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Departamento de Biologia Animal

Effects of domestic sewage on characteristics of mangrove communities and their functioning in East Africa

Gil Pessanha Penha-Lopes

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Centro de Oceanografia, Laboratório Marítimo da Guia, Departamento de Biologia Animal, Faculty of Sciences of University of Lisbon

Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels

Institute of Biology, University of Southern Denmark, Denmark

University of Dar es Salaam, Faculty of Aquatic Sciences and Technology, Dar es Salaam, Tanzania

Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, Moçambique

Kenya Marine and Fisheries Research Institute, Mombasa, Kenya

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PRIOR NOTE

In accordance with Paragraph 1 of “Artigo 40, Capítulo V, do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no Diário da República – II Série No. 153, de 5 de Julho de 2003”, it is clarified that full scientific articles already published or in press (5), and submitted (4) for publication in peer-reviewed scientific journals, were used in the elaboration of this dissertation. Having such works been conducted in collaboration, the candidate acknowledges that he was fully involved in the planning, sampling, in all laboratory processes, data analysis and discussion of the results of all the works, as well as in their preparation and submission for publication, with the exception of the article "Behavioural responses of mangrove fiddler crabs (*Uca* spp.) to urban sewage loadings: results of a mesocosm approach", in which the candidate was not the leading author.

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Abstract

In the last 15 years, several studies investigated the effects of domestic contamination in mangrove ecosystems, however, only few have recognized mangrove fauna species as effective bioindicators and biomonitors of anthropogenic contamination. The present work intends to better understand the effect of high loadings of organic matter on fauna inhabiting natural mangrove ecosystems or mangrove mesocosms as well as their ecosystem functioning.

Our results demonstrated that identifying infauna organisms to high taxonomic levels may not be a reliable tool as most of the community changes must occur at a functional group level and consequently at genus and species levels. Although there was a decrease in their diversity indexes at the peri-urban mangrove sites, only Oligochaeta group has shown to be a potential reliable indicator. In peri-urban areas, the reproductive season of crustaceans was extended, the percentage of ovigerous female increased and reproductive potential and embryo quality improved. Nevertheless, negative indicators included high percent of shrimp parasitation in natural mangrove creeks as well as lower survival and growth rates of fiddler crabs and gastropods in constructed mangrove wetlands (CMW).

Most recent studies on mangrove sewage filtration have focussed on the role of trees and sediment with associated microbes and microalgae. In our study, we have found that key mangrove macrofauna decreased feeding activity, and consequently top sediment disturbance, although slightly increasing sediment turnover, through burrow construction and maintenance.

Carbon dioxide and methane production and carbon oxidation pathways were also evaluated at different sewage concentrations and vegetation conditions and in the absence or presence of biogenic structures. Our results clearly show impacts of sewage on the partitioning of electron acceptors in mangrove sediment and confirm the importance of biogenic structures for biogeochemical functioning, by increasing organic matter mineralization, and consequently CMW efficiency.

Keywords: Bioindicators, Biomonitors, Ecosystem engineering, Benthic fauna, Wastewater pollution, Mangroves, East Africa

Resumo

As florestas de mangal estão localizadas em zonas costeiras tropicais. Embora apresentem uma relevância ecológica global, tais como sumidouro de carbono e áreas de viveiro de peixes e crustáceos, os mangais fornecem igualmente alimentos, fibras, madeira, produtos químicos e medicamentos às populações humanas locais. No entanto, durante as últimas décadas, os ecossistemas costeiros foram sofrendo forte pressão antropogénica devido a actividades de desflorestação e despejo de contaminantes, levando a uma redução anual da sua área em 1-2%.

O rápido aumento da população, e consequente urbanização e industrialização em zonas costeiras, resultaram em graves problemas ambientais e socioeconómicos. No entanto, determinar quando contaminação resulta em poluição exige em particular a análise das comunidades biológicas. Desta forma, torna-se importante a identificação precoce de biomonitores e/ou bioindicadores específicos de contaminantes. Por outro lado, as alterações na estrutura da comunidade e em especial das suas actividades podem conduzir a profundas alterações da sua função no ecossistema, afectando significativamente a sua robustez e sustentabilidade. Nos últimos 15 anos, vários estudos debruçaram-se no efeito da contaminação doméstica em florestas de mangal e comunidades faunísticas associadas, no entanto, poucos têm reconhecido espécies de fauna como eficazes bioindicadores e biomonitores de contaminação antropogénica.

Estes sistemas mostraram recentemente um potencial para o tratamento de águas residuais, minimizando a poluição costeira. Estudos têm demonstrado que os sedimentos destes ecossistemas são muito eficientes na remoção de nutrientes sem aparentes impactos negativos sobre as árvores de mangal ou comunidades de invertebrados bentónicos. Estações de tratamento de esgoto utilizando plantas de mangal estão agora a ser instaladas e estudadas, principalmente devido ao seu baixo custo e alta eficiência, embora tenha já sido demonstrado que cargas de água residual acima da capacidade do sistema e fracas condições hidrodinâmicas levam geralmente à eutrofização do sistema, e consequentemente a condições de hipoxia ou anóxia. Nestas condições é esperada uma redução de diversidade e biomassa em sistemas naturais, assim como elevadas taxas de mortalidade em sistemas artificiais. Esta situação poderá alterar significativamente o funcionamento dos ecossistemas devido à redução da actividade das espécies com maior potencial de bioturbação do sedimento, afectando potencialmente a saúde e eficiência de filtração do sistema, quer natural quer artificial. Enquanto a maioria dos estudos concentrou-se sobre o potencial das plantas e sedimentos, incluindo micróbios e microalgas, na degradação da matéria orgânica e filtração dos nutrientes, poucos têm estudado o potencial filtrador da macrofauna em locais peri-urbanos ou em sistemas artificiais sujeitos a elevadas concentrações de esgoto.

A secção I fornece uma breve descrição do projecto PUMPSEA, dos ecossistemas de mangal e comunidades faunísticas associadas. O efeito da poluição antropogénica em zonas costeiras tropicais (mangais) e a potencialidade destes sistemas para o tratamento de esgotos serão também brevemente descritos. Serão também ilustrados os processos da degradação da matéria orgânica assim como o papel da fauna (*bioturbação*) como um possível instrumento para aumentar o potencial filtrador destes sistemas.

A fim de cumprir os objectivos do presente estudo, nove estudos foram realizados resultando cada um em um manuscrito. Esta dissertação incorpora estes documentos, cada um constituindo um capítulo completo (Secção II), estando estes aceites ou publicados (5), submetidos (4) em revistas científicas internacionais.

No capítulo 1 do presente estudo, desenvolvem-se as possíveis diferenças na estrutura da comunidade da infauna em zonas de mangal peri-urbanas impactadas por esgotos doméstico e zonas não poluídas ao longo da África Oriental, utilizando análises multivariadas ou de diversidade básicas.

Pela primeira vez parâmetros reprodutivos de espécies de mangal foram avaliados como indicadores de contaminação de matéria orgânica e nutrientes. No capítulo 2 apresentam-se parâmetros reprodutivos, incluindo fecundidade, fertilidade e qualidade dos embriões (medido através da análise de ácidos gordos) de populações de caranguejo violinista, uma das espécies mais comuns em zonas de mangal, *Uca annulipes* (H. Milne Edwards, 1837), que habitam em locais peri-urbanos (contaminados) e mangais relativamente pristinos no sul de Moçambique.

Dado que a maioria dos contaminantes são descarregados para os canais dos mangais, um estudo multidisciplinar foi realizado nos mesmos locais que o estudo anterior utilizando populações de camarões residentes (*Palaemon concinnus* (Dana, 1852)). No capítulo 3, a estrutura populacional, parâmetros reprodutivos (como curvas de maturação, fecundidade, fertilidade e qualidade dos embriões), infecção parasitária e rácio de RNA / DNA foram usados como indicadores para testar a qualidade do habitat.

No capítulo 4, o efeito de diferentes condições do esgoto e vegetação na sobrevivência e bioturbação de caranguejos em unidades artificiais de mangal (mesocosmos) foi avaliada. O comportamento destas espécies de caranguejo foi também utilizado como um potencial indicador de contaminação de águas residuais (capítulo 5). O efeito da concentração de esgotos e presença de vegetação na sobrevivência, crescimento e comportamento da *Terebralia palustris* foi avaliado no capítulo 6. No mesmo capítulo, o papel

destes gastrópodes na dinâmica do sedimentos foi avaliada e as suas consequências na biogeoquímica do sedimento discutida. Sabendo que estes gastrópodes são capazes de se alimentar de uma grande variedade de fontes alimentares, no capítulo 7, a distribuição, o comportamento e as estratégias alimentares deste gastrópodes foram avaliadas no campo, a fim de melhor compreender o seu potencial nos sistemas artificiais de mangal com o objectivo de filtrar os esgotos domésticos.

No capítulo 8, que teve por objectivo estudar os efeitos dos esgotos domésticos nos isótopos estáveis de diferentes compartimentos das unidades de tratamento de esgotos (sedimento, microalgas bentónicas, árvores de mangal e fauna), os isótopos estáveis foram reconhecidos como bons indicadores de poluição antropogénica. O seu estudo permitiu também analisar a dieta das duas espécies de macrofauna estudadas (*Uca annulipes* e *Terebralia palustris*) sob diferentes cargas de efluentes domésticos e vegetação, a fim de compreender a importância destas espécies no funcionamento destas estações de tratamento.

O capítulo 9 incidiu sobre a emissão de dióxido de carbono e metano sob períodos de imersão e emersão, com especial ênfase na importância de estruturas biogénicas (como raízes aéreas e galerias de caranguejo), bem como nas vias de oxidação de carbono mais utilizadas em diferentes condições de esgoto e vegetação. Ambos os processos são importantes para compreender totalmente os efeitos de descargas de matéria orgânica sobre os ecossistemas de mangal assim como fornecer conhecimentos básicos para o desenvolvimento futuro de estações de tratamento de esgotos biológicas, utilizando como modelo os ecossistemas de mangal.

Os resultados mais significativos desta secção são também descritos. O presente estudo demonstrou que a identificação de organismos pertencentes à infauna a níveis taxonómicos elevado não pode ser um instrumento fiável como indicador de poluição doméstica, uma vez que as alterações são geralmente sentidas na alteração dos grupos funcionais, e consequentemente só podem ser reconhecidas ao nível do género ou espécie. Uma ligeira diminuição dos índices de diversidade e dos organismos pertencentes ao grupo Oligochaeta em locais peri-urbanos, poderão ser considerados como potenciais bioindicadores de contaminação antropogénica. Em zonas peri-urbanas, a época reprodutiva de crustáceos foi prolongada, tendo a percentagem de fêmeas ovígeras aumentado assim como o seu potencial reprodutivo e a qualidade dos embriões. No entanto, indicadores negativos incluíram elevados níveis de parasitismo de camarões em mangais peri-urbanos,

bem como reduzida taxa de sobrevivência e crescimento de caranguejos e gastrópodes nos sistemas artificias.

Neste estudo foi observada uma redução significativa da actividade alimentar de caranguejos e gastrópodes, diminuindo significativamente a quantidade de sedimento processado durante as actividades de alimentação, embora aumentando ligeiramente o volume de sedimento revolvido durante a construção e manutenção das galerias de caranguejo.

Os nossos resultados mostraram também claramente os impactos dos efluentes sobre as vias de oxidação de carbono no sedimento e confirmaram a importância das estruturas biogénicas na biogeoquímica e metabolismo do sedimento, e consequentemente na eficácia das estações de mangal para o tratamento de esgotos.

Uma discussão geral considerando todos os estudos realizados, as principais conclusões extraídas deste estudo e as contribuições feitas para aumentar o conhecimento de potenciais bioindicadores e biomonitores de esgoto doméstica utilizando a fauna de mangal, bem como as suas implicações no funcionamento do ecossistema em sistema contaminados artificiais são o conteúdo da Secção III. Nesta mesma secção será feita uma reflexão sobre as questões levantadas durante este estudo e propostas de estudos a realizar no futuro próximo.

Palavras-chave: Bioindicadores, Biomonitores, Bioturbação, Fauna bentónica, Esgoto, Mangais, África Oriental

List of papers

This dissertation comprises the following scientific manuscripts:

Penha-Lopes, G., Xavier, S., Okondo, J., Cannicci, S., Fondo, E.N., Ferreira, S., Macamo, C., Macia Jr, A., Mwangi, S., Paula, J., submitted. Effects of urban wastewater loading on macro and meioinfauna assemblages in equatorial and subtropical mangroves of East Africa. Western Indian Journal of Marine Science

Penha-Lopes, G., Torres, P., Narciso, L., Paula, J., accepted. Comparison of fecundity, embryo loss and fatty acid composition of mangrove crab species in sewage contaminated and pristine mangrove habitats in Mozambique. J. Exp. Mar. Biol. Ecol.

Penha-Lopes, G., Torres, P., Cannicci, S., Narciso, L., Paula, J., submitted. Monitoring anthropogenic sewage pollution on mangrove creeks in southern Mozambique: a test of *Palaemon concinnus* Dana, 1852 (Palaemonoidae) as a biological indicator. Environ. Pollut.

Penha-Lopes, G., Bartolini, F., Limbu, S., Cannicci, S., Paula, J., in press. Are fiddler crabs potentially useful ecosystem engineers in mangrove wastewater wetlands? Mar. Pollut. Bull.

Bartolini, F., Penha-Lopes, G., Limbu, S., Paula, J., Cannicci, S., in press. Behavioural responses of mangrove fiddler crabs (*Uca* spp.) to urban sewage loadings: results of a mesocosm approach. Mar. Pollut. Bull.

Penha-Lopes, G., Bartolini, F., Limbu, S., Cannicci, S., Mgaya, Y., Paula, J., in press. Ecosystem engineering potential of the gastropod *Terebralia palustris* (Linnaeus, 1767) in mangrove wastewater wetlands - a controlled mesocosm experiment. Environmental Pollution Series A, Ecological and Biological

Penha-Lopes, G., Bouillon, S., Mangion, P., Macia Jr, A., Paula, J., in press. Population structure, density and food sources of *Terebralia palustris* (Potamididae: Gastropoda) in a low intertidal *Avicennia marina* mangrove stand (Mozambique). Estuar. Coast. Shelf Sci., doi:10.1016/j.ecss.2009.1004.1022

Penha-Lopes, G., Mangion, P., Limbu, S., Kristensen, E., Dehairs, F., Bouillon, S., Paula, J., submitted. Stable Isotopes as indicators of sewage and trophic changes in constructed mangrove wastewater wetlands. Mar. Pollut. Bull.

Penha-Lopes, G., Kristensen, E., Flindt, M., Mangion, P., Bouillon, S., Paula, J., submitted. The role of biogenic structures on the biogeochemical functioning of mangrove constructed wetlands sediments – a mesocosm approach. Environ. Pollut.

SECTION I

General Introduction

Opening remarks

Ecological damage due to anthropogenic activities is threatening marine eco-systems and coastal resources around the world. Growing population, increasing urbanisation and rapid industrialisation have resulted in serious environmental and socio-economic crises in coastal cities (Sharma, 2000; Mohammed, 2002a; Diaz and Rosenberg, 2008). However, determining when contamination results in pollution requires not only chemical but also biological measurements (Chapman, 2007). Some of these contaminants are known to cause the destruction of entire coastal shallow-water communities, affecting animal physiological functioning, behaviour, reproductive success, and outright mortality in associated invertebrates and fishes (Peters et al., 1997; Kennish, 2002). This way it becomes important to identify early bioindicators or biomonitors of contaminants in natural conditions. On the other hand, changes in community structure or in species behaviour and activities may lead to changes in the ecological role of fauna, which may affect significantly ecosystems health and sustainability.

The following section of this introduction provides a brief description of the project PUMPSEA and an overview of anthropogenic pollution in coastal zones, mainly in tropical regions, mangrove ecosystems and their associated fauna communities and early warning indicators of pollution used globally and in mangrove forest. Furthermore, the role of wastewater wetlands and the potential of mangroves to treat sewage are also briefly described. The organic matter degradation and nutrient cycling processes will also be illustrated and the ecosystem engineering role of fauna as a potential tool to increase these processes will also be clarified. Finally, the aims and objectives of this study are outlined, and the rationale driving to the investigations conducted is explained.

PUMPSEA project

This PhD thesis was done within the PUMPSEA (Peri-urban mangrove forests as filters and potential phytoremediators of domestic sewage in east Africa) project that was built based on the preliminary observation and studies that mangroves filtrate discharged wastewater and prevent coastal pollution, although this ecosystem service has not been conveyed to coastal management, nor has the filtration capacity been fully exploited. In this light, the overall objective of this project was to demonstrate the ecological and economical service that peri-urban mangroves provide by mitigating coastal pollution through sewage-filtration, and to offer innovative solutions for the exploitation and management of this service. The project examined two innovative ways in which mangrove filtration can be utilised to preclude coastal sewage pollution: (1) facilitating sewage filtration by conserving filtering mangroves and replanting mangroves in deforested areas exposed to sewage (´strategic reforestation and conservation'), and (2) using constructed mangrove wetlands for sewage treatment. It also developed an implementation plan for the exploitation of the developed technology and know-how, based on analysis of governance, policy, cost and financing options. At the same time, several studies tried to better understand the impact of sewage contamination on ecosystem health and processes, including microbial, fauna and flora diversity and abundance as well as sediment biogeochemical processes. This work took place in peri-urban mangrove areas of Maputo (Mozambique), Dar es Salaam (Tanzania) and Mombasa (Kenya) and pristine (control) mangrove areas nearby, and involved several African and European scientific teams and expertise fields.

Anthropogenic pollution

In peri-urban areas, wastewater loadings rich in labile organic matter and nutrients are common and under weak hydrodynamic conditions usually lead to eutrophication. Accumulation of particulate organic matter will encourage microbial activity and the consumption of dissolved oxygen in bottom waters leads to hypoxic or even anoxic conditions (Gray et al., 2002; Diaz and Rosenberg, 2008). The worldwide distribution of coastal oxygen depletion is associated with major population centers and watersheds that deliver large quantities of nutrients (Figure 1). Low oxygen levels, and consequently production of toxic components (such as sulphite and ammonia) may decrease faunal diversity and biomass due to emigration of mobile species or high mortality of less mobile species in natural ecosystems (Diaz and Rosenberg, 1995). This could also significantly affect ecosystem functioning (Biles et al., 2002; Le Hir et al., 2007), and coupled with a potential decrease in activity and behaviour of the more resistant species (Diaz and Rosenberg, 1995) potentially reduce the ecosystem health and functioning.

In tropical developing countries the lack of technical solutions for sewage treatment, combined with an increase in population growth and migration to coastal areas, has lead to a rapid increase in urban wastewater production, which is putting breakpoint pressures on already inadequate sewage systems (e.g., Mohammed, 2002b).

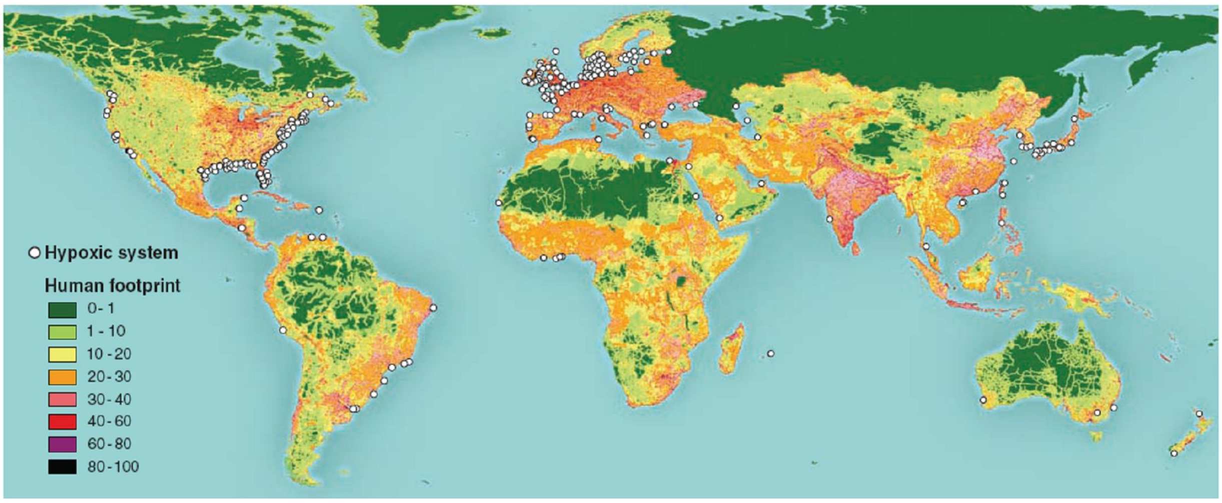


Figure 1 – Global distribution of 400-plus systems that have scientifically reported accounts of being eutrophication-associated dead zones. Their distribution matches the global human footprint in the northern hemisphere, while for the Southern hemisphere, the occurrence of dead zones is only recently being reported. (adapted from Diaz and Rosenberg, 2008)

As a result, peri-urban coastal areas receive extensive amounts of untreated sewage, which is typically discharged into creeks lined by mangrove forests. Although this uncontrolled drainage of raw sewage into peri-urban coastal areas is a factual result, there is hardly any understanding of its consequences regarding ecosystem health (Holguin et al., 2001; Adeel and Pomeroy, 2002).

Mangrove habitats

Mangroves can be defined as trees and shrubs that grow in saline (brackish) coastal habitats in the tropics and subtropics (see Figure 2), where water temperature does not go below 20ºC. The word is used also more broadly to refer to the habitat and entire plant assemblage, for which the terms mangrove swamp and mangrove forest are also used. Mangrove areas are characterized by multiple substrate conditions but usually fine sediments, often with high organic content, low energy wave action, freshwater runoff, prolonged hydroperiod, salinity, anoxic conditions, and accumulation of toxic substances (Hogarth, 2007). Species composition is strongly influenced by these coastal settings because they are linked to differences in mangrove tree species' capability to become established and grow. True mangroves comprise some 55 species in 20 genera, belonging to 16 families, although the Avicenniaceae and Rhizophoraceae comprise most of the species (Hogarth, 2007; Ellison, 2008).

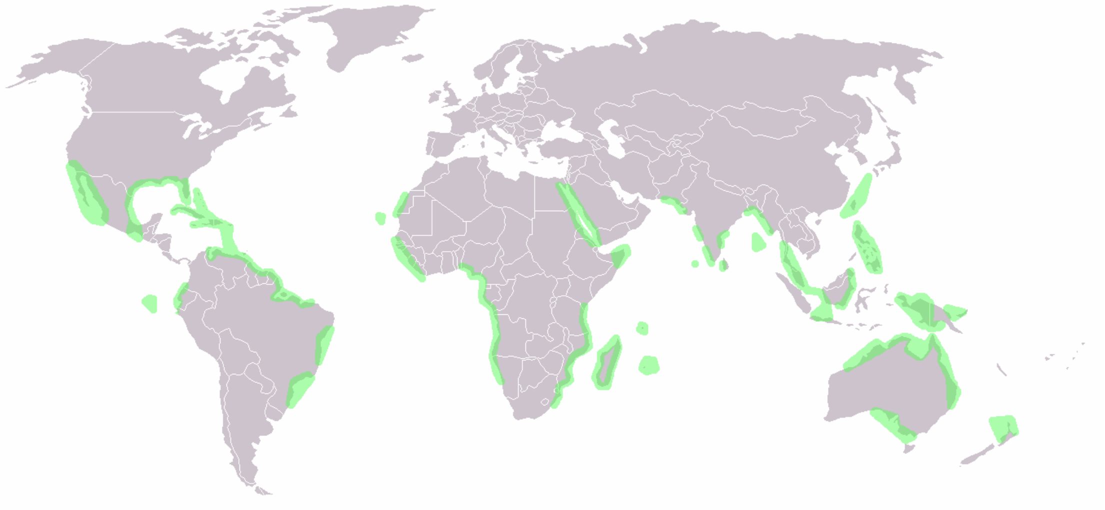


Figure 2 – World Distribution Map of Mangroves.

These ecosystems accomplish a number of functions and services (Duke et al., 2007). These forests are one of the most productive systems (> 2 t ha^{-1} yr^{-1}), and as a result they play a key role in nutrient cycling of coastal zones and global carbon cycling, and showed to act both as source and sink areas (Kristensen et al., 2008; Alongi, 2009). Its structure and productivity are crucial components of estuarine habitats that support ecosystem services such as nursery areas for fish, prawns and crabs (Sheridan and Hays, 2003; Crona and Rönnbäck, 2005). Human communities living near mangroves also rely on the provision of a variety of food, timber, chemicals and medicines derived from mangrove forests or associated plants (Ewel et al., 1998; Glaser, 2003; Stone, 2006). After the 2004 tsunami, mangrove forests proved also their effectiveness in protecting coastlines (Dahdouh-Guebas et al., 2005; Kathiresan and Rajendran, 2005).

Despite the great social, economic, and environmental importance of mangroves in tropical ecosystems (Rönback et al., 2007; Nagelkerken et al., 2008; Walters et al., 2008), during the last decades, mangrove ecosystem have been reduced at a rate of 1-2% due to anthropogenic influence through deforestation and dumping activities (Duke et al., 2007; Kruitwagen et al., 2008). Also, environmental monitoring and assessment of these systems are still lacking (Peters et al., 1997). Most studies done in these forests have pursued the quantification in situ (water, sediment samples as well as in organisms) of anthropogenic contaminants such as heavy metals, organotins, organochlorine pesticides and polychlorinated biphenyls, among others (Uma Devi and Prabhakara Rao, 1989a; b; De Wolf et al., 2001; Mohammed, 2002b; Kruitwagen et al., 2008), while the classification of pollution using key fauna communities or species as bioindicators has been neglected.

Key mangrove fauna

Mangrove ecosystems harvest a unique set of associated faunal species (Vannini et al., 2002; Lee, 2008). A unique and diverse assemblage of benthic invertebrates, including gastropods, polychaetes, oligochaetes and burrowing brachyuran crabs, such as sesarmids and fiddler crabs, are considered the most important functional groups in this ecosystem (Cannicci et al., 2008). This is mainly due to their high biomass, significantly affecting vegetation structure by leaf consuming and propagule predation, regulation of benthic microalgae biomass and productivity, sediment properties, mainly through their ecosystem engineering activities, and controlling energy flow between mangroves and nearby ecosystems (Cannicci et al., 2008; Kristensen et al., 2008).

Gastropods

Gastropods play an important ecological role in natural systems through organic matter consumption (Slim et al., 1997; Fratini et al., 2004), bioturbation effects on nutrient cycling (Biles et al., 2002), regulation of meiofauna and microphytobenthos biomass (Carlén and Ólafsson, 2002; Pape et al., 2008), changes in the dynamics of suspended material (Kamimura and Tsuchiya, 2004) and effects on sediment stability (Orvain et al., 2003; 2004; 2006). In mangrove forests more than 30 species of gastropods can be found (Hogarth, 2007), and total densities can achieve values higher than 200 m^{-2} (Houbrik, 1991).

The mudwhelk gastropod *Terebralia palustris* (Linnaeus, 1767) is a key epifaunal species in mangrove forests around the world (Soemodihardjo and Kastoro, 1977; Nishihira, 1983; Houbrik, 1991; Slim et al., 1997; Dahdouh-Guebas et al., 1998; Fratini et al., 2004; Pape et al., 2008). Studies have shown that it is important for the nutrient cycling by consuming large amounts of *Avicennia marina* (Forsk.) and *Rhizophora mucronata* Lam. litter, mangrove propagules, carrion, detritus, benthic diatoms and bacteria (Nishihira, 1983; Rambabu et al., 1987; Dahdouh-Guebas et al., 1998; Fratini et al., 2000; Fratini et al., 2004). It can also regulate microphytobenthic primary productivity and meiobenthos community through feeding and/or crawling activities (Schrijvers et al., 1996; Schrijvers et al., 1998; Carlén and Ólafsson, 2002; Pape et al., 2008). This intertidal snail seems able to discriminate between different food items and can potentially use both air-borne and water-borne odours to locate food (Fratini et al., 2001; Fratini et al., 2008). A spatial separation between young and older individuals was first observed by Soemodihardjo and Kastoro (1977). Due to anatomical differences in the structure of the radula, only large (shell height >5 cm) *T. palustris* actively feed on fallen mangrove leaves, propagules and fruits, while small individuals (shell height <5 cm) are usually detritivorous or deposit feeders (Nishihira, 1983;

Houbrik, 1991; Slim et al., 1997; Dahdouh-Guebas et al., 1998; Fratini et al., 2004; Pape et al., 2008).

Crabs

Brachyuran crabs, primarily fiddler crabs (family Ocypodidae) and leaf-eating sesarmid crabs (family Grapsidae), dominate the mangrove fauna in number and biomass (Kristensen, 2008), and these burrowing decapods are key components of Indo-Pacific mangroves (Lee, 1998).

More than 97 species of fiddler crabs (genus *Uca*), although not all present in mangrove ecosystems, establish dense populations reaching more than 150 individuals m^{-2} on intertidal flats (Rosenberg, 2001; Skov and Hartnoll, 2001; Skov et al., 2002; Cannicci et al., in press), making them important grazers on microalgae and bacteria (Bouillon et al., 2002; Reinsel, 2004). As many as 36 species of sesarmid crabs have been found in mangrove forests of Thailand (see references in Thongtham et al., 2008) and densities are much lower than for fiddler crabs, reaching average values of 6 m^{-2} (Cannicci et al., in press) and mainly consuming organic matter, dead fauna and leaves (Thongtham and Kristensen, 2005; Cannicci et al., 2008; Lee, 2008; Thongtham et al., 2008).

Fiddler crabs of genus *Uca* (Ocypodidae) are characterised by strong sexual dimorphism and male asymmetry and are a well known group due to the fact that one of the male chelae is very enlarged and carried horizontally across the front, like a fiddle. This bigger chelae is used for protection, aggressive and female courtship behaviours (Eshky et al., 1995). Fiddler crabs are known to inhabit and seal their burrows during immersion periods (De la Iglesia et al., 1994), while feeding, waving (as a female courtship behaviour), and gallery maintenance activity during emersion period during day time (Eshky et al., 1995). These crabs have been the subject of a wide variety of studies, including taxonomic (Crane, 1975; Rosenberg, 2001), reproductive parameters and behaviour (e.g., Hyatt, 1977; Christy, 1982; Backwell and Passmore, 1996; Backwell et al., 1999; Litulo, 2004a; c; b; 2005d; c; b; 2006; Torres et al., 2008; 2009), visual and acoustic display (Salmon, 1965; Salmon and Atsaides, 1968; Cannicci et al., 1999), morphometric relations and dynamics (e.g., Huxley, 1924; Huxley and Callow, 1933; Levinton and Judge, 1993; Takeda and Murai, 1993), aggressive, foraging and burrowing behaviour (Murai et al., 1982; Weissburg, 1992; Wolfrath, 1992; Weis and Weis, 2004) and a few studies on toxicity, environmental monitoring and pollution (e.g., Devi, 1987; Weis and Kim, 1988; Ismail et al., 1991; Das and Chakraborty, 2004; Mokhtari et al., 2008).

Infauna

Diversity and abundance studies of infauna in Indo-Pacific mangrove forests are still scarce (Sasekumar, 1974; Guerreiro et al., 1996; Fondo and Martens, 1998; Metcalfe and Glasby, 2008) mainly due to limited taxonomic expertise or resources availability in developing countries (Thorne and Williams, 1997). Polychaeta species represent more than 90% of the biomass of macroinfauna and more than 60 species were already observed in Australian mangroves (Metcalfe and Glasby, 2008). Regarding meiofauna, total densities can easily reach 2.46 x 106 m^{-2} and nematods are usually the most abundant group representing more than 80% of the total density (Dye, 1983; Ólafsson, 1995) and more than 90 genera belonging to more than 30 families were recorded (Olafsson et al., 2000).

Several descriptive studies have been done on the temporal and spatial distribution of the infauna (Dye, 1983; Alongi, 1987; Ólafsson, 1995; Olafsson and Elmgren, 1997) along with studies on trophic and non-trophic relations between macro and meiofauna as well as with macroepifauna and flora (Dye and Lasiak, 1986; Olafsson and Elmgren, 1991; Olafsson and Moore, 1992; Schrijvers et al., 1995; Schrijvers et al., 1996; Schrijvers et al., 1997; Schrijvers et al., 1998; Paula et al., 2001; Olafsson, 2003), food source to pelagic and visiting fauna or avifauna (Sutherland et al., 2000; Lee, 2008), nutrient re-cycling (Kristensen et al., 2005; Lillebø et al., 2007) and the effects of physical and biogeochemical disturbances (Alongi and Christoffersen, 1992; Banta et al., 1999; Kristensen and Kostka, 2005).

