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Behavioural responses of the mangrove fiddler crabs (*Uca annulipes* and *U. inversa*) to urban sewage loadings: Results of a mesocosm approach

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ABSTRACT

The study aimed at investigating the effects of sewage loadings on the behaviour of two fiddler crabs species maintained in a system of experimental mesocosms, built in a mangrove area in Tanzania and inundated with different seawater/sewage mixtures. Our results show that sewage loads led to a modification of the overall activity budget of the crab community as a result of increased hypertrophic conditions (high COD, increased chlorophyll-a concentrations). During their activity period, crabs inside contaminated mesocosms seemed to satisfy their feeding demand faster than those of the control cells, spending a significant longer time in other activities like courtship and territorial defence. Apart from being a good biological indication of ecosystem eutrophication, such a reduced foraging activity by fiddler crabs also depresses their sediment bioturbation activity, important factor for the health of mangrove systems, suggesting practical implications regarding the efficiency of mangrove-based wetlands for treatment of domestic sewage.

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1. Introduction

Anthropogenic pressure on tropical coasts has been steadily increasing over the recent years, prompting a strong impact on coastal ecosystems, and on mangrove forests in particular (Alongi, 2002; Duke et al., 2007; Ellison, 2008). Although human impacts on mangroves can be ascribed to a variety of different activities (Alongi, 2002), one of the most common and increasingly important stress factors derives from the pouring of untreated wastewaters directly into these tidal forests. Wastewaters affecting mangroves can have a number of origins, such as shrimp farming (Trott et al., 2004), agricultural activities (Meziane and Tsuchiya, 2002) and urban sewage and industrial effluents (Tam and Wong, 1995, 1996; Yu et al., 1997). As a consequence of the variety of sources and loadings, wastewater impact on mangrove communities is not always the same, and, as a matter of fact, it is often still far from being fully understood.

Sewage loading, in particular, determines an enrichment of organic matter and available nutrients within the mangrove environment, and can stimulate the growth of both aerobic and anaerobic bacteria (Meziane and Tsuchiya, 2002; Tam, 1998), as well as macroalgae and benthic diatoms. Thus, sewage represents a potential enriched nutrient source that can support higher levels of primary and secondary production (Meziane and Tsuchiya, 2002). However, it must be emphasized that a higher secondary production can still represent an overall ecological impoverishment, via the development of high biomass of few dominant opportunistic species (Wear and Tanner, 2007).

Fiddler crabs, as well as several other mangrove crabs, are well known to play a key role on the ecological processes of mangrove ecosystems, by means of their continuous soil processing and reworking, due to both foraging activity and burrow excavation (Cannicci et al., 2008; Kristensen, 2008; Lee, 2008). The fiddler crabs of the genus *Uca* are indeed the main microalgal and bacteria feeders within Indo-Pacific mangroves (Cannicci et al., 2008; Kristensen and Alongi, 2006) and they can be considered a key taxa to understand the consequence of sewage exposure on mangrove benthic species. In fact, the discharge of sewage in their habitat can affect their biology and behaviour both directly, thorough O_2 depletion and changes in water salinity and pH, and indirectly, via the overgrowth of their preferred food sources, as showed by Meziane and Tsuchiya (2002) for an Okinawan population of *Uca vocans*. However, although previous studies clarified the symptomatology derived from the exposure of fiddler crabs to several chemicals related with human activities (Devi, 1987; Ismail et al., 1991; Reddy et al., 1997; Souza et al., 2008), only limited information is available regarding the biological and behavioural consequences of sewage exposure and specifically not dealing with mangrove populations of *Uca*.

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About the role played by contaminants in *Uca* population biology, Bergey and Weis (2008) found that a *Uca pugnax* population from a New Jersey site characterized by high levels of organic contaminants but also heavy metals, had lower population density, lower recruitment, reduced reproductive season and lower survivorship of early benthic phases, when compared to a population from a far less stressed environment.

In parallel with the use of biochemical and physiological stress parameters as biomarkers of anthropogenic pollution, recently many authors suggested the detection of changes in behavioural traits, of both fiddler crabs and other crustaceans (Ungherese and Ugolini, 2009) as a useful diagnostic tool and a reliable early-warning biomarker. As an example, Culbertson and colleagues (2007) used a purely behavioural approach to assess the effect of chronic exposure to oil pollution in *U. pugnax* populations. Spilled oil affected the burrowing activity of crabs, which avoided the deepest oiled layer of sediments and established low population density. A lowered foraging activity as well as a delayed escaping response were also observed, compared to a control population from a pristine site.

Based on this scientific frame we used a mesocosm experimental approach to study how different loadings of domestic sewage could change the behavioural patterns of two East African mangrove species, *Uca annulipes* and *Uca inversa*. Considering, in fact, their highly stereotyped pattern of activity through the surface activity period (Eshky et al., 1995), we hypothesized that the altered food abundance could trigger some changes in the average time budget of the crab population, ultimately ending in an alteration of their ecological role as bioengineers within the mangrove systems.

2. Material and methods

2.1. Experimental setup

To test the effect of several domestic sewage loadings on the various components of the mangrove ecosystem, a controlled experimental facility was settled at Jangwani Beach (6°41′S, 39°13′E; Dar es Salaam, Tanzania) where a structure of 36 $3 \times 3 \times 0.8$ m plots was built with concrete and equipped with a flushing–flooding system.

