values than the values obtained at the water from pristine creeks (Fig. 2).

3.2. Population structure, maturation and parasitism

Shrimp standard length was significantly ($t = 1.89$; $p = 0.05$) higher at Costa do Sol (5.18 ± 0.06 mm) population than at both pristine locations (4.41 ± 0.13 and 4.13 ± 0.10 mm for Saco and Ponta Rasa, respectively) for the entire year (Fig. 3, Table 1). Average size of ovigerous females was slightly bigger than non-ovigerous females ($t = 9.45$; $p = 0.06$) and males ($t = 250.51$; $p = 0.0001$). The size of all 3 groups decrease significantly ($p < 0.05$) according to the sequence $\text{CS} > \text{S} > \text{PR}$, except non-ovigerous female at S and PR (Fig. 4A).

The relative abundance of sexes strongly differed among locations (chi-square = 92.15; $df = 2$; $p < 0.001$, chi-square test), with

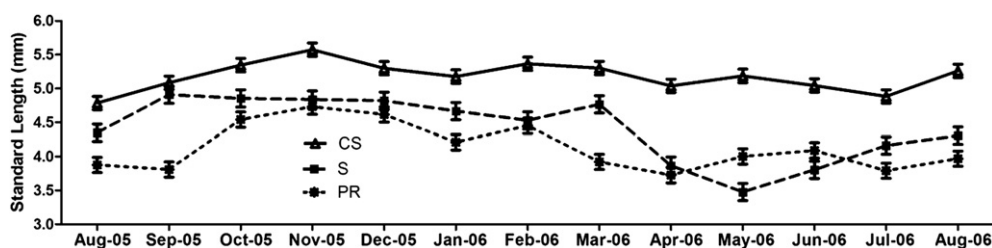


Fig. 3. Average standard length (\pm SE) of *Palaemon concinnus* population at the peri-urban (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, in the south of Mozambique, during a period of 12 months.

Table 1

Results of the three-way PERMANOVA conducted on size of *Palaemon concinnus*. Costa do Sol (impact) and Saco and Ponta Rasa (control). Factors are as follows: impact vs control (asymmetrical, fixed and orthogonal), location (random and nested in 'impact vs control' – just at the wet season) and sex/ovigerous (fixed and orthogonal). Degrees of freedom, df, mean squares, MS, F and its probability value, p, are shown for each factor.

Source	df	Total no. of specimens		
		MS	F	p
Impact vs control (I vs C)	1	54,453.00	22,052.00	0.0001
Location (I vs C)	1	20,426.00	40,424.00	0.0484
Sex/ovigerous	2	34.35	181.33	0.0035
I vs C × sex/ovigerous	2	20,739.00	11.27	0.0749
Location (I vs C) × sex/ovigerous	2	0.16816	0.3328	0.718
Res	237	0.5053		

males much more abundant in Saco and Costa do Sol and females more abundant in Ponta Rasa (Fig. 4B). Costa do Sol presented the highest ovigerous female frequency along the year, followed by Ponta Rasa and then Saco. Merging both pristine populations in a single group representing the “control locations” we found strong significant differences in frequency of ovigerous females between the controls and the impacted locations (chi-square = 519.11; df = 2; p < 0.001; chi-square test; Fig. 5A).

The maturation index (Fig. 5B), calculated based on the SL at which 50% of the females are ovigerous at the time of sampling, showed Ponta Rasa with the lowest values of 5.3 mm, followed by Costa do Sol (6.15 mm) and Saco (6.25 mm).

Although parasites were never observed in the cephalothorax of ovigerous females at all 3 locations, the percentage of infected

males and non-ovigerous females at Costa do Sol was almost always greater than 30%, sometimes reaching more than 60% in medium size classes (Fig. 5C). The frequency of parasitized shrimps was significantly lower in pristine mangroves (chi-square = 179.19; df = 1; p < 0.001; chi-square test). The value was always below 20%, and mostly between 5% and 10%. The frequency of parasite infection did not differ between sexes (chi-square = 1.01; df = 1; p = ns; chi-square test) and it varied with time but with no specific temporal pattern, although oscillations were similar between Costa do Sol and Saco shrimp populations (Fig. 6).

3.3. Physiological stress

The PERMANOVA and post hoc tests showed a significant (t = 2.71; p < 0.03, Table 2) lower RNA/DNA ratio in PR (1.10 ± 0.24 µg mg⁻¹ of freeze-dried dorsal muscle) compared with S and CS (1.56 ± 0.22 and 1.55 ± 0.29 µg mg⁻¹ of freeze-dried dorsal muscle, respectively). During the wet season, the overall average RNA content was significantly (p < 0.001) different in shrimps inhabiting all 3 mangroves, increasing from PR to S to CS, with average values of 0.77 ± 0.09, 1.06 ± 0.18 and 1.61 ± 0.19 µg mg⁻¹ of freeze-dried dorsal muscle, respectively (Table 2). DNA content was not significantly different between locations (ranging from 0.68 to 1.04 µg mg⁻¹ of freeze-dried muscle); therefore, the overall lower RNA/DNA ratio in PR shrimp populations resulted from lower RNA content.