Ecological indicators

During the past decades, researchers have been trying to find appropriate bioindicators and biomonitors from each ecosystem for the different types of contaminants that they are subjected to. According to Market (2004): “A bioindicator is an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment), while a biomonitor, on the other hand, is an organism (or part of an organism or a community of organisms) that contains information on the quantitative aspects of the quality of the environment”.

Studies of contaminant-induced alterations in fauna species abundance and diversity were developed and are nowadays commonly used in order to give an indication of anthropogenic contamination (e.g., Bigot et al., 2006; Saunders et al., 2007). For example, fauna community from different ecosystems (from deep sea to estuaries) has already demonstrated to be significantly affected (both in diversity and abundance) by different types of pollutants such as heavy metals and hydrocarbons (Inglis and Kross, 2000; Heininger et al., 2007), as well as organic matter and nutrients (Beier and Traunspurger, 2001; Rossi and

Underwood, 2002; Hyland et al., 2005; Chambers et al., 2006; Saunders et al., 2007), including in mangrove ecosystems (Yu et al., 1997).

Positive indicators, also named opportunistic species, from polychaete worms, such as *Capitella capitata* (Méndez et al., 1997) to fishes, such as carp *Cyprinus carpio* (Tsai, 1975 in Smith and Suthers, 1999), tend to increase abundance in polluted areas out competing local species. Negative indicators are usually key fauna species in the environment that tend to decrease biomass, show reduced growth rates (e.g., Crouau and Moia, 2006; LeBlanc, 2007; Amara et al., 2009), as well as low reproduction output and embryo quality (e.g., Smith and Suthers, 1999; Elumalai et al., 2005; Crouau and Moia, 2006). They are efficient indicators of contamination due to a general reallocation of resources favouring tolerance to stress, by combating against contaminants, low oxygen levels or repairing damage (Diaz and Rosenberg, 1995; Wu, 2002). Changes in morphological structures and physiological processes have been also an alternative indication of organisms (including foraminifera, bivalves and crustaceans) condition when facing stress or pollution (e.g., Le Cadre and Debenay, 2006; Norkko et al., 2006).

Biochemical indicators have also become central in such monitoring studies. They are key components of synthetic or metabolic pathways, reflecting metabolic activities directly or indirectly linked to growth, reproduction or survival, and thus physiological condition (Dahlhoff, 2004). Biochemical changes usually occur before those in organism growth or reproduction become discernable, and thus may help us early identify effects of stressors, such as pollution, and thus prevent the loss of critical ecosystem functions. RNA/DNA ratio has been especially valuable as a biochemical estimator of organism condition in studies of marine invertebrates and fishes (Dahlhoff, 2004; Gilliers et al., 2004; Amaral et al., 2009; in press). The principle is that RNA content is correlated with new protein synthesis, usually interpreted as being beneficial to the organism, reflecting active metabolic rates canalized to growth and reproduction. As DNA content remains relatively constant in an individual due to be a function of chromosome number, higher RNA/DNA ratios are expected where, or when, conditions are favourable (Dahlhoff, 2004).

Parasites are useful bioindicators particularly if anthropogenic pollution is to be monitored, due their strong impact on fauna communities (Poulin, 1999). They have been widely accepted as a good indicator of anthropogenic pollution due a generally decrease of host defence mechanism and fitness, thereby increasing the host susceptibility (Sures, 2004; Sures, 2006), affecting host abundance, tolerance to pollution, behaviour and mortality (Lafferty, 2008). Pollution can either increase parasitism if, for example, host defence mechanisms are negatively affected, thereby increasing host susceptibility, or by simply increasing the population densities of suitable intermediate or final hosts (Lafferty and Kuris, 1997). On the other hand it can also decrease parasitism provided that: (i) infected hosts suffer more from environmental exposure than do uninfected hosts; (ii) parasites are more susceptible to the particular pollutant than their host; or (iii) pollution drives the necessary intermediate and final hosts to become extinct. Nevertheless, the effects of pollution can vary between parasite species and developmental stages as well as between host species (Sures, 2004).

Stable isotope (SI) signatures have been successfully used to trace organic matter and nutrients fluxes within mangrove ecosystem food web (Bouillon et al., 2008), and several studies have focussed on the role of primary producers and microbial community as well as on the diet of invertebrates within these forests (e.g., Bouillon et al., 2002; 2004a; 2004b; Penha-Lopes et al., in press). Based on the small (0–1‰) or high (2.6 to 3.4‰) fractionation of carbon and nitrogen SI, respectively, between an organism and its dietary sources, analysis of $\delta^{13}C$ can help in elucidating the role of organic matter sources and identifying the diet of consumers, while nitrogen isotope signatures are used to assess the trophic levels of consumers (McCutchan et al., 2003; Bouillon et al., 2008). Recently, the analysis of carbon and nitrogen SI ratios on some key ecosystem components have also been proposed as a versatile approach for assessing and monitoring anthropogenic contamination on ecosystems, being considered good biomonitors (Cabana and Rasmussen, 1996; Costanzo et al., 2001; Vizzini et al., 2005; Cole et al., 2006). Although carbon has shown to be able to track sewage-sludge contamination (depleted in ^{13}C) in mesocosms simulating estuarine food web (Gearing et al., 1991), nitrogen has been considered globally (from deep sea to coastal areas) the most robust and reliable indicator of sewage and animal wastes contamination (Van Dover et al., 1992; Heikoop et al., 2000; Vizzini and Mazzola, 2006; Chang et al., 2009). SI analysis on sediment, primary producers and invertebrates from pristine and contaminated areas with wastewater nutrients and organic matter, derived from animal waste and sewage disposal, have shown their high potential as good anthropogenic pollution early biomonitors, even at low concentrations (McClelland et al., 1997; McClelland and Valiela, 1998; Struck et al., 2000; deBruyn and Rasmussen, 2002; Cole et al., 2004; Cole et al., 2005). However, most of biomonitors but specially bioindicators have been identified in developed regions of the world and are almost inexistent for developing countries, especially when considering organic matter and nutrient contamination (Peters, 1997).

Mangrove macrofauna ecological indicators

There is a lack of clear understanding of the response of key mangrove species to anthropogenic sewage contamination in natural environments, mainly because sewage might be diffuse and is usually a mixture of different types of contaminants with spatial and temporal variations. The majority of pollution assessment studies in mangrove forests are mostly focussed on local community diversity and structure, such as the one done by Yu et al (1997), in Futian mangroves, P.R.C., experimentally treated with municipal wastewater, where eighty-four species of ground-dwelling fauna were studied, or Cannicci et al. (in press) where only major key species of macro-epifauna, such as gastropods and crabs, were studied in peri-urban and pristine mangrove of East Africa. This last study has found that East African mangrove crab and mollusc populations are significantly affected by domestic wastewater, with an increase in density and biomass of the former group and a decrease in the last one in peri-urban mangroves compared with relative pristine mangroves. A similar pattern of mollusc depopulation was also observed by Yu et al. (1997). Another indication of a negative impact of sewage on mangrove gastropod populations is provided by a preliminary study on fauna distribution in the disturbed mangrove system of Maruhubi, Zanzibar (Machiwa and Hallberg, 1995).

Infauna studies in developing countries are limited due to restricted taxonomic expertise or resources availability. Therefore, emphasis should be placed on cost-effective techniques such as taxonomic minimalism (Thorne and Williams, 1997), which is also currently the case for mangrove meiofauna studies (Nagelkerken et al., 2008). This way, in order to save time and resources, the effect of environmental or anthropogenic disturbance on meio and macrofauna communities have been effectively detected with multivariate analysis at high taxonomic levels, from family to phylum (Thorne and Williams, 1997; Chapman, 1998; Kennedy and Jacoby, 1999; Savage et al., 2001; Gesteira et al., 2003). Another approach is to select specific groups, such as nematods and polychaets, and identify specimens to lower taxonomic levels or separate them into functional groups, another clear indication of the degree of contamination (Raffaelli and Mason, 1981; Levin et al., 1996; McPherson et al., 2002; Gyedu-Ababio and Baird, 2006; Gillet et al., 2008). This way, is urgently needed to develop easy but efficient tools and biological indices to monitor and manage near shore marine environments in developing countries such as East African countries.

However, community structure and dynamics is merely an expression 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 survival and growth, physiological conditions and reproductive output, that might be more or less sensitive and used as an early warning to determine deterioration in ecosystem health (Attrill and Depledge, 1997). Although in other marshes this approach is common, in mangrove ecosystems is rare. Only recently, the physiological stress (using RNA/DNA ratio of claw muscle) of key crab species inhabiting a peri-urban and two pristine mangroves was assessed and results indicated that RNA/DNA ratio of *U. annulipes* decrease significantly at the contaminated mangrove, indicating stress, and may thus be a useful indicator of pollution (Amaral et al., 2009). Although reproductive studies of key mangrove species became recently very common on mangrove habitats (Litulo, 2004a; c; 2005d; a; 2006; 2007; Penha-Lopes et al., 2007; Torres et al., 2007; Mokhtari et al., 2008; Torres et al., 2008; 2009), none has, till this moment, used it as a proxy for sewage pollution. Also, species survival and growth was not assessed in contaminated conditions.

It thus becomes urgent to better understand the effects of sewage contamination in key mangrove species by studying its population structure, survival and growth, changes in reproductive parameters and/or behaviour in contaminated and pristine conditions. Although not profuse, a multidisciplinary approach to investigate the impacts of sewage pollution on several aspects of a single species, usually mainly focussed on polychaetes and fishes, started to be used as indicators of anthropogenic impact and are now widely accepted (e.g., Smith and Suthers, 1999; Amara et al., 2009; Durou et al., accepted), but once again inexistent for mangrove habitats.

Biomonitors may reflect heavy metal or other contaminants by accumulating it in the organism tissues. In mangrove habitats this approach has already been widely used (Saha et al., 2006; Kruitwagen et al., 2008), such as in fiddler crabs (Uma Devi and Prabhakara Rao, 1989a; b), *Littoraria scabra* gastropods (De Wolf et al., 2001), and oysters (Zanette et al., 2006; Mtanga and Machiwa, 2007) inhabiting contaminated zones. Another biomonitor tool already used at natural mangroves ecosystem contaminated with sewage or agriculture wastes is the stable isotope analysis technique, where the nitrogen derived from sewage was found in mangrove tree tissues (Fry et al., 2000; Costanzo, 2003; Pitt et al., 2009) and associated crabs species (Pitt et al., 2009). To our knowledge no study using SI has been conducted in constructed wastewater wetlands, and controlled conditions, in order to validate data obtained from the field.

Wastewater Wetlands

The main wastewater treatment goal in developing countries is protection of public health through preventing transmission of waterborne diseases and eutrophication of surface waters. Stabilization pond systems, septic tanks, activated sludges, trickling filters, anaerobic systems and land application systems have been used to treat sewage due to their low cost of installation and maintenance, and optimum climatic conditions for ponds found in tropical areas where many developing countries are located (Kivaisi, 2001). The most widely used treatment systems are stabilization ponds. However, when effluents are released without further treatment back into environment, they can lead to eutrophication of downstream ground and surface water. Wetlands are potential alternative or supplementary systems for wastewater treatment. In combination with established stabilization ponds, the use of wetlands for processing nutrient and organic-rich wastewater has proved effective to prevent coastal pollution in developing countries, mainly due to low running cost (low energy consumption and training requirements) and high filtration efficiency (Kivaisi, 2001; Crites et al., 2006).

The primary productivity of wetland ecosystems is typically high in the tropics, due to the ample light, temperatures, water and nutrient supply (mainly near peri-urban centers). Many natural and constructed tropical wetlands have net primary productivity of more than 1000 g C m^{-2} yr^{-1} which is greater than most other ecosystems (Alongi, 2009). Incoming nutrients support the growth of vegetation, which converts inorganic chemicals into organic materials, the basis of the wetland food chain. However they are characterized by high organic matter accumulation due to a reduced rate of decomposition as a consequence of anaerobic conditions (Kristensen et al., 2008). Finally, they also allow multi-purpose sustainable utilization such as swamp fisheries, biomass production, seasonal agriculture, water supply, public recreation, wild life conservation and scientific study (Kivaisi, 2001). However, previous studies have identified current limitations to widespread adoption of CW technology for wastewater treatment in developing countries (see Kivaisi, 2001). These include large land requirements, lack of knowledge of tropical wetland ecology and native wetland species, prevalence of mixed domestic:industrial wastewaters, and limited knowledge and experience with CW design and management.

Mangrove potential as wastewater wetlands

Mangrove forests have recently shown a potential as natural wastewater treatment facility in China, by removing nutrients and organic matter efficiently (Wong et al., 1997; Yang et al., 2008). Growth and productivity in natural and pristine mangrove forests are usually nutrient limited (Hogarth, 2007). Consequently, discharge of moderate sewage loadings results in enhanced growth of trees (Mohamed et al., 2008) as well as stimulation of benthic primary producers and microheterotrophs (Tam, 1998; Meziane and Tsuchiya, 2002). Field trials have shown that sediments of these ecosystems are very efficient in removing nutrients from sewage (Tam and Wong, 1995; 1996), without apparent impacts on mangrove trees (Wong et al., 1997) or significant effect on the benthic invertebrate communities (Yu et al., 1997).

However, possible harmful effects due to toxic materials and pathogens in wastewater (Al-Sayed et al., 2005) and anthropogenic degradation of natural wetlands have forced managers to use constructed wetlands (Kivaisi, 2001). CWs for wastewater treatment involve the use of engineered systems that are designed and constructed to utilize natural processes. These systems are designed to mimic natural wetland systems, using wetland plants, soil, and associated microorganisms to remove contaminants from wastewater effluents (Kivaisi, 2001). This way sewage filtering of constructed wetlands is now addressed in several tropical and subtropical countries (Kivaisi, 2001; Stottmeister et al., 2003), and more recently mangroves have also been also considered as an effective alternative (PUMPSEA, 2008; Yang et al., 2008).

Ecosystem engineering

Bioturbation is generally referred as a key process in the transport of various particles and compounds by the activity of benthic organisms in terrestrial soils and aquatic sediments (Rhoads, 1974; Aller, 1982; Kristensen and Kostka, 2005). Although this topic was addressed by Charles Darwin at the end of his life (Meysman et al., 2006), only recently has been recognized to affect several major ecological processes, being considered also one of the most significant events in the evolution of marine ecosystems (Lohrer et al., 2004; Meysman et al., 2006; Solan et al., 2008). The physical, chemical and biological effects of sediment and water transport by benthic fauna (or biogenic structures) plays an important role for the biogeochemical and ecological functioning of soils and sediments (Meysman et al., 2006). It is well known that benthic organisms affect the stability, erodability and particle transport of aquatic sediments through locomotion, irrigation and feeding activities as well as construction of biogenic structures, such as burrows, tracks and mounts. The actual effect on the ecosystem depends on the type of bioturbation, its range, intensity and period (Solan and Wigham, 2005). The extent of particle reworking or redistribution is species specific and depends on the organism’s mobility, burrowing and feeding mode, as well as activity rhythms, animal size and abundance (Rhoads, 1974; Rhoads and Boyer, 1982; Solan and Kennedy, 2002).

Most studies on sewage filtration have focussed on the role of plants and sediment with associated microbes and microalgae (e.g., Wong et al., 1995; Wong et al., 1997; Tam, 1998) and none have dealt with macrofaunal performance under severe conditions. However, it was shown that wastewater loadings above the system capacity and under weak hydrodynamic conditions usually lead to eutrophication and consequently hypoxic conditions (Gray et al., 2002), which could affect significantly fauna survival and growth rates, as well as behaviour and ecosystem functioning, of more sensitive species in constructed wetlands, affecting the system sustainability and filtration efficiency. It is important to understand that fauna does not only suffer the consequences of sewage contamination in these natural ecosystems, being able to consume organic matter detritus, increase its horizontal and vertical transport as well as its degradation rate and nutrient cycling (Aller, 1994; Kristensen et al., 2005). Although it is also known that pelagic species of fishes and other vertebrates can induce severe sediment disturbance (Fleeger et al., 2006; Lohrer et al., 2008), most of the previous studies have demonstrated the influence of diversity and abundance (and biovolume) of benthic worms (such as polychaets and oligochaetes), crustaceans and molluscs in the sediment structure as well as microbial and biogeochemistry dynamics (Krantzberg, 1985; Kristensen, 2000; Solan et al., 2004; Mermillod-Blondin et al., 2005; Ieno et al., 2006).

Ecosystem engineers have recently been addressed as powerful agents to restore ecological systems when managed well (Byers et al., 2006). Bioturbation activities influence the functioning of sediments, causing dramatic changes in nutrient dynamics and organic matter decomposition, and thus affect ecosystem health, productivity and filtration capacity (Kristensen and Kostka, 2005). However these processes depend on the type of organisms and environmental conditions, once each species may change behaviour and consequently bioturbation activity in different conditions (e.g., food quality and quantity, water current, sediment granulometry). According to Solan and Wigham (2005) there are 7 types of fauna bioturbation, although here only 2 will be described in detailed:

Epifauna bioturbators are organisms whose activities occur predominantly at the top sediment surface, although they have a negligible contribution to particle transport other than redistribute fine particles randomly over very short distances along the surface. These include non-burrowing crabs (or crab's non burrowing activities) as well as gastropods (such as *Terebralia palustris*).

Regenerators are organisms that excavate holes, transferring sediment at depth to the surface where it is removed by the overlying water currents. The excavated sediment is replaced by surficial sediments from both of the infilling of surface sediment and the collapse of burrow walls. Within this group we can allocate fiddler crabs (McCraith et al., 2003).

Displacement of sediment at the surface or from deep layers by epibenthos and burrowing fauna, respectively, will expose new surfaces to oxygen, increasing old and refractory organic matter degradation by efficient aerobic bacteria. At the same time, labile organic carbon is buried into anoxic layers full of starved anaerobic bacteria, that will degrade this fresh substrate easily and rapidly (Kristensen et al., 1995; Kristensen and Holmer, 2001; Kristensen and Kostka, 2005). Epifauna bioturbators may also stimulate benthic bacterial biomass and activity through faecal pellet production and mucus secretion (Solan and Wigham, 2005). Furthermore, the continuous mixing of the top layer by these organisms, will enhance carbon mineralization and nutrient cycling by improving sediment aeration and drainage (Aller, 1994), as well as by increasing surface areas for microbial activity (Solan and Wigham, 2005).

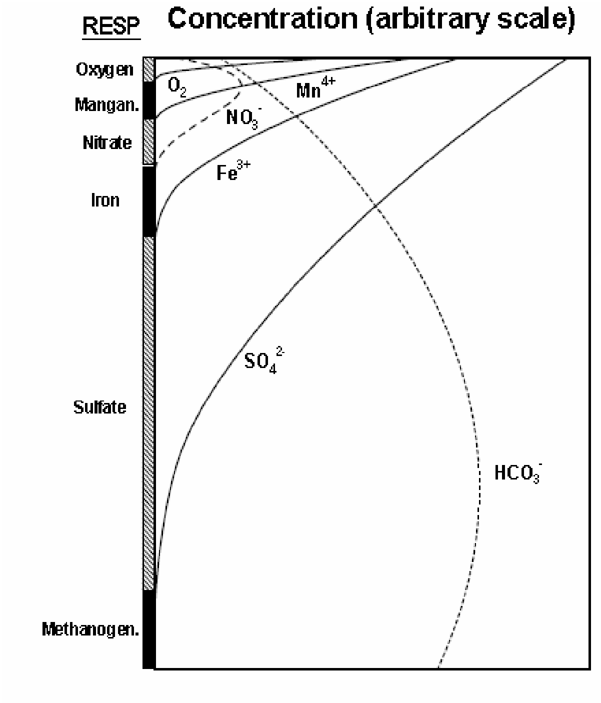
Also, by limiting microalgal biomass indirectly by subduction below the sediment surface (Carlén and Ólafsson, 2002; Pape et al., 2008), bioturbators suppress the development of dense algal mats, and consequently avoid the development of near surface anoxic zones (Kristensen and Alongi, 2006; Marsden and Bressington, 2009). Microphytobenthic primary production can potentially be enhanced when the biomass is grazed below the carrying capacity by deposit-feeding invertebrates (Blanchard et al., 2001). Consequently, faunal bioturbation may increase nutrient cycling and sediment capacity to degrade organic matter.

Although many studies have measured bioturbation activities in deep sea and coastal areas (e.g., Moodley et al., 1998; Kristensen et al., 1999; Stora et al., 1999), temperate estuaries or lagoons (e.g., Rysgaard et al., 1995; Petersen et al., 1998; Mazik and Elliott, 2000; Gerino et al., 2007; Cardoso et al., 2008), saltmarshes (e.g., Gribsholt et al., 2003; Gribsholt and Kristensen, 2003; Botto et al., 2006), rare are the ones done in mangroves (Kristensen et al., 1991; Lee, 1998; Nielsen et al., 2003; Amouroux and Tavares, 2005; Ferreira et al., 2007), especially in developing countries such as in East Africa.

Organic matter degradation and microbial pathways

The actual rates of decay of organic matter depend primarily on its quality, age, and ambient temperature (Kristensen, 2000; Canfield et al., 2005). Both the sedimentation rate and the remineralisation rate are interdependent processes closely coupled with the microbial community, and are affected (and affect) by the sediment properties and environmental conditions (Aller et al., 1996; Burdige, 2006). Oxygen availability is a very important parameter that affects the distribution of the processes occurring in the sediment, due to its role in microbial respiration as the most energy delivering electron acceptor (Canfield et al., 2005; Glud, 2008). In the sediment surface layer, availability of oxygen is high, due to the high diffusion rates between top sediment and water or air. Also in this top layer, the abundance of both nutrients and sun light (restricted photic zone of the sediment: 1-3cm) allows microalgae to contribute around 20-35% of the total primary production in soft-bottom areas (Kristensen et al., 2005). On the other hand, oxygen produced in the top layer is utilised to reoxidize reduced compounds (e.g., H_2S and Fe^{2+}) that are brought from deeper layers by diffusion or mixing, which, in turn, restricts oxygen availability to benthic respiration (Kristensen, 2000).

As the oxic zone in coastal sediment is usually limited to the upper centimetre (Canfield et al., 2005), a large fraction of the organic matter will be buried (more or less undecomposed) into anoxic layers. Here organic matter will start being degraded by hydrolysing and fermenting anaerobic bacteria into smaller compounds easier to degrade by respiratory bacteria. In this anoxic zone, microorganisms use different electron acceptors than oxygen (NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-}) following an order that depends on the Gibbs free energy, availability and efficiency of the electron acceptors, quality of organic matter, sediment characteristics, environmental conditions and bioturbation (Canfield et al., 2005). Although sulphate reduction is one of the least favourable respiration processes, the high concentration of SO_4^{2-} in seawater (300-1000 times higher than O_2) is responsible for its deep vertical distribution and thus quantitative importance. Moreover, in organic-rich sediments sulphate reduction represents the main respiratory pathway of organic matter mineralization (Holmer and Kristensen, 1994b; Holmer et al., 2001). Although some of these can occur as pure chemical processes, most of the reactions in the sediment are mediated by microorganisms. Due to electron donor stratification, different predominance of microbial groups is observed throughout the sediment column (Canfield et al., 2005) (Figure 3).



Organisms

Hydrogen bacteria
Mn reducers
Denitrifyers
Fe reducers

Sulfate reducers

Acetogens

Methanogens

Figure 3 - Scheme of the diagenetic processes occurring throughout sediment depth with the distribution of organism (on the right) based on different respiratory mechanisms (on the left). Adapted from Canfield et al. (2005).

The sediment biogeochemistry and microorganism diversity, dynamics and processes vary not only with environmental conditions but also according to the level of anthropogenic pressure on these coastal shallow-water regions. Increased anthropogenic loading of nutrients into the coastal zone is now a worldwide problem and the expected changes would include the extension of the anoxic layer to the surface, as well as subsequent shift in carbon mineralization decomposition pathways (Kristensen, 2000; Valdemarsen et al., in press-a).

A continuous loading of organic rich wastewater at a level above the system capacity usually leads to severely reduced O_2 penetration (Gray et al., 2002) and OM accumulation in sediments (Holmer and Kristensen, a; b; Valdemarsen et al., in press-a) which may induce negative effects on sediment associated flora and fauna due to stimulated SR and toxic sulfide accumulation (Hargrave et al., 2008). However, benthic dwelling invertebrates as well as flora are known to create important physical and biogeochemical modifications in sediment ecosystems (Kristensen et al., 2005). On a global scale, faunal ecosystem engineering affects the top 15cm of sediment layer (Boudreau, 1998), while flora roots are known to affect sediment dynamic much deeper in the sediment (Alongi, 2005). The burrowing, feeding, irrigation, construction and locomotory activities of benthic invertebrates and root development and functioning have significant implications for the physicochemical environment, and consequently to microorganism diversity, biomass and activity (Kristensen, 2000; Alongi, 2005; Canfield et al., 2005; Kristensen et al., 2005). By creating a mosaic of oxic/anoxic boundary layers throughout the sediment they influence significantly remineralisation pathways and rates counteracting the negative effects of organic matter loading. This ecosystem engineer service provided by infauna was already observed in organic-enriched sediments (Hansen and Kristensen, 1998; Heilskov and Holmer, 2001; Nickell et al., 2003; Nielsen et al., 2003; Heilskov et al., 2006; Kinoshita et al., 2008; McHenga and Tsuchiya, 2008; Lindqvist et al., 2009; Valdemarsen et al., in press-b).

Flora and fauna are known to simultaneously stimulate sulphate reduction through root exudates and transport of labile detritus to subsurface layer during bioturbation activities (Alongi, 1998; Kristensen, 2000; Kristensen and Alongi, 2006; Ferreira et al., 2007). Also, they increase the importance of aerobic and suboxic pathways on carbon mineralization, as iron reduction, by increasing the redox potential (Kristensen et al., 2000; Nielsen et al., 2003; Kristensen and Alongi, 2006), and denitrification by expanding the oxic/anoxic layer where coupled nitrification-denitrification can occur (Hansen and Kristensen, 1998; Heilskov and Holmer, 2001; Purvaja et al., 2004). This way, highly contaminated mangrove ecosystems might be important emission sites for greenhouse gases (Alongi, 2009). Denitrification (Barnard et al., 2005) and methanogenesis (Knowles, 2005) produce nitrous oxide, a known greenhouse gas that is 200 to 300 times more powerful than CO_2 and very high rates (>150 $\mu mol\ m^{-2}\ d^{-1}$) were already found in nutrient enriched mangroves (Corredor et al., 1999; Munoz-Hincapié et al., 2002). Methane, produced during methanogenesis, is also a greenhouse gas but less strong than N_2O.

Aims and importance of this study

This study aimed at evaluating the impacts of domestic sewage contamination on key mangrove species or communities as well as to better understand their role in ecosystem functioning, with particular focus on sediment biogeochemistry.

Most of biomonitors, but specially bioindicators, have been identified in developed regions of the world and are almost inexistent for developing countries, especially when considering organic matter and nutrient contamination (Peters, 1997). It is thus urgent to develop easy but efficient tools and biological indices to monitor and manage near shore marine environments in developing countries such as East African countries. In order to achieve this goal, in one or more countries was chosen one contaminated mangrove surrounding a highly populated city with two pristine mangrove ecosystems. Also, in Dar es Salaam, Tanzania, a mesocosms facility was built in order to assess, in controlled conditions, fauna endurance when subjected daily to severe sewage concentrations. Community diversity, abundance and distribution, and population structure, survival and growth rates, behaviour, as well as feeding and reproductive activities and physiological condition of key species were examined and related to habitat quality.

Ecosystem engineering of mangrove key species is limited and focussed on few species (sesarmid crabs and gastropods) and activities, such as leaf consumption (Fratini et al., 2004; Cannicci et al., 2008; Lee, 2008). Although many studies have assessed fiddler crabs influence on sediment biogeochemistry in other ecosystems, such as saltmarshes (Gribsholt et al., 2003; Gribsholt and Kristensen, 2003; Kristensen, 2008), very little is known on their role in mangrove forest. *Terebralia palustris* is one of the most important species regulating litter export from mangrove forest (Fratini et al., 2004; Cannicci et al., 2008), however, its role as a sediment bioturbator has not been seriously assessed. In this study, fiddler crab and gastropods ecosystem engineering will be assessed in situ and controlled conditions and at different sewage and vegetation conditions.

Also, very few studies have measured carbon mineralization, microbial pathways and/or greenhouse gas emission in constructed wetlands (von Sperling, 1996; Kivaisi, 2001; Yang et al., 2008; Maltais-Landry et al., 2009), and especially with focus on the abundant biogenic structures commonly present in mangrove natural systems (pneumatophores and fauna burrows). In this study, all these processes were evaluated to better understand the functioning of mangrove wetlands and their potential as constructed wetlands.

Benthic invertebrate populations are an essential component of estuarine and coastal ecosystems, especially in mangrove forests, by actively maintaining healthy sediment conditions, and consequently the entire ecosystem, as well as by supporting higher trophic levels (such as economically important fisheries). The generalized clearance and deterioration of nearshore ecosystems are jeopardizing the ecological persistence of such populations, putting at risk the stability and functioning of ecosystems and that of local communities (Costanza et al. 1997, Walther et al. 2002, Hogarth 2007). Together with the fact that mangroves have been recognized as filters for a wide range of contaminants (Wong et al., 1995; Wong et al., 1997; Yang et al., 2007; Yang et al., 2008), it becomes urgent the need for effective conservation, protection, restoration and sustainable managing programmes for such ecosystems and resources (Duke et al., 2007; Rönback et al., 2007; Nagelkerken et al., 2008).

This study intends to provide reliable information on the ecosystem health and its potential as sewage filtering system so intelligent, responsible and informed policies, strategies and actions can be made in order to identify in an early stage potential deleterious contamination of mangrove forests, as well as promote the conservation of natural forests and creation of constructed wetlands to decrease anthropogenic contamination of natural ecosystems.

Structure of the dissertation

In order to accomplish the objectives of this study, nine specific investigations were conducted and each resulted in a scientific manuscript (paper). This dissertation incorporates these papers, each constituting a full chapter (Section II), being either in press (5), or under review (4) in peer refereed international scientific journals.

Benthic infaunal community studies are a useful tool in terms of determining whether or not chemical contamination is resulting in impacts to resident populations. In chapter 1 of this study, possible differences in infauna structure, using some basic (easy and inexpensive) metrics, between peri-urban mangroves impacted by sewage dumping and non-urban sites (where evident wastewater dumping was not present) along East Africa, was assessed.

For the first time reproductive parameters of mangrove species was also assessed as proxy of organic matter and nutrient contamination. In chapter 2 the reproduction potential was measured in terms of fecundity, fertility and embryo quality (measured through fatty acid analysis) of the fiddler crab *Uca annulipes* (H. Milne Edwards, 1837), one of the most common crabs in mangrove forests sediments, between a contaminated peri-urban and two pristine mangrove creeks in southern Mozambique. Due to the fact that most contaminants are discharged to mangrove creeks, a multidisciplinary study a multi-disciplinary study was done in the same sites as the previous study to a common and local shrimp population, *Palaemon concinnus* (Dana, 1852), known to inhabit mangrove creeks. In chapter 3, population structure, reproductive parameters (such as maturation curves, fecundity, potential fertility and embryo quality), parasite infection, and RNA/DNA ratio were used as proxies to test for habitat quality.

In chapter 4, the effect of sewage and different vegetation conditions on fiddler crabs survival and ecosystem engineering was assessed in constructed mangrove wetlands (CMW). The behaviour activities of these crabs were also used as a potential proxy of sewage contamination (chapter 5). The effect of sewage concentration and presence of vegetation on *Terebralia palustris* survival, growth and behaviour was assessed in chapter 6. In the same chapter, the role of these gastropods on sediment disturbance was calculated and its biogeochemical consequences discussed. Knowing that *Terebralia palustris* is able to feed on a wide variety of food sources, in chapter 7, the distribution, feeding behaviour and strategies of this gastropod was assessed in the field in order to better understand their potential role in mangrove constructed wetlands.