The experimental setup was designed in order to enable to disentangle the effect of two factors, vegetation type and sewage loading, on various biological and behavioural characteristics of mangrove macrofauna. Plots were divided into three groups in terms of vegetation condition: bare, *Avicennia marina* or *Rhizophora mucronata* saplings (Fig. 1). These two species were chosen since they are the two dominant mangroves along the East African coast, forming almost pure monospecific associations in the landward and seaward belts, respectively. Saplings were planted in early February 2006, with a density of 2.7 m^{-2}, if present. In late August 2006 the commonest macrofaunal species present in the neighbouring forest, the mud whelk *Terebralia palustris* and the fiddler crabs *U. annulipes* and *U. inversa*, were introduced to reproduce mangrove communities as similar as possible to the natural ones. Crabs were introduced in the mesocosms at a density that reflected minimum natural densities (Hartnoll et al., 2002). The density of both males and females of *U. inversa* and of males of *U. annulipes* was 5 ind. m^{-2}, while for *U. annulipes* females 3 ind. m^{-2} were introduced, for a total of 18 crabs m^{-2} and an overall total of 162 crabs per mesocosm.

After an initial period, in which the whole system of plots was flooded with seawater (as to allow stabilization of the introduced populations), from October 2006 sewage loadings were applied. A simulated floodtide was created with a 12-hour periodicity and

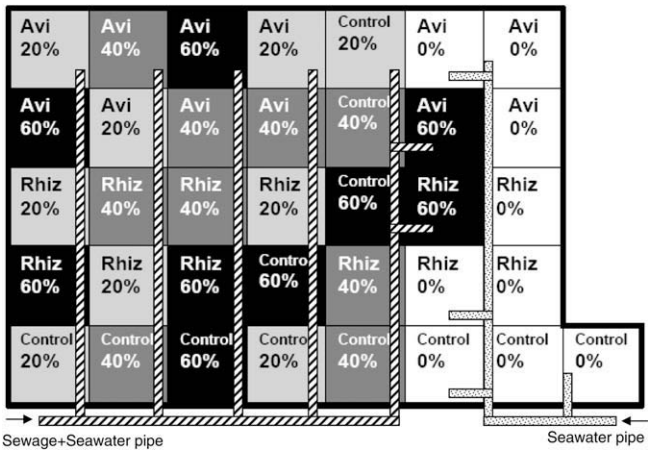


Fig. 1. Scheme of the 36 mesocosms at the experimental site. Within each module, representing a single concrete plot, is reported the vegetation type (Control: bare; Avi: *Avicennia marina*; Rhiz: *Rhizophora mucronata*) and the sewage concentration (0%, 20%, 40% and 60%). A schematic disposition of the pipes system used to flood the plots is shown.

the system was inundated by a water column of 10 cm high, from 12:00 pm to 12:00 am, so that the simulated low tide occurred for the first 6 h under light, coinciding with the activity period of the selected fiddler crabs, and the remaining 6 h under darkness. Seawater only (i.e. 0% of sewage), as a control, or a mixture of seawater with three different percentage of sewage (20%, 40% and 60%) were introduced into four groups of randomly selected plots (Fig. 1). The sewage came from a tourist receptive structure nearby and it was constantly accumulated in a proper storage pond for primary treatment before being used in the mesocosms. The distribution of the different treatments throughout the system of mesocosms was randomized except for the plots flooded with sea water, that were grouped on the same side of the structure. This deployment was necessary to avoid the contamination of the control plots, served by a different inlet pipe (see Fig. 1).

2.2. Observation and sampling techniques

The behavioural observations were carried out 6 months after the experimental system was established and fully working, in March 2007. The young trees, at the moment of the behavioural observations, were 50–100 cm tall. At that moment *Rhizophora* saplings had just started to produce prop roots and plots with *Avicennia* trees already presented pneumatophores, proportional to the small age of the plants. The main chemical parameters of the different seawater/sewage mixture flushed into the system, at the beginning of the 12-hour permanence, are reported in Table 1. It can be noticed that sewage was basically characterized by increased amounts of nutrients, with no presence of heavy metals or other toxic compounds. This is not surprisingly since the sewage came directly from the sanitary sewer of a hotel, not being linked to any urban pipelines.

Two plots for each combination of sewageXvegetation were simultaneously observed, while one temporal replicate per each natural spring and neap tide was used for each treatment. Although the crabs were not exposed to the natural tide rhythm for 6 months, the sampling was done on both natural spring and neap tides as temporal replication, due to the possibility of crabs still showing endogenous activity rhythms (which was not the case).

Because of the small size of the mesocosms and the height of the outside walls, the use of the binocular by a distant observer (as suggested by various authors) was not possible. Instead, the

Table 1
Chemical composition of the four sewage–sea water mixtures used to flush the experimental mesocosms (COD: Chemical Oxygen Demand). Averages ± SE are shown.