3.4. Fecundity, egg development and egg loss

The number of eggs tended to increase with animal size for all seasons, locations and egg stages. PERMANOVA and post hoc pair-wise tests revealed that females sampled at PR were slightly smaller than those collected at S and CS (Table 3, Fig. 7A, B). The PERMANOVA test performed on the number of eggs carried by each female, standardised to her SL, showed a significant difference between peri-urban and pristine locations (or impact vs control in Table 3, Fig. 7C, D). Post hoc pair-wise tests showed that females colonising the impacted location of Costa do Sol were producing more eggs at both egg stages than the ones collected in the two control locations (t = 323.3; p < 0.001). Nevertheless, it also showed that at the impacted location there was significant egg loss, with females carrying significantly fewer eggs at stage IV than at stage I (t = 3.43; p < 0.001). In the dry season, Costa do Sol and Ponta Rasa ovigerous females presented similar number of eggs/SL at both egg stages.

3.5. Fatty acids analysis

In general, by analysing the data from all 3 sampled zones, we find a common background in fatty acid used during embryogenesis by *Palaemon concinnus*, with most fatty acids being consumed during embryo development in all populations (Tables 4 and 5). The principal saturated fatty acids (SFA) comprising shrimp embryos were palmitic (16:0) and stearic (18:0) acids, while the primary monounsaturated fatty acids (MUFA) were oleic (18:1n – 9) acid, followed by vaccenic (18:1n – 7) and palmitoleic (16:1n – 7) acids (Table 5). The most abundant polyunsaturated fatty acids (PUFA) were eicosapentaenoic (EPA, 20:5n – 3) acid, followed by linoleic (18:2n – 6) acid. DHA/EPA ratio tended to decrease at all locations throughout embryo development, although at Costa do Sol this ratio was significantly higher, followed by Ponta Rasa and Saco. At all locations, 18:1n – 7/18:1n – 9 remained similar between embryo stages and locations (Table 5).

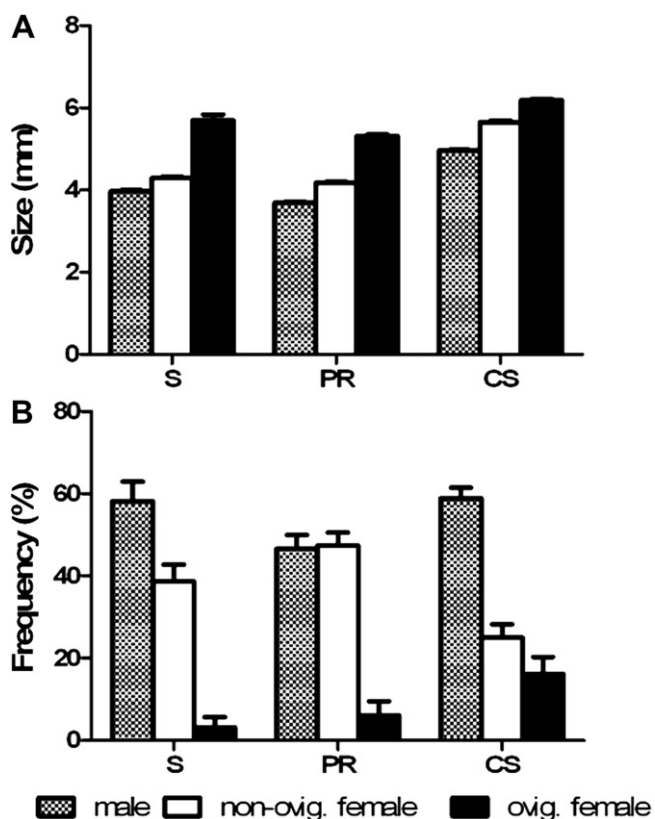


Fig. 4. Annual average (±SE) size (A) and frequency (B) of males (grey), non-ovigerous females (white) and ovigerous females (black) of *Palaemon concinnus* population at the peri-urban (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, in the south of Mozambique. n = 250–1895. (S) Saco; (PR) Ponta Rasa; (CS) Costa do Sol.