In chapter 8, we have aimed to validate the effects of anthropogenic sewage on the stable isotopes of different CMW compartments (sediment, microphytobenthos, mangrove trees and fauna species) as indicators of sewage exposure. We also have used stable isotopes to examine potential diet shift of two key macrofauna species (*Uca annulipes* and *Terebralia palustris*) under different domestic sewage loadings and vegetation conditions, in order to understand the importance of these macrofauna species on CMW functioning.

In chapter 9, we have focused on carbon gas (i.e. CO_2 and CH_4) emissions under immersion (sediment-water) and emersion (sediment-air) periods, with special emphasis on the importance of biogenic structures (pneumatophores and crab burrows), as well on the most important carbon oxidation pathways used. Both these processes are important to fully comprehend the effects of organic discharge on mangrove forests and to provide basic knowledge for future development of sustainable wastewater wetlands. We provide rough net budgets of carbon gas emission in constructed mangrove wetlands under different sewage and vegetation treatments and discuss the long-term implications for the ecosystem functioning and health.

A general discussion considering all the investigations conducted, the main conclusions drawn from this study and the contributions made to increase the knowledge of mangrove fauna bioindicators and biomonitors as well as their ecological functioning in contaminated and pristine natural and artificial system are the contents of Section III, together with the issues that have arose and on which further research is necessary.

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SECTION II

Chapter 1

Effects of urban wastewater loading on macro and meioinfauna assemblages in equatorial and subtropical mangroves of East Africa

Gil Penha-Lopes[1, 2*], Susana Xavier[1], Julius Okondo[3], Stefano Cannicci[4], Esther Fondo[3], Susana Ferreira[5], Célia Macamo[5], Adriano Macia[5], Steven Mwangi[3] and José Paula[1]

[1] Centro de Oceanografia - Laboratório Marítimo da Guia,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211
[2] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[3] Kenya Marine and Fisheries Research Institute, P.O. Box 81651, Mombasa, Kenya
[4] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy
[5] Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C.P. 257 Maputo, Moçambique

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[1] Centro de Oceanografia - Laboratório Marítimo da Guia,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211
[2] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[3] Kenya Marine and Fisheries Research Institute, P.O. Box 81651, Mombasa, Kenya
[4] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy
[5] Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C.P. 257 Maputo, Moçambique

Abstract

East African peri-urban coastal areas are one of the destinations of untreated sewage. To consider an environment polluted we need to correlate the levels of contamination with the ecosystem health and biological components provide an integrated measure of pollution. Benthic infaunal community studies are a useful tool in terms of determining whether or not chemical contamination is resulting in impacts to resident populations. An ACI (After Control/Impact) unbalanced design was followed, comparing the community structure of macro and meio-infauna recorded in one contaminated mangrove swamp with those characteristic of two nearby relative pristine mangroves of similar ecological traits in Kenya and Mozambique. At macroinfauna communities, Oligochaeta density decreased significantly in contaminated mangroves of both countries. While crustaceans increased and molluscs decreased at Mozambiquean contaminated mangrove, at Kenya a clear decrease of polychaetes was also observed. Meiofauna responded in opposite ways, increasing at the Mozambiquean peri-urban mangrove and decreasing at Kenya one. Diversity indexes calculated using meiofauna major groups did present lower number of taxa in contaminated sites. The differences observed between countries may have to do with the fact that Costa do Sol and Mikindani mangrove systems seemed to be completely different systems, plus the

fact that Mikindani is subjected to other contaminants (e.g., heavy metals) other than sewage-derived nutrients and detritus.

Keywords: infauna, mangrove, ecological indication, pollution, East Africa

1. INTRODUCTION

To consider an environment polluted, the levels of contamination need to be correlated with the ecosystem health (Chapman, 2007a). Biomonitoring and bioindication are promising methods of observing the impact of external factors on ecosystems and their development over a long period, or differentiating between a polluted from a non polluted site (Underwood, 1992a; 1994a). Both macro and meiofauna are good biologic candidates to assess the quality of a aquatic ecosystem and both communities exhibit repeatable patterns of response to environmental stressors (Chapman, 2007b).

Meiofauna has the advantage of being close associated with the sediment matrix, thus changes in interstitial chemistry quickly lead to changes in meiofaunal abundance and diversity (Kennedy and Jacoby, 1999a). Short generation times and asynchronous reproduction of the majority of meiofaunal species have the advantage that all stages in the life cycle are exposed to the pollutant, which results in a short time response by the community to a pollution event (Coull and Chandler, 1992). Meiofauna may also persist where macrofauna is absence and in such cases, the characteristic composition of the residual assemblage may help with diagnosing pollutant types (Kennedy and Jacoby, 1999b). Macrofauna, on the other hand, has longer life expectancy allowing it to integrate environmental conditions over longer periods of time, as well as accumulates contaminants that are transferred to higher levels of the food web (Markert et al., 2004).

Both communities were already used successfully to assess anthropogenic contamination *in situ* at rivers basin areas (Beier and Traunspurger, 2001; Chambers et al., 2006; Heininger et al., 2007; Saunders et al., 2007), intertidal zones (Yu et al., 1997; Frouin, 2000; Rossi and Underwood, 2002; Solis-Weiss et al., 2004; Bigot et al., 2006; Sutherland et al., 2007; Wear and Tanner, 2007), ocean floor (La Rosa et al., 2001), as well as in mesocosms subjected to specific contaminants (Austen et al., 1994; Austen and McEvoy, 1997; Gyedu-Ababio and Baird, 2006), although most studies have focussed on heavy metals. Sewage effluents can affect the receiving benthos in a variety of ways by changing salinity regimes and introducing contaminants (e.g., such as metals, pesticides and hydrocarbons), and increasing detritus and nutrient availability, and consequently more frequent eutrophication and anoxic events, affecting severely the local community structure and dynamic (Pearson and Rosenberg, 1978; Wu, 2002; Diaz and Rosenberg, 2008).

East African peri-urban coastal areas receive extensive amounts of untreated sewage, which is typically discharged into creeks usually lined by mangrove areas (Mohammed, 2002; Kruitwagen et al., 2008). These forests accomplish a number of ecosystem functions and services (Duke et al., 2007), including their great potential as natural wastewater treatment areas (Wong et al., 1995; 1997). Preliminary results from field trials or at peri-urban mangrove systems (compared with pristine areas) have shown that sewage loading did not produce any harmful effect on the higher plant communities (Wong et al., 1997) while its role on the benthic macrofauna community structure is insignificant (Yu et al., 1997) or severe affect faunal diversity and/or biomass (Machiwa and Hallberg, 1995; Cannicci et al., in press). Nevertheless, the influence of anthropogenic sewage in mangrove associated meiofauna is inexistent. More importantly, it is known that changes in community structure and dynamic, as well as behaviour, can lead to a significant decrease in the bioturbation potential and consequently a loss of ecosystem functioning (Mazik and Elliott, 2000; Coleman and Williams, 2002; Solan et al., 2004; Bartolini et al., accepted; Penha-Lopes et al., accepted-a; Penha-Lopes et al., accepted-b).

In order to save time and resources, the effect of environmental or anthropogenic disturbance on meio and macrofauna communities have been effectively detected with multivariate analysis at high taxonomic levels, from family to phylum (Thorne and Williams, 1997; Chapman, 1998; Kennedy and Jacoby, 1999b; Savage et al., 2001; Gesteira et al., 2003). This is even more important in developing countries where limited taxonomic expertise or resources are available, and therefore emphasis should be placed on cost-effective techniques such as taxonomic minimalism (Thorne and Williams, 1997), which is also currently the case for mangrove meiofauna studies (Nagelkerken et al., 2008).

The present study was integrated in PUMPSEA Project (funded by European Commission: FP6, INCO-CT2004-510863), which global aim was to demonstrate the ecological and economical ecosystem service performed by mangroves as filtering discharged wastewater, and thereby limiting coastal sewage pollution, and to positively improve coastal zone management within East Africa. The present study aimed at investigating the possible differences in infauna structure between peri-urban mangroves impacted by sewage dumping and non-urban sites where evident wastewater dumping was not present, along East Africa, using some basic (easy and inexpensive) metrics to be used in developing countries. These studies are urgently needed to develop easy but efficient tools and biological indices to monitor and manage near shore marine environments.

2. MATERIALS AND METHODS

2.1. Study area and sampling design

Appropriately replicated Before/After sampling designs are perhaps the most reliable methods for detecting an environmental impact (Underwood, 1992b; 1994b). However, in the absence of such data, with appropriate spatial and temporal replication it has been possible to examine differences between potentially disturbed and control locations after the ‘‘disturbance’’ (Chapman et al., 1995). For these reasons, we followed a ACI (After Control/Impact) unbalanced design, comparing the ecological features recorded in one contaminated mangrove swamp with those characteristic of two nearby pristine mangroves of similar ecological traits, in two countries, Kenya and Mozambique.

Mangrove areas and sampling design were extensively described in Cannicci et al (in press), however a brief description will be done here. Three sampling sites were chosen at the southern Kenya coast (Figure 1), Mikindani (impacted site), Gazi Bay and Shirazi creek (pristine sites). The Mikindani mangrove system is located within the Tudor creek, which surrounds the city of Mombasa. This mangrove has been exposed to sewage for more than a decade, primarily affected by the sewage from Mikindani residential estate and also highly affected by part of Mombasa city sewage (Mohammed, 2008). The sewage runs through the mangrove forest in canals and is discharged directly into the nearby mangrove forest at Mikindani in Tudor creek. The mangroves are periodically dozed with sewage every tidal cycle the loading exponentially reduces with distance from source (PUMPSEA, 2008).

At Mozambique, the sampling was conducted in three different mangrove ecosystems, a peri-urban mangrove, Costa do Sol, and two pristine mangroves, Saco and Ponta Rasa, at Inhaca Island (Figure 1). Costa do Sol mangrove is located at Maputo Bay, ~7 Km north of Maputo city. The seasonal river Quinhenganine discharges in the swamp after passing through the city. The mangrove is bordered by a residential area, and has been receiving domestic sewage, aquaculture residuals and solid dumps of various sources during the last decades (PUMPSEA, 2008). Costa do Sol presents higher concentrations of nutrients, especially nitrites and nitrates in relation to Saco and Ponta Rasa mangroves (PUMPSEA, 2008).

At each site, a stratified random sampling approach was used, since the chosen mangroves showed a clear zonation pattern, with a division in obvious vegetation belts, which are known to be characterised by different ecological features and, thus, colonised by different faunal assemblages (Macnae, 1968; Hogarth, 2007). The considered belts were: a landward sandy belt dominated by *Avicennia marina* (Forssk.) Vierh. trees, representing the zone flooded at spring tides only (from now on called the *Avicennia* belt); and the seaward muddy belt dominated by *Rhizophora mucronata* Lam. trees, flooded twice a day by high tides (from now on called the *Rhizophora* belt). At Costa do Sol site (southern Mozambique) only a wide *A. marina* dominated area is present and we concentrated our efforts on this belt alone, comparing it with the corresponding landward *Avicennia* belts of the Mozambican control sites.

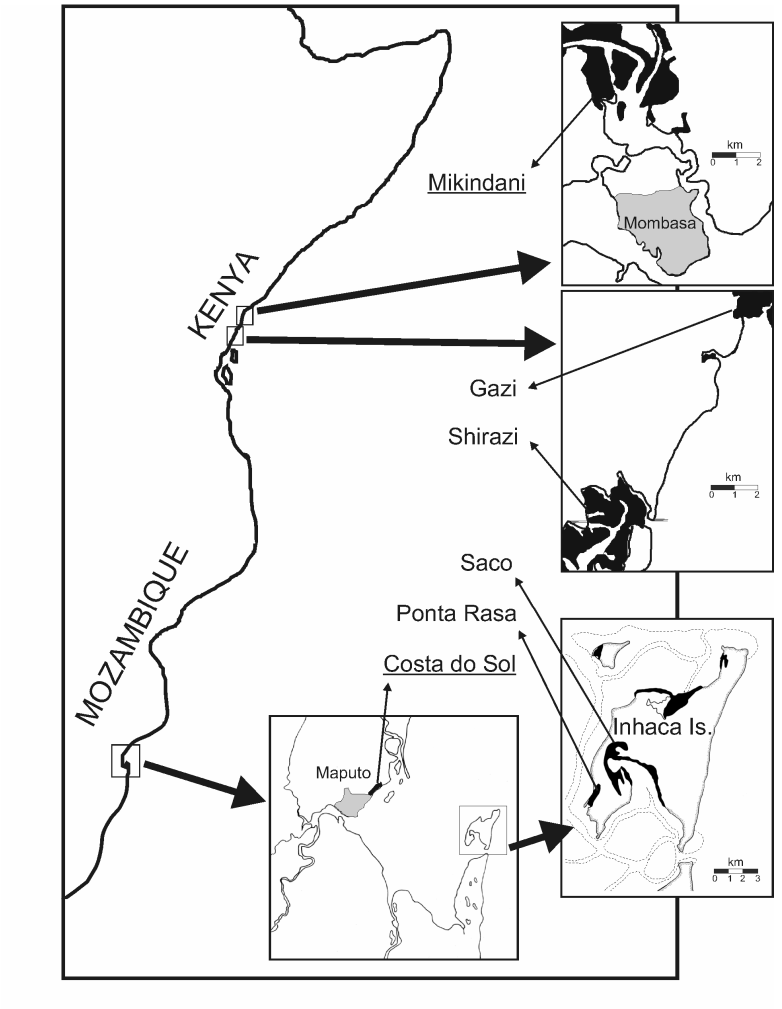

Figure 1 – Map of the sampling sites in Kenya and Mozambique, at East Africa coast.

2.2. Sampling

At each sampling site, two *A. marina* and two *R. mucronata* study areas were selected (50 m apart). In each area, two sub-areas (1 m of diameter) were delimited and separated more than 5 m apart. In each sub-area three replicates for meiofauna (corer 5 cm Θ and 20 cm depth) and another three for macrofauna (corer 15 cm Θ and 20 cm depth) were taken and preserved in 10% formalin. Two temporal replicates were performed: a dry season sampling campaign occurred during the months of July and August 2005 at the Mozambican locations and in October 2005 at the Kenyan locations. A second campaign took place at wet season, in April 2006 in Kenya and in February 2006 in Mozambique. Meiobenthos was extracted from sediment and retained on a 63µm sieve after washing the sample with tap water through a 500 µm sieve to remove coarse root material and macrobenthos. The material retained on the fine sieve was thoroughly mixed with Ludox HS40® (density 1.15 g.cm^{-3}) The supernatant was washed on the 63µm sieve with tap water (Burgess, 2001) and the material retained was preserved in 4% formalin, to which a small amount of Rose Bengal stain was added to aid counting. Macro and meiobenthos were counted and identified to the levels of phylum, order or class.

2.3. Data analysis

The data were analysed using univariate and non-parametric multivariate techniques contained in the PRIMER (Plymouth Routines In Multivariate Ecological Research) package (Clarke and Gorley, 2006). For meiofauna groups, generic diversity was assessed by using the Shannon-Wiener diversity index (H'), Pielou's evenness index (R') Margalef's species richness index (d). The nematode to copepod ratio was also calculated by dividing the number of nematodes in a sample by the number of copepods. These indices were handled statically by a two-way ANOVA. For multivariate representation of community structure a non-metric multi-dimensional scaling (NMDS) was used, based on lower triangular similarity matrices constructed using the Bray-Curtis similarity measure on square root for macrofauna and fourth root for meiofauna transformed data (Clarke and Green, 1988; Clarke, 1993). For macrofauna, a 4-way Anova was used for each of the major groups to statiscally test differences between sites. While for meiofauna distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001) was employed to test the null hypotheses of no differences among faunal assemblages on sites across sampling time (Anderson and Ter Braak, 2003). A SNK test (Post-hoc test) was used in conjunction with ANOVA to determine which specific group pair(s) were statiscally different from each other when significant differences between samples were observed. Species contributing to dissimilatiries between sites were investigated using similarities percentages procedure (SIMPER) (Clarke, 1993).

3. RESULTS

3.1. Macrofauna

Four major macrofauna groups where recorded: Polychaeta, Oligochaeta, Mollusca and Crustacea. At *Avicennia* belt in both countries, macrofauna densities was similar between contaminated and pristine mangroves (Table 1 - A), except for polychaets, which presented a very low abundance at Mikindani, and crustaceans that were completely absent from Saco mangrove and Costa do Sol during the wet season (Figure 2). Oligochaets and molluscs assemblages were highly variable at small spatial scales (area).

At Kenyan contaminated mangrove, Mikindani, a clear decrease in abundance of oligochaetes at both tree belts and polychaets from *Avicennia* belt was observed when compared with pristine mangroves (Figure 2, Table 1 - B and SNK tests). MDS plots on Kenya data shows that Mikindani can be easily separated from pristine locations at both belts and seasons (although more strongly at the wet season), while both pristine locations are barely separable (Figure 3). SIMPER analysis between sites corroborates the previous results, since Oligochaeta was the group that presented higher contribution to differences between sites for both belts (dissimilarity 36-45 %), thus better explains the separation between the peri-urban mangroves from pristine ones.

At Mozambique, a clear decrease of molluscs and oligochaets at Costa do Sol was observed and while most fauna groups decreased during the wet season, polychaets increased their density (Figure 2, Table 1 - C and SNK tests). The MDS plots separated well all three sampling sites (Figure 3). SIMPER analysis showed that Mollusca and Crustacea (both making nearly 66%) are the groups responsible to separate Costa do Sol mangrove (peri-urban) from Saco (dissimilarity 81%), while Mollusca (49%) is the group that more contributed for the dissimilarities (39%) between Costa do Sol and Ponta Rasa mangroves.

3.2. Meiofauna

Ten major taxa were recorded: Copepoda, Foraminifera, Halacaroidea, Insecta, Mollusca, Nematoda, Oligochaeta, Polychaeta, Tardigrada and Turbellaria. Nematodes were the dominant group (43-98%) in all samples with copepods usually the second most abundant taxon. Halacaroidea and Turbellaria groups were also quite abundant in many samples (Figure 4). At *Avicennia* belt in both countries, significant interactions were found between seasons and sites (Table 2 - A), with a general significant decrease of the most abundant meiofauna groups at Kenya contaminated mangrove while the opposite was observed at Mozambique (Figure 4).

In Kenya significant interactions were found between Season x Site and Belt x Site (Table 2 - B). At non-urban mangroves, while nematods were more abundant at *Rhizophora* belt, Halacaroidea and Turbellaria individuals decreased significantly (Figure 4) when compared with *Avicennia* belt (SNK tests).

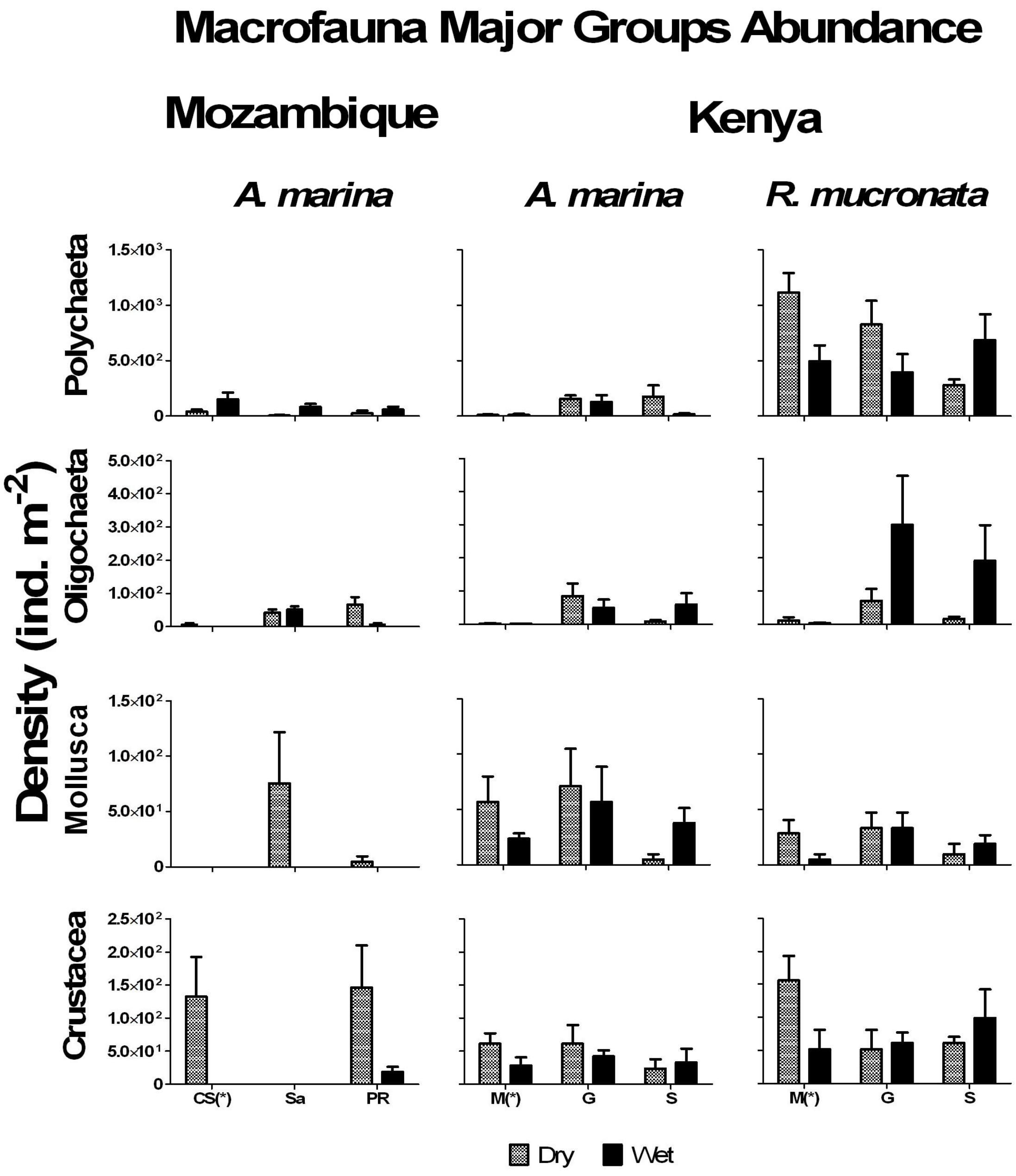

Figure 2 – Average (±SE) of the macrofauna major groups identified in contaminated and pristine mangrove sites in Kenya and Mozambique at dry and wet seasons per square meter.

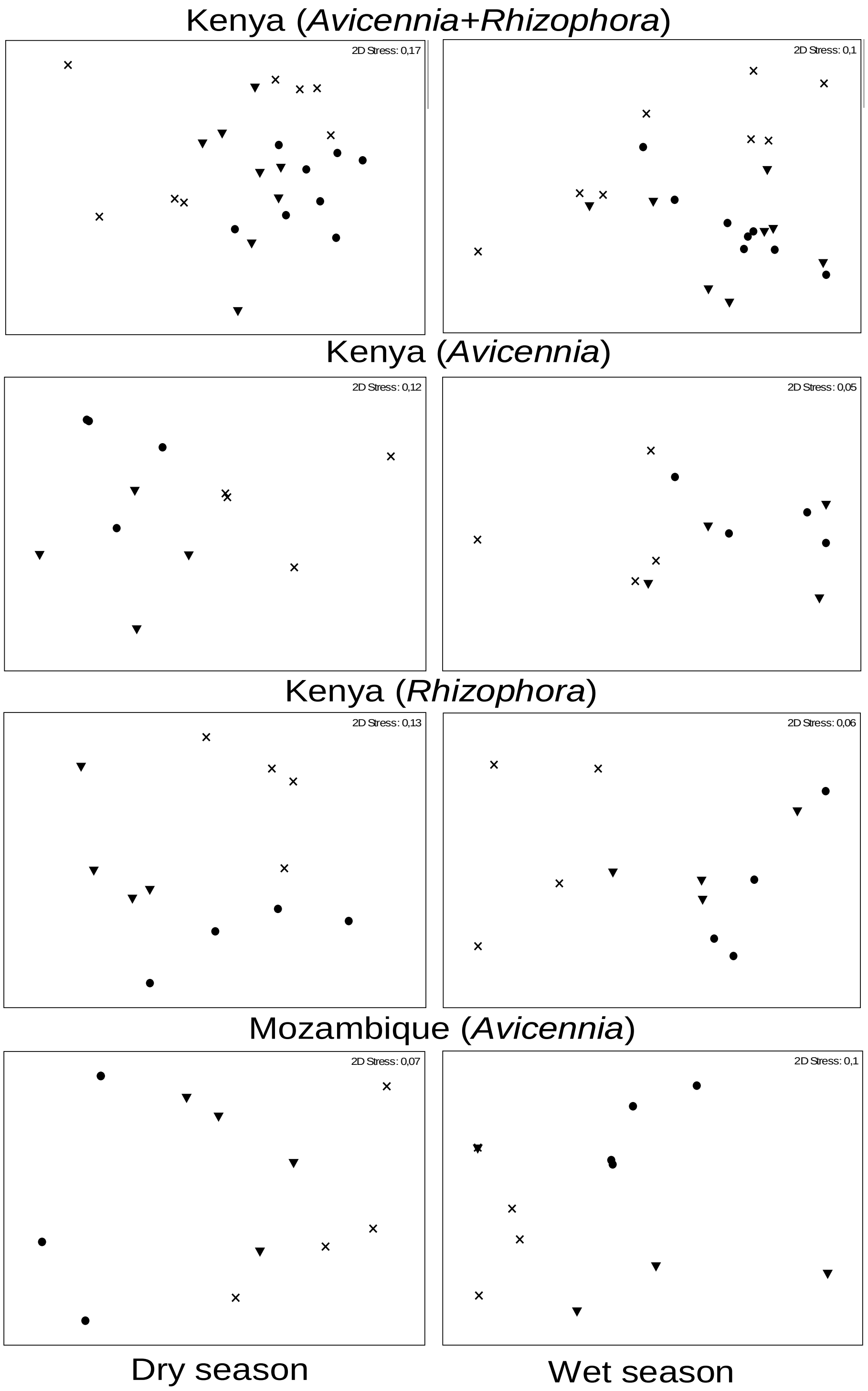

Figure 3 - Non-metric multidimensional scaling (NMDS) ordination of root transformed macro-infaunal abundance data from three sampling sites, in both *Avicennia* and *Rhizophora* belts, in Kenya ((x) Mikindani (●) Gazi (▼) Shirazi) and Mozambique ((x) Costa do Sol (●) Saco (▼) Ponta Rasa).

Table 1 - Results of the four 4-way ANOVA on macro-infauna major groups density data, log (x+1) transformed, estimated for the three sampling sites: A - in both countries (*Avicennia* belt only); B - Kenya (*Avicennia* and *Rhizophora* belt) and C - Mozambique (*Avicennia* belt). Factors: season (fixed and orthogonal), country (fixed and orthogonal), site (random and orthogonal) and area (random and nested in site). The degrees of freedom, DF, Variance, MS, and value of F ratio are showed for each of the four tests.

		Crustacea		**Polychaeta**		**Oligochaeta**		**Mollusca**	
	df	**MS**	**F**	**MS**	**F**	**MS**	**F**	**MS**	**F**
A - Kenya and Mozambique									
Season	1	0,62	4,68	0,13	0,48	0,04	0,04	0,16	0,65
Country	1	0,10	0,17	0,12	0,10	9,84	2,65	1,49	5,56
Site	4	0,58	**157,81**[b]	1,17	**9,25**[b]	3,71	3,18	0,27	1,09
Area	6	0,00	0,02	0,13	0,61	1,17	**4,52**[b]	0,25	**3,38**[a]
Season x Country	1	0,78	2,26	3,38	**12,07**[a]	0,29	0,30	0,17	0,69
Season x Site	4	0,35	**14,07**[b]	0,28	0,84	0,98	3,52	0,25	1,74
Season x Area	6	0,02	0,12	0,33	1,59	0,28	1,08	0,15	1,99
Residual	24	0,21		0,21		0,26		0,07	
Total	47								
B - Kenya									
Season	1	0,17	0,34	1,60	8,61	3,59	1,17	0,01	0,02
Belt	1	1,03	4,74	33,03	17,20	5,09	12,92	0,44	11,16
Site	2	0,09	1,00	0,59	1,36	18,28	**27,53**[a]	0,37	4,21
Area	3	0,09	0,49	0,44	2,24	0,66	1,06	0,09	0,96
Season x Belt	1	0,00	0,01	0,04	0,02	2,39	1,96	0,01	0,22
Season x Site	2	0,52	6,27	0,19	0,39	3,06	2,03	0,32	4,05
Season x Area	3	0,08	0,44	0,48	2,44	1,51	2,40	0,08	0,87
Belt x Site	2	0,22	2,15	1,92	**12,06**[a]	0,39	0,20	0,04	0,23
Belt x Area	3	0,10	0,54	0,16	0,82	2,00	**3,18**[a]	0,17	1,86
Season x Belt x Site	2	0,12	5,75	1,82	7,15	1,22	7,76	0,04	0,34
Season x Belt x Area	3	0,02	0,11	0,25	1,30	0,16	0,25	0,12	1,28
Residual	24	0,19		0,20		0,63		0,09	
Total	47								
C - Mozambique									
Season	1	0,34	1,37	2,43	**27,48**[a]	2,33	3,84	0,27	0,86
Site	2	0,25	0,89	0,31	4,09	1,02	**274,01**[b]	0,64	**16,94**[a]
Area	3	0,28	**30,56**[b]	0,08	0,36	0,00	0,02	0,04	1,04
Season x Site	2	0,25	0,89	0,09	0,26	0,61	**26,73**[a]	0,31	**10,97**[a]
Season x Area	3	0,28	**30,56**[b]	0,34	1,61	0,02	0,09	0,03	0,78
Residual	12	0,01		0,21		0,24		0,04	
Total	23								

(a = $p < 0.05$; b = $p < 0.01$)

Both these meiofauna groups were also almost absence in the peri-urban mangrove and at significantly lower densities when compared with pristine mangroves (SKN tests).

While MDS plots (Figure 5) at Kenya, merging both *Avicennia* and *Rhizophora* belts, show no significant differences between assemblages, data at *Avicennia* belt shows Mikindani separated, although with some overlapping, from both pristine mangroves, and mainly at wet season. At the *Rhizophora* belt, the MDS plot shows all mangroves more or less overlapping at the wet season but very well separated at the dry season (Figure 5).

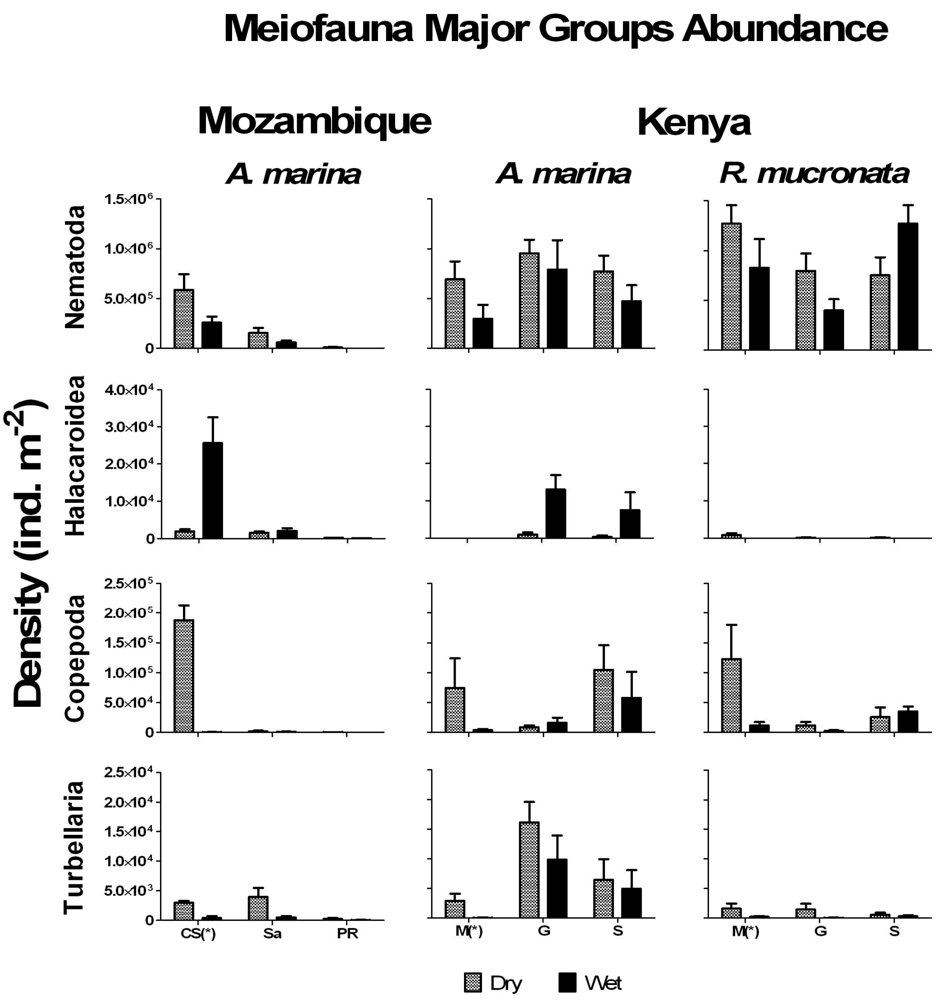

Figure 4 – Average (±SE) of the most abundant meiofauna major groups identified in contaminated and pristine mangrove sites in Kenya and Mozambique at dry and wet seasons per square meter.