Sewage concentration (%)	Salinity	COD (mg/l)	NH_3–N (mg/l)	PO_4 (mg/l)	NO_x (mg/l)	Chl-a (mg/m^3)
0	39.1 ± 0.6	274 ± 23	0.37 ± 0.03	1.29 ± 0.11	0.09 ± 0.03	7.8 ± 1.2
20	24.5 ± 0.9	535 ± 26	1.72 ± 0.13	3.38 ± 0.20	0.17 ± 0.06	23.7 ± 5.0
40	20.5 ± 0.8	758 ± 25	2.15 ± 0.17	4.81 ± 0.30	0.19 ± 0.07	29.9 ± 5.4
60	18.9 ± 1.4	1055 ± 29	2.71 ± 0.24	6.38 ± 0.43	0.13 ± 0.04	21.8 ± 3.6

observer watched the crabs inside each mesocosm standing motionless close to it. Although some crabs retreated in their burrows when the observer approached, after few minutes a normal and intense activity on the surface was restored. Each of two observers checked crabs behaviour inside six plots every hour over a full 6-hour diurnal low tide period (12 pm–6 pm), registering the exhibited behaviour of every crab in each single plot.

To record the activity of all the crabs in a plot, observation of the instantaneous activity was carried out rather than following few focal animals for a longer period. This kind of survey allowed to extrapolate what was the most frequent behaviour in each mesocosm at every hour, thus reasonably asserting the most dominant activities throughout the six sampling hours. The information obtained for each plot, therefore, was analogous to six independent snapshots of the activity patterns throughout the entire artificial low tide period. Although a conspicuous number of animals was observed at the same time (few minutes), these were fairly distributed within the plots and the record of a single animal more than once during the fast observations was a negligible source of mistake.

The main traits of *Uca* behavioural patterns are well known and related to environmental cyclic phenomena (Nobbs, 2003; Weis and Weis, 2004). They are active during low tide when, outside their burrow, spend most of the time foraging, in social behaviours, especially males fighting for territorial defence and displaying with the enlarged claw, or maintaining their den (Caravello and Cameron, 1991). Accordingly to the literature and to hours of preliminary observations carried out both on wild populations and on the plots, we recognised and described nine main behaviours, which are listed and explained in Table 2.

2.3. Microphytobenthos abundance

An environmental parameter closely linked to fiddler crabs feeding activity, the chlorophyll-a concentration in the surface sediment, was also measured in plots flushed with 0% and 60% sewage mixture, as a measure of microalgal abundance. During the behavioural activity period of the crabs, four 15 × 20 cm cages were randomly placed in each plot to avoid crabs depletion of the sediment by foraging on it, and samples were collected every three hours (12:00 pm, 3:00 pm and 6:00 pm).

After measured the wet weights, sediment samples were placed in vials containing 5 ml of 90% ethanol and incubated for a 24 h period at 4 °C. Each sample was then spun down at 3000 rpm for 10 min and the supernatant analysed for Chlorophyll-a (Chl-a) by the spectrophotometric method of Lorenzen (1967).

2.4. Statistical analysis

Behavioural data were analysed by mean of analysis of variance (ANOVA) using a three-way full factorial design (factors: species, vegetation type and sewage%).

Behavioural frequencies observed were standardized across the different crabs densities of the plots using percentage frequency of observed behaviours and transformed to $x' = \arcsin(\%)$ before the analysis that was performed at the first, third and fifth hours of the emersion period. The homogeneity of variances was assessed using Cochran's test and if found significant a permutational analysis of variance (PERMANOVA) (Anderson, 2001) was carried out.

Student-Newman–Kelus (SNK) tests were used for multiple comparisons of means when the ANOVA presented significant differences.

ANOVAs were performed using GMAV 5 program (University of Sydney, Australia) and the permutational analysis of variance by using FORTRAN program PERMANOVA (Anderson, 2005).

Chlorophyll-a concentration data were analysed using Student *t*-test or Welch *t*-test for those datasets with unbalanced variances.

3. Results

3.1. Overall behavioural patterns

No significant differences were observed between the 2 neap and the 2 spring tides, thus the data were pooled and considered as four independent temporal replicates. The results of the analysis of variance are reported in detail in Table 3, while a synthesis of the relative frequencies of the main behaviours observed throughout the six hours of observations for the four sewage loads and the two species is provided in Fig. 2.

As expected, there were some clear trends for the dominant behavioural patterns exhibited by the two species, independently of the experimental treatments. In particular, as soon as the crabs exited the burrows to start their surface activities after the high tide, the feeding behaviour was the most frequent, and after this the other behaviours tended progressively to increase. Waving

Table 2
List and description of the main behaviours exhibited by the two species of fiddler crabs.

Behaviour	Detail
Burrow maintenance	Digging activity for den enlarging, restoration after high tide, or *ex-novo* establishment.
Courtship	Female chased by male trying to get her into his own den for mating
Display	Standing high on the legs, usually close to burrow entrance
Feeding	Easily identified by the constant movement of the minor claws from the substratum towards the mouth parts with progressive production of small pseudopellets of processed sediment
Fighting	Aggression or reciprocal males' offence by means of the major claw
Grooming	Cleaning activity of body surface and of major claw, in the case of males, from mud residuals. Often observed when exiting the den.
Inactive	No activity shown
Wandering	Continuous wandering activity, apparently without a defined goal
Waving	Typical male display towards females to attract them, by a rotating (*U. annulipes*) or up–down movement (*U. inversa*) of the major claw.