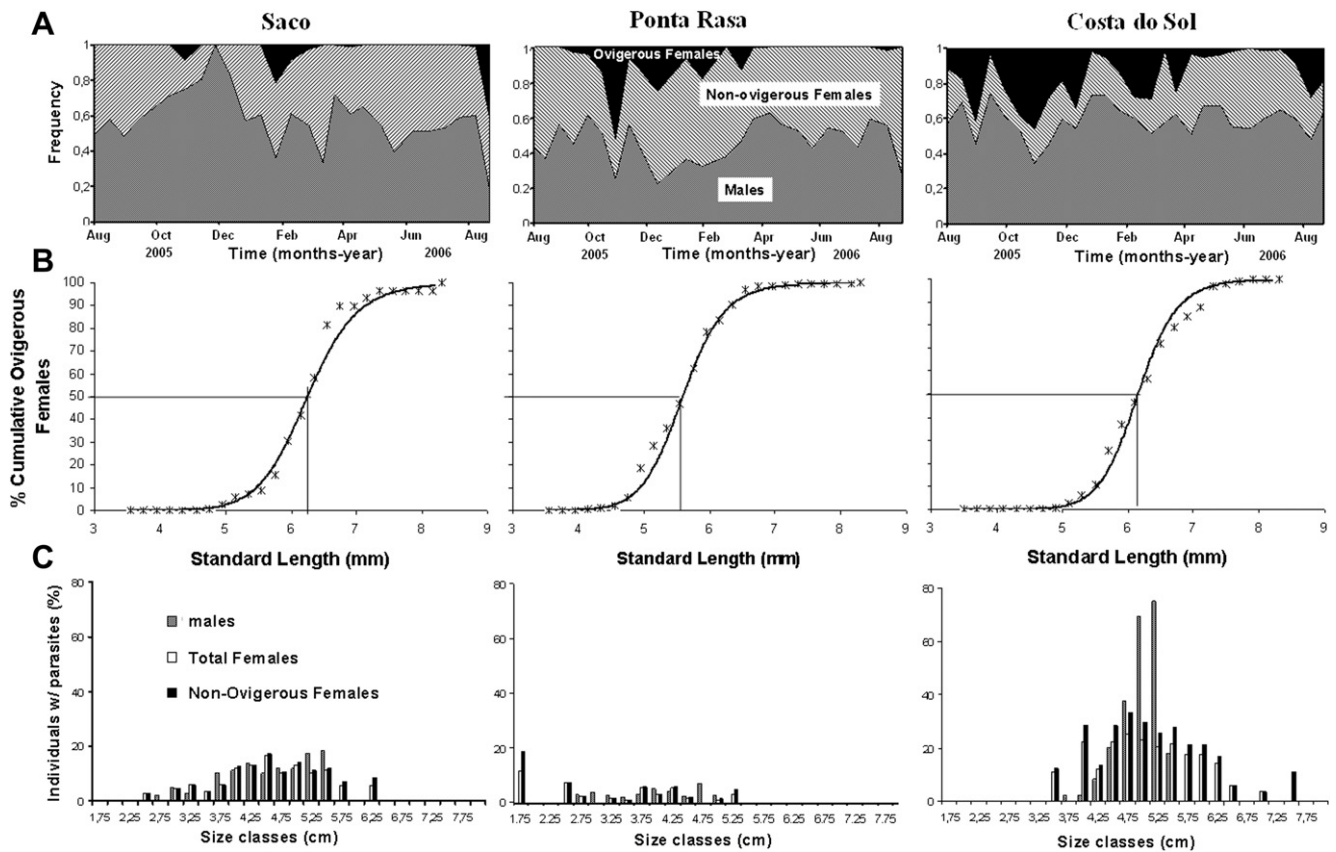


Fig. 5. A) Frequency dynamic (every 15 days) of males (grey), non-ovigerous (white) and ovigerous females (black); B) Logistic function fitting the cumulative proportion of ovigerous females; and C) Percentage, per size class, of males, non-ovigerous females and total females infected with parasite *Pseudione elongata africana* in the cephalothorax of *Palaemon concinnus* population at the peri-urban (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, in the south of Mozambique, during a period of 12 months.

4. Discussion

4.1. Population structure and reproduction

Non-opportunistic fauna species tend to decrease abundance and/or size near sewage outfalls (Smith and Suthers, 1999; Bigot et al., 2006), possibly due to increased mortality, slower growth, dispersal of larger fish away from the sewage, increase in predation or preferential recruitment. Shrimp species are usually used as biomonitors of different types of contaminants (Gokoglu et al., 2008; Tang et al., 2009) but no study has, to our knowledge, used them as reliable bioindicators. Also, while several studies have addressed shrimp fecundity, only a few have addressed, in detail, embryo production and brood loss for all size ranges in particular

species (e.g., Calado & Narciso, 2003; Penha-Lopes et al., 2007; Torres et al., 2007), but none has used it as a proxy of anthropogenic pollution.

Although we have not analysed population dimension and productivity, which also could be a good proxy for organic loading into the systems (Cross et al., 2006), the overall size distribution and dynamics during the sampling period have shown that CS presented the largest shrimps, independent of sex and female maturity. Loading of organic detritus and stimulation of primary productivity, bacteria and microalgae may have supplied shrimp with a more abundant and diverse food source, increasing growth rates and maximum length. These results were found to occur in Sudanese fairy shrimp, *Streptocephalus proboscideus*, being fed agro-industrial waste products (Ali and Brendonck, 1993).