At Mozambique, MDS plot for the *Avicennia* belt showed that all three mangroves are clearly separated (Figure 5). PERMANOVA results support the MDS results, since significant differences were obtained between Sites (Table 2). SNK post-hoc test showed significant differences between all three mangrove communities assemblages. Meiofaunal composition differences between Saco and Ponta Rasa (dissimilarity = 68%) were mainly due the contribution of nematodes and foraminifers. Nematodes and halacarids were responsible for nearly 50% of the dissimilarity between Saco and Ponta Rasa and Costa do Sol (dissimilarity

= 76%) were. Considering differences between Saco e Costa do Sol (dissimilarity = 38%), copepods, nematodes and polychaets were the groups that contributed ~50% for dissimilarity between this two sites.

Table 2 – Results PERMANOVA on Bray-Curtis distances for abundance data of major meiofauna groups recorded at the three sampling sites: A - in both countries (*Avicennia* belt only); B - Kenya (*Avicennia* and *Rhizophora* belt) and C - Mozambique (*Avicennia* belt). The factors used in all the analysis were: Season (fixed and orthogonal), Country (fixed and orthogonal), Site (random and nested in country), Area (random and nested in site), and Belt (random and orthogonal). (Degrees of freedom (df), sum of squares (SS), mean squares (MS) and F-ratio value (F)).

Source	**df**	**SS**	**MS**	**F**	**P**
A - Kenya and Mozambique					
Season	1	3057,22	3057,22	2,01	0,13
Country	1	16668,02	16668,02	2,81	0,05
Site	**4**	**23711,77**	**5927,94**	**11,44^b**	**0,00**
Area	6	3108,06	518,01	1,03	0,44
Season x Country	1	966,28	966,28	0,64	0,67
Season x Site	**4**	**6073,49**	**1518,37**	**2,44^a**	**0,02**
Season x Area	6	3725,55	620,93	1,23	0,23
Residual	24	12098,73	504,11		
Total	47	69409,13			
B – Kenya					
Season	1	2204,75	2204,75	2,42	0,14
Belt	1	2411,14	2411,14	2,40	0,14
Site	2	1375,53	687,77	1,37	0,31
Area	**3**	**1508,28**	**502,76**	**2,71^b**	**0,01**
Season x Belt	1	562,64	562,64	1,01	0,45
Season x Site	**2**	**1819,37**	**909,69**	**3,49^a**	**0,03**
Season x Area	3	780,79	260,26	1,41	0,19
Belt x Site	**2**	**2006,55**	**1003,28**	**6,42^b**	**0,00**
Belt x Area	3	468,41	156,14	0,84	0,58
Season x Belt x Site	2	1109,39	554,70	2,05	0,14
Season x Belt x Area	3	810,03	270,01	1,46	0,17
Residual	24	4438,78	184,95		
Total	47	19495,68			
C - Mozambique					
Season	1	2267,61	2267,61	1,03	0,43
Site	**2**	**20912,57**	**10456,28**	**19,54^b**	**0,00**
Area	3	1605,25	535,08	0,72	0,70
Season x Site	2	4418,25	2209,12	2,41	0,10
Season x Area	3	2754,61	918,20	1,23	0,29
Residual	12	8942,77	745,23		
Total	23	40901,05			

(a = $p < 0.05$; b = $p < 0.01$)

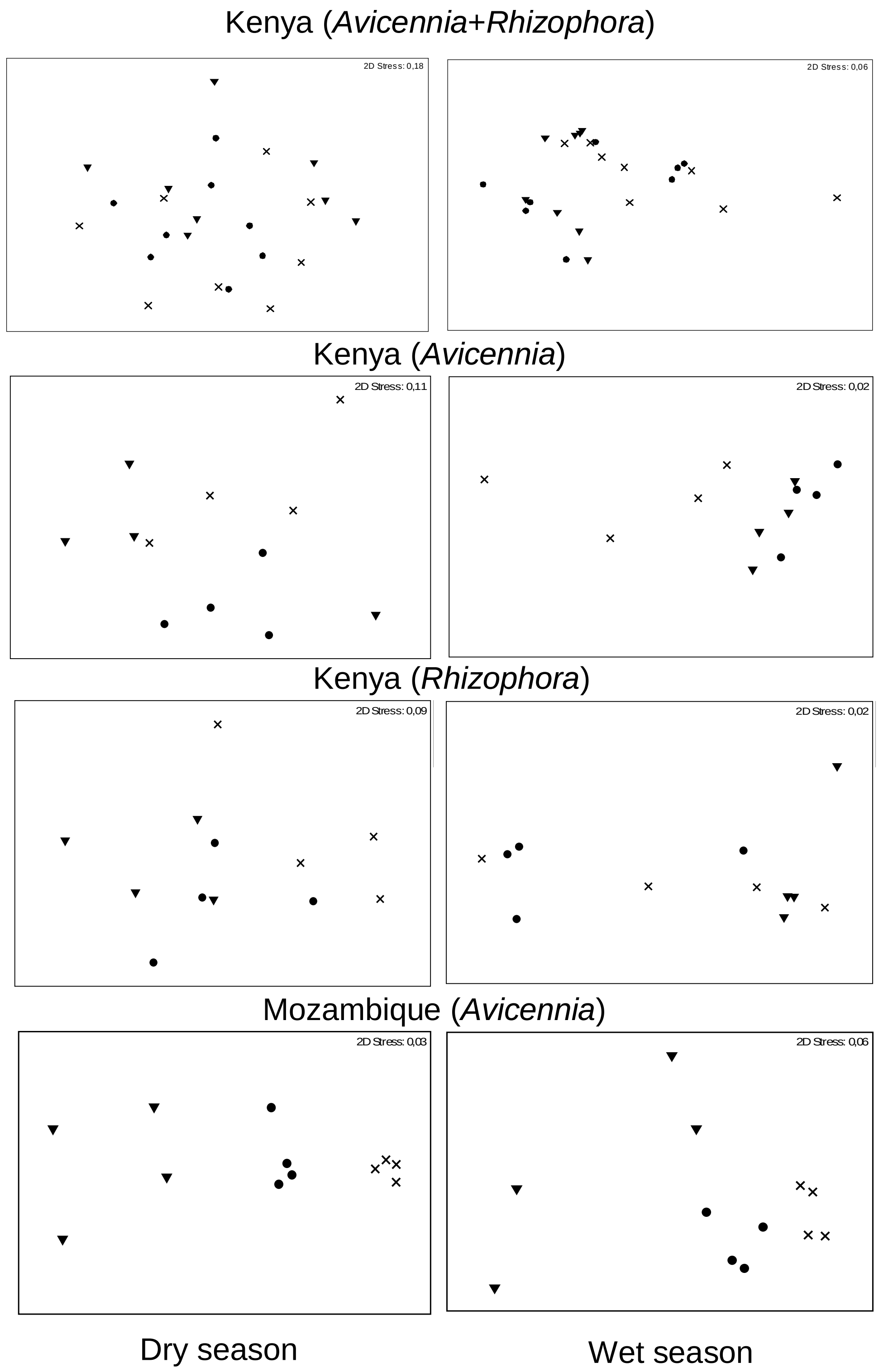

Figure 5 - Non-metric multidimensional scaling (NMDS) ordination of root transformed meiofaunal abundance data from three sampling sites, in both *Avicennia* and *Rhizophora* belts, in Kenya ((x) Mikindani (●) Gazi (▼) Shirazi) and Mozambique ((x) Costa do Sol (●) Saco (▼) Ponta Rasa).

In Mozambique, Costa do Sol (peri-urban) mangrove presented similar diversity indices, calculated for major meiofauna groups (Table 3), than both pristine mangroves, except for a much higher nematode/copepod ratio during the wet season. In Kenya, Margalef's index at Mikindani for both tree belts, and in particular at the wet season, was significantly lower compared to both pristine mangroves (Table 3). Shannon diversity index obtained at Mikindani was only significantly different from the other two mangroves at the *Rhizophora* belt and only at the wet season. The nematode to copepod ratio, in Kenya, presented similar values for contaminated mangrove compared to the ones obtained for pristine areas, except at dry season where Gazi presented ratios significantly different from Mikindani at the *Avicennia* belt, and both other mangroves at the *Rhizophora* belt (Table 2).

Table 3 – A comparison of average (±SE) of richness (d), evenness (J') and diversity (H') indices and nematode/copepod ratio of meiofauna communities between contaminated and pristine locations in both seasons, in Kenya and Mozambique (different letter indicate significant differences between season for the same site, while different number indicate significant differences between sites for the same season).

Kenya *A. marina*	**Season**	**Margalef's index (d)**	**Pielou's index (J')**	**Shannon index (H')**	**Nematod/Copepod Ratio**
Mikindani	**Dry**	0.69±0.07	0.19±0.06	0.33±0.10	**177±82**^{a}
	Wet	**0.60±0.04**^{a}	0.16±0.05	0.21±0.05	288±106
Shirazi	**Dry**	0.86±0.08	0.16±0.03	0.31±0.06	301±239
	Wet	**0.86±0.06**^{b}	0.14±0.03	0.32±0.05	410±130
Gazi	**Dry**	0.76±0.08	0.13±0.02	0.25±0.03	**881±295**^{b}
	Wet	**0.98±0.06**^{b}	0.14±0.03	0.30±0.05	410±131
Kenya ***R. mucronata***					
Mikindani	**Dry**	**1.05±0.12**$^{(1)}$	0.15±0.03	**0.31±0.05**$^{(1)}$	**151±60**^{a}
	Wet	**0.61±0.05**$^{(2)a}$	0.07±0.01	**0.12±0.02**$^{(2)a}$	445±47
Shirazi	**Dry**	1.00±0.08	0.11±0.02	0.23±0.04	**189±94**^{a}
	Wet	**0.81 ±0.07**^{b}	0.10±0.01	**0.20±0.03**^{b}	283±113
Gazi	**Dry**	0.94±0.04	0.12±0.01	0.25±0.04	**638±86**^{b}
	Wet	**0.99±0.04**^{b}	0.12±0.02	**0.25±0.04**^{b}	467±87
Mozambique ***A. marina***					
Costa do Sol	**Dry**	1.03±0.02	0.35±0.03	0.75±0.06	**3±1**$^{(1)}$
	Wet	1.03±0.07	0.29±0.05	0.58±0.09	**291±26**$^{(2)a}$
Saco	**Dry**	0.91±0.08	0.42±0.07	0.76±0.14	102±53
	Wet	1.05±0.12	0.29±0.05	0.52±0.10	**85±43**^{b}
Ponta Rasa	**Dry**	1.47±0.34	0.54±0.14	0.77±0.18	10±5
	Wet	4.39±3.24	0.65±0.22	0.97±0.40	**1±0**^{b}

4. DISCUSSION AND CONCLUSION

Human-induced polluting events can exert different effects on exposed populations, generating changes in community structure and ecosystem functioning (Parker et al., 1999). Infaunal species are considered to be key organisms to detect ecosystem changes as well as human impacts and contamination, due to their short life span and low mobility (Warwick and Clarke, 1993; Chapman, 2007), although it is also necessary to consider the bioavailability and hence toxicity of contaminants to the different fauna groups (Markert et al., 2004). Fauna assemblages are also influenced by several factors, such as hydroperiod, sediment characteristics and abiotic conditions (e.g., temperature and salinity) (Alongi, 1987a; Coull, 1999; Lee, 2008). Abundances of infauna in soft sediments are patchy at a range of spatial scales, from meters up to several kilometres (Morrisey et al., 1992; Chapman and Tolhurst, 2004; 2007) and they also undergo strong temporal changes linked to natural and unpredictable fluctuations in the ecological factors of the systems they inhabit (Underwood and Chapman, 1996).

Anthropogenic stress tends to exacerbate this spatial and temporal variability by increasing variability in the abundance of individual taxa or through changes in the taxonomic composition of samples taken from within and among affected sites (Warwick and Clarke, 1993). For this reason, in order to use infauna community multivariate analysis we need nested sampling designs incorporating various spatial and temporal scales to disentangle these natural variations from those due to the human impact (Warwick and Clarke, 1993; Cannicci et al., in press). Although the present study showed high spatial variability between samples (area), it presented a clear distinction between communities inhabiting pristine and contaminated mangrove forests at both regions. Nematods, together with copepods and Turbellaria organisms, as well as larger individuals such as polychaets and oligochaetes comprised more than 90% of all infauna as was previously observed in most mangrove areas, and densities are within the range observed in previous studies (e.g., Alongi, 1987b; Fondo and Martens, 1998; Nagelkerken et al., 2008). Meiofauna reacted in opposite ways to anthropogenic contamination, increasing at the Mozambiquean but decreasing at Kenyan peri-urban mangroves. Only Oligochaeta showed to decrease in contamination conditions in both countries, confirming the high sensitivity and efficiency of this group to be widely used on bioassessement assays as bioindicators of organic pollution as well as heavy metals (Chapman, 2001; Markert et al., 2004; Lin and Yo, 2008). At Mozambique there was a significant decrease of molluscs and an increase of crustaceans and most meiofauna groups (such as Nematoda, Halacaroidea and Copepoda) at the peri-urban mangrove, probably explained by an increase in sediment organic matter, microphytobenthos and bacteria.

A parallel study done on macroepifauna also found that the peri-urban systems were richer than the mangroves not affected by urban sewage in terms of fiddler and sesarmid crabs diversity and abundance, known to feed on benthic microalgae and bacteria, or on both substratum and leaf litter, respectively (Cannicci et al., in press). The same study also documented a complete eradication of certain mollusc's species (e.g., *Terebralia palustris*) from the contaminated mangroves, probably due to the low tolerance to this sort of pollution. In Kenya, a decrease in Oligochaeta, Polychaeta, Halacaroidea and Turbellaria groups mainly at the *Avicennia* belt in peri-urban mangrove, indicated that higher loadings of organic matter or other contaminants were present and affected local communities (e.g., Danovaro et al., 1995; Gillet et al., 2008). The fact that the dumping of sewage at Mikindani affected primarily the landward *Avicennia* belt, before flowing through the *Rhizophora* belt, could help explain this differences, and since both soils and vegetation of this landward belt can efficiently absorb the overload of nutrients (Tam and Wong, 1995; Wong et al., 1997), the landward belt of Mikindani is probably acting as a first phytoremediating system, mitigating the effect of wastewater dumping to the seaward (*Rhizophora*) belt of the forest. This was also observed by Cannicci et al (in press) that found that macro epibenthic fauna was more severely affected at the landwards (*Avicennia*) belt.

In environmental monitoring, multivariate techniques have traditionally required detailed taxonomic data, but that is no longer the case. For a variety of benthic organisms (macrobenthos, meiobenthos and reef corals), aggregation trials suggest that little information is lost by this approach based on the assumption that anthropogenic stresses modify community structure at a higher taxonomic level than that at which natural environmental variables impact on community structure (Warwick, 1988). Taxonomic minimalism thus facilitates the discrimination of human and natural causes. In the present study, the MDS analysis clearly indicated a separation between peri-urban mangroves and control sites for both meio and macrofauna groups at the two countries, tree belt and seasons. Nevertheless, a low taxonomic resolution may not be sensitive enough for changes in functional groups within the same taxa, as well as changes in biomass or behaviour, known to have significant effects on ecosystem functioning.

Diversity indexes have been widely used in many ecosystems (Kennedy and Jacoby, 1999b; Markert et al., 2004), including mangrove forests (e.g., Bosire et al., 2004) as ecological indicators. The values obtained in this study are significantly lower than the ones obtained in other studies due to the fact that higher taxa levels are being used, although the diversity indexes calculated did present lower diversity measures in contaminated sites. At Kenya, Margalef's and Shannon index indicated Mikindani as having lower diversity when compared with pristine mangroves, although nematode/copepod ratio indicates lower contamination stress (Raffaelli and Mason, 1981), while at Costa do Sol this last index is the only one indicating a severe contamination but only during wet seasons, where rains is though to bring contaminants from terrestrial to the mangrove creek.

Very little is known about species interaction among meio and macrobenthos and much is yet to be learnt from process shaping community structure and function in these complex and productive environments (Lee, 2008), mainly when subjected to intensive anthropogenic stress. In a recent compilation done by Oláfsson (2003), most studies indicate that the overall effects of macrofauna originating from processes such as predation, physical disturbance, competition for food and biogenic structures affect significantly meiobenthic community structure, diversity and dynamic. This way, the impact of sewage contamination on infauna community is more complex than the direct effect of the contaminants on organisms.

Mangrove infauna species also serve as important food sources for local and mobile nekton that enters the mangrove at high tide, including commercial important fish and crustaceans, as well as several bird species that feed on these forests habitats (Lee, 2008; Nagelkerken et al., 2008). Both groups are also very important on nutrient cycling and organic matter degradation (such as litter), by direct feeding on the detritus as well as performing bioturbation activities (sediment reworking and bioirrigaton) and constructing biogenic structures that stimulate microbial activity and efficiency (Schrijvers et al., 1995; Kristensen, 2000; Cannicci et al., 2008). This will ultimately play an important role for the biogeochemical and ecological functioning of mangrove sediments (Kristensen, 2008; Lee, 2008), and consequently on mangrove nutrient and carbon dynamic (Kristensen et al., 2008; Penha-Lopes et al., submitted), influencing the natural mangrove potential for pollution buffering and as systems for potential sewage treatment (Penha-Lopes et al., in prep).

The results obtained in the present study were able to differentiate peri-urban from pristine mangroves in both countries using univariate, multivariate and diversity indexes analysis, although meio and macro community responded different in each country. Only Oligochaeta individuals decreased significantly in both contaminated mangroves, making them a reliable indicator for East African mangroves. Other taxonomic groups should be identified to lower levels in order to provide a better understanding of community changes. Although the multidimensional scaling analysis, as well as diversity indexes, showed that identifying organisms at high taxonomic levels are indeed useful tools for environmental impact assessment, more research is needed in order to upgrade knowledge, especially in what concerns bioindicators in these habitats and mainly in East Africa region.

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Chapter 2

Comparison of fecundity, embryo loss and fatty acid composition of mangrove crab species in sewage contaminated and pristine mangrove habitats in Mozambique

Gil Penha-Lopes*[1], Paulo Torres[1], Luis Narciso[1], Stefano Cannicci[2] and José Paula[1]

[1] Laboratório Marítimo da Guia, IMAR,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, Nº 939, 2750-374 Cascais, Portugal
Gil Penha-Lopes (*Corresponding author): gil.penha-lopes@fc.ul.pt;
Tel: (+351) 214 869 211; Fax: (+351) 214869720
[2] Dipartimento di Biologia Evoluzionistica "Leo Pardi", Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy

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Gil Penha-Lopes*[1], Paulo Torres[1], Luis Narciso[1], Stefano Cannicci[2] and José Paula[1]

[1] Laboratório Marítimo da Guia, IMAR,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, Nº 939, 2750-374 Cascais, Portugal
Gil Penha-Lopes (*Corresponding author): gil.penha-lopes@fc.ul.pt;
Tel: (+351) 214 869 211; Fax: (+351) 214869720
[2] Dipartimento di Biologia Evoluzionistica "Leo Pardi", Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy

Abstract

The effects of anthropogenic activities combined with the lack of technical solutions for sewage treatment have lead to serious contamination problems in the coastal ecosystems of East Africa. However, not all contaminants can be considered pollutants. Determining when contamination results in pollution requires not only chemical but also biological measurements. Because benthos integrates conditions over time, macrobenthic organisms are considered good bioindicators to assess local environmental quality. Crabs constitute one of the most important macrofauna taxa in terms of abundance, species richness and biomass in mangrove ecosystems. In the present study, the reproductive potential and quality of *Uca annulipes* (H. Milne Edwards, 1837) population inhabiting a peri-urban mangrove, subjected to domestic sewage discharges, was compared to populations inhabiting pristine mangroves. Fecundity, egg quality (fatty acids composition) and potential fertility were evaluated and compared by sampling a representative fraction of ovigerous females captured in each of the mangrove habitats at two seasons (February to March, 2006 – wet season; and August to September, 2006 – dry season). Most of the measured reproductive parameters of *U. annulipes* were different at Maputo peri-urban mangrove when compared to nearby pristine locations. Although we cannot prove that sewage discharge done at Costa do Sol mangrove was the main factor influencing the reproductive dynamics of *U. annulipes* populations, at this peri-urban mangrove this fiddler crab species extended its reproductive season, increased fecundity, as well as improved embryo quality, mainly regarding the concentration of SFA and MUFA, in relation to the pristine mangrove populations.

Keywords: embryonic development, fatty acids, indicator species, pollution, reproduction, *Uca annulipes*

1. Introduction

In East Africa, the lack of technical solutions for sewage treatment, combined with an increase in population growth and migration to coastal areas, has lead to a rapid increase in urban wastewater production, which is putting breakpoint pressures on already inadequate sewage systems (e.g., Mohammed, 2002). As a result, peri-urban coastal areas receive extensive amounts of untreated sewage, which are typically discharged into creeks lined by mangrove forests. Although this uncontrolled drainage of raw sewage into peri-urban coastal areas is a factual result, there is hardly any understanding of its consequences regarding ecosystem health (Holguin et al., 2001; Adeel and Pomeroy, 2002).

Determining when contamination results in pollution requires not only chemical but also biological measurements (Chapman, 2007). Studies of contaminant-induced alterations in fauna species abundance and diversity were developed and are nowadays commonly used (e.g., Bigot et al., 2006; Saunders et al., 2007). However, other fauna fitness parameters have been less used in classifying ecosystems as polluted, such as growth (e.g., LeBlanc, 2007), physiological stress (e.g., Elumalai et al., 2007), and reproduction and embryogenesis (e.g., Elumalai et al., 2005). Ecotoxicological studies using reproductive parameters as indication measurements are scarce for populations inhabiting organic contaminated mangrove ecosystems (Peters et al., 1997), especially for mangrove crabs.

Crabs have already proven to be suitable organisms for use in ecotoxicological studies due to their biological and ecological characteristics, making them able to integrate environmental ecological condition over time (LeBlanc, 2007). The fecundity of mangrove crabs vary significantly according to the species, populations, size, latitude, habitat structure and food availability (Koga et al., 2000; Hemni, 2003; Torres et al., 2009). Bergey and Weis (2008), have also observed that fiddler crabs inhabiting contaminated saltmarshes (by both organic matter and heavy metals) decrease their reproductive season and present lower survival rates of early larval stages.

Over the years, many biomarkers have been developed and claimed to be efficient in providing an early warning of deleterious effects on biological systems and estimating biological effects due to contaminants (Lam and Gray, 2003). Other than just a simple biomarker, the embryos' fatty acids (FA) content and dynamics may provide information on the population life history traits, feeding ecology and habitats. Fatty acids have been recently used as biomarkers in ecotoxicology studies, since some specific fatty acids only exist in particular contaminants or pathogenic groups (Seguel et al., 2001). Also, it constitutes an important parameter that impact larval quality and survival, since newly spawned eggs FA composition is a useful parameter that helps determining the nutritional requirements of larvae, especially in crustaceans (Heming and Buddington, 1988).

Crabs of the Ocypodidae family, such as fiddler crabs (genus *Uca*), are key components of Indo-Pacific mangroves (Lee, 1998), usually occurring at very high densities, high number of species and total biomass in the intertidal zones of mud-sandy sediments (Skov et al., 2002). These deposit feeders tend to burrow and rely for food on organic matter, microalgae and bacteria (Cannicci et al., 2008). These burrowing and feeding activities have shown to increase soil aeration, thus preventing the formation of phytotoxins and increasing organic matter degradation, resulting in more productive ecosystems (Kristensen, 2008).

Uca annulipes is a key species of mangrove fauna and has been widely studied, mainly regarding its distribution and abundance (Skov and Hartnoll, 2001; Skov et al., 2002), behaviour (Zeil, 1998; Cannicci et al., 1999; Litulo, 2005a), ecological role as engineers (Kristensen, 2008), reproduction (e.g., Litulo, 2004a; b; Skov et al., 2005), dispersal (Paula et al., 2004) and its physiology (e.g., Jadhav et al., 2000; 2001). The first toxicity studies using *U. annulipes* as a biomonitor and bioindicator were done with heavy metals (Devi, 1987; Devi and Rao, 1989; Ismail et al., 1991). Only recently this species has been studied as an organic pollution indicator. Cannici et al. (in press) have found that *U. annulipes* significantly increase its density at nutrient contaminated mangroves, when compared to pristine locations, explained by a likely increase of benthic diatoms and meiobenthos, in which they feed on. Despite the relevance of such crabs in mangrove ecosystems and their potential as habitat quality evaluation tools, knowledge is lacking on their responses to stress, namely organic pollution, regarding reproductive parameters.

The goal of this study was to compare the reproductive potential and quality of *Uca annulipes* population inhabiting a peri-urban mangrove (Costa do Sol), subjected to domestic sewage discharges, with populations from two pristine mangroves (Saco and Ponta Rasa). Several reproductive parameters were measured, such as fecundity, potential fertility, egg loss, and embryo quality (fatty acids composition) as potential proxy responses to organic pollution. We hypothesize that an increase of organic loading at Costa do Sol stimulates microbenthic primary production and sewage-derived detritus leading to an increase in egg production as well as an increase in embryo nutritional quality. Brood loss was also evaluated at all mangrove areas and could depend on sewage contamination, fecundity and embryo nutritional value.

2. Materials and methods

For detecting an environmental impact appropriately, replicated Before/After sampling designs is one of the most reliable methods (Underwood, 1992; 1994). However, in the absence of such data, with appropriate spatial and temporal replication it has been possible to examine differences between potentially disturbed and control locations after the ‘‘disturbance’’ (Chapman et al., 1995). For these reasons, in the present study it was followed by an ACI (After Control/Impact) unbalanced design, comparing the ecological features recorded in one contaminated mangrove swamp to those characteristic of two nearby pristine mangroves of similar ecological traits.

2.1 Study area

This study was conducted in the south of Mozambique, during the wet (February to March, 2006) and dry (August to September, 2006) seasons. 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 (Kalk, 1995). Sampling was performed in three different mangrove ecosystems located at similar latitudes in southern Mozambique: a peri-urban mangrove, Costa do Sol (CS), at Maputo Bay (25º55’S, 32º35’E), and two pristine mangroves (26ºS, 32ºE), Saco (S) and Ponta Rasa (PR), located at Inhaca Island (Fig. 1). Sites were chosen based on preliminary results obtained within the PUMPSEA project that showed that CS was significantly more contaminated than both other systems analysed (see description bellow), and some studies done on these same sites have already demonstrated effectively the effect of pollution in many other ecosystem compartments, such as macroepifauna abundance and diversity (Cannicci et al., in press). Although mangrove ecosystems should tend to have similar morphological (e.g., hydrology and sediment) and biological (e.g., flora and fauna species density and diversity) properties, all three mangroves present particular characteristics (see below), but they were similar enough to be used as impacted Vs controls (PUMPSEA, 2008; Cannicci et al., in press).

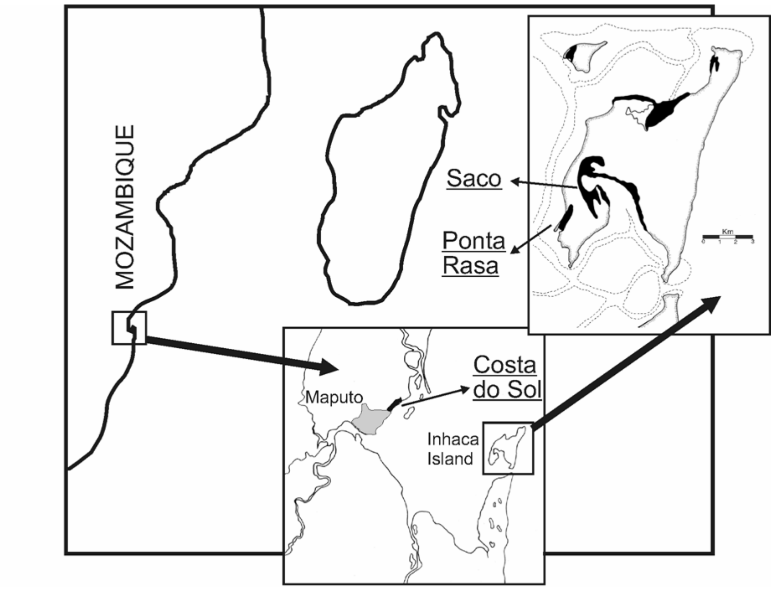

Figure 1 – Location of study sites in southern Mozambique.

Both pristine mangroves are found at Inhaca Island, a small island at ~32 km east of Maputo, constituting a barrier between Maputo bay and the Indian Ocean: the west and south coasts face Maputo bay, while the east coast is exposed to the Indian Ocean. Maximum tidal amplitude is ~3.7 m and average water temperature and salinity varies between 20 to 33 ºC and 22 to 39, respectively (Kalk, 1995). No rivers are present in the island, hence the main freshwater supply to mangrove ecosystems results from diffused ground water flow and rainfall, although the water mass around Inhaca reflects the discharge on estuaries into Maputo Bay (Paula et al., 1998). There are few human communities in the south part of the island, and the absence of industries contributes to the preservation of pristine areas in relation to those of Maputo Bay (Kalk, 1995). One of the pristine locations is Saco, a mangrove that covers an area of ~2.1 km^2, located in a small, enclosed and shallow bay in the south of the island (Fig. 1). While *Avicennia marina* borders the entire bay, *Rhizophora mucronata*, *Ceriops tagal* and *Bruguiera gymnorrhiza*, line channel banks and creeks, dominating the vegetation (Kalk, 1995). Ponta Rasa mangrove is the smallest of the island, covering ~0.2 km^2, and is located in the southwest coast, facing Maputo Bay (Fig. 1). The creek is densely bordered by *R. mucronata*, while sparser patches of *C. tagal* and *B. gymnorrhiza* dominate higher areas of the mangrove, and the uppermost sandier zone is characterized by *A. marina*. Both pristine mangroves present similar abundance and diversity of epimacrofauna, such as crabs and molluscs (Cannicci et al., in press).

Costa do Sol mangrove (peri-urban) is characterized by a small and shallow seawater swamp located ~7 km north of Maputo city center, but within urban boundaries. Maximum tidal amplitude is ~3.5 m, and water temperature and salinity vary between 18 to 35 ºC and 20 to

35, respectively (Litulo, 2005a). The river Quinhenganine discharges in the swamp after passing through the city. *Avicennia marina* and small patches of *R. mucronata* dominate the vegetation (Litulo, 2005a). A residential area surrounds the mangrove that has been receiving domestic sewage, aquaculture residuals and solid dumps from various sources (diffuse and point sources) during the last decades (PUMPSEA, 2008). Several pollution indicators have been studied in the three mangroves as part of the PUMPSEA (2008) project. Higher organic contents as well as concentrations of nutrients, especially nitrites and nitrates, characterized Costa do Sol (0.49±0.52 µM 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). As a direct result, the benthic microalgae community and organic matter is significantly more exuberant and abundant at Costa do Sol (PUMPSEA, 2008). This mangrove also presents much higher abundances of both total and faecal coliform bacteria, such as *Escherichia coli*, *Vibrio cholerae* and *Salmonella* spp., which is reflected in the frequent observation of levels above the international recommended acceptable limits (PUMPSEA, 2008).