Table 3
Results of three-way full factorial analysis of variance (ANOVA; *PERMANOVA) for three behaviours (feeding, waving and wandering) and for the densities of active crabs during three hours of the entire low tide period.

Behaviour	Factor	DF	First hour			Third hour			Fifth hour		
			MS	F	P	MS	F	P	MS	F	P
Feeding	Species – Sp	1	243.112	0.520	0.472	6208.517	4.479	0.010*	5480.445	10.460	0.002
	Vegetation – Veg	2	251.050	0.540	0.585	1516.883	1.094	0.353*	3.216	0.010	0.994
	Sewage% – Sew	3	3409.275	7.330	0.000	7538.308	5.438	0.000*	2016.231	3.850	0.013
	SpXVeg	2	726.140	1.560	0.217	116.671	0.084	0.997*	911.111	1.740	0.183
	SpXSew	3	339.116	0.730	0.538	1829.222	1.320	0.232*	317.024	0.610	0.614
	VegXSew	6	609.406	1.310	0.264	1121.991	0.809	0.657*	174.931	0.330	0.917
	SpXVegXSew	6	637.328	1.370	0.239	426.354	0.308	0.995*	212.719	0.410	0.873
	RES	72	465.286			1386.296			523.926		
	TOT	95									
Waving	Species – Sp	1	137.580	0.670	0.416	3750.529	9.210	0.003	9231.439	34.430	0.000
	Vegetation – Veg	2	159.582	0.780	0.464	83.191	0.200	0.816	847.467	3.160	0.048
	Sewage% – Sew	3	404.372	1.970	0.127	1119.530	2.750	0.049	239.635	0.890	0.449
	SpXVeg	2	4.366	0.020	0.979	234.507	0.580	0.565	146.308	0.550	0.582
	SpXSew	3	17.054	0.080	0.969	75.315	0.180	0.906	351.579	1.310	0.277
	VegXSew	6	217.543	1.060	0.396	222.331	0.550	0.772	137.899	0.510	0.796
	SpXVegXSew	6	56.108	0.270	0.948	254.200	0.620	0.710	75.214	0.280	0.944
	RES	72	205.756			407.211			268.116		
	TOT	95									
Moving	Species – Sp	1	44.768	0.072	0.943*	99.321	0.320	0.575	28.066	0.720	0.399
	Vegetation – Veg	2	1108.930	1.774	0.177*	115.501	0.370	0.693	21.831	0.560	0.574
	Sewage% – Sew	3	1360.399	2.177	0.075*	1278.305	4.090	0.010	71.151	1.830	0.150
	SpXVeg	2	110.000	0.176	0.954*	122.030	0.390	0.678	7.632	0.200	0.823
	SpXSew	3	44.768	0.072	0.998*	173.728	0.560	0.646	26.460	0.680	0.568
	VegXSew	6	720.391	1.153	0.321*	149.093	0.480	0.824	59.643	1.530	0.180
	SpXVegXSew	6	110.000	0.176	0.999*	41.097	0.130	0.992	8.436	0.220	0.970
	RES	72	625.000			312.832			38.962		
	TOT	95									
Surface activity	Species – Sp	1	5119.915	15.610	0.000	1261.075	5.530	0.022	15921.344	51.300	0.000
	Vegetation – Veg	2	219.521	0.670	0.515	389.830	1.710	0.188	237.146	0.760	0.470
	Sewage% – Sew	3	882.842	2.690	0.053	283.307	1.240	0.301	12.338	0.040	0.989
	SpXVeg	2	182.623	0.560	0.576	761.828	3.340	0.041	435.689	1.400	0.252
	SpXSew	3	289.555	0.880	0.454	161.145	0.710	0.551	653.534	2.110	0.107
	VegXSew	6	177.027	0.540	0.776	337.825	1.480	0.197	88.981	0.290	0.942
	SpXVegXSew	6	94.670	0.290	0.941	457.288	2.000	0.076	109.762	0.350	0.906
	RES	72	327.958			228.150			310.347		
	TOT	95									

frequency increased in the early hours of the low tide, successively in some case it decreased in some other it maintained almost constant rates until the end of the activity period. The burrow maintenance, as well as the observed inactive crabs, assumed the highest occurrence approaching the last hours of the activity interval. Grooming behaviour, even though scarcely observed, was interestingly exhibited mostly during the first hour, that is when the crabs exited their burrow and needed to 'clean' the major claw, in the case of males, and the rest of the carapace from mud residuals attached to it.

3.2. Behavioural differences between the two species

Some differences between the activity patterns of the two fiddler crab species considered were evident. First of all, the beginning and conclusion of the activity period of the two species were weakly out of phase (Fig. 3). In particular *U. inversa* reached a peak of surface activity at the beginning of the low tide interval, then this value started to decrease soon after the second hour. On the other hand, for *U. annulipes* the highest number of presences on the surface was attained in the final part of the non flooded period, around the fifth hour.