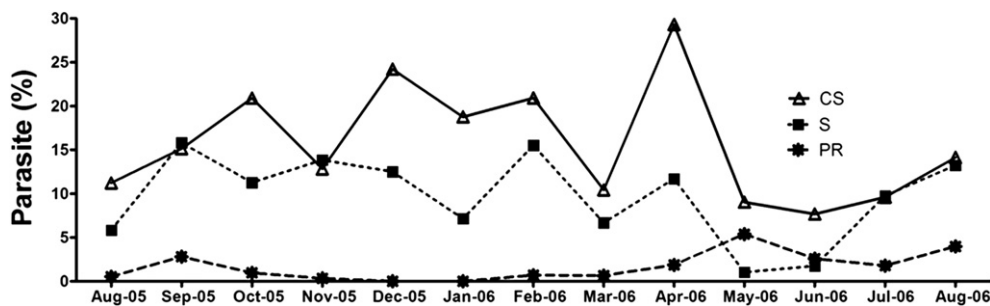


Fig. 6. Monthly parasitic frequency of *Palaemon concinnus* population by *Pseudione elongata africana* at the peri-urban (Costa do Sol – CS) and pristine (Saco – S and Ponta Rasa – PR) mangroves, in the south of Mozambique, during a period of 12 months.

Table 2

Results of the two-way PERMANOVA conducted on RNA/DNA ratio, RNA and DNA content (μg) per weight of muscle (g) of *Palaemon concinnus* individuals during the wet season at Costa do Sol (impact) and Saco and Ponta Rasa (control). Factors are as follows: impact vs control (asymmetrical, fixed and orthogonal) and location (random and nested in 'impact vs control' – just at the wet season). Degrees of freedom, df, mean squares, MS, F and its probability value, p, are shown for each factor.

A	df	RNA/DNA			RNA			DNA		
		MS	F	p	MS	F	p	MS	F	p
Impact vs controls (I vs C)	1	0.37	0.37	0.50	0.77	0.77	0.16	0.08	0.08	0.33
Location (I vs C)	2	0.58	0.29	0.04	0.34	0.17	0.00	0.11	0.05	0.08
Res	17	1.09	0.06		0.41	0.02		0.25	0.01	
Total	20	2.12			3.01			0.79		

Although most crustaceans reduce growth when energy is diverted to reproductive processes (Hartnoll, 2006), in this study it seems that anthropogenic contamination supplied enough food source to sustain higher growth rates, higher percentage of ovigerous females and a continuous reproductive season. At the peri-urban mangrove, nearly 40% of all females were ovigerous while at the pristine mangroves these percentages rarely went above 10–15%. A higher percentage of ovigerous females, mainly during the wet and warmer season, as commonly observed for other palaemonid species inhabiting temperate estuaries (Cartaxana, 1994), increased the population average size at CS due their higher length when compared to males and non-mature females (Cartaxana, 1994; Penha-Lopes et al., 2007). It is commonly accepted that mature females possess larger bodies that allow them to carry bigger egg clutches, as was found in the present study and for other hippolytid shrimp populations inhabiting mangrove creeks (Penha-Lopes et al., 2007; Torres et al., 2007) or temperate coastal zones (Calado and Narciso, 2003).

Also, ovigerous females at Costa do Sol possessed a larger brood per female size when compared with females collected at the pristine mangroves, although this was more evident at the highest reproductive (wet) season. The same was observed for *Uca annulipes* fiddler crab at Costa do Sol (Penha-Lopes et al., 2009b) and fish

Table 3

Results of the three-way (A – wet season) and two-way (B – dry season) PERMANOVA conducted on standard length (SL) and the ratio between the number of eggs and SL (# eggs/SL) for females collected at the study locations. Factors are as follows: impact vs control (asymmetrical, fixed and orthogonal), location (random and nested in 'impact vs control' – just at the wet season) and stage of the embryos (stage, as a proxy of fecundity vs potential fertility, fixed and orthogonal). Degrees of freedom, df, mean squares, MS, F and its probability value, p, are shown for each factor.

Source	df	SL			# Eggs/SL		
		MS	F	p	MS	F	p
A – wet seasons							
Impact vs controls (I vs C)	1	6.77	6.77	0.67	4251.70	4251.70	0.00
Stage – St	1	0.10	0.10	0.66	594.55	594.55	0.17
Location (I vs C)	1	1.46	1.46	0.02	0.02	0.02	0.98
I vs C \times St	1	0.09	0.09	0.67	477.11	477.11	0.25
Location (I vs C) \times St	1	0.54	0.54	0.14	42.38	42.38	0.42
Res	175	43.64	0.25		12,502.00	71.44	
Total	180	61.35			21,028.00		
B – dry seasons							
Impact vs controls – I vs C	1	121.67	121.67	0.22	121.67	121.67	0.22
Stage – St	1	193.92	193.92	0.10	193.92	193.92	0.10
I vs C \times St	1	80.51	80.51	0.30	80.51	80.51	0.30
Res	104	7713.90	74.17		7713.90	74.17	
Total	107	8217.40			8217.40		