2.2 Sampling

Ovigerous females were collected throughout the wet and dry seasons, during day-time at low tide, in Saco, Ponta Rasa and Costa do Sol mangroves, by means of haphazardly excavating with a shovel to a depth of 30 cm. Crabs were placed in individual plastic bags and were brought to laboratory in cool boxes.

2.3 Fecundity and embryonic development

Carapace width (CW) and carapace length (CL) of females were measured using Verenier callipers. In this study we used the same fecundity and potential fertility concepts already employed by Penha-Lopes 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 fertility, on the other hand, is calculated based on the number of newly hatched larvae from a single female. In this study we have not measured fertility, but potential fertility, once we have considered the brood size (number of embryos) just before hatching (stage IV) and not the actual number of newly hatched larvae. In the laboratory, for every egg stage, 10 eggs from each of 10 haphazardly chosen females were selected, placed in Petri dishes with seawater (salinity of 33±1) and their diameter measured immediately under a microscope with a calibrated micrometer eyepiece to the nearest 0.01 mm. Egg volume (V) (mm^3) was then calculated using the formula for spheres $V= 4/3(\pi R^3)$.

Eggs were separated with diluted bleach, placed between 2 transparent sheets and photographed under a digital camera (Nikon D50) at a distance of 38.1 cm with a 50 mm 2.8 Sigma lens. Egg counting was done using Image J software (Abramoff et al., 2004), after adjustments and calibrations, which counted the egg particles on photographs with an error inferior to 3%. The error estimation was done by manual counting of all the eggs present in each photograph and comparing it with the output given by the software for 15% of all the photographs taken for each embryonic stage, population and season.

Two ratios were developed: "*Fecundity per CW* ratio" and "*Potential Fertility per CW* ratio", for embryonic stage I and IV, respectively. *Fecundity per CW* is calculated dividing the number of embryos at stage I divided by the female CW, while *Potential Fertility per CW* uses the egg number at stage IV instead.

2.4 Fatty acids analysis

To determine the fatty acids (FA) profile of *U. annulipes* eggs at each developmental stage, three replicates (different egg batches at the same stage of development) were used. Freeze-dried samples were ground in a Potter homogenizer with chloroform-methanol-water (2:2:1.8) (Bligh and Dyer, 1959). An internal standard FA (C19:0) was added to the extracts. After saponification and esterification of the lipid extracts (Metcalfe and Schmitz, 1961), the fatty acid methyl esters (FAME) were injected into capillary column (30 m fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas-liquid chromatograph (GLC). Helium was used as a carrier gas at a flow rate of 1mL/min; oven temperature was 180ºC for 7 min, and then increased to 200ºC (with a temperature gradient of 4ºC/min) over a period of 71 min. Both the injector and the FID detector were set at 250ºC. GLC data acquisition and handling were performed using a Varian integrator 4290 connected to the GLC. Peak quantification was carried out with a Star Chromatography workstation. Peak identification was performed using well-characterized cod liver oil chromatograms and specific standards as a reference.

2.5 Statistical analysis

A PERMANOVA, permutational multivariate analysis of variance (Anderson and Robinson, 2001), asymmetrical design was used to test the null hypothesis that there were no differences in crabs dimension and in both the *Fecundity per CW* and *Potential Fertility per CW* among peri-urban and non-urban sites. Similarity matrices were computed using

Euclidean distance on untransformed data. All analyses were based on 9,999 permutations of residuals within a reduced model (Anderson and Ter Braak, 2003) and type III sums of squares to cope with the unbalanced design (Anderson et al., 2008). Thus, two three-way univariate PERMANOVA tests were applied to determine whether there were differences in both CW and in the ratio between the number of eggs and the CW with Impact vs Control (asymmetrical, fixed and orthogonal), site (random and nested in 'Impact vs Control') and Fecundity vs Potential Fertility (fixed and orthogonal) as factors. The PERMDISP (Anderson et al., 2006) technique was used to test the homogeneity of multivariate dispersions. All analyses were performed using PRIMER v. 6.1 (Clarke and Gorley, 2006) and the PERMANOVA+ for PRIMER routines (Anderson et al., 2008).

To compare egg volume, and fatty acids composition between populations a one-way ANOVA test was used (followed by Tukey test in case of significant difference), while for comparing data for each population but between seasons or embryonic stages a t-test was used. All the results were considered statistically significant at $p<0.05$ levels.

3. Results

In this study, while ovigerous females were very abundant during the wet season in all three locations, they were not found at Inhaca Island during the dry season. Even at Costa do Sol during the dry season, ovigerous females were present at low density making them very difficult to obtain. No significant differences were found on the crab morphological correlations between embryonic stages, between populations and seasons ($F_{7,158}=1.61$; $p=0.14$ for slopes, and $F_{7,165}=1.95$; $p=0.06$ for interceptions). Since correlations were similar, it was possible to calculate a general equation for all *U. annulipes* populations:

$CW = 0.58*CL+0.04$ ($r = 0.90$; $p<0.0001$)

The high correlation between CW and CL, allowed us to proceed using just one of the dimension (CW).

3.1 Fecundity and embryonic development

PERMANOVA revealed that there were no differences in the females´ dimensions across both sites and for what concern the samples collected to study Fecundity and Potential Fertility (Table 1, Fig. 2-A). On the other hand, the test performed on the number of eggs carried by each females, standardised by her CW, showed a positive interaction between the factor Impact vs Control and the factor Stage (Table 1, Fig. 2-B). Post hoc pair-wise tests showed that females colonising the impacted site of Costa do Sol were producing more eggs at stage I than the ones collected in the two control sites ($t=8.72$; $P<0.001$), and, moreover, that at the impacted site there was a significant egg loss, with females carrying significantly less eggs at stage IV than at stage I, regardless of their size (t=9.64; P<0.001).

In the dry season, Costa do Sol populations did not present significant brood loss, (F=0.53; P=0.47; one-way PERMANOVA test). Regarding the egg volume at initial and final stages, similar results were obtained for the three crab populations in both seasons. During the wet season, average embryo growth (from stage I to IV) for Costa do Sol, Saco and Ponta Rasa was 87.4%, 94.9%, and 97.3%, respectively, while during the dry season was 69.6% for the Costa do Sol population.

Table 1 – Results of the three-way PERMANOVA conducted on Carapace Width (CW) and the ratio between the number of eggs and CW (# eggs/CW) for females collected at the study sites. Factors are as follows: Impact vs Control (asymmetrical, fixed and orthogonal), site (random and nested in 'Impact vs Control') 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

		CW			# eggs/CW		
Source	df	MS	F	P	MS	F	P
Impact vs Controls - I vs C	1	34.05	43.55	0.10	46942.00	19.34	0.15
Stage - St	1	15.85	8.09	0.21	136530.00	1361.00	0.02
Location(I vs C)	1	0.78	0.58	0.46	2427.30	0.90	0.35
I vs C x St	1	0.83	0.42	0.63	90136.00	898.56	0.02
Location (I vs C) x St	1	1.96	1.45	0.24	100.31	0.04	0.85
Res	136	1.36			2693.60		
Total	141						

3.2 Fatty acids analysis

In general, *Uca annulipes* showed a similar consumption pattern in the FA used during embryogenesis (Tables 2 and 3) for all populations. Total saturated fatty acids (SFA), mainly composed of palmitic (16:0) and stearic (18:0) acids, monounsaturated fatty acids (MUFA), primarily composed of palmitoleic acid (16:1n-7) followed by vaccenic (18:1n-7) and oleic (18:1n-9) acids, decreased through embryo development in all populations. Total polyunsaturated fatty acids (PUFA), mainly composed of eicosapentaenoic (EPA - 20:5n-3) acid, followed by eicosatetraenoic (20:4n-3) and linoleic (18:2n-6) acids, remained almost constant during embryogenesis (Table 2 and 3).

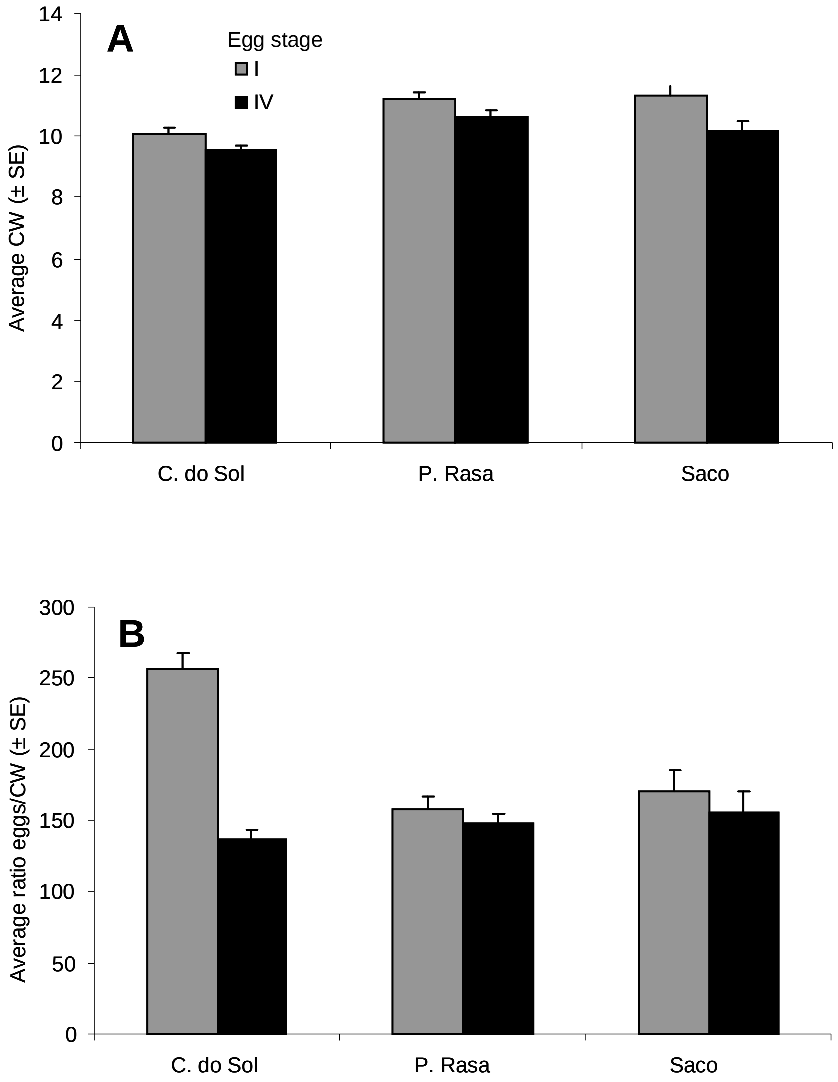

Figure 2 – Average (±SD) dimensions (A) and number of carried eggs, standardised on carapace dimensions (B) observed for the females carrying eggs of stage I and IV collected in the study locations.

Although the general FA dynamic was similar, during the wet season, Costa do Sol populations presented some significant differences when compared to pristine populations. Starting by total FA of newly laid eggs, the peri-urban population presented the highest value, although only significantly different from Saco population, while at the last embryonic stage no difference was observed between the 3 populations (Table 4). Most of the SFA at embryonic stages I and IV were much more abundant at Costa do Sol population, leading to significantly higher concentration of total SFA of newly laid eggs at Costa do Sol when compared to the other two pristine mangrove populations (Table 4). For MUFA, the same results were obtained, although significant differences between peri-urban and pristine mangrove populations were maintained during the whole embryonic development. During the dry season, while the overall FA dynamics at Costa do Sol was similar to the one observed at the wet season, its concentration was nearly half lower, at both egg stages. At Costa do Sol the EPA/DHA ratio of both egg stages was significantly higher than the one found in embryos of pristine locations (Table 4).

Table 2 - Embryo development FA consumption rate (%), between stage I and IV, in U. annulipes 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).

	Consumption (%)			
Fatty acids	***Saco***	***Ponta Rasa***	***Costa do Sol (wet season)***	***Costa do Sol (dry season)***
14:0	69.3	79.0	62.4	63.3
15:0	78.2	83.6	78.2	83.5
16:0	69.1	69.0	62.5	60.1
17:0	60.1	77.1	45.4	51.3
18:0	49.6	52.3	37.9	42.7
Σ SFA	67.0	66.5	58.8	57.6
16:1n-7	71.4	74.7	72.5	61.5
18:1n-9	50.7	64.3	46.5	51.8
18:1n-7	54.2	60.6	48.5	51.5
Σ MUFA	63.5	69.2	61.7	57.6
18:2n-6	35.1	69.1	31.3	32.8
18:3n-3	62.3	74.8	39.1	8.04
20:4n-6	-17.1	30.4	-20.8	-11.5
20:5n-3	41.2	45.4	5.7	0.0
22:5n-3	28.9	28.6	8.3	-58.8
22:6n-3	21.8	21.4	-30.4	-26.6
Σ PUFA	38.2	51.1	17.3	8.4
Σ HUFA	35.2	42.7	2.7	-2.1
Total FA	62.8	65.8	56.6	51.5

4. Discussion

Our findings indicate a clear difference in the reproductive fitness of *Uca annulipes* between polluted and unpolluted mangroves. Females at Costa do Sol (peri-urban mangrove) breeds continuously, usually (wet season) with higher number of eggs at stage I and superior nutritional quality, followed by high brood loss, while at pristine mangroves populations only reproduce during wet season. Litulo (2004b) had already previously reported a continuous breeding season for *Uca annulipes* at Costa do Sol (peri-urban mangrove), while other *Uca* populations inhabiting sub-tropical pristine sites (Torres et al., 2009), as well as *U. annulipes* inhabiting temperate mangrove systems (Mokhtari et al., 2008), are restricted to warm seasons. Spawning at the rainy season (wet season) is known to provide a selective advantage to intertidal brachyuran populations, since periods of higher rainfall rate can cause changes in the salinity of water and promote an increase of nutrients concentration, favouring primary productivity increase and development of planktonic larvae (Mantelatto et al., 2003). At Costa do Sol, a continuous flow of domestic sewage, composed of freshwater rich in nutrients will boost benthic primary production and detritus abundance, two main food sources of fiddler crabs (Meziane and Tsuchiya, 2002). This seems to be favouring detritivorous feeding, and consequently gonad development and larval release, allowing females to bread continuously. Vergamini and Mantelattto (2008) have also found that other crab species (*Panopaeus americanus*) have changed to a continuous and effective reproduction as a strategy to establish and maintain a stable population living associated with stressful energy demanding conditions in a human-impacted mangrove in Brazil. This potential increase in *U. annulipes* total embryo annual production could contribute to the highest density of these fiddler crabs at Costa do Sol (Cannicci et al., in press), although return of released larvae into the same estuary is known to be rare (Paula et al., 2004). This increase in fiddler crab reproductive potential, and consequently abundance, has a positive consequence for the ecosystem by increasing the bioturbation activity of these crabs (see Kristensen, 2008) and consequently the zooremediation performed at mangroves loaded with high organic input (Penha-Lopes et al., accepted; Bartolini et al., submitted).

At all locations, fecundity was similar to that described for fiddler crabs (e.g., Litulo, 2004b; 2005c; b; 2006; Torres et al., 2009) with an expected increase as the females grow larger. At Costa do Sol, ovigerous females also presented a higher brood mass of newly extruded embryos when compared to pristine locations during the wet season. This finding may be related with the higher organic content found at the peri-urban mangrove, especially at the rainy season, that probably leads to the increase of egg number to the abdomen maximum carrying capacity. However, in late development stages (IV) the potential reproductive output of each female was not significantly different from the populations inhabiting pristine sites, due to the egg loss observed during embryonic development. There are numerous reports of brood loss in crustaceans, such as egg loss at oviposition, aborted development, mechanical losses due to abrasion with the substratum, maternal cannibalism, bacterial and fungal infections, embryo predation and parasitism (see review by Kuris, 1991). At Costa do Sol, other crustacean species are being severely affected by a high degree of parasitism infection (Penha-Lopes et al., unpublished), that is affecting crustacean reproductive potential.

Table 3 - Average (±SD) of embryos volume, reproduction ratio and fatty acid composition (µg mg-1dw) on each developmental stage. Different superscript letters represent significant differences between embryonic stages for the same population and season (t-test; 2-tails), while different numbers represent significant differences between seasons for the same embryonic stages for Costa do Sol (t-test; 2-tails) (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).

	Saco Wet season		**Ponta Rasa Wet season**		**Costa do Sol Wet season**		**Costa do Sol Dry season**	
	Stage I	**Stage IV**	**Stage I**	**Stage IV**	**Stage I**	**Stage IV**	**Stage I**	**Stage IV**
Volume (mm^3)	0.014±0.002^{a}	0.029±0.004^{b}	0.014±0.003^{a}	0.027±0.003^{b}	0.015±0.002^{a}	0.028±0.003^{b}	0.016±0.002^{a}	0.027±0.003^{b}
Reprod. ratio (Eggs / CW)	169±16^{a}	155±14^{a}	157±9^{a}	147±7^{a}	256±11^{a}	136±7^{b}	214±65^{a}	225±76^{a}
FA (µg. g^{-1} dw)	187.98±26.3^{a}	70.0±7.59^{b}	235.31±42.66^{a}	78.55±16.51^{b}	267.26±9.72^{a,1}	115.88±39.80^{b,1}	103.81±31.05^{a,2}	50.36±14.22^{b,2}
Fatty acids								
12:0	0.14±0.04^{a}	0.00±0.00^{b}	0.19±0.22^{a}	0.00±0.00^{b}	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
13:0	0.35±0.06^{a}	0.03±0.06^{b}	0.59±0.14^{a}	0.06±0.07^{b}	0.25±0.06^{a}	0.00±0.00^{b}	0.06±0.05	0.00±0.00
14:0	9.23±1.08^{a}	2.84±0.30^{b}	9.63±1.32^{a}	2.02±0.50^{b}	12.13±1.92^{a}	4.56±1.41^{b}	4.18±1.35^{a}	1.53±0.52^{b}
15:0	10.89±2.41^{a}	2.37±0.07^{b}	5.57±0.62^{a}	0.92±0.14^{b}	5.00±2.30^{a}	1.09±0.07^{b}	0.13±0.04^{a}	0.02±0.04^{b}
16:0	58.23±5.64^{a}	17.98±2.37^{b}	67.91±5.16^{a}	21.09±5.04^{b}	88.29±2.74^{a}	33.09±9.49^{b}	31.73±9.63^{a}	12.65±3.82^{b}
17:0	5.25±4.62	2.09±0.10	4.42±0.88^{a}	1.01±0.20^{b}	4.08±0.76^{a}	2.23±1.61^{b}	0.43±0.13^{a}	0.21±0.07^{b}
18:0	10.75±1.00^{a}	5.42±0.77^{b}	15.13±1.60^{a}	7.22±1.19^{b}	16.02±1.37^{a}	9.94±4.16^{b}	5.63±1.51	3.23±1.10
20:0	1.06±0.10	0.80±0.13	1.55±0.05^{a}	0.68±0.10^{b}	1.39±0.28	1.09±0.86	0.24±0.06	0.19±0.06
22:0	0.62±0.36	0.30±0.03	0.74±0.16^{a}	0.38±0.05^{b}	0.68±0.10	0.66±0.50	0.32±0.05	0.30±0.06
∑SFA	96.53±11.79^{a}	31.85±3.31^{b}	105.74±6.63^{a}	33.40±10.14^{b}	127.83±6.55^{a}	52.67±17.95^{b}	42.72±12.82^{a}	18.13±5.67^{b}
Iso 15:0	1.69±0.42^{a}	0.35±0.08^{b}	3.16±0.36^{a}	0.60±0.12^{b}	1.14±0.29^{a}	0.31±0.09^{b}	0.09±0.08^{a}	0.00±0.00^{b}
Anteiso 15:0	0.75±0.20^{a}	0.16±0.02^{b}	1.43±0.24^{a}	0.26±0.06^{b}	0.27±0.06^{a}	0.00±0.00^{b}	0.15±0.04^{a}	0.00±0.00^{b}
Iso 16:0	0.91±0.16^{a}	0.23±0.05^{b}	1.89±0.17^{a}	0.46±0.11^{b}	0.56±0.13^{a}	0.12±0.18^{b}	0.13±0.10	0.15±0.04
Anteiso 16:0	0.08±0.07	0.00±0.00	0.18±0.03^{a}	0.03±0.03^{b}	0.06±0.05	0.00±0.00	0.03±0.02	0.00±0.00
Iso 17:0	1.49±0.25^{a}	0.39±0.07^{b}	2.84±0.42^{a}	0.77±0.17^{b}	1.45±0.36^{a}	0.31±0.18^{b}	0.82±0.12^{a}	0.15±0.10^{b}
∑BFA	4.92±0.98^{a}	1.13±0.22^{b}	9.49±1.11^{a}	2.14±0.47^{b}	3.48±0.77^{a}	0.74±0.32^{b}	1.22±0.36^{a}	0.30±0.14^{b}
14:1*n*-5	0.03±0.05	0.00±0.00	0.15±0.27	0.00±0.00	0.19±0.13	0.00±0.00	0.07±0.05	0.00±0.00
16:1*n*-7	39.86±5.67^{a}	11.42±1.53^{b}	45.16±6.86^{a}	11.42±3.01^{b}	73.04±1.62^{a}	20.12±2.10^{b}	31.65±9.46	12.20±3.48
16:1*n*-5	0.73±0.08^{a}	0.22±0.03^{b}	1.20±0.18^{a}	0.36±0.09^{b}	0.69±0.13^{a}	0.21±0.09^{b}	0.31±0.10	0.15±0.04
17:1*n*-8	1.20±0.22^{a}	0.39±0.03^{b}	2.12±0.36^{a}	0.51±0.11^{b}	1.06±0.31^{a}	0.26±0.01^{b}	0.26±0.18	0.14±0.18
18:1*n*-9	7.45±0.96^{a}	3.67±0.64^{b}	17.91±8.82^{a}	6.40±0.95^{b}	18.67±6.75^{a}	9.99±5.15^{b}	6.58±2.15	3.17±1.09
18:1*n*-7	8.89±1.07^{a}	4.07±0.48^{b}	13.17±2.87^{a}	5.19±0.97^{b}	12.12±0.90^{a}	6.24±1.78^{b}	5.53±1.70	2.69±0.69
18:1*n*-5	0.28±0.04^{a}	0.07±0.06^{b}	0.45±0.08^{a}	0.11±0.02^{b}	0.48±0.09^{a}	0.12±0.17^{b}	0.13±0.05	0.02±0.04
19:1*n*-8	0.18±0.04^{a}	0.00±0.00^{b}	0.18±0.04^{a}	0.06±0.01^{b}	0.36±0.06^{a}	0.00±0.00^{b}	0.16±0.05^{a}	0.00±0.00^{b}
19:1*n*-10	0.36±0.08	0.26±0.05	0.14±0.03	0.13±0.01	0.29±0.15	0.08±0.11	0.14±0.11	0.03±0.04
20:1*n*-9	1.91±0.12	1.50±0.08	2.27±0.51	1.00±0.17	1.91±0.55	2.69±1.41	0.32±0.12	0.12±0.04
20:1*n*-7	1.82±0.49^{a}	0.71±0.21^{b}	1.23±0.18^{a}	0.37±0.09^{b}	1.38±0.29^{a}	0.66±0.43^{b}	0.15±0.05	0.10±0.04
20:1*n*-5	0.51±0.27^{a}	0.00±0.00^{b}	0.96±0.04^{a}	0.33±0.04^{b}	0.43±0.05^{a}	0.00±0.00^{b}	0.27±0.12	0.14±0.10
22:1*n*-11	1.03±0.27	0.99±0.17	1.25±0.21^{a}	0.59±0.14^{b}	1.18±0.25	2.06±0.85	0.62±0.13	0.65±0.55
22:1*n*-9	0.80±0.09^{a}	0.41±0.06^{b}	0.73±0.02^{a}	0.31±0.07^{b}	0.74±0.04	0.69±0.25	0.38±0.08	0.32±0.09
∑MUFA	65.04±7.08^{a}	23.72±2.64^{b}	86.92±18.74^{a}	26.78±5.28^{b}	112.53±9.30^{a}	43.11±12.32^{b}	46.57±14.35^{a}	19.73±6.38^{b}
16:4*n*-3	1.15±0.28^{a}	0.56±0.12^{b}	1.23±0.34^{a}	0.66±0.14^{b}	1.71±0.03^{a}	0.56±0.48^{b}	0.85±0.13^{a}	0.28±0.08^{b}
18:2*n*-6	2.87±0.87	1.87±0.57	6.19±3.78^{a}	1.92±0.47^{b}	4.40±1.67	3.03±2.08	1.11±0.08	0.74±0.17
18:3*n*-3	1.02±0.41	0.38±0.11	2.69±1.19^{a}	0.68±0.16^{b}	1.67±1.44	1.01±1.07	0.17±0.01	0.16±0.05
18:4*n*-3	0.75±0.26^{a}	0.31±0.05^{b}	0.68±0.28^{a}	0.17±0.05^{b}	0.96±0.39	0.50±0.27	0.38±0.14	0.43±0.18
20:4*n*-6	0.35±0.10	0.41±0.13	0.56±0.07^{a}	0.39±0.07^{b}	0.48±0.07	0.58±0.48	1.22±0.66	1.36±0.33
20:3*n*-3	0.52±0.01^{a}	0.38±0.11^{b}	0.63±0.22^{a}	0.24±0.05^{b}	0.47±0.05	0.44±0.03	0.24±0.05	0.22±0.06
20:4*n*-3	3.96±1.66	2.25±0.23	5.75±2.84	2.98±0.63	3.38±0.95	3.24±0.90	2.03±0.65	1.95±0.71
20:5*n*-3	7.80±3.15	4.58±0.43	11.42±6.20	6.24±1.51	8.20±2.93	7.73±3.63	6.21±1.24	5.70±0.06
21:5*n*-3	0.28±0.12	0.16±0.02	0.45±0.24	0.26±0.10	0.31±0.11	0.29±0.10	0.14±0.07	0.12±0.08
22:4*n*-6	0.48±0.22	0.32±0.03	0.40±0.39	0.23±0.04	0.21±0.05	0.00±0.00	0.14±0.24	0.46±0.09
22:5*n*-3	0.38±0.12	0.27±0.10	0.35±0.32	0.25±0.07	0.24±0.07	0.22±0.32	0.07±0.12	0.07±0.16
22:6*n*-3	1.92±0.84	1.50±0.16	2.74±1.66	2.16±0.63	1.35±0.43	1.76±0.71	0.77±0.13	0.81±0.06
∑PUFA	21.50±8.09	13.29±1.51	33.15±14.58	16.23±3.77	23.41±6.70	19.36±9.07	13.33±3.52	12.20±2.03
∑HUFA	15.44±6.15	10.00±1.07	21.92±11.59	12.55±2.95	14.36±4.43	13.98±6.04	10.82±3.16	10.59±1.55
∑(*n*-3)/∑(*n*-6)	4.66±0.41	3.72±0.72	3.70±0.71^{a}	5.29±0.66^{b}	3.65±0.91	4.98±1.73	3.07±0.37	2.92±0.53
18:1*n*-7/18:1*n*-9	1.21±0.19	1.12±0.10	0.80±0.18	0.82±0.15	0.69±0.17	0.67±0.17	0.85±0.09	0.86±0.06
EPA/DHA	4.14±0.30^{a}	3.06±0.10^{b}	4.24±0.53^{a}	2.92±0.26^{b}	6.02±0.95^{a}	4.33±0.30^{b}	8.08±0.29	7.06±0.56
EPA/ARA	22.32±6.44^{a}	12.21±4.63^{b}	20.24±10.09	15.88±2.60	16.89±4.75	16.20±7.09^{b}	5.74±2.78	4.34±0.44

Table 4 - One-way ANOVA to investigate variation within each stage of embryonic development between mangrove populations during the wet season. Significant p-values ($p<0.05$) are indicated in bold. Only the quantitatively most important fatty acids (FAs) are represented. (S) Saco ; (P) Ponta Rasa ; (C) Costa do Sol. (FA - fatty acids; SFA - saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; HUFA - highly unsaturated FA).

ANOVA results	**Stage I**			**Stage IV**		
	***F*-ratio**	***P***	**Tukey test**	***F*-ratio**	***P***	**Tukey test**
Total FA	**5.49**	**0.04**	S ≠ C	4.86	0.06	-
14:0	3.38	0.10	-	**11.36**	**0.01**	S,P ≠ C
15:0	**8.26**	**0.02**	S ≠ P,C	**217.08**	**0.00**	S ≠ P,C
16:0	**32.12**	**0.00**	S,P ≠ C	**7.54**	**0.02**	S ≠ C
17:0	0.14	0.87	-	2.98	0.13	-
18:0	**13.12**	**0.01**	S ≠ P,C	4.38	0.06	-
Σ SFA	**10.32**	**0.01**	S,P ≠ C	4.06	0.08	-
16:1n-7	**34.93**	**0.00**	S,P ≠ C	**16.70**	**0.03**	S,P ≠ C
18:1n-9	2.85	0.14	-	**6.20**	**0.04**	S ≠ C
18:1n-7	4.40	0.07	-	3.82	0.08	-
Σ MUFA	**10.43**	**0.01**	S ≠ C	**8.83**	**0.02**	S,P ≠ C
18:2n-6	1.39	0.32	-	1.43	0.31	-
18:3n-3	1.74	0.25	-	0.86	0.47	-
20:4n-3	1.18	0.37	-	2.77	0.14	-
20:5n-3	0.62	0.57	-	2.47	0.16	-
22:6n-3	1.20	0.36	-	1.44	0.31	-
Σ PUFA	0.91	0.45	-	1.44	0.31	-
Σ HUFA	0.78	0.50	-	1.30	0.34	-
EPA/DHA	**7.91**	**0.02**	S,P ≠ C	**23.86**	**0.00**	S,P ≠ C
Σn-3/Σn-6	1.91	0.23	-	2.51	0.16	-

Although parasitism and bacteria and fungal infection could be explaining *U. annulipes* higher brood loss at the contaminated site, in this case it seems to be related to the incapacity of the female to retain all the newly laid eggs in excess in its body-cavity, which increase significantly in volume during embryogenesis. No relation with embryo nutritional value seemed to have occurred (see bellow).

The average embryo volume obtained for *U. annulipes* populations were within the range found for other brachyuran species (Wear, 1974) and were not significantly different between all locations. In general, the FA consumption pattern during embryonic development for all studied populations, namely saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), were essentially similar to that reported for other fiddler crabs (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 *de novo* or obtained by dessaturation 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 fiddler crab embryos, followed by the monounsaturated 16:1n-7, both very common in mangrove sediments (Meziane and Tsuchiya, 2002). 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, marker of bacteria (Meziane and Tsuchiya, 2002), and oleic (18:1n-9) acid, marker of fungus (Chen et al., 2001), suggest that *U. annulipes* consumes these organism during oogenesis. This is supported by previous research done on *Uca annulipes* fatty acid composition (e.g., Meziane et al., 2002; Meziane et al., 2006). The EPA/DHA and 18:1n-7/18:1n-9 ratios (2.92-8.08 and 0.67-1.21, respectively) also corroborate these findings, placing these populations in a medium-low trophic level, as DHA is highly conserved throughout the food chain, and 18:1n-9 is the major FA in marine animals (Auel et al., 2002; Dalsgaard et al., 2003). The EPA/DHA ratio also suggests that the fiddler crabs 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 the higher microbenthic algal abundance at Costa do Sol, which is also indicated by the higher concentration of palmitoleic acid (diatom trophic marker), registered in this population embryos. Higher energy content (as fatty acids) at newly hatched embryos reflects a better maternal nutrition during oogenesis. At Costa do Sol, the higher values of SFA and MUFA in the embryos are probably caused by a more abundant and different microphytobenthos and bacteria community present in the sediment. In the dry season, the lowest values obtained seem to indicate that the dry sediment, combined with a lower amount of nutrients brought by the river, due to a strong decrease in rainfall, may not be enhancing microphytobenthos and bacteria growth, usually found during the wet season.

The present study, as the research made by Cannicci et al (in press), seems to indicate that the degree and type of contamination of Costa do Sol is not severe enough to cause negative effects on the crabs community inhabiting this ecosystem. Although peri-urban mangroves of many cities of the tropics are extensively used for sewage dumping, these ecosystems (including the fauna component) appear naturally resilient to the introduction of a degree of sewage substances such as organic matter (e.g., Wong et al., 1995; Wong et al., 1997; Cannicci et al., in press).