Rates of both feeding and waving behaviour were significantly different between the two species (Table 3, SNK test) except during the first hour, when they were equally high and similarly low, respectively. *U. annulipes* was characterized by lower foraging rates and by a more intense displaying activity that rose after the low frequencies of the first hour reaching a maximum and maintaining it asymptotically in the last part of the day. *U. inversa*, instead, kept low levels of this behaviour, with a peak at the third hour.

3.3. Effect of vegetation type on crab behaviour

The vegetation treatment (Fig. 4) did not strongly affect the activity patterns observed in the mesocosms. In fact, the only influence found is a weak tendency to a lower waving activity in the plots without young trees. This tendency becomes significant (Table 3, SNK test) in the final part of the sampling period.

3.4. Effect of sewage load on crab behaviour

Examining the trend of the feeding ratio among different sewage loadings throughout the entire 6-hour period (Fig. 5), it can be noted that significantly higher foraging rates were exhibited on average by both species of crabs present in the control plots (Table 3, SNK test). Although the lowest registered feeding frequencies belong almost always to the populations living in the 60% sewage mesocosms, no statistically relevant differences were observed among the three different sewage mixtures (SNK test). On the contrary, the waving activity (Fig. 5), estimated considering only males, had low values at the beginning of the emersion period, increasing till the third of fourth hour and then slightly decreasing approaching the sunset. The waving activity period was longer

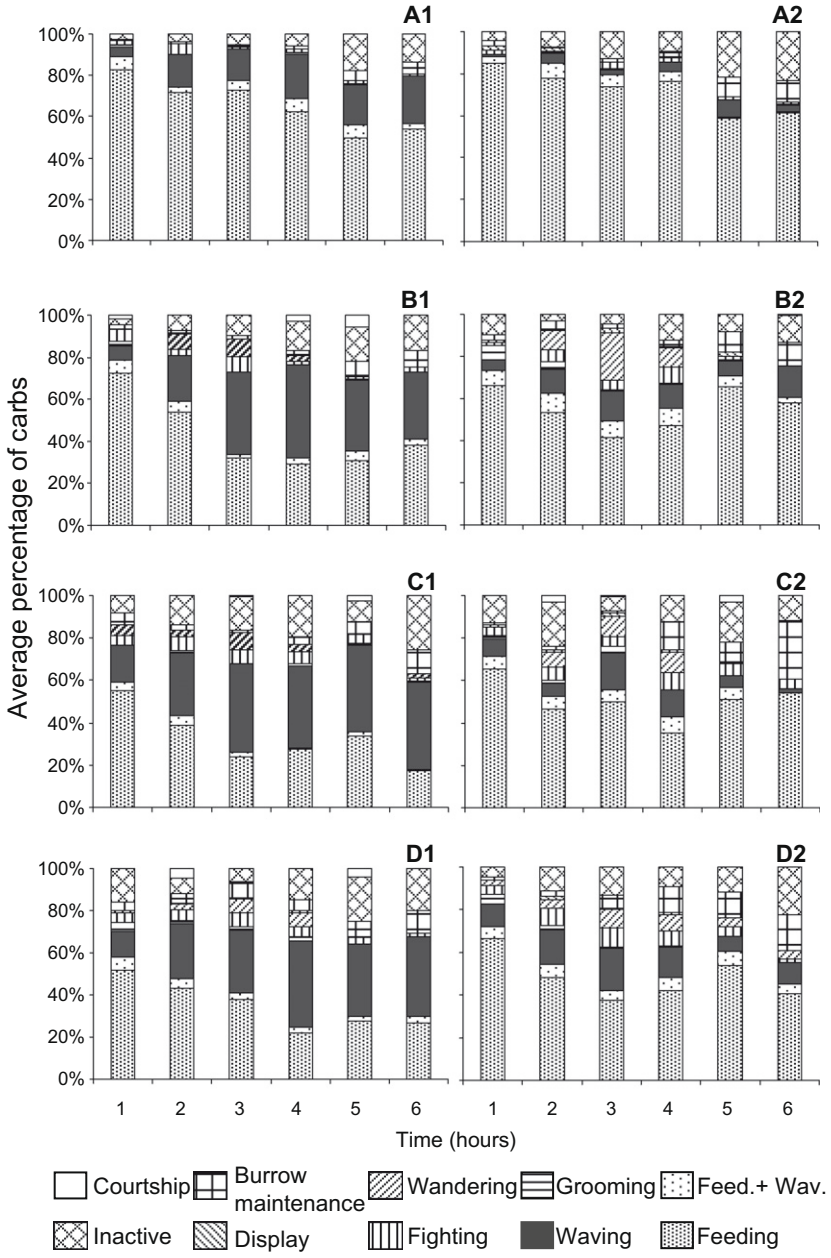


Fig. 2. Percentage crabs observed performing the listed behaviours, averaged over vegetation treatment, for different sewage loadings and different species, throughout the six-hour activity period ($n = 4$). 1 *Uca annulipes*; 2 *U. inversa*. (A) 0%; (B) 20%; (C) 40% and (D) 60%.

with increasing sewage concentrations throughout the entire sampling period, however, significant differences were only observed between 0% and 60% conditions at the third hour of the emersion period (Table 3, SNK test).

The crabs wandered mostly in the central part of the low tide interval (second, third and fourth hours) but interestingly this kind of restlessness was almost never present in the control plots (Fig. 5). During the third hour this activity reached a peak in all the polluted plots, that significantly differed from the controls, where it kept being not observed at all (Table 3, SNK test).