species inhabiting near sewage outfall locations (Smith and Suthers, 1999). Continuous reproduction of *Palaemon concinnus* and *Uca annulipes* fiddler crab at Costa do Sol, as well as *Panopeus americanus* mud crab inhabiting contaminated mangrove in Brazil (Vergamini and Mantelatto, 2008), was not registered in shrimp populations inhabiting nearby pristine mangroves. Continuous reproduction, as well as higher fertility, may be due to the higher nutrient and food availability for the *P. concinnus* population found at this peri-urban mangrove.

Lower salinities have also been suggested as one of the main causes for higher reproduction in crab species inhabiting southern Mozambique mangroves (Litulo, 2004) and mangrove creeks receiving high discharges from domestic wastewater typically experience a decrease in salinity during the wet season (see Fig. 2). Also, CS wastewater may also contain a large variety and/or quantity of endocrine disruptors (such as hormone-like substances), which could affect fish reproduction (Jobling and Tyler, 2002; Markert et al., 2004). However, increases in reproductive activity could also be considered a strategy to establish and maintain a stable population in human-impacted mangroves, as suggested by Vergamini and Mantelatto (2008).

The brood loss reported in the present study for *P. concinnus* is basically nonexistent for the pristine locations but observed for the peri-urban populations. Brood loss in crustaceans may be induced by numerous factors: aborted development, mechanical loss due to abrasion, maternal cannibalism, embryo predation and parasitism (see Kuris, 1991), but has also been recently considered as a potential proxy for anthropogenic perturbations (Ford et al., 2003; Penha-Lopes et al., 2009b). For example, resorption of oocytes is also a common response of fish species inhabiting contaminated estuaries (Jobling et al., 2002). Production of large egg clutch (stage I) sizes due to higher amounts of available food sources and limited space available to hold the growing embryos (Lardies and Wehrmann, 2001) was also considered to explain higher brood loss during embryo development of fiddler crabs inhabiting Costa do Sol compared with the same pristine mangroves (Penha-Lopes et al., 2009b) once egg volumes increased significantly in size during development (50–150%).

Maturation index was tested as an indicator of domestic sewage pollution by potentially reducing or increasing the size at which 50% of the females were ovigerous, however, it was similar at all 3 mangrove locations in this study.

4.2. Fatty acid

Fatty acid consumption patterns during embryonic development were similar to that reported for other decapods (Figueiredo et al., 2008a,b; Torres et al., 2008) with some expected variation as FA content varies between and within populations. SFA were always metabolized at a higher rate than unsaturated fatty acids (UFA) with MUFA being preferentially used for energetic purposes. It is important to note that SFA are non-essential and can be synthesized or obtained by desaturation of MUFA and polyunsaturated fatty acids (PUFA). Therefore, their pattern of consumption may either suggest a selective retention during embryonic development or partial utilization and replacement.

The palmitic acid (16:0) was by far the most common fatty acid found in these shrimp embryos (from 10 to 20%), followed by the monounsaturated 18:1n-9 (7–15%), 16:1n-7 (5–10%), and highly unsaturated fatty acids 20:5n-3 (5–10%) which all very common in mangrove systems (Meziane and Tsuchiya, 2002; Penha-Lopes et al., 2009b). The high values of the palmitoleic (16:1n-7) and eicosapentaenoic (20:5n-3) acids, both trophic markers of diatoms (Meziane and Tsuchiya, 2000), cis-vaccenic (18:1n-7) acid, a marker of bacteria (Meziane and Tsuchiya, 2002),