The present work supports the possibility of using reproductive parameters as a bioindicator of organic pollution in situ, using a key mangrove crab species in East Africa. However, we do not exclude the possibility of other factors observed at Costa do Sol be exerting some influence in *U. annulipes* reproductive dynamics as lower salinities, different hydrological regimes, other contaminants, as well as muddier sediment when compared to pristine locations (PUMPSEA, 2008; Cannicci et al., in press). Future research should focus on other key mangrove species, as well as to better understand the reproductive parameter responses to different types and levels of stressors in order to better monitor impacts on ecosystems.

5. Conclusion

Reproduction parameters of *U. annulipes* were different in Maputo peri-urban mangrove when compared to nearby pristine locations. Although Costa do Sol mangrove may be contaminated to some degree, *U. annulipes* reproductive performance does seem to be enhanced by increasing its breeding season, fecundity, as well as egg quality, mainly regarding the concentration of SFA and MUFA.

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Chapter 3

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

Gil Penha-Lopes*[1], Paulo Torres[1], Stefano Cannicci[2], Luis Narciso[1] and José Paula[1]

[1] Laboratório Marítimo da Guia, IMAR,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, Nº 939, 2750-374 Cascais, Portugal
Gil Penha-Lopes (*Corresponding author): gil.penha-lopes@fc.ul.pt;
Tel: (+351) 214 869 211; Fax: (+351) 214869720
[2] Dipartimento di Biologia Evoluzionistica "Leo Pardi", Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy

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[1] Laboratório Marítimo da Guia, IMAR,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, Nº 939, 2750-374 Cascais, Portugal
Gil Penha-Lopes (*Corresponding author): gil.penha-lopes@fc.ul.pt;
Tel: (+351) 214 869 211; Fax: (+351) 214869720
[2] Dipartimento di Biologia Evoluzionistica "Leo Pardi", Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy

Abstract

The shrimp *Palaemon concinnus* Dana, 1852 was selected as a potential bioindicator of organic pollution for East Africa mangroves due its high abundance and fidelity to mangrove creeks. A multidisciplinary approach was carried out in southern Mozambique to investigate the impacts of sewage pollution on this shrimp, one of the least studied species of Eastern African mangrove environments. Population structure, average size, parasite infection rate, RNA/DNA ratio and a set of reproductive parameters, including maturation curves, fecundity and potential fertility, were used as bioindicators for habitat quality comparing a contaminated peri-urban and two pristine mangrove creeks. The domestic sewage discharges showed to affect positively the shrimp population, by increasing the number of ovigerous females and shrimp average size, fertility and reproductive output. Moreover, RNA/DNA ratio did not indicate any physiological stress by the individuals inhabiting the contaminated mangrove. However, a higher level of parasitation by a Bopyridae isopod, *Pseudione elongata* that leads to the physiological reproductive death of the host indicates some degree of stress on the host shrimp at the peri-urban mangrove. The identification of the effects of sewage on *P. concinnus* increases the choice of possible bioindicators in East African mangrove environments.

Keywords: *Palaemon concinnus*, indicator species, pollution, reproduction, embryonic development, parasitism, Mozambique

1. Introduction

During the last decades, mangrove ecosystems have suffered strong anthropogenic disturbances through deforestation and dumping activities (Duke et al., 2007; Kruitwagen et al., 2008). Despite the great social, economic, and environmental importance of mangroves in tropical coasts (Nagelkerken et al., 2008; Walters et al., 2008), environmental monitoring and assessment of these systems is still limited (Peters et al., 1997). Most studies performed in these forests have pursued the quantification in situ (water, sediment samples as well as in organisms) of anthropogenic contaminants such as heavy metals, organotins, organochlorine pesticides and polychlorinated biphenyls, among others (De Wolf et al., 2001; Mohammed, 2002; Kruitwagen et al., 2008).

However, determining when contamination results in 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 nowadays commonly used in the investigation of environmental assessment (e.g., Warwick, 1993; Bigot et al., 2006; Saunders et al., 2007), including mangroves (Cannicci et al., 2009), and have been considered as the most important level for impact assessment studies (Warwick, 1992). However, community structure and dynamics is merely an expression 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 to determine deterioration in ecosystem health (Attrill and Depledge, 1997). Positive indicators, also named opportunistic species, tend to increase abundance in polluted areas out competing local species (Tsai, 1975 in Méndez et al., 1997; Smith and Suthers, 1999). Negative indicators are usually key fauna species in the environment that tend to decrease biomass, show reduced growth rates (e.g., Crouau and Moia, 2006; LeBlanc, 2007; Amara et al., 2009), as well as low reproduction output and quality (e.g., Smith and Suthers, 1999; Elumalai et al., 2005; Crouau and Moia, 2006). They are efficient indicators of contamination due to a general reallocation of resources favouring tolerance to stress, by combating against contaminants, low oxygen levels or repairing damage (Diaz and Rosenberg, 1995; Wu, 2002). Morphological structures and physiological processes (such as RNA/DNA ratios and cytological modifications) have been also an alternative indication of organisms (including foraminifera, bivalves and crustaceans) condition when facing stress or pollution (e.g., Le Cadre and Debenay, 2006; Norkko et al., 2006; Amaral et al., 2009a). Parasite infection have also been recently considered as a good indicator of anthropogenic pollution due a decrease of host defence mechanism and fitness, thereby increasing the host susceptibility (Sures, 2004; Sures, 2006), affecting host abundance, tolerance to pollution, behaviour and mortality (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 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 (Cannicci et al., 2009; Bartolini et al., accepted-a; accepted-b; Penha-Lopes et al., accepted-a; accepted-b)

Although not profuse, a multidisciplinary approach to investigate the impacts of sewage pollution on single species, although mainly focussing on polychaetes and fishes, started to be used as indicator of anthropogenic impact and is now widely accepted (e.g., Smith and Suthers, 1999; Amara et al., 2009; Durou et al., accepted), but never applied to 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 to mangrove creeks, although it is one of the least studied species in mangroves habitats. Only a few studies have focussed on geographic distribution, taxonomic or morphological variations (Dutt and Ravindra.K, 1974; De Grave, 1999) although is probably a potential food source for commercial species that migrate into the mangrove during high tides (Nagelkerken et al., 2008).

The purpose of this article is to examine whether or not marked differences in anthropogenic disturbance decreased the estuarine habitat quality for *Palaemon concinnus*. We used several different approaches: population structure and average size, reproductive parameters (such as maturation curves, fecundity and potential fertility), 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

Appropriately replicated Before/After sampling designs are perhaps the most reliable methods for detecting an environmental impact (Underwood, 1992; 1994). However, in the absence of such data, with appropriate spatial and temporal replication it has been possible to examine differences between potentially disturbed and control locations after the ‘‘disturbance’’ (Chapman et al., 1995). For these reasons, we followed a ACI (After Control/Impact) unbalanced design, comparing the ecological features recorded in one contaminated mangrove swamp with those characteristic of two nearby pristine mangroves of similar ecological traits in Mozambique. Mangrove areas, sampling design and site selection were described in detail in previous studies (Amaral et al., 2009b; Cannicci et al., 2009; Penha-Lopes et al., accepted-a; accepted-b).

The sampling was conducted in three different mangrove ecosystems, a peri-urban mangrove, Costa do Sol, and two pristine mangroves, Saco and Ponta Rasa, at Inhaca Island (Figure 1). Costa do Sol mangrove is located at Maputo Bay, ~7 Km north of Maputo city. 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 of various sources during the last decades (PUMPSEA, 2008). Costa do Sol presents significantly higher concentrations of pathogens, organic matter and nutrients, especially nitrites and nitrates in relation to Saco and Ponta Rasa mangroves (PUMPSEA, 2008).

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 *P. concinnus* specimens were collected with the help of a 1mm mesh net, and water surface temperature and salinity were measured. Wet season was considered from December to May and dry season from June to November (Figure 2 and Kalk, 1995). 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 (Kalk, 1995).

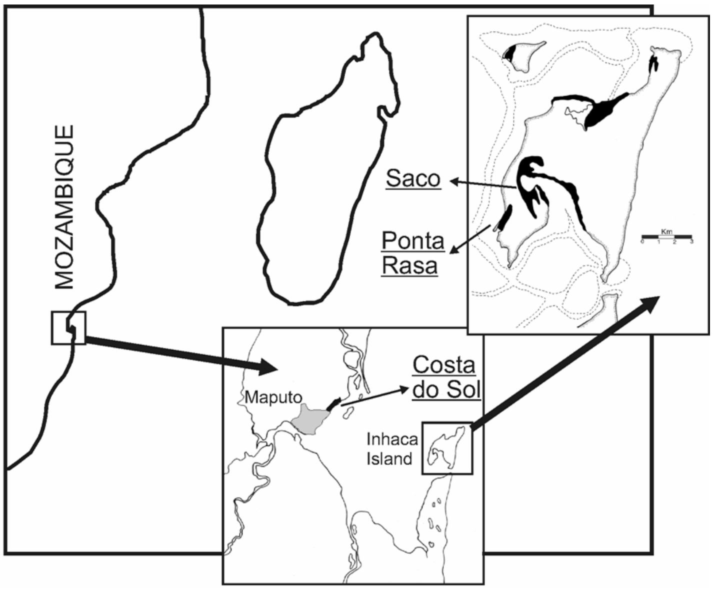

Figure 1 – Location of study sites in southern Mozambique.

2.2 Population structure, parasitism and maturation

Shrimps 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 of pleopods). 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) and Aiken & Waddy (1980), 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:

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

With the logarithmic transformation given by:

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

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 of this equation was made by correlation analysis of variables of P and SL after linearization. Size at sexual maturity (SL50) was estimated as the negative of the ratio of the coefficient (SL50 = - (a/b)) by substituting P=0.5 in Eq. 1.

2.3 Condition indices (RNA/DNA)

During the January-February 2006 campaign for all sampling sites and August 2006 campaign only for CS, 7 ovigerous female shrimps in intermoult stage were haphazardly selected and evaluated in situ by the hardness of the carapace, measured and not preserved in formalin. The individuals were immediately transported to the laboratory in cool boxes and body muscle was removed and was promptly freeze-dried and stored at -80 º C until nucleic acid quantification. Nucleic acid quantification was conducted based on the Schmidt-Thannhauser method, according with the procedures of Amaral et al. (in press-b). Analyses were performed on ~15 mg (freeze-dried weight) of 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 / 280 and 260 / 230 nm ratios, concurrently to sample analysis.

2.4 Fecundity and Embryo development

In this study we used the same fecundity and potential fertility concepts already 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, known to account for the highest reproductive activity in crustaceans in these subtropical mangroves (Torres et al., 2009; Penha-Lopes et al., accepted-c), and dry season (except for Saco where no ovigerous females were found).

2.5 Statistical analysis

The PERMANOVA test, permutational multivariate analysis of variance (Anderson and Robinson, 2001), with asymmetrical design, was used to test the null hypothesis of: 1) no difference in RNA/DNA ratio, RNA content and DNA content, and 2) in both the *Fecundity per SL* and *Potential Fertility per SL* among peri-urban and non-urban sites and sampling seasons. Similarity matrices were computed using Euclidean distance on untransformed data. All analyses were based on 9,999 permutations of residuals within a reduced model (Anderson and Ter Braak, 2003) 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 frequency of males, females and ovigerous females as well as differences in the relative frequency of parasites males and females at impacted and control locations were determined using chi-square test.

3. Results

3.1 Environmental conditions

Water column temperatures in all three mangrove creeks increased slowly form August 05 to March 06, decreasing slowly in the pristine mangroves but dramatically at CS during February and April06 (Figure 2). Salinity ranged from 35 to 40 in all mangroves, while at CS a decrease to values near 10 occurred at the start of the rainy season (January 06), increasing afterwards till April and remaining with slightly lower values till September 06 (Figure 2).

3.2 Population structure, maturation and parasitism

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

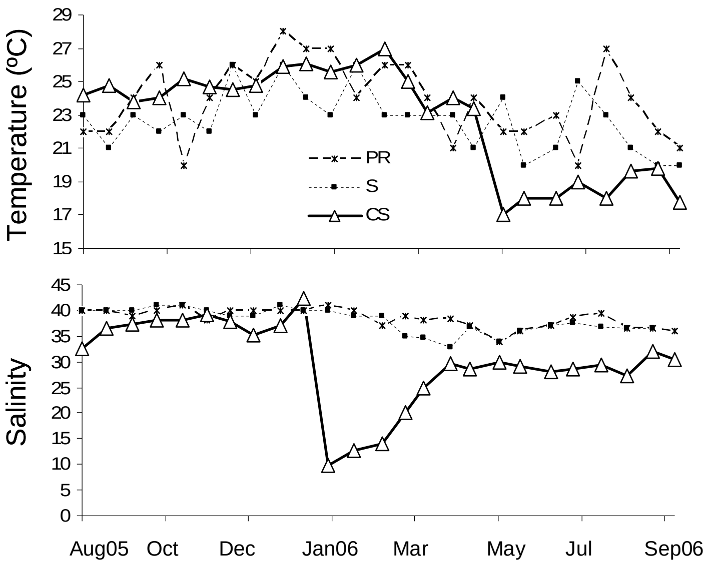

Figure 2 – Temperature and salinity measured at the low tide in all three mangrove creeks during a period of 12 months. Values are given as average (±SE).

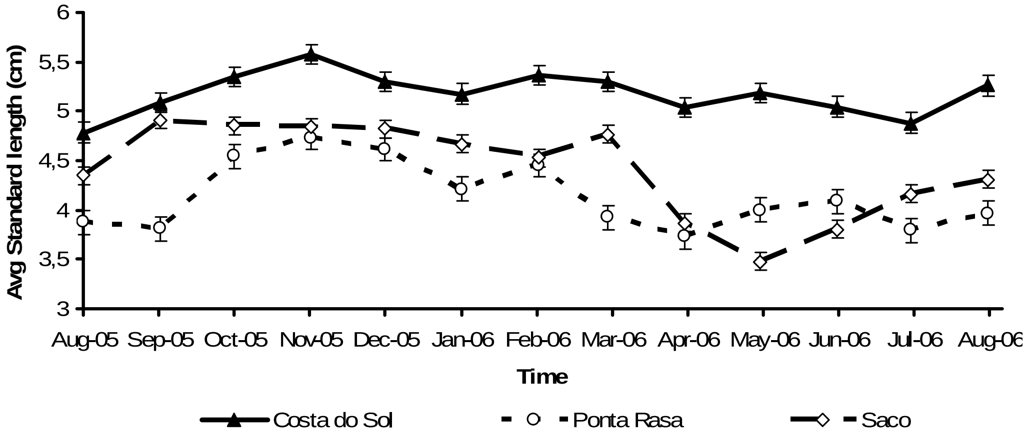

Figure 3 – Average standard length (±SE) of *Palaemon concinnus* population at the contaminated (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, south Mozambique, during a period of 12 months.

Table 1 - Results of the three-way PERMANOVA conducted on size of *Palaemon concinnus*. 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.

		Size of specimens		
Source	df	MS	F	P
Impacted (I) vs Control (C)	1	54453.00	22052.00	**0,0001**
location(I vs C)	1	20426.00	40424.00	**0,0484**
Sex/ovigerous	2	34,35	181,33	**0,0035**
I vs C x Sex/ovigerous	2	20739.00	11,27	0,0749
location(I vs C) x Sex/ovigerous	2	0,16816	0,3328	0,718
Residuals	237	0,5053		

The relative abundance of sexes strongly differed among sites (chi-square = 92.15; df = 2; p < 0.001), with males much more abundant in Saco and Costa do Sol and females more abundant in Ponta Rasa. Costa do Sol presented the higher ovigerous female frequency along the year, followed by Ponta Rasa and then Saco, merging these latter two sites in a single group representing the "control sites". We found significant differences in frequency of ovigerous females between the controls and the impacted sites (chi-square = 519.11; df = 2; p < 0.001; Figure 7-A).

The maturation index (Figure 7-B), calculated based on the SL at which 50% of the females are ovigerous at the time of sampling, presented Ponta Rasa with the lowest values of 5.3 mm, followed by Costa do Sol (6.15 mm) and Saco (6.25 mm).

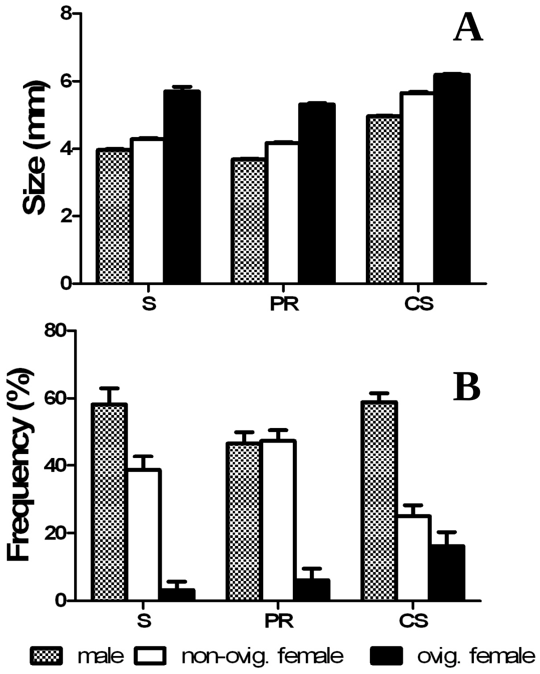

Figure 4 – Annual average size and frequency of males (grey), non-ovigerous females (white) and ovigerous females (black) of *Palaemon concinnus* population at the contaminated (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, south Mozambique. Values are given as average (±SE, n = 250-1895)

Although parasites were never observed in the cephalothorax of ovigerous females at all 3 sites, the percentage of infected males and non-ovigerous females at Costa do Sol was almost always superior to 30%, sometimes reaching more than 60% in medium size classes (Figure 7-C). The frequency of parasitized shrimps was significantly lower in pristine mangroves (chi-square = 179.19; df = 1; $p < 0.001$), always bellow 20%, and mostly between 5 and 10%. The frequency of parasite infection did not differed between sexes (chi-square = 1.01; df = 1; p = ns) and it varied with no specific temporal pattern, although oscillations were similar between Costa do Sol and Saco (Figure 5).

3.3 Physiological stress

The PERMANOVA and post hoc tests showed a significant ($p<0.03$) lower RNA/DNA ratio in PR compared to S and CS (Table 2, Figure 6). At the dry season, RNA/DNA ratio at CS was slightly higher but not significantly different from the ratio obtained at the wet season (Student's t = 2.03, df = 12, p =0.551). The overall average of RNA content was significantly ($p<0.001$) different from shrimps inhabiting all 3 mangroves, increasing from PR to S and then CS, with average values of 0.77±0.09, 1.06±0.18 and 1.61±0.19 µg per mg of freeze-dried dorsal muscle, respectively (Table 2). DNA content was not significantly different (Table 2) although higher in contaminated (1.04±0.15 µg per mg of freeze-dried muscle) than in pristine mangroves (0.68±0.08 and 0.71±0.11 µg per mg of freeze-dried muscle, for S and PR, respectively). Therefore, the overall lower RNA/DNA ratio in PR shrimp populations resulted from lower RNA content. At the dry season, CS population presented significantly ($p<0.01$) lower RNA as well as DNA content (1.25±0.16 and 0.69±0.11 µg per mg of freeze-dried muscle, respectively) than at the wet season.

3.4 Fecundity, Egg Development and Egg loss

The number of eggs tended to increase with animal size at all seasons, locations and egg stages. PERMANOVA and post hoc pair-wise tests revealed that females sampled at PR were slightly smaller that those collected at Saco and CS (Table 3, Figure 8-A,B). The PERMANOVA test performed on the number of eggs carried by each female, standardised by SL, showed a significant difference between Impact vs Control (Table 3, Figure 8-C,D). Post hoc pair-wise tests showed that females colonising the impacted site of Costa do Sol were producing more eggs at both egg stages than the ones collected in the two control sites ($t=323.3$; $P<0.001$), and, moreover, that at the impacted site there was a significant egg loss, with females carrying significantly less 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.

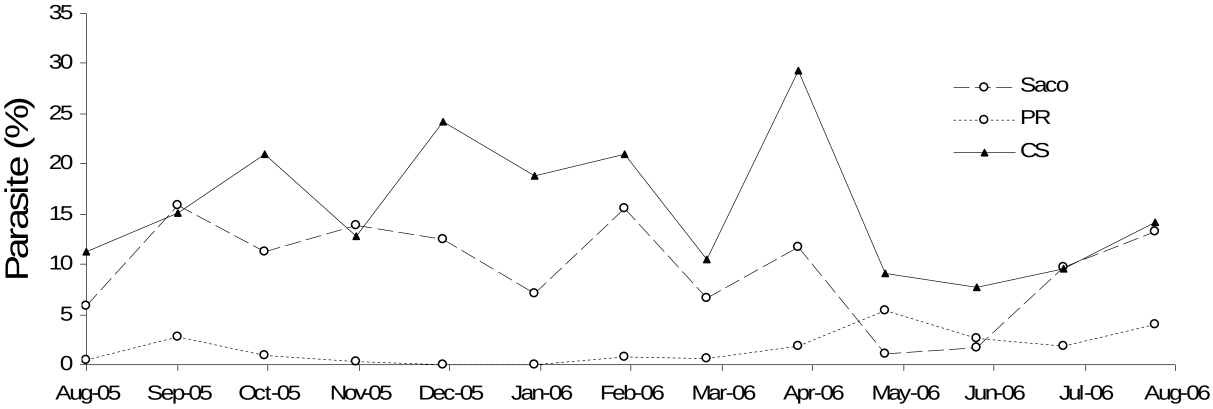

Figure 5 – Monthly parasitic frequency of *Palaemon concinnus* population by *Pseudione elongata africana* at the Peri-urban (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, at southern Mozambique, during a period of 12 months.

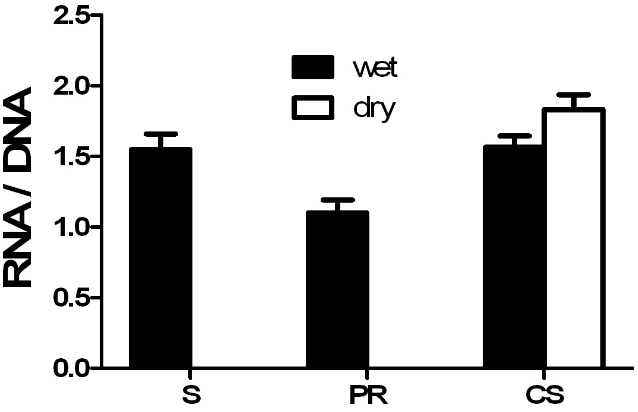

Figure 6 - Mean RNA/DNA (±SE) ratios of *Palaemon concinnus* in three different Mozambican mangrove ecosystems, in the rainy and dry seasons.

Table 2 – Results of the two-way PERMANOVA conducted on RNA/DNA ratio, RNA and DNA content (μg) per weight of muscle (g). Factors are as follows: Impact vs Control (asymmetrical, fixed and orthogonal) and site (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		RNA/DNA			RNA			DNA		
Source	df	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		

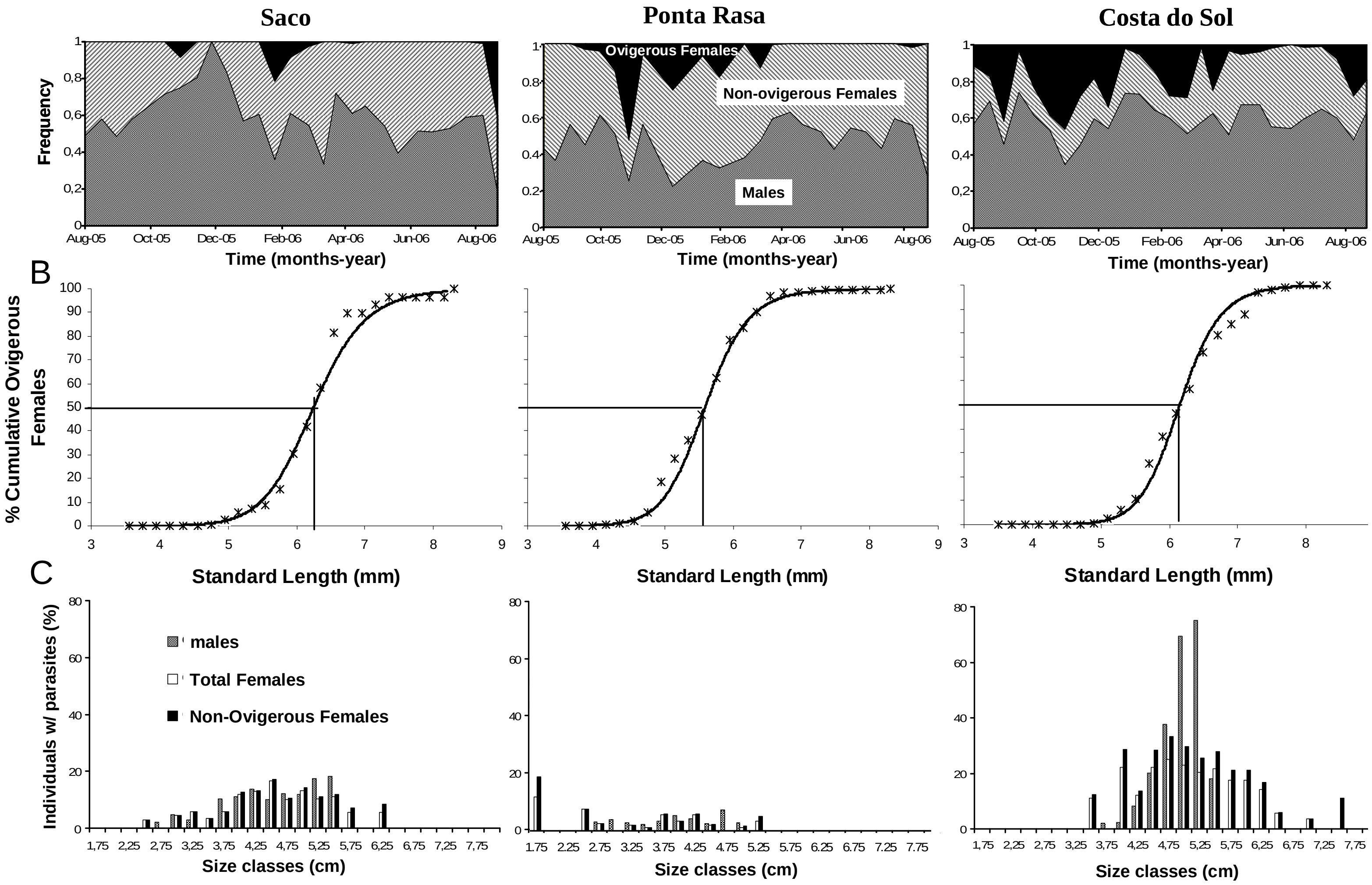

Figure 7 – A) Frequency variation (every 15 days) of males (grey), non-ovigerous females (white) and ovigerous females (black); B) Logistic function fitting the cumulative proportion of ovigerous females; and C) Percentage, per size class, of males, non-ovigerous females and total females infected with parasite *Pseudione elongata africana* in the cephalothorax of *Palaemon concinnus* population at the contaminated (Costa do Sol) and pristine (Saco and Ponta Rasa) mangroves, south Mozambique.

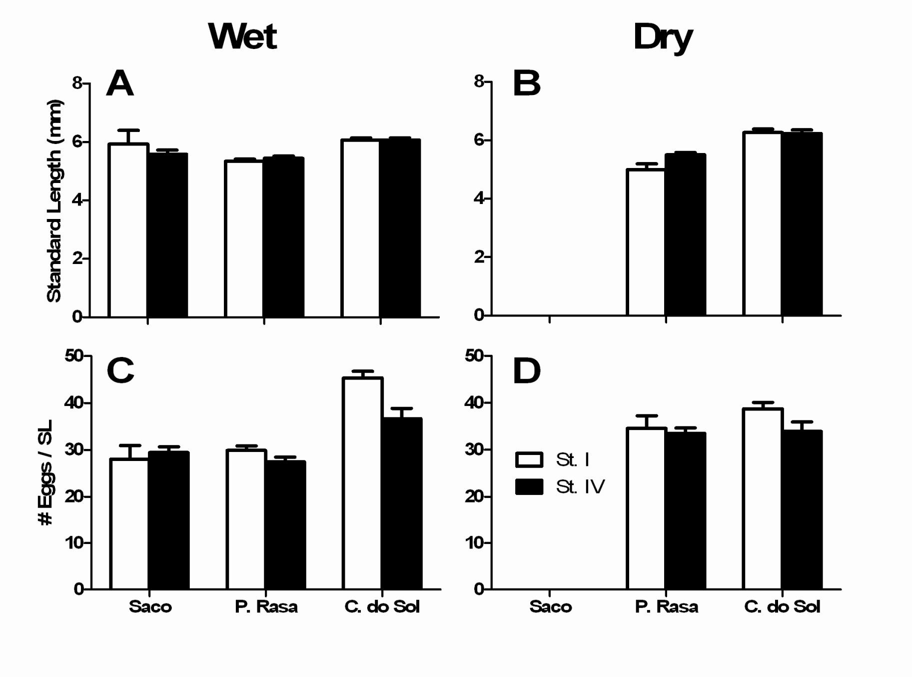

Figure 8 – Average (±SD) dimensions (A) and number of carried eggs, standardised on carapace dimensions (B) observed for the females carrying eggs of stage I and IV collected in the study locations.

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 sites. Factors are as follows: Impact vs Control (asymmetrical, fixed and orthogonal), site (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.

A - Wet seasons		SL			# eggs/SL		
Source	df	MS	F	P	MS	F	P
Impact vs Controls - I vs C	1	6.77	2.59	0.67	**4251.70**	**104920.00**	**0.00**
Stage - St	1	0.10	0.31	0.66	594.55	8.39	0.17
Location(I vs C)	1	1.46	5.87	**0.02**	0.02	0.00	0.98
I vs C x St	1	0.09	0.30	0.67	477.11	6.89	0.25
Location (I vs C) x St	1	0.54	2.17	0.14	42.38	0.59	0.42
Res	175	0.25			71.44		
Total	180						

B - Dry seasons		SL			# eggs/SL		
Source	df	MS	F	P	MS	F	P
Impact vs Controls - I vs C	1	23432.00	62738.00	**0.00**	121.67	16403.00	0.22
Stage - St	1	13391.00	35854.00	0.06	193.92	26144.00	0.10
I vs C x St	1	1791.00	47952.00	0.03	80513.00	10855.00	0.30
Res	104	0.37			74173.00		
Total	107						

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; Northington and Hershey, 2006) and possible explanations include increased mortality, slower growth, dispersal of larger fish away from the sewage, increase in the predation or preferential recruitment. Shrimp species are usually used as biomonitors of different types of contaminants (Gokoglu et al., 2008; Opuene and Agbozu, 2008; Tang et al., 2009) but no study has use 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 the all size range in particular species (e.g., Calado & Narciso, 2003; Penha-Lopes et al., 2007; Torres et al., 2007), and none has used it as a proxy of anthropogenic pollution in mangrove habitats.

Although we have not analysed population dimension and productivity, which could have also been a good proxy of organic loading into the systems (deBruyn et al., 2003; Cross et al., 2006), the overall size distribution and dynamics during the sampling period have shown that the peri-urban site presented the largest shrimps, independent of sex and female maturity. Loading of organic detritus and stimulation of primary productivity, bacteria and microalgae, (Hillebrand et al., 2000; Savage et al., 2004), may have supplied the shrimp with more abundant and diverse food sources, thus increasing growth rates and maximum length. Similar results were found to occur in Sudanese fairy shrimp, *Streptocephalus proboscideus*, being fed agro-industrial waste products (Ali and Brendonck, 1993).