3.5. Chlorophyll-a measurement

The chlorophyll-a concentration measured in the sediment of the bare mesocosms reached significantly higher values in the impacted than in the pristine plots (12 h: Welch *t*-test for unbalanced variances; $t = -2.13$, df = 10 and $p < 0.05$; 15 h: *t*-test for unbalanced variances; $t = -3.12$, df = 11 and $p < 0.01$; 18 h: Student *t*-test; $t = -3.87$, df = 6 and $p < 0.01$), independently of the hour of the day (Fig. 6), although such difference was less pronounced at 12:00, namely soon after the emptying of the plots.

4. Discussion

Although some differences between the activity patterns of *U. inversa* and *U. annulipes* were observed, the two species basically showed a similar stress response to sewage loading. These results seem to confirm a recent study carried out within the same research frame and in the same experimental system by Penha-Lopes and colleagues (2009). These authors found similar mortality rates as well as similar levels of top sediment reworking between the two species exposed to similar sewage loadings,

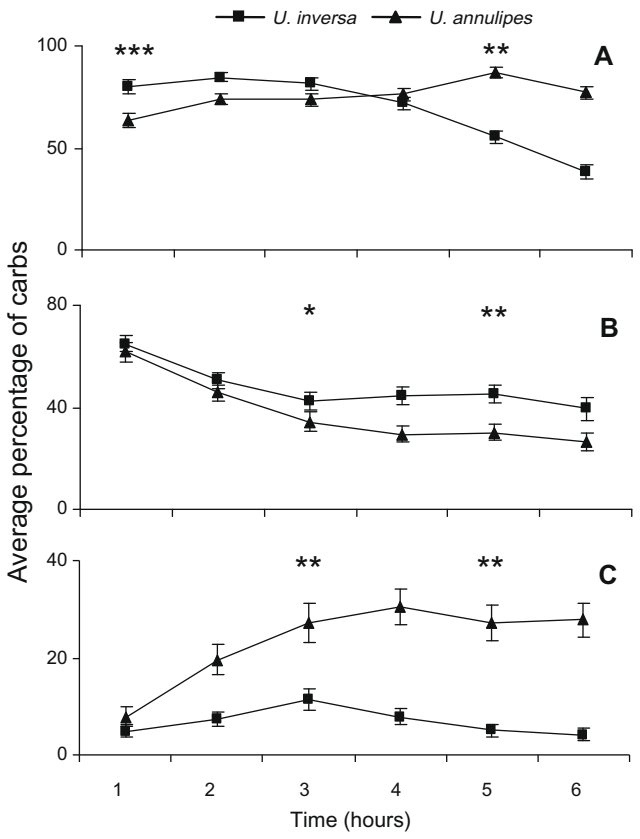


Fig. 3. Comparison between activity patterns of *U. annulipes* and *U. inversa*. (A) Observed active crabs (normalized as percentage of the maximum number observed), averaged over sewage and vegetation treatments, at each of the six hours. Feeding (B) and waving (C) rates of the two species averaged over sewage and vegetation treatments. Error bars indicate SE ($n = 4$).

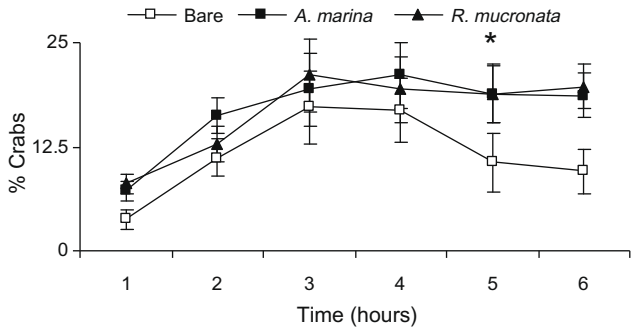


Fig. 4. Waving activity for the three vegetation types, averaged over species factor (*U. annulipes* and *U. inversa*) and sewage treatment, during the entire low tide activity period. *:$0.01 < p < 0.05$; **: $p < 0.01$. Error bars indicate SE ($n = 4$).