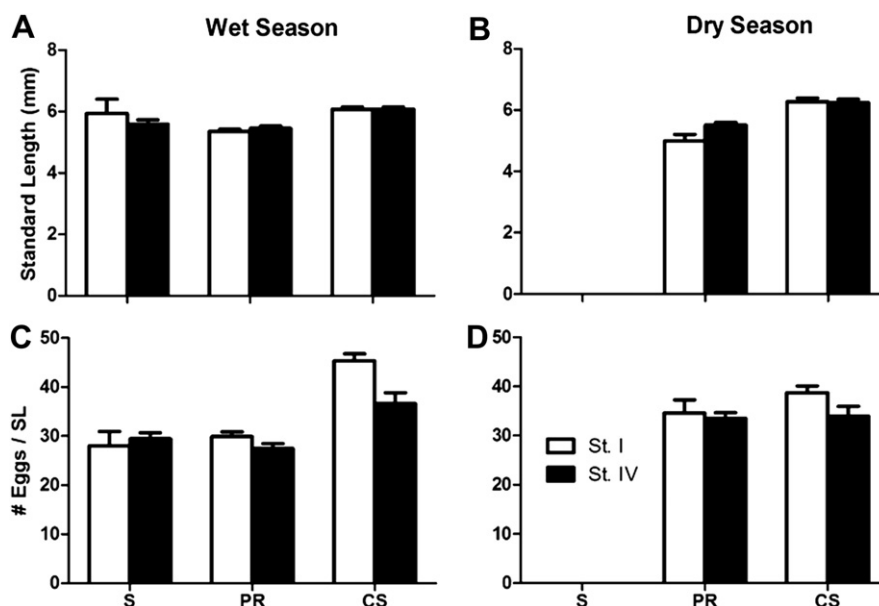


Fig. 7. Average (\pm SD) shrimp carapace length (A, B) and number of carried eggs standardised on carapace dimensions (C, D), observed for the females carrying eggs on stage I and IV collected in the study locations at both sampling seasons. n = 20–30.

and oleic (18:1n – 9) acid, a marker of fungus (Chen et al., 2001), suggest that *P. concinnus* consumes these organism during oogenesis.

The EPA/DHA and 18:1n – 7/18:1n – 9 ratios (0.2–3.9 and 0.3–0.6, respectively) also corroborate these findings, placing these populations in a medium trophic level, as DHA is highly conserved throughout the food chain, and 18:1n – 9 is the major FA in marine animals (Dalsgaard et al., 2003). The EPA/DHA ratio also suggests that the shrimps inhabiting the peri-urban mangrove (with higher ratio) are situated in a lower trophic level than the ones in pristine mangroves. This could be explained by a higher primary

productivity (both at sediment and water column) at Costa do Sol suggested by Cannicci et al. (2009), which is also indicated by the higher concentration of palmitoleic acid (diatom trophic marker), registered in the embryos of this population. At Costa do Sol, the higher values of total FA, SFA and MUFA in the embryos are probably caused by a more abundant and/or different micro-phytobenthos and bacteria community present in the sediment. Nevertheless, the higher energy content (as fatty acids) in newly hatched embryos reflects better maternal nutrition during oogenesis.

4.3. Physiological conditions (RNA/DNA)

The RNA/DNA ratio has been used an alternative measure of the physiological condition of ecologically relevant organisms (e.g., Norkko et al., 2006; Amaral et al., 2009b) and has furthermore been described as being well correlated with growth and nutritional condition of such organisms. At the same locations of the present study, Amaral et al. (2009b), using RNA/DNA ratio of male *U. annulipes* claw muscle as an indication of physiological stress, stated that this species presented a much lower RNA/DNA ratio at Costa do Sol when compared to Saco and Ponta Rasa populations. The authors state that a lower rate of new protein synthesis indicate that individuals are facing stress. However, an increased protein synthesis might sometimes reflect a stress response as well (Dahlhoff, 2004), invalidating, in such cases, the bioindicator potential of the RNA/DNA ratio.

Our results indicate that PR presents a significantly lower RNA/DNA ratio compared to S and CS, mainly due to an increase of RNA content from PR towards CS shrimp populations. By analysing the RNA/DNA ratio, but mainly RNA content, we must assume that CS populations present the lowest stress, being well correlated with the larger size and nutritional condition of such organisms, compared with pristine locations.

4.4. Parasitisation

In addition to pollutants, parasites have been found to cause endocrine disruption in fishes (Jobling and Tyler, 2003). In the

Table 4

Embryo FA initial concentration ($\mu\text{g g}^{-1}$ dw) and consumption rate (%) between stage I and IV of embryonic development in *P. concinnus* populations from all studied mangroves. Only the quantitatively most important FAs are represented (FA – fatty acids; SFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; HUFA – highly unsaturated FA; ARA – 20:4n – 6; EPA – 20:5n – 3; DPA – 22:5n – 3; DHA – 22:6n – 3).

Initial (consumption – %)	Ponta Rasa		Costa do Sol
	I–IV	I–IV	I–IV
14:0	48.47	53.48	69.89
15:0	27.96	68.62	68.67
16:0	39.16	52.50	51.72
17:0	22.12	34.70	79.44
18:0	38.20	57.05	65.45
Σ SFA	39.36	54.22	61.02
16:1n – 7	–23.37	64.30	75.51
18:1n – 9	54.04	50.49	62.82
18:1n – 7	38.11	52.95	65.96
Σ MUFA	36.73	58.60	67.67
18:2n – 6	52.95	66.71	54.18
18:3n – 3	56.19	66.31	60.91
20:4n – 6	–93.03	82.63	78.85
20:5n – 3	20.42	18.32	72.15
22:5n – 3	63.43	63.27	87.64
22:6n – 3	–8.31	43.37	49.10
Σ PUFA	35.41	61.83	64.12
Σ HUFA	28.54	47.05	66.00
Total FA	32.31	54.45	62.80

Table 5

Average (\pm SD) fatty acid composition ($\mu\text{g mg}^{-1}$ dw) of *Palaemon concinnus* embryos at different developmental stages, location and during the wet season. Different superscript letters and numbers represent significant differences on FA concentration at the same embryonic stage for different population and between embryonic stages for the same population, respectively (FA – fatty acids; SFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; highly unsaturated FA; EPA – 20:5n – 3; DHA – 22:6n – 3; ARA – 20:4n – 6).