Although most crustaceans reduce growth when energy is diverted to reproductive processes (Anger, 2001; Hartnoll, 2006), in our case it seems that anthropogenic contamination supplies enough food sources to sustain higher growth rates, higher percentage of ovigerous females and longer reproductive seasons. At the contaminated mangrove, nearly 40% of all females were ovigerous while at the pristine mangroves these percentages were rarely observed above 10-15%. Higher percentage of ovigerous females, mainly during the wet and warmer seasons, as commonly observed for other palaemonid species inhabiting temperate estuaries (Cartaxana, 1994), increased the population average size at Costa do Sol 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 eggs clutches, as was found in the present study and for other hippolytid shrimp populations inhabiting mangroves 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 the fiddler crab *Uca annulipes* at Costa do Sol (Penha-Lopes et al., accepted-c) and fish species inhabiting near sewage outfalls locations (Smith and Suthers, 1999). Continuous reproduction of *Palaemon concinnus* and *Uca annulipes* at Costa do Sol (Penha-Lopes et al., accepted-c), as well as *Panopeus americanus* mud crab inhabiting contaminated mangrove in Brazil (Vergamini and Mantelatto, 2008), has not been registered in populations inhabiting nearby pristine mangroves. Continuous reproduction, as well as increment in fertility, may be due the higher nutrient and food availability to the shrimp population found at the contaminated mangrove. Lower salinity has also been considered as one of the main responsible factors for higher reproduction activity in crab species inhabiting southern Mozambique mangroves (Litulo, 2004; 2006), and usually mangrove creeks suffering high discharges from domestic wastewater have lower salinity during the wet season (see Figure 2). Also, Costa do Sol wastewater may also contain a large variety and/or quantity of endocrine disruptors (such as hormone-like substances), which could affect the fish reproductive activity (Jobling and Tyler, 2002; Markert et al., 2004). However, improvement of reproductive activity could also be considered as a strategy to establish and maintain a stable population living associated with stressful energy demanding conditions in human-impacted mangroves (Vergamini and Mantelatto, 2008).

The brood loss reported in the present study for *P. concinnus* is basically restricted for the pristine sites but significant 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 disturbances (Ford et al., 2003). For example, resortion of oocytes is also a common response of fish species inhabiting contaminated estuaries (Jobling et al., 2002). Production of big egg clutch (stage I) size, due to higher amount of food sources available and limited space available to hold the growing embryos (Lardies and Wehrtmann, 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., accepted-c).

Maturation index was tested as an indicator of domestic sewage pollution by potentially reducing or increasing the size at which 50% of the females are ovigerous, however this potential bioindicator was similar between contaminated and pristine mangroves studied.

4. 2 Physiological condition (RNA/DNA)

The RNA/DNA ratio has been an alternative indication of the physiological condition of ecologically relevant organisms in a natural context (e.g., Norkko et al., 2006; Amaral et al., 2009a), 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 *Uca 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 indicated that individuals were 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.

Ponta Rasa presented a significant lower RNA/DNA compared to Saco and Costa do Sol. However this is due an increase of RNA content from PR to CS, while DNA content was significantly higher at CS than at both populations inhabiting pristine mangroves. By analysing only RNA content, we must assume that CS populations presented the lowest stress, being well correlated with the larger size and nutritional condition of such organisms, compared with pristine locations. Although higher cellular DNA content among closely related species has been documented in several crustacean species (e.g., Gregory et al., 2000; Amaral et al., 2009b), within the same species it has not been demonstrated. Although samples were re-analysed, in order to sort out methodological faults in laboratory, it is known that larger shrimp ovigerous females were collected at CS, which could be supported by larger muscle fibres (Govind et al., 1986; Rhodes, 1986), possibly originated by larger muscle cells (e.g., Penney et al., 1983). Therefore, a similar weight of freeze-dried muscle tissue may contain fewer cells, and thus lowering DNA content in *P. concinnus* inhabiting the peri-urban mangrove. Although a similar explanation was used by Amaral et al (2009b) to explain less DNA content in fiddler crabs with larger claws, only histological and genetic studies could clarify this issue.

4.3 Parasitism

Not only pollutants were found to cause endocrine disruption on fishes, but also parasites (Jobling and Tyler, 2003). In the present study, the isopod *Pseudione elongata africana* was not found in a single ovigerous female, clearly indicating that they inhibit shrimp reproduction or no longer can parasite a mature female. However, *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 (O´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.

Peri-urban mangrove parasite infection rates for both males and non-ovigerous females were usually much higher than the ones obtained at Saco and especially Ponta Rasa, indicating that hosts at Costa do Sol 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 indicators of pollutants, because this complex seems to meet all of the requirements suggested by Kennedy (1997) to use 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.

Nevertheless, one interesting detail is that while Costa do Sol presented the highest parasite percentage, it is also where we observed the longest reproductive period, highest ovigerous female rate and larger egg clutches. This may indicate that, while on one side Costa do Sol provides "resources" to boost reproductive fitness (positive indication) at the same time increases the parasite rate in males and non-ovigerous females, reducing the reproductive potential of this population. 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 shown to affect in a "positive way" mangrove ecosystems, due the nutrient limitations that these ecosystem have. Growth, fertility and reproductive output are some of these examples presented in this study. *Palaemon concinnus* - *Pseudione elongata* interrelationship is one of the factors studied that indicated some degree of stress (negative effect) on the host shrimp at Costa do Sol. The identification of the effects of sewage on *P. concinnus* increases the choice of possible bioindicators in East African coastal mangroves.

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Chapter 4

Are fiddler crabs potentially useful ecosystem engineers in mangrove wastewater wetlands?

Gil Penha-Lopes [1, 2*], Fabrizio Bartolini [3], Samwel Limbu [4], Stefano Cannicci [3], Erik Kristensen [5] and José Paula [1]

[1] Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211
[2] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050, Brussels, Belgium
[3] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy
[4] University of Dar es Salaam, Faculty of Aquatic Sciences and Technology, P.O. Box 35064, Dar es Salaam, Tanzania
[5] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark

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Gil Penha-Lopes[1, 2*], Fabrizio Bartolini[3], Samwel Limbu[4], Stefano Cannicci[3], Erik Kristensen[5]
and José Paula[1]

[1] Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211

[2] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050, Brussels, Belgium
[3] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy
[4] University of Dar es Salaam, Faculty of Aquatic Sciences and Technology, P.O. Box 35064, Dar es Salaam, Tanzania
[5] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark

ABSTRACT

The effect of different organic-rich sewage concentration (0%, 20% and 60% diluted in seawater) and absence or presence of mangrove trees on the survival, bioturbation activities and burrow morphology of fiddler crabs species was assessed. After 6 months, males of both species always showed higher survival (~80%) when compared to females (~20%). Crabs inhabiting pristine conditions achieved higher survival (67-87%) than those living in sewage-exposed mesocosms (40-71%). At 60% sewage loading, fiddler crabs processed less sediment (34-46%) during feeding and excavated slightly more sediment (45-80%) than at pristine conditions. While percentual volume of the burrow chambers increased (13-66%) at contaminated mesocosms for both vegetation conditions, burrows were shallower (~33%) in bare cells loaded with sewage. The results show that fiddler crabs presented moderate mortality levels in these artificial mangrove wetlands, but mainly in sewage impacted cells. However, they still function as ecosystem engineers through bioturbation activities and burrow construction.

Keywords: sewage, survival, bioturbation, ecosystem engineer, fiddler crabs, mangroves

1. INTRODUCTION

Constructed freshwater wetlands are now a worldwide accepted technology for water pollution control, mainly due to low running cost (low energy consumption and training requirements) and high filtration efficiency (Crites et al., 2006) Mangrove forests have recently shown a potential as natural wastewater treatment facility in China, by removing nutrients and organic matter efficiently (Wong et al., 1997; Yang et al., 2008). Growth and productivity in natural and pristine mangrove forests are usually nutrient limited (Hogarth, 2007). Consequently, discharge of moderate sewage loadings results in enhanced growth of trees (Mohamed et al., 2008) as well as stimulation of benthic primary producers and microheterotrophs (Tam, 1998; Meziane and Tsuchiya, 2002). The effect on macrofauna biomass and diversity seems to be dependent on the degree of contamination, proximity to the effluent and sensitivity of the species (Yu et al., 1997; Wear and Tanner, 2007; Cannicci et al., in press).

The benthic fauna in mangrove forests is typically dominated by burrowing decapods, such as fiddler crabs (*Uca* spp.). These crabs establish dense populations on intertidal flats (Skov and Hartnoll, 2001; Skov et al., 2002) making them important grazers on microalgae and bacteria (Bouillon et al., 2002; Reinsel, 2004), as well as key ecosystem engineers (Kristensen, 2008). The effects of *Uca* spp. on mangrove habitat characteristics have been extensively studied (for a review see Cannicci et al., 2008; Kristensen, 2008). Their crawling and foraging activities process a significant amount of the top 2 cm sediment layer during a single low tide period (Dye and Lasiak, 1986). As a consequence, they disturb the microbenthic primary producers and meiofauna communities in the top sediment (Ólafsson and Ndaro, 1997; Kristensen and Alongi, 2006). In addition, these crabs actively dig and maintain burrows in the sediment which function as a refuge from predation and adverse environmental conditions (e.g., high tide), provide water for the crabs' physiological needs (at low tide), and are used for moulting and reproduction (Crane, 1975). These biogenic structures may easily reach and pass 10 cm depth for adult mangrove fiddler crabs (Lim and Heng, 2007). Through their crawling, foraging and burrow construction and maintenance activities, *Uca* species increase sediment drainage, soil redox potential, translocate sediment, organic matter and nutrients, change sediment erosion threshold, and increase the sediment surface area (Wolfrath, 1992; Botto and Iribarne, 2000; Kristensen, 2008). Consequently, they stimulate microbial metabolism, organic matter degradation and nutrient cycling, as well as flora productivity (Gribsholt et al., 2003; Nielsen et al., 2003; Kristensen and Alongi, 2006), which may also potentially counteract adverse effects of anthropogenic eutrophication

(Nielsen et al., 2003; McHenga and Tsuchiya, 2008).

Stimulation of microalgal and bacterial growth in sewage contaminated mangrove environments has apparently no effect (Yu et al., 1997) or increases fiddler crab abundance and/or biodiversity when compared to pristine mangrove areas (Cannicci et al., in press). This is probably caused by an enhanced reproductive performance of crabs due to the surplus of food availability (Penha-Lopes et al., submitted), particularly in areas where environmental conditions (such as wind and water currents) restrict offshore dispersion of the pelagic larvae (Paula et al., 2001). However, under poor hydrodynamic conditions or in closed wastewater wetlands, organic enrichment above the ecosystem capacity may lead to eutrophication and consequently hypoxic conditions (Gray et al., 2002). Low oxygen levels can result in faunal migration to normoxic zones, lower survival and activity, and smaller burrow structures of marine benthic organisms. As a consequence, a decrease in the ecological role of bioturbation is observed (Diaz and Rosenberg, 1995). Among all benthic animals, crustaceans are considered one of the most sensitive species to hypoxia, only surpassed by fish. First their activity is decreased under hypoxia, and secondly exhibit high mortality if low oxygen concentrations are maintained for extended periods (Gray et al., 2002).

Knowledge on the ecological responses of mangrove associated fauna to urban sewage loadings is necessary. Understanding of direct and indirect effects of severe sewage contamination on crab survival and bioturbation activities is fundamental to comprehend the effects of organic discharge on mangrove forests and to develop sustainable mangrove wastewater wetlands. In this study, we addressed the effects of domestic sewage loading and vegetation type on (1) the survival, (2) bioturbation activity due to feeding and burrowing, and (3) burrow morphology of two fiddler crabs species, *Uca annulipes* (H. Milne Edwards, 1837) and *Uca inversa* (Hoffmann, 1874), in constructed mangrove mesocosms.

2. MATERIALS AND METHODS

2.1 Mesocosm setup

Mangrove mesocosms were constructed in the upper intertidal zone of the Jangwani mangrove forest, Dar es Salaam, Tanzania. The system consisted of 27 cells (9 m^2 each) separated by 1-m tall cement walls and protected from tidal influence by a soil embankment. The sediment inside the cells was composed of the original sandy mangrove substratum from the area. Each cell was equipped with in- and outflow pipes for tidally simulated flooding and drainage with seawater or sewage mixtures. A daily tide with 12 h inundation (0.1 m water depth) starting at 23:00 and 12 h exposure starting 11:00 was applied. Sewage from a nearby hotel facility was subjected to primary treatment in a 86 m^3 storage pond before entering the mesocosm system. For complete details of the mesocosm construction and performance see PUMPSEA (2008).

The cells were either kept as unvegetated controls (bare), planted with *Rhizophora mucronata* Lam. or *Avicennia marina* (Forsk.) mangrove trees. These two species were chosen since they dominate mangrove areas along the Eastern African coast. Mangrove saplings were planted (at a density of 2.8 m^{-2}) on selected cells in early February 2006. Epifauna was introduced to all cells in late August, including males and females of two of the most abundant crab species in the area (*Uca annulipes* and *U. inversa*) and the common mangrove gastropod, *Terebralia palustris* (Linnaeus, 1767). The fauna was randomly collected from the Kunduchi mangrove forest immediately prior to introduction. A density of 5 ind. m^{-2} per sex and species was applied, except for *Uca annulipes* females with only 3 ind. m^{-2}. These are at the low range of natural densities in the area (Skov et al., 2002).

The mesocosm system was inundated exclusively with seawater from February to early October 2006. Subsequently, sewage loadings of 0, 20 and 60% mixed in seawater were applied. Three replicate cells were assigned for each sewage loading and vegetation treatment. The chosen sewage loadings are known to enhance growth of *A. marina* and *R. mucronata* (PUMPSEA, 2008). The chemical characteristics and biological oxygen demand (BOD) of sewage-seawater mixtures are presented in Table 1 and chlorophyll a concentration at the sediment surface is presented in Table 2.

Table 1 - Chemical characteristics and oxygen uptake of the sewage-sea water mixtures used in the experimental mesocosms from October 2006 to April 2007. (DO: Dissolved Oxygen; BOD: Biological Oxygen Demand) (N = 45). Values are given as averages ± SE (adapted from PUMPSEA, 2008). Different letters indicate significant difference – $p < 0.05$ – between sewage loading treatments

Sewage loading	**Salinity (‰)**	**DO_{day} (µM)**	**DO_{night} (µM)**	**BOD (µM h^{-1})**	**NH_4^+ (µM)**	**NO_3^- (µM)**	**PO_4^{3-} (µM)**
0%	39.1 ± 0.6^a	308 ± 133	197 ± 17^a	5.1 ± 2.6^a	26 ± 2^a	3.6 ± 1.4	42 ± 4^a
20%	24.5 ± 0.9^b	312 ± 227	10 ± 5^b	12.9 ± 2.9^a	123 ± 9^b	7.1 ± 2.9	109 ± 6^b
60%	18.9 ± 1.4^c	225 ± 256	9 ± 1^b	21.3 ± 1.2^b	194 ± 17^{c42}	5.0 ± 1.4	206 ± 14^c

Table 2. Sediment surface chlorophyll-a (Chl-a (µg.g^{-1})) concentration at different vegetation and sewage concentration treatments in April 2007 is shown (N = 12). Values are given as averages ± SE (adapted from PUMPSEA, 2008).

	Chl-a (µg.g^{-1})		
	0%	**20%**	**60%**
Bare	13.9±3.9	12.5±6.1	23.1±9.4
A. marina	5.4±1.1	17.1±12.0	14.0±3.5
R. mucronata	8.1±2.7	10.1±4.0	14.7±7.0

2.2 Sediment oxidation level

The relative difference in Fe (III) and Fe(II) concentration is an excellent indicator of sediment oxidation level (Canfield et al., 2005). Two sediment cores were taken for solid phase Fe analysis from only one random cell per treatment due to logistic constraints. In the laboratory cores were sliced into the following depth intervals: 0-1, 1-2, 2-3, 3-4, 4-6, 6-8 and 10-12 cm before any further handling. Solid phase Fe was extracted by a modified version of the HCl technique of Lovley & Phillips (1987). Briefly, 100-300 mg subsamples were extracted in 5 ml of 0.5 M HCl for 30 min on a shaking platform. After centrifugation (1500 rpm) for 10 min, 50 µl of the supernatant were transferred to 2 ml Ferrozine solution for Fe(II) analysis and to 2 ml Ferrozine solution containing the reducing agent hydroxylamine (10 g l^{-1}) for total-Fe (Fe(II)+Fe(III)) analysis. The reactive amorphous Fe(III) oxide concentration was then operationally defined as the difference between total Fe and Fe(II).

2.3 Crab survival

The survival of crabs was checked 6 months after the start of sewage discharge into the mesocosm cells (end of March 2007). The density of crabs was determined by counting the number of active burrows in each cell. In addition, visual counting of active crabs on the surface of each cell was done to obtain the species and sex ratio (Skov et al., 2002). Although some crabs retreated into their burrows when the observer approached, the crab population on the surface was restored with normal activities after 5 minutes (Bartolini et al., submitted). Counting was conducted early in the afternoon (after flushing out the cells) to obtain the maximum number of active crabs outside their burrows (Bartolini et al., submitted). The counting procedure was replicated twice and the highest burrow and crab counts where used to estimate survival.

2.4 Bioturbation activities

Fiddler crabs feed at the sediment surface by sorting bacteria and microalgae from sediment grains placed in the mouth cavity by the minor chaela. The non-ingested particles are shaped into small (up to 3 mm) irregular balls, with colour and grain size similar to the surrounding surface sediment (‘feeding pellets’). When crabs construct and maintain burrows, they form mounds (usually pellets with a diameter of 5-7 mm) near the opening. New mounds have a black-grey coloration typical of reduced sediment because they consist of subsurface sediment (‘burrowing pellets’) extracted deep in burrows (Botto and Iribarne, 2000).

The two bioturbation modes performed by crabs (feeding and burrowing) were studied in only control (bare) and *A. marina* mesocosm cells at 0% and 60% sewage loadings due to logistical limitations. Since crabs were active until sunset (around 18:00) during emersion periods, all feeding and burrowing pellets produced were collected from around 19:00 to 23:00 in the haphazardly chosen cells. The sediment surface was smoothened when cells were flushed with water during inundation, at 23:00, which removed all irregularities and eliminated the bioturbation effects from the previous emersion period. This sampling procedure was repeated in two different tidal periods. Sampled sediment pellets were dried at 60ºC for 48h and weighed (0.01g). The amount of collected sediment due to bioturbation was correlated to the maximum number of surface active crabs in each sampled cell and compared between treatments.

About 30 random active crab holes were chosen in each examined cell for burrow resin casts. However, due to difficulties in obtaining complete casts, only between 8 and 28 were considered successful. The chosen burrow openings were, after 18:00, filled with a resin and hardening/catalyser solution until water started to emerge from the hole as an indication that the whole burrow was filled with resin. When the casts had solidified for about 2 h, they were dug up for subsequent measurement of burrow dimensions. Entrapped crabs in the burrow casts were identified to species and sexed when possible. However, due to the low number of identified casts, only data combining all burrows will be presented.

Uca burrows are usually considered simple L or J shaped structures (e.g., Lim and Diong, 2003). However, for the present purpose we used a classification that divided burrows into 3 sections: an upper neck with entrance to the surface, a middle chamber and a deep extension leg. The distinction between the 3 sections were identified from the ratio between burrow (B_p) and surface opening perimeter (O_p): $R = B_p/O_p$. The chamber section was defined as burrow regions with $R > 1.45$, while the neck and extension leg were defined as burrow sections above and below the chamber, respectively, with $R < 1.45$.

Several additional burrow characteristics were also determined: total depth, wall surface area, total volume, volume of burrow sections, and angle of the different sections to horizontality. Total depth and angles were measured from lateral photographs of the burrow casts positioned with horizontal burrow entrance. The wall surface area was estimated from the length of tape with known width needed to cover the casts completely. The burrow volume was determined by dividing the weight of cast sections by the resin density (1.22 g cm^3). Although burrow depth, surface area and total volume was intended to be standardize to the burrow opening perimeter in order to minimize sampling bias due to differences in crab sizes, regression slopes were not different from zero and goodness of fitness (r^2) was always below 0.1. This way data was not standardized and even when data was standardized similar results were obtained (data not shown).

2.5 Statistical analysis

Differences in survival of the two *Uca* species between treatments were tested by a three-way ANOVA. A two-way ANOVA was applied to compare: pellet production by crab feeding and burrowing, burrow depth, surface area, total volume and percent volume of the 3 burrow sections between treatments (Vegetation vs Sewage loading), as well as chlorophyll-a concentration. A one-way ANOVA was applied to compare salinity, oxygen levels and nutrients of seawater and sewage solution. Prior to analyses, the homogeneity of variances was assessed using Cochran's test and transformation of data was performed. While *Uca* survival data and percent volume of the burrows sections were transformed to x' = ArcSin (x), *Uca* bioturbation data was transformed to x' = log (x+1). When appropriate, Student–Newman–Keuls (SNK) tests were used as post hoc tests for multiple comparisons of the means.

3. RESULTS

3.1 Mesocosms conditions

The sewage concentrations discharged into the contaminated mesocosms (20% and 60%) was lower in salinity, and higher in biological oxygen demand (BOD) than the seawater used to flush the pristine mesocosms (Table 1). As a consequence dissolved oxygen was low particularly during the night, while ammonium and phosphate concentrations were high in sewage treated cells. Chlorophyll a concentration in the top sediment tended to increase with sewage dosing and decrease with vegetation presence, although microalgae patchiness did not allow to find significant differences between treatments (Table 2).

Solid phase iron profiles differed among treatments (Figure 1). Total iron (Fe(II)+Fe(III)) content was about twice as high in planted (0-14 cm depth integrated: ca. 250 μmol cm^{-2}) than in bare cells (0-14 cm depth integrated: 100-140 μmol cm^{-3}) and was independent of contamination level. Fe(II) always dominated over Fe(III) in contaminated cells. Fe(III) never exceeded 20 μmol cm^{-3} and was almost absent below 2 cm depth in 60% sewage treated cells, irrespective of vegetation. Fe(II) approached, and occasionally exceeded, 20 μmol cm^{-3} in these cells, except at depths below 5 cm in bare 60% cells. Fe(II) and Fe(III) did not change significantly with depth in pristine (0%) cells. In general, Fe(III) was higher and Fe(II) lower in these cells than those exposed to 60% sewage. Fe(III) was particularly high at all depths, and in the vegetated 0% cells presented concentrations ranging from 5 to 30 μmol cm^{-3}.

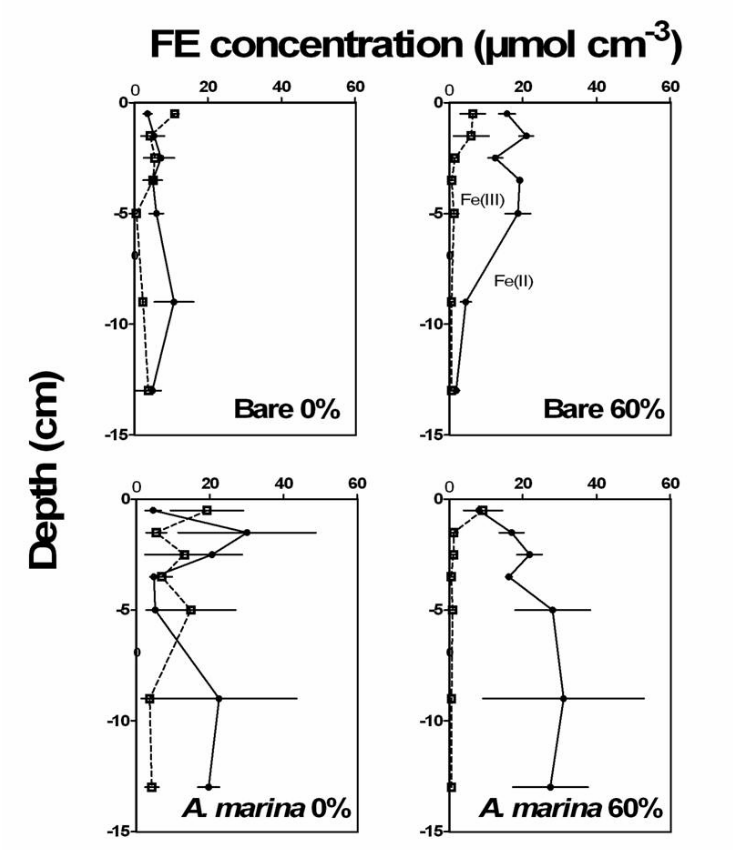
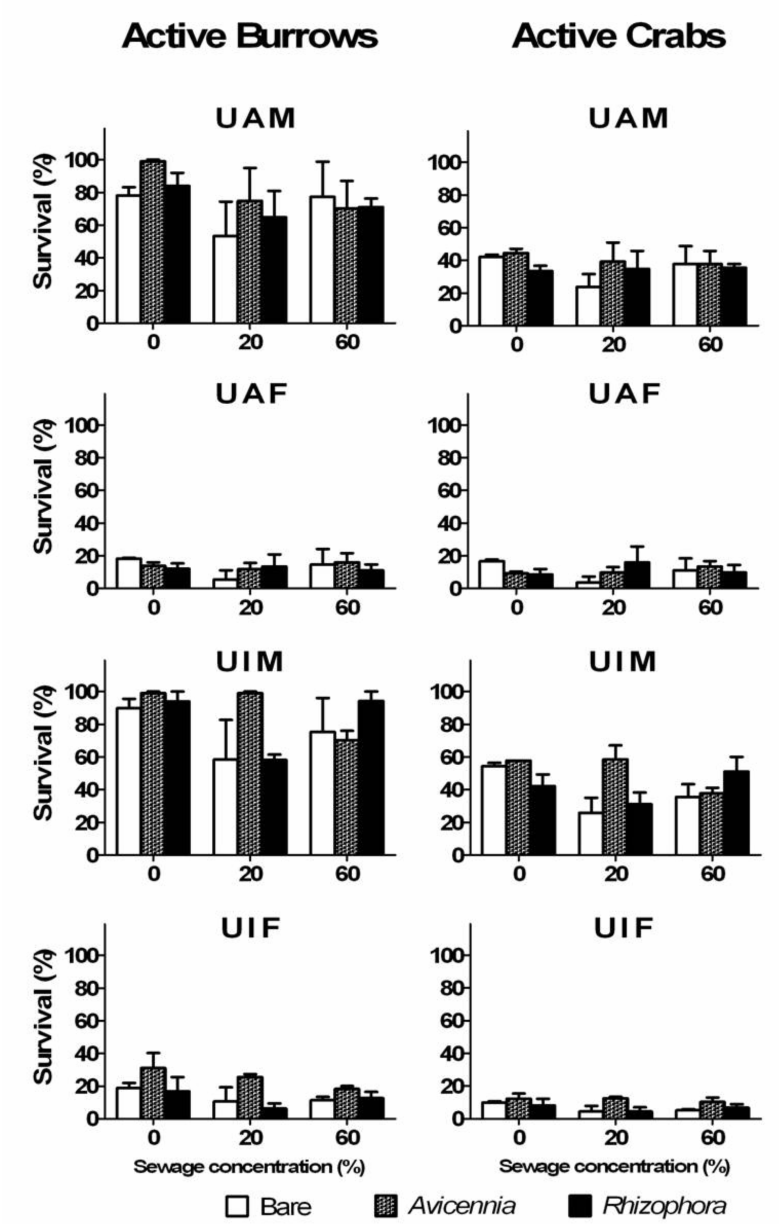


Figure 1 – Vertical profiles of solid phase iron in both vegetation and sewage loading conditions. Solid and dotter lines represent Fe(II) and Fe(III) concentration, respectively. Values are given as mean ± S.E (n = 2).

3.2 Crab survival

When active crabs are considered the only survivors in each cell (minimum estimate), male and female survivorship was estimated to 40% and 10%, respectively. However, from the



numbers of active burrows, male survivorship estimate increased to approximately 80% and females to 20% (

Figure **2**). While total survival was similar between species (males plus females), a significant decrease (Table 3) was observed in survival with increasing sewage loading, from 67-87% (0% sewage) to 40-71% (20 and 60% sewage). Vegetation, on the other hand, showed only a marginally significant positive effect on survival.

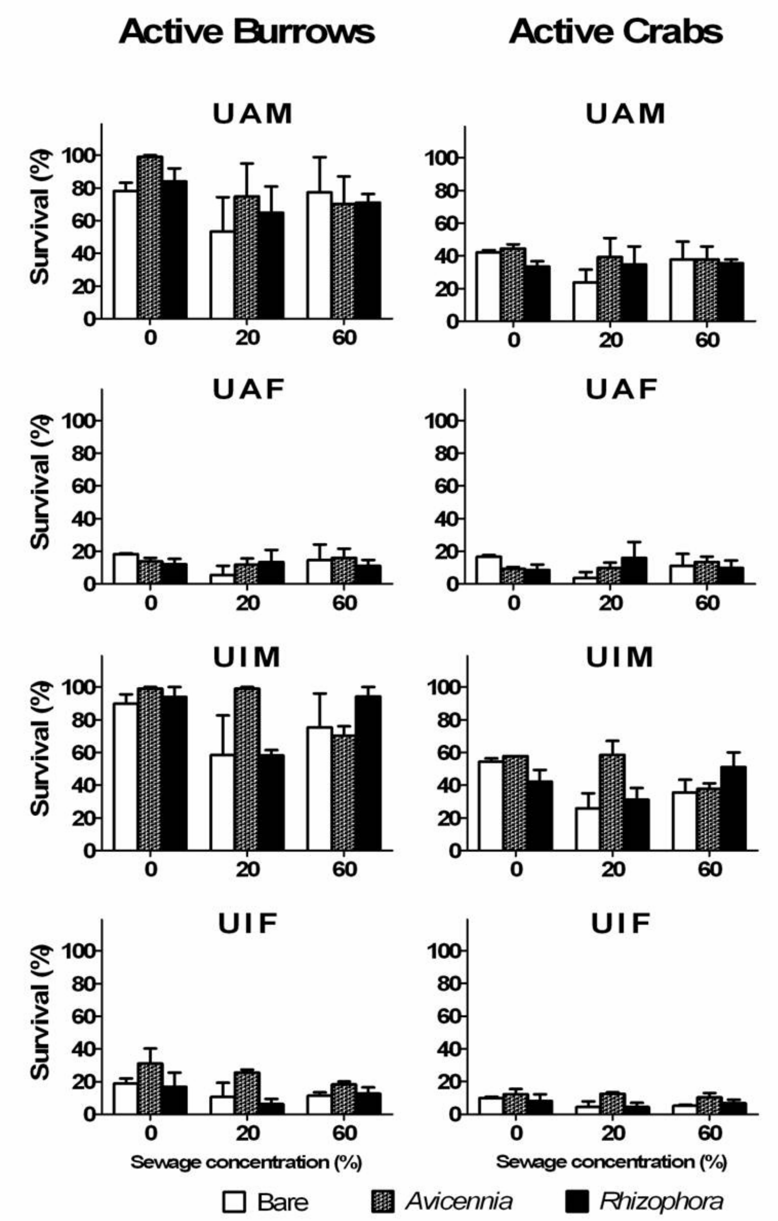


Figure 2 – Survival rates based on active burrows and active crabs of *U. annulipes* and *U. inversa* males and females after 6 months (April 07) for all vegetation factors and 0%, 20% and 60% sewage concentration treatments. Values are given as average (±SE), n = 3. UAM – *Uca annulipes* males; UAF – *U. annulipes* females; UIM – *U. inversa* males; UIF – *U. inversa* females.

3.3 Bioturbation activities

Each fiddler crab processed daily approximately 13 g dw during feeding in pristine (0%) cells, and showed a significant decrease by 34-46% at higher sewage concentration (Figure 3, Table 3). Conversely, there was no significant effect of vegetation on the amount of feeding pellets produced. The amount of sediment handled by crabs for burrow maintenance under pristine conditions (17 to 20 g dw per active crab^{-1} day^{-1}) was of similar magnitude to that processed by feeding. Crabs exposed to 60% sewage handled 45-80% more burrow sediment (Figure 3), but due to high variability among cells no significant differences were evident (Table 3). As for feeding activity, there was no apparent effect of vegetation on burrow maintenance.