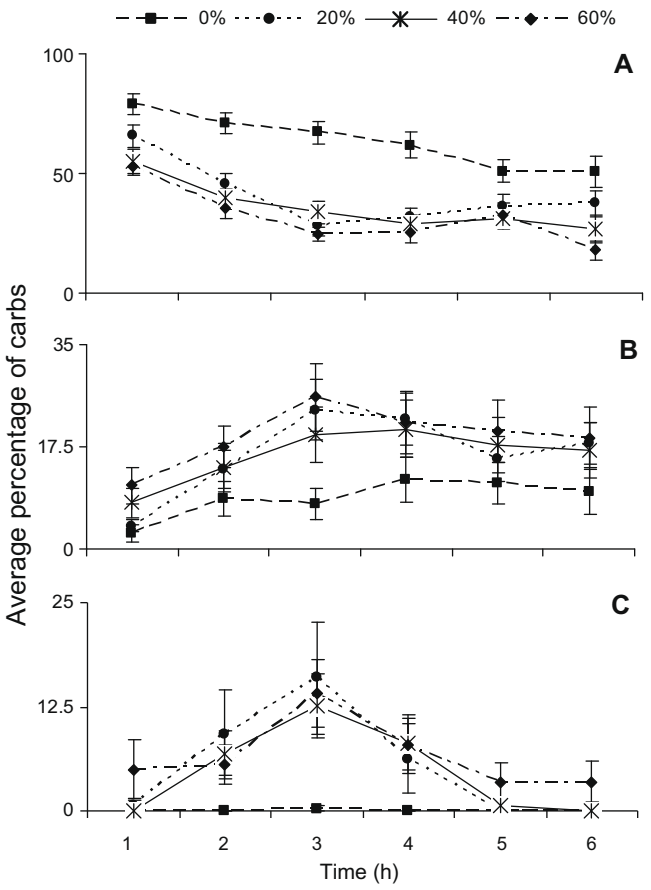


Fig. 5. Effect of sewage concentrations on the behaviour of crabs, throughout the six-hour activity period, averaged over species factor (*U. inversa* and *U. annulipes*) and vegetation treatment. (A) Feeding; (B) Waving; (C) Walking. Error bars indicate SE ($n = 4$).

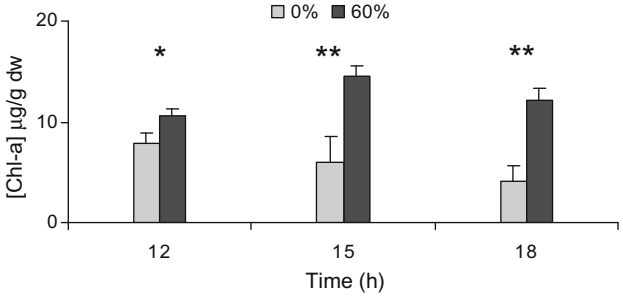


Fig. 6. Chlorophyll-a concentrations on the top 1 cm sediment layer in treated plots (60% sewage concentration) compared to non contaminated ones, throughout 6 h. Error bars indicate SE.

leading to the conclusion of no differences in sewage impact on the crabs biologies.

The observed asynchrony at the beginning of the surface activity of *U. annulipes*, that emerged from the burrows slightly later than the other species, is, however, difficult to discuss, since no studies do exist specifically on daily time budget of these two species. In natural ecosystems, in fact, these two species do share common areas inside specific mangrove belts, often reaching densities well higher than the ones we used to populate the mesocosms (Skov et al., 2002), thus excluding strong competition for food and space.

Our observations showed that foraging is the main surface activity of *U. annulipes* and *U. inversa*, agreeing with previous studies dealing with the time budget of several other *Uca* species (Eshky et al., 1995; Weis and Weis, 2004). The frequency of this behavioural pattern tended to decrease along the artificial low tide, while, conversely, the frequency of all the other behavioural patterns increased after an initial minimum.

The vegetation of the plots did not strongly affect the surface activity of the crabs and the only significant difference observed was a weak lower rate of waving activity in the bare plots with respect to the vegetated ones. This difference could be related to the presence of both physical and visual obstacles, such as pneumatophores, stilt roots and leaves of the saplings. This would be consistent with some of the observations made by Nobbs (2003) on *Uca*

elegans, an Australian species, which was supposed to avoid vegetated areas that may hinder visibility during visual communication. However, the lack of differences on some results when comparing between bare and planted mesocosms needs to be carefully interpreted by considering that at this stage mangrove trees were still young (small canopy). It is possible that if these observations were done later in the experiment (with more developed plants) or with mature trees, vegetated and bare conditions would have showed more enhanced differences.

Within the mesocosms impacted by different sewage/seawater mixtures, the foraging time exerted by fiddler crabs was strongly reduced when compared to the control plots. Within the impacted plots the high amounts of chemical oxygen demand (COD), which indirectly measures the amount of organic compounds in the water, ammonium and phosphate, testified of hypertrophic conditions occurring in those mesocosms. This rise in nutrients bursts benthic microalgae growth (as showed by the increase in chlorophyll-a concentrations on the top sediment) and bacteria (Tam, 1998), which are known to be the main food source for fiddler crabs (Dye and Lasiak, 1986; Meziane and Tsuchiya, 2002). This seems to be the major explaination of their shift in activity patterns. In other terms, *U. annulipes* and *U. inversa* proved to limit their feeding rates up to a similar amount of microalgae in both the control and the impacted mesocosms, thus, since the food concentration was higher in the impacted plots, their intake rate was higher and they reached that limit in a shorter period.