	Ponta Rasa Wet season		Saco Wet season		Costa do Sol Wet season	
	Stage I	Stage IV	Stage I	Stage IV	Stage I	Stage IV
FA ($\mu\text{g g}^{-1}$ dw)	317 \pm 21 ^a	215 \pm 4 ^a	380 \pm 11 ^a	173 \pm 81	494 \pm 126 ^a	221 \pm 18
Fatty acids						
14:0	15.2 \pm 1.8 ^{a,1}	7.8 \pm 0.7 ²	20.3 \pm 0.3 ^{b,1}	9.5 \pm 4.6 ^{2,2}	23.5 \pm 6.9 ^{b,1}	7.1 \pm 1.2 ²
15:0	4.2 \pm 1.2	3.1 \pm 0.2	5.5 \pm 0.3 ¹	1.7 \pm 0.7 ²	5.8 \pm 1.7	1.8 \pm 0.2
16:0	66.4 \pm 12.1 ^a	40.4 \pm 3.6	70.3 \pm 2.4 ^{a,1}	33.4 \pm 15.5 ²	111.7 \pm 30.1 ^{b,1}	38.4 \pm 6.6 ²
17:0	0.6 \pm 0.0 ^a	0.5 \pm 0.1	0.9 \pm 0.1 ^a	0.6 \pm 0.3	5.1 \pm 2.4 ^{b,1}	1.0 \pm 0.4 ²
18:0	19.2 \pm 3.7 ¹	11.9 \pm 0.9 ²	23.6 \pm 0.4 ¹	10.1 \pm 4.9 ²	35.4 \pm 9.3 ¹	12.2 \pm 2.1 ²
Σ SFA	107.9 \pm 18.9 ^{a,1}	65.4 \pm 5.4 ²	123.1 \pm 2.6 ^{a,1}	56.4 \pm 2.9 ²	183.5 \pm 50.5 ^{b,1}	71.5 \pm 5.5 ²
16:1n – 7	10.9 \pm 8.4 ¹	13.5 \pm 2.0	38.0 \pm 0.4 ^{a,2}	13.6 \pm 6.6 ^b	57.4 \pm 16.7 ^{a,2}	14.1 \pm 1.7 ^b
18:1n – 9	44.8 \pm 10.7 ¹	20.6 \pm 2.4 ²	41.5 \pm 2.5 ¹	20.5 \pm 9.7 ²	51.9 \pm 10.5 ¹	19.3 \pm 3.8 ²
18:1n – 7	14.0 \pm 0.4 ¹	8.6 \pm 0.9 ²	17.2 \pm 0.3 ¹	8.1 \pm 3.9 ²	30.4 \pm 10.0 ¹	10.4 \pm 0.7 ²
Σ MUFA	79.9 \pm 5.0 ^{a,1}	50.5 \pm 5.1 ²	110.2 \pm 2.7 ^{b,1}	45.6 \pm 21.8 ²	148.0 \pm 36.8 ^{b,1}	47.8 \pm 7.5 ²
18:2n – 6	13.1 \pm 2.4 ^{a,1}	6.2 \pm 0.5 ²	41.8 \pm 29.6 ^{b,1}	13.9 \pm 7.8 ²	15.1 \pm 1.7 ^{a,1}	6.9 \pm 2.0 ²
18:3n – 3	3.2 \pm 1.0 ^{a,1}	1.4 \pm 0.7 ²	3.4 \pm 0.0 ^{a,1}	1.1 \pm 0.6 ²	6.3 \pm 1.0 ^{b,1}	2.5 \pm 0.5 ²
20:4n – 6	0.3 \pm 0.3 ^a	0.7 \pm 0.3	1.0 \pm 0.0 ^a	0.2 \pm 0.2	7.2 \pm 3.9 ^{b,1}	1.5 \pm 1.1 ²
20:5n – 3	18.3 \pm 1.9 ^a	14.6 \pm 0.7	19.7 \pm 9.4 ^a	16.1 \pm 3.7	67.2 \pm 23.9 ^{b,1}	18.7 \pm 2.8 ²
22:5n – 3	2.3 \pm 1.1	0.9 \pm 0.1	1.5 \pm 0.3	0.6 \pm 0.2	2.3 \pm 0.7	0.3 \pm 0.3
22:6n – 3	8.6 \pm 1.2 ^a	9.3 \pm 0.4	10.5 \pm 2.0 ^b	5.9 \pm 2.1	15.7 \pm 4.8 ^{b,1}	8.0 \pm 1.3 ²
Σ PUFA	73.4 \pm 2.8 ^{a,1}	47.4 \pm 3.2 ²	102.5 \pm 24.0 ^{b,1}	39.1 \pm 8.1 ²	131.3 \pm 35.7 ^{b,1}	47.1 \pm 8.1 ²
Σ HUFA	49.2 \pm 2.8 ¹	35.2 \pm 1.6 ²	47.0 \pm 5.8 ¹	24.9 \pm 10.0 ²	99.6 \pm 31.2 ¹	33.9 \pm 5.6 ²
$\Sigma(n-3)/\Sigma(n-6)$	2.0 \pm 0.7	4.4 \pm 0.2	2.8 \pm 1.2	3.8 \pm 1.3	4.1 \pm 0.3	3.9 \pm 0.2
18:1n – 7/18:1n – 9	0.3 \pm 0.1	0.4 \pm 0.1	0.4 \pm 0.2	0.4 \pm 0.1	0.6 \pm 0.3	0.5 \pm 0.3
EPA/DHA	2.1 \pm 0.0 ^a	1.6 \pm 0.6 ^a	0.2 \pm 0.0 ^b	0.5 \pm 0.2 ^b	3.9 \pm 0.0 ^b	2.3 \pm 0.0 ^c
EPA/ARA	22.5 \pm 6.4	14.4 \pm 1.4	18.3 \pm 8.8	13.5 \pm 2.60	37.3 \pm 17.1	14.8 \pm 7.7

present study *Pseudione elongata africana* isopod parasite was not found in a single ovigerous female, indicating that they inhibit shrimp reproduction or no longer can parasitize a mature female. *Pseudione elongata africana* belongs to isopods of the family Bopyridae. These organisms display a free swimming epicaridean larva that attaches itself to an intermediate host (a calanoid copepod), later metamorphosing into a microniscus (Dale and Anderson, 1982). This larva metamorphoses to a free swimming cryptoniscus, leaves the copepod and acts as the infective stage of the definitive host, usually decapods (e.g., Calado et al., 2008). Bopyrids are known to interfere with gonad maturation of their hosts and the decapod reproductive potential is always substantially reduced (Ó'Brien and Van Wyk, 1985) although it is commonly accepted that bopyrid infection leads to the 'reproductive death' of the host (Vanwyk, 1982), exactly the situation in the present study. The potential mechanisms by which the parasite is able to castrate the host have been suggested in literature but no factual evidences have been demonstrated.

Peri-urban mangrove parasite infection rates in both males and non-ovigerous females were usually much higher than the ones obtained at S and especially PR, indicating that hosts at CS are more vulnerable, thereby increasing their susceptibility. Other explanations could include a lower abundance of parasites or intermediate hosts (the calanoid copepod) in pristine mangroves. This particular host–parasite relationship may be used as an indicator of pollutants, because this complex seems to meet all of the requirements suggested by Kennedy (1997) to use freshwater fish parasites as bioindicators: the host must be abundant and easily accessible; parasite species, despite their over dispersed distribution, must show a high prevalence and abundance in host population; parasites should be easily identified and not laborious to remove and count; information on the ecology and biology of both host and parasite should be available.

Although contrasting, one interesting detail is that, while Costa do Sol presents the highest parasite percentage, it also had the longest

reproductive period, highest ovigerous female percentage, and largest egg clutches. This may indicate that, while on one side Costa do Sol provides "resources" to boost reproductive fitness, on the other side and at the same time increases the parasite percentage in males and non-ovigerous females, which seems to function as a regulatory factor. This way, parasites reduce the potential amount of total reproductive females regulating, directly, this population structure and, indirectly, the mangrove community and its functioning, by affecting interspecific competition, energy flow and biodiversity levels (see for example Hudson et al., 2006). However, the opposite can also be argued, saying that reproduction potential is increased as a strategy to compensate the reduction in the reproduction output caused by the parasite.

Similar multi-disciplinary studies should be undertaken on other species of mangrove biota that are exposed to sewage pollution increasing the choice of possible bioindicators in mangrove habitats.

5. Conclusion

Domestic sewage discharges at low concentrations have already been shown to increase fauna mangrove population parameters, due to the nutrient limitations that these ecosystems present. Shrimp size, fertility and embryo quality, as well as a host–parasite interaction, increased significantly at the peri-urban location. Although at first some of these parameters could be considered positive (*P. concinnus* population structure and reproduction) or negative (parasite infection rate) bioindicators, such classification system would need to be elucidated, and its consequenced better addressed. Although a higher secondary productivity of this shrimp population could be considered a real indicator of the mangrove condition, at the same time could lead to a different mangrove system dynamic (inter-species interactions) that could result in a general positive or negative condition. Nevertheless, the identification of the effects of sewage on *P. concinnus* population increases the choice of possible bioindicators in East African coastal water.

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