Interestingly enough, the differences in terms of feeding time were not proportional to the increase of sewage in the mixture, rather a threshold effect was observed. This threshold seemed to be very low, since a relatively small alteration of nutrients concentration in the mesocosms, i.e. the addition of 20% of sewage, had a strong effect on the crabs feeding demand. These latter results are important in view of the fact that fiddler crabs are recognised to be important ecosystem engineers (Kristensen, 2008). They showed, in fact, to actively alter sediment biogeochemistry by means of their bioturbative activities, performed both below (within-burrowing activity) and above (feeding and crawling activities) the sediment (Cannicci et al., 2008). Moreover, it was recently showed by Kristensen and Alongi (2006) that without the active feeding of *Uca* species the microalgal communities, which in normal conditions provide the heterotrophic microbial community with labile organic carbon, may overgrow and establish very dense and continuous layers. In such altered situation, anoxic conditions may occur with high rates of iron and sulphate respiration leading to high concentration of sulphide, thus negatively affecting mangrove saplings settlement and growth. Thus, a massive introduction of nutrients in the environment, such as the case of sewage loading, would totally prevent such a beneficial action exerted by the crabs, since we showed that an increase in food availability strongly limited their foraging activity. The indirect evidences coming from our behavioural observations well integrate with the results obtained by Penha-Lopes et al. (2009), who proved a lesser amount of sediment processing due to a depressed foraging activity of the crabs, here demonstrated. Such a scenario, would testify for a subtle detrimental effect on the ecosystem determined by sewage contamination via a process mediated by key macrobenthic species (Dahdouh-Guebas et al., 2005).

However, no difference in the number of crabs active on the surface was observed at any time of the period between impacted and control mesocosms, meaning that the induced effect of the trophic abundance is not an overall reduction of the activity period but rather a modification of the crabs' time budget. Indeed, a direct outcome of the increased amount of food in the impacted plots seemed to be the extension of the actual period spent in the social activities, usually limited by temporal constraints related to feeding.

In the present study the main behavioural pattern enhanced in frequency is the waving activity of the males. The escalation of this male behaviour with sewage concentration may be interpreted as a possible substitute activity elicited by the strong modification of the time budget of the crabs as well as by the stressing conditions due to environmental modifications. Similarly, the wandering behaviour observed only in the plots flooded with sewage mixtures can be read as a peculiar activity stimulated by the particular conditions within the treated mesocosms. In these plots, and mostly in the central hours of the activity period, many crabs were observed wandering frenetically and without any apparent purpose or goal. Most of them walked rapidly forward and backward along the concrete walls, surprisingly not triggering any aggressive reaction by other males, repeatedly encountered during this relentless wandering phase. Many studies have described such behavioural pattern in several ocypodids, interpreting this so called "droving" activity as a strategy to implement feeding efficiency (Gherardi et al., 2002; Murai et al., 1983), as a reproduction linked behaviour (Takeda, 2003) or as a strategy for water loss limitation (Yoder et al., 2005). The fact that this wandering behaviour was almost absent in the control mesocosms at any time of the activity period, could have two possible interpretations. Firstly, it could be seen as an escape response from some mesocosms stressful conditions and directly related to the sewage impact suffered by the crabs, such as lower salinity or pH alteration of the interstitial water faced within their dens. This would be consistent with the very stereotyped kind of movements observed, often along the walls of the plots. Recent research, carried out comparing populations of *U. annulipes* leaving in a pristine mangrove forest with others dwelling in a forest affected by raw domestic sewage discharge in Mozambique, (Amaral et al., 2009) confirmed a stressing effect of chronic exposure of *U. annulipes* to domestic sewage, showing an alteration of the RNA/DNA ratio inside the muscular tissue of the crabs. A further support to the interpretation of the enhanced wondering activity as determined by stress condition would came from the study on the differential survival among treated and uncontaminated mesocosms, carried out by Penha-Lopes et al. (2009). In particular, after six months the experiment was running, the treatment with sewage was observed to reduce the survival of the two species with a rate of about 22% when compared to the plots flushed with sea water only.

It must be considered however a second possible explanation to this particular activity, consistent with the logic of a time budget modification which could implicate an enhanced explorative attitude of replete animals. At the present stage these hypotheses need further analyses and studies to be consistently supported.

5. Conclusions

The present results show that behavioural patterns of *U. annulipes* and *U. inversa* are strongly affected by domestic sewage dumping and have the potential to be used as bioindicators. Once a deep understanding of the changes in behaviour is obtained the time budget could be included in a battery of biomarkers used for biomonitoring areas of suspected pollution. In fact, the activity of these East African fiddler crabs can easily be studied due to the high density of their populations and a comparison between the time budget of populations occurring in purportedly impacted vs. pristine sites can be made. Moreover, it was demonstrated that behavioural changes related to sewage impact had a threshold effect and started immediately at low sewage loadings, making these ocypodid crabs quite sensitive indicators.

However, the main conclusions to be depicted by this study deal with the impact of sewage loading on mangrove macrofauna communities and, on a broader extent, on the overall mangrove system. In fact, although the fiddler crabs communities dwelling in the

mesocosms were able to feed and perform social behaviours, apparently confirming the results of Yu et al. (1997) which found no significant impact of wastewater on the macrofaunal community of a Chinese mangrove forest, clear signals of an incipient stress on the system were described. Actually, feeding rates of crab communities did not increase with the growing availability of microalgae, responding to an increase in nutrients. On the contrary, they reduced their feeding activities earlier, thus failing to perform those bioturbating and algal removal activities so important for the overall health of mangrove sediments. Thus, although preliminary, the present results show that our understanding of the mangrove communities response to sewage loadings is far from being understood and further research is strongly needed before proposing constructed mangrove wetlands as possible phytoremediation areas, as recently suggested (Wong et al., 1997; Wu et al., 2008; Yang et al., 2008).

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