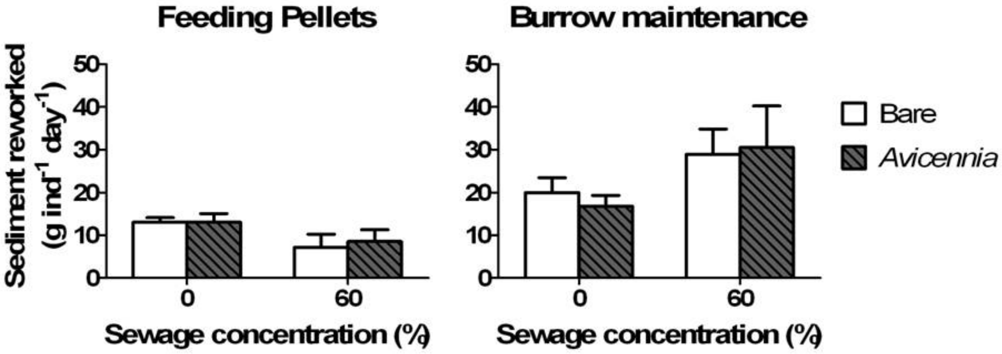


Figure 3 – Weight of sediment (g dw) processed by fiddler crabs feeding and burrowing activities at different sewage loadings and presence or absence of *A. marina* trees. Values are given as average (±SE), n = 3

Burrow morphology appeared visually affected by both vegetation and sewage loading (Figure 4). However, only burrow depth and relative volume of burrow sections were affected significantly (Figure 5 and 6, Table 3). Burrow surface area (22 to 29 cm^2), volume (34 to 48 cm^3), and section angles (neck: 64 to 72º; chamber: 42 to 56º; and extension leg: 52 to 55º), were similar under all vegetation and sewage treatments. Burrows in bare sediment were significantly 30% ($p < 0.01$) deeper under pristine than contaminated conditions (~10.6 cm), while no such difference was evident in the presence of vegetation (~14 cm deep). While sewage also affected the relative volume of all 3 burrow sections significantly, no vegetation effect could be detected. The volume of these sections was significantly ($p < 0.05$) greater (30%) in bare than planted cells under pristine conditions.

About 38% larger chambers ($p < 0.001$) were found at sewage contaminated mesocosms than at pristine ones in the presence of trees. Sewage did not influence relative burrow chambers in bare cells. When chamber volume increased both neck and extension leg volumes tended to decrease (17 to 35 %), although no significant differences were noted.

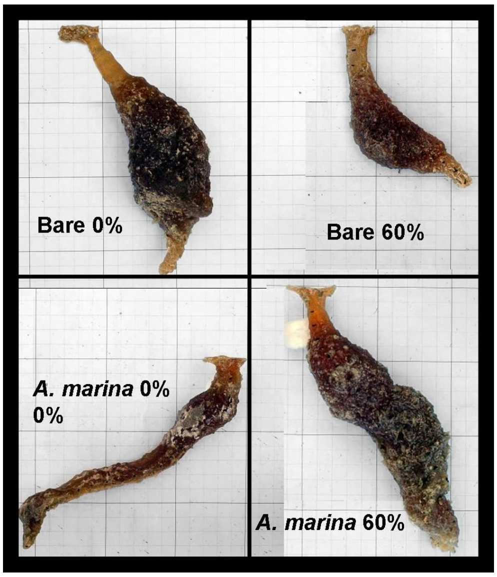


Figure 4 – Photos of illustrative burrow casts collected in all treatment types.

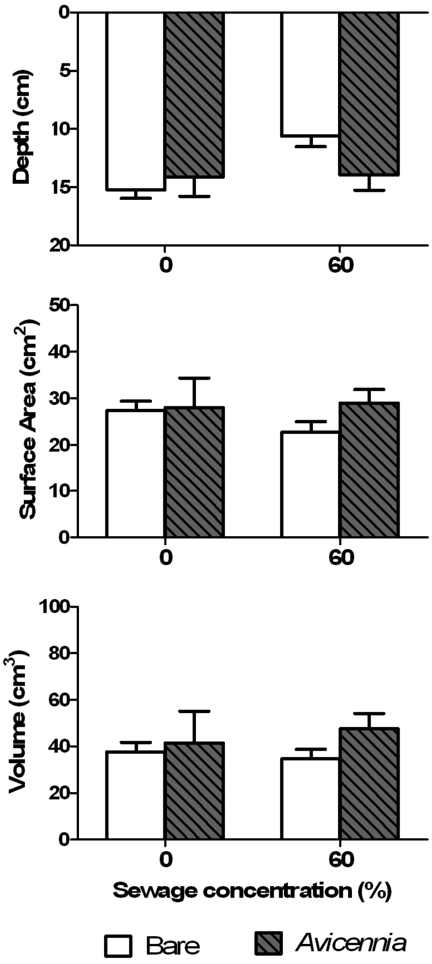


Figure 5 – Fiddler crab burrow depth, total volume and surface area for all active burrows for bare and *Avicennia marina* and 0%and 60% sewage loading treatments. Values are given as average (±SE) (n_{B0} = 28; n_{B60} = 16; n_{A0} = 8; n_{A60} = 15).

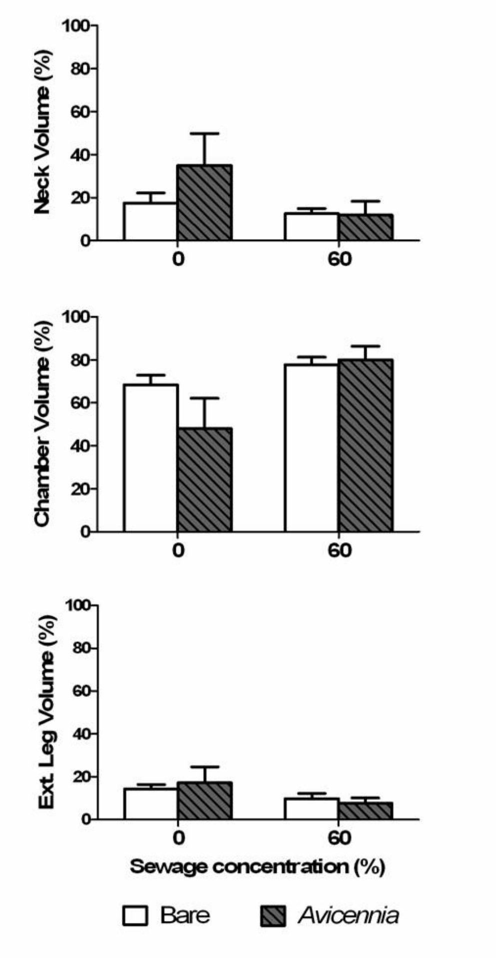


Figure 6 – Volumetric percentage of burrow neck, chamber and extension leg constituting fiddler crab's burrow for bare and *Avicennia marina* and 0% and 60% sewage loading treatments. Values are given as average (±SE) (n_{B0} = 28; n_{B60} = 16; n_{A0} = 8; n_{A60} = 15).

4. DISCUSSION

4.1 Crab survival

U. annulipes and *U. inversa* can survive for at least 6 months under extreme conditions with respect to daily inundation periods of 12 h, low salinity and dissolved oxygen levels in the water column, as well as in sediments. However, some mortality occurred, mainly concerning females of both species. Although crab survival was negatively affected by sewage exposure, female survival was apparently always low even at pristine conditions. This indicates either that: (1) low activity of females on the sediment surface may underestimate their real abundance (Skov et al., 2002), which occurs particularly during the breeding season usually more strongly during the wet season when the sampling was performed (Litulo, 2005 and references therein), or (2) females are more sensitive than males to the artificial conditions of the wetland with respect to the long and constant inundation cycles.

Table 3 – A) Results of the 3-way ANOVA on genus *Uca* survival rates (Oct 06 to April 07) (ArcSin transformation). B) 2-way ANOVA on sediment processed (expressed as DW) log (x+1) transformed for total sediment processed for feeding and during the burrow maintenance activity in different conditions. C) 2-way ANOVA on crab burrow depth, total surface area and volume at both sewage load and vegetation type; and D) 2-way ANOVA on volume (%) of neck, chamber and extension leg burrow sections at both sewage load and vegetation type (transformation ArcSin %). Factors: Species (fixed and orthogonal), Vegetation (fixed and orthogonal) and Sewage Concentration (fixed and orthogonal). The degrees of freedom, DF, Variance, MS, and values of F ratio are showed for each of the tests.

A	Survival Rate (April 07 – Oct 07)		
Factors	Df	MS	F
Species (sp)	1	0.08	0.12
Vegetation (ve)	2	2.14	**3.39**[a]
Sewage (se)	2	3.92	**6.21**[b]
Species x Vegetation	2	0.15	0.25
Species x Sewage	2	0.09	0.14
Vegetation x Sewage	4	0.99	1.57
spXveXse	4	0.37	0.59
RES	36	0.63	
TOT	53		

B		Feeding Pellets		Burrow Maintenance	
Source	DF	MS	F	MS	F
Vegetation	1	2.95	0.09	3.70	0.02
Sewage	1	163.30	**4.91** [a]	767.10	3.47
Interaction	1	3.00	0.09	33.01	0.15
RES	20	33.22		221.30	221.31
TOT	23				

C)		Depth		Surface area		Volume	
Source	DF	MS	F	MS	F	MS	F
Vegetation	1	17.56	0,99	107.10	0,79	278.60	0,50
Sewage	1	79.29	**4,46**[a]	168.00	1,23	967.10	1,73
Interaction	1	67.04	3,77	47.14	0,35	30.80	0,06
RES	63	17.76		136.20		558.90	
TOT	66						

D)		Vol. neck		Vol. chamber		Vol. extension leg	
Source	DF	MS	F	MS	F	MS	F
Vegetation	1	0,10	1,59	0,14	2,37	0,00	0,02
Sewage	1	0,27	**4,31**[a]	1,10	**18,28**[b]	0,07	**4,89**[a]
Interaction	1	0,13	2,04	0,31	**5,15**[a]	0,01	0,53
RES	63	0,06		0,06		0,01	
TOT	66						

(a = $p < 0.05$; b = $p < 0.001$)

Table 4 –Total Survival and survival based on active burrows counts or active crabs counts corrected with previous studies (see discussion section for further explanation). Burrow counting overestimate in about 30% while visual counting underestimate in about 40%, so all raw data obtained was multiplied by 0.7 or 1.4, respectively. Values are given as averages ± SE. (N = 3)

			UAM		UAF		UIM		UIF	
	Sewage (%)	Total survival	Active Burrows (corrected)	Active Crabs (corrected)	Active Burrows (corrected)	Active Crabs (corrected)	Active Burrows (corrected)	Active Crabs (corrected)	Active Burrows (corrected)	Active Crabs (corrected)
Bare	0	67.3±3.6	54.7±6.2	59.1±1.8	12.8±0.4	23.3±1.5	62.9±4.1	76.2±2.7	13.3±2.1	14.0±0.9
	20	39.6±7.5	37.3±14.8	33.2±11.0	3.9±3.9	5.2±5.2	40.9±17.0	36.3±12.7	7.4±6.1	6.2±4.8
	60	59.2±11.5	54.1±15.1	52.9±15.3	10.2±6.8	15.6±10.4	52.8±14.5	49.8±10.9	8.0±1.4	7.3±1.0
Avicennia	0	87.6±5.7	69.3±0.7	62.2±3.6	9.7±1.5	13.0±1.5	69.3±0.7	80.9±0.0	21.8±6.5	17.1±4.5
	20	71.7±7.3	52.4±14.1	55.0±16.3	8.3±2.6	13.8±4.6	69.3±0.7	81.9±12.0	17.9±1.3	17.6±1.0
	60	55.0±6.4	49.2±11.8	52.9±11.2	11.2±4.0	19.0±4.6	49.2±6.9	52.9±4.8	12.8±1.3	14.5±3.7
Rhizophora	0	76.5±4.1	58.9±5.6	46.7±4.8	8.5±2.3	12.1±4.6	65.9±7.2	59.1±10.0	11.9±6.0	11.4±5.8
	20	49.8±7.1	45.5±11.1	48.7±15.3	9.5±5.1	22,5±13.5	40.9±3.9	43.6±10.0	4.4±2.3	6.2±3.6
	60	61.6±6.0	49.7±3.7	49.8±3.1	7.7±2.7	13.8±6.2	66.0±4.0	71.6±12.6	8.9±2.7	9.3±3.1

Most studies on the effect of hypoxia on crabs have targeted mainly commercial and/or non-burrowing species. These have shown that most crabs do not survive more than a few hours or days, depending mainly on the species and hypoxia levels (Diaz and Rosenberg, 1995). Crabs do not possess efficient anaerobic metabolism and they are more dependent on their O_2 transport system than most other benthic invertebrates (Das and Stickle, 1993). However, both *U. annulipes* and *U. inversa* are known to inhabit and seal their burrows during immersion periods (De la Iglesia et al., 1994). They must therefore to some extent have the capacity to withstand the potentially hypoxic conditions that develop inside burrows for several hours. In our experiment, they adapted rapidly to the “artificial 12 h daily tides” used in the mesocosms, and remained inside burrows during immersion periods as under natural conditions. By doing so, they avoided hypoxia in the water column of contaminated cells, but had a higher risk of hypoxic exposure inside burrows. Under normal conditions they capture an air bubble inside while sealing their burrows, which provides sufficient oxygen for breathing until the next emersion period (De la Iglesia et al., 1994). The high organic matter availability and high microbial metabolism in contaminated mesocosms may be responsible for low oxygen levels in the water column and sediment (Canfield et al., 2005). The associated higher microbial oxygen consumption in burrow walls (Gribsholt et al., 2003; Nielsen et al., 2003) may augment hypoxia inside burrows and coupled with release of sulfide increase stress and may cause death by asphyxiation or drowning (Diaz and Rosenberg, 1995). The risk of a fatal outcome is higher during moulting as this oxygen sensitive process usually takes place inside burrows (Das and Stickle, 1993).

Also, fiddler crab males were observed to wander around for longer periods in contaminated mesocosms (Bartolini et al., submitted) and high agonistic interactions between males may have also been a factor increasing crab mortality. 'Hypoxic events are known to increase cannibalism in the crab *Callinectis sapidus*, leading to high mortality rates (Aumann et al., 2006).

The present survival results strongly depend on the approach to estimate abundance. A known number of individuals were introduced initially to the cells while the subsequent enumeration was done by indirect approaches. Some studies have found that fiddler crab burrows numbers overestimate (30%) the actual crab abundance while visual counting of active crabs, on the other hand, underestimates (40%) the real crab density (Skov and Hartnoll, 2001; Skov et al., 2002). If we correct our data according to these studies, we obtain similar survival using both methods (Table 4) at an average of 50% for males and 12% for females. This is a clear indication that crabs resist stressful conditions for extended periods of hypoxia, but conclusions must be taken with caution because this study was only performed for a 6-month period. The estimated low survival of females, coupled with chronic exposure to hypoxic conditions and toxic metabolites may lead to collapse of the crab population due to limited reproductive activity and larval development (Tankersley and Wieber, 2000; Wu, 2002; Bergey and Weis, 2008), which may jeopardize the fiddler crab sustainability in closed systems or heavily contaminated mangrove forests.

4.2 Bioturbation activities

The amount of sediment processed by crabs during feeding activities depends strongly on sewage loadings, while the presence of young trees has no significant influence. The effect of sewage concentration is probably caused by the higher nutrient availability in contaminated sediment and water, which enhances (>70%) microalgal production as a food source for these crabs (Bouillon et al., 2002; Meziane and Tsuchiya, 2002; Reinsel, 2004). The lack of effect by trees is probably due to the limited shading by the small canopy of young *A. marina* trees (107.8 ± 7.8 cm height), leading to similar microphytobenthos growth in both bare and planted cells. Foraging is the primary activity taking place during the first hours after crabs exit their burrows (Eshky et al., 1995) until they get satiated (Bartolini et al., submitted) followed by a sequence of activities in a hierarchical temporal series (Eshky et al., 1995). If their intake efficiency remains constant the crabs need less time to be satiated at higher microalgae and bacteria densities in the sediment. Consequently, there is a decrease of the amount of feeding pellets produced and thus bioturbation potential. Alternatively, feeding rates can be lowered under hypoxic condition in the water column due to decreased metabolism of the crabs (Wu, 2002). A decrease in metabolic activity of more than 20% has been observed for important commercial crabs species (*Callinectes similis* and *C. sapidus*) under long-term (28 days) hypoxic conditions (Das and Stickle, 1993). However, a parallel behaviour study done at these mesocosms demonstrated that crab metabolism is not reduced but a significant modification of the period of the sequential activities is achieved (Bartolini et al., submitted). The lesser time spent feeding allows the crabs to devote more time and effort on other behaviours, such as gallery construction and maintenance. We actually observed a slight increase in the amount of “deep” sediment brought to the surface under high sewage loadings. Other studies have shown the opposite effect, e.g. ghost shrimps, with a decrease of the burrowing activity and sediment turnover with increasing nutrient availability (e.g., Berkenbusch and Rowden, 1999). However, just as here, this was probably linked with lower demand to process sediment for obtaining food, as the burrowing activity of ghost shrimps is always linked to feeding.

The burrow structure of *U. annulipes* and *U. inversa* in our mesocosm cells was slightly different from the typical L or J funnel-bent chamber, as observed for *U. annulipes* populations in Singapore (Lim and Diong, 2003; Lim and Heng, 2007; Lim and Rosiah, 2007).

The majority of burrows in our system exhibited an extra narrow elongation (extension leg) below the chamber. Otherwise, the general burrow morphology is within the dimensions already registered for fiddler crabs (Lim and Diong, 2003; Lim and Heng, 2007; Lim and Rosiah, 2007). The trend for shallower burrows with larger chambers in contaminated cells was probably a consequence of low redox conditions in the sediment (high Fe(II)). Shallow burrows are more likely to maintain oxic conditions by facilitating the diffusion of air between the gallery and atmosphere (Lim and Diong, 2003). Similarly, shallow *Uca pugnax* burrows have also been reported from oil contaminated salt marshes (Culbertson et al., 2007). The depth of crab burrows may therefore be a good proxy for environmental stress.

The decrease in burrowing depth as a consequence of hypoxia is usually not associated with concurrent change in burrow volume (Diaz and Rosenberg, 1995; Weissberger et al., in press). Burrow volume therefore appears to be a key parameter that crabs need to keep constant to assure that sufficient air is trapped inside burrows to survive during immersion periods. Crabs seem to compensate shallower burrows by constructing larger chambers when reduced sediment conditions demand shallower burrowing depth. Consequently, the total burrow volume is maintained with minimum increase in burrow surface area, and thus sediment-air interfaces with potential for oxygen uptake (Gribsholt et al., 2003; Nielsen et al., 2003).

The similarity in burrow depth between pristine and contaminated planted cells indicates that mangrove trees compensate for the reducing effect of nutrient loading as observed in the bare cells. *Avicennia marina* roots are known to translocate O_2 to deep sediment layers providing Fe(III) to iron reducers (Alongi, 2005; Kristensen and Alongi, 2006), which is clearly evident in planted pristine cells (Fig. 1). However, simultaneous exudation of labile DOC from roots also stimulates sulfate reducers forcing reduced conditions with high Fe(II) levels to prevail near the sediment surface (Kristensen and Alongi, 2006). This was actually what we observed in our planted cells. The presence of roots and pneumatophores therefore seems to have stabilized the sediments allowing deeper fiddler crab burrows when compared to unvegetated zones (Lim and Heng, 2007; Lim and Rosiah, 2007). It is puzzling, though, that this root induced stabilization in contaminated cells is not associated with higher Fe(III) concentrations in deep sediment. Except for the reducing effect of sewage, we have no explanation for this discrepancy.

4.3 Implications for wetland functioning

The role of mangrove wastewater wetlands is to remove, through biological, chemical and physical processes, organic matter and nutrients from sewage to acceptable levels before it is discharged into surrounding aquatic ecosystems (Yang et al., 2008). Bioturbation significantly influence the functioning of sediments, causing dramatic changes in nutrient dynamics and organic matter decomposition, and thus affect ecosystem health, productivity and filtration capacity (Kristensen and Kostka, 2005).

We found that fiddler crab survival, feeding intensity, reworking activity and biogenic structures were slightly affected by high sewage loadings. The observed decrease in feeding activity due to luxury growth of microalgae in contaminated sediments, may potentially lead to establishment of thick “algal mats” (Kristensen and Alongi, 2006), causing extended anoxia and sulfidic conditions in near-surface sediment (Kristensen and Alongi, 2006; Marsden and Bressington, 2009). However, through a simultaneous increase in deposition of excavated sediment on top of the microalgae at the surface, crabs prevent the development of algal mats and maintain higher benthic primary productivity (Blanchard et al., 2001). The activities of crabs may therefore improve mangrove system health and productivity (Kristensen and Alongi, 2006) and consequently its filtration capacity. Displacement of sediment from deep layers by crabs will also expose new surfaces to oxygen, increasing old and refractory organic matter degradation by efficient aerobic bacteria. At the same time, labile organic carbon is buried into anoxic layers full of starved anaerobic bacteria, that will degrade this fresh substrate easily and rapidly (Kristensen et al., 1995; Kristensen and Holmer, 2001; Kristensen and Kostka, 2005). In fact, the turnover time of an average burrow (volume ~40 cm^3) when assuming a daily amount of 10 g excavated sediment per burrow is roughly 8 days. Furthermore, by creating juxtaposition of oxic and anoxic habitats, fiddler crabs have the potential to enhance nitrification-denitrification processes in the sediment and thus augment removal of fixed nitrogen from discharged sewage (Canfield et al., 2005).

A decrease in burrow depth at contaminated bare cells, could influence the sediment redox profile (Weissberger et al., in press) and consequently carbon mineralization pathways in sub-surface sediment layers (Canfield et al., 2005). However, at planted cells (expected in a functional mangrove wetland) burrow depth was kept constant allowing the introduction of oxygen into subsurface sediment and promoting conditions for oxic respiration and microbial Fe(III) reduction (Kristensen and Holmer, 2001; Kostka et al., 2002; Gribsholt et al., 2003). By not decreasing the average burrow wall surface area in contaminated conditions, sediment microbial metabolism might be enhanced and nutrient cycling continuously occur, promoting removal of nutrients from sediment exposed to organic waste contamination as has been shown for fiddler (Nielsen et al., 2003) and grapsid crabs (McHenga and Tsuchiya, 2008).

It must be emphasized, however that the low abundance of crabs used in the present experiment will not provide the optimal functioning and filtering capacity of mangrove wastewater wetlands (Nielsen et al., 2003). We only applied ~10 crabs m^{-2}, while much higher densities (>100 m^{-2}) can easily be found in natural habitats (Skov et al., 2002). The increased impact of a crab population of 100 crabs m^{-2} in these wetlands can be stressed by the following scenario: the system reworking by feeding (10 g dw crab^{-1} day^{-1}) and burrowing (25 g dw crab^{-1} day^{-1}) of 100 crabs m^{-2} replaces the upper 0.3 cm every 6 days, instead of every second month as in the present situation. This also means that the sediment is reworked to ~10 cm depth by fiddler crab burrows every 6 months at natural crab densities.

This study demonstrates that both fiddler crab species studied are efficient ecosystem engineers in sewage enriched mangrove sediment. However, the relatively high mortality of females renders their populations unstable and recruitment must be assured to maintain a sustainable system. The high bioturbation levels under contaminated conditions indicate that these crabs have the potential to enhance organic matter decomposition, as known for other macrobenthic species (see Kristensen and Kostka, 2005), and thus cause a rapid turnover and ultimate removal of organic and inorganic nutrients. While this study only examined one functional type of organism, it is important that future studies address the effect of multispecies epifaunal and infaunal assemblages, as well as their density and interactions on mangrove wastewater wetland biogeochemistry and filtration efficiency.

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Chapter 5

Behavioural responses of mangrove fiddler crabs (*Uca* spp.) to urban sewage loadings: results of a mesocosm approach

Fabrizio Bartolini [a*], Gil Penha-Lopes [b], Samwel Limbu[c], José Paula [b], Stefano Cannicci [a]

[a] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze Italy

[b] IMAR – Laboratório Marítimo da Guia, Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Av. N.S. Cabo 939, 2750-374 Cascais, Portugal.

[c] University of Dar es Salaam, Faculty of Aquatic Sciences and Technology, P.O. Box 35064, Dar es Salaam, Tanzania

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Behavioural responses of the mangrove fiddler crabs (*Uca* spp.) to urban sewage loadings: results of a mesocosm approach

Fabrizio Bartolini[1], Gil Penha-Lopes[2], Samwel Limbu[3], José Paula[2], Stefano Cannicci[1]

1 - Dipartimento di Biologia Evoluzionistica “Leo Pardi” – University of Florence, Italy
2 - Laboratório Marítimo da Guia, Faculty of Sciences of the University of Lisbon, Av. Nossa Sra Cabo, 939, 2750-374 Cascais, Portugal
3 - Faculty of Aquatic Science and Technology (FAST) – University of Dar es Salaam, Tanzania

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 Chl-a concentrations). Along 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 of 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.

Keywords: Sewage, East Africa, Mangrove, Fiddler Crab, Behaviour, Ecosystem Engineer.

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 (Tam, 1998; Meziane and Tsuchiya, 2002), 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 (Kristensen and Alongi, 2006; Cannicci et al., 2008) 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 *U. 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*.

About the role played by contaminants in *Uca* population biology, Bergey and Weis (2008) found that a *U. pugnax* population from a New Jersey site characterised 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, *U. annulipes* and *U. 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 3x3x0.8 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 (Figure 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 the system was inundated by a water column of 10 cm high, from 12:00 pm till 12:00 am, so that the simulated low tide occurred for the first 6 hours under light, coinciding with the activity period of the selected fiddler crabs, and the remaining 6 hours 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 (Figure 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 seawater 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 figure 1).

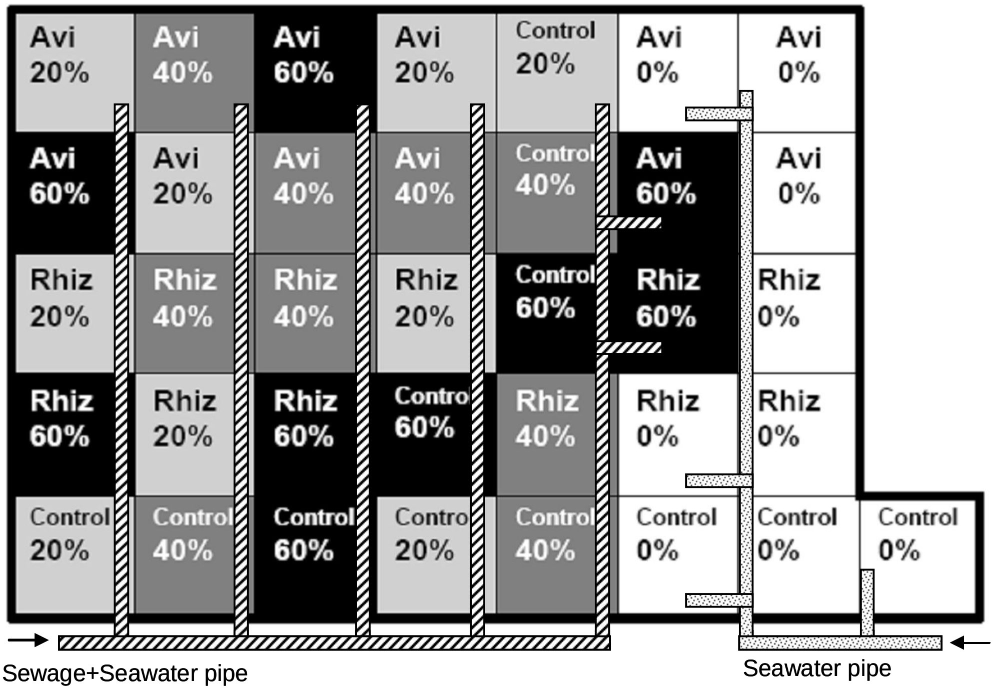

Figure 1. Scheme of the 36 mesocosms at the experimental site. Within each module, representing a single concrete plot, is reported the vegetation type (Control: bare; Avi: *Avicennia marina*; Rhiz: *Rhizophora mucronata*) and the sewage concentration (0%, 20%, 40%, 60%). A schematic disposition of the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

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 to 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.

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

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 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 (12pm-6pm), 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 6 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 9 main behaviours, which are listed and explained in Table 2.

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.

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 15x20 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, 6:00 pm).

After measured the wet weights, sediment samples were placed in vials containing 5ml of 90% ethanol and incubated for a 24 hours period at 4 °C. Each sample was then spun down at 3000 rpm for 10 minutes and the supernatant analyzed 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, 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 1st, 3rd and 5th 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 analyzed 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 figure 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 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.

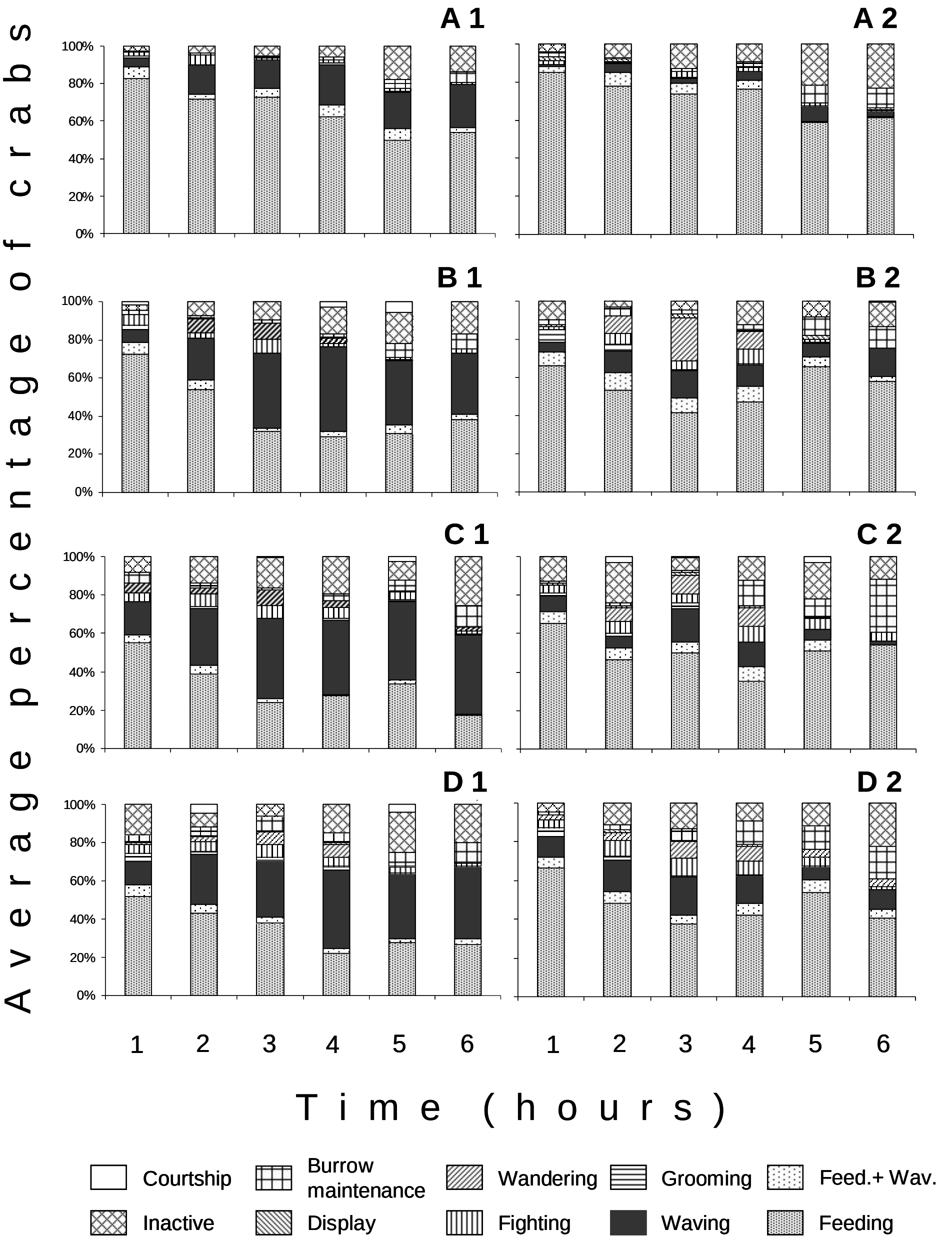

Figure 2. Percentage crabs observed performing the listed behaviours, averaged over vegetation treatment, for different sewage loadings and different species, throughout the six-hour activity period (n=4). 1 *Uca annulipes*; 2 *U. inversa*. A 0%; B 20%; C 40%; D 60%.

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 (Figure 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 5th 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. *Uca 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. *Uca inversa*, instead, kept low levels of this behaviour, with a peak at the third hour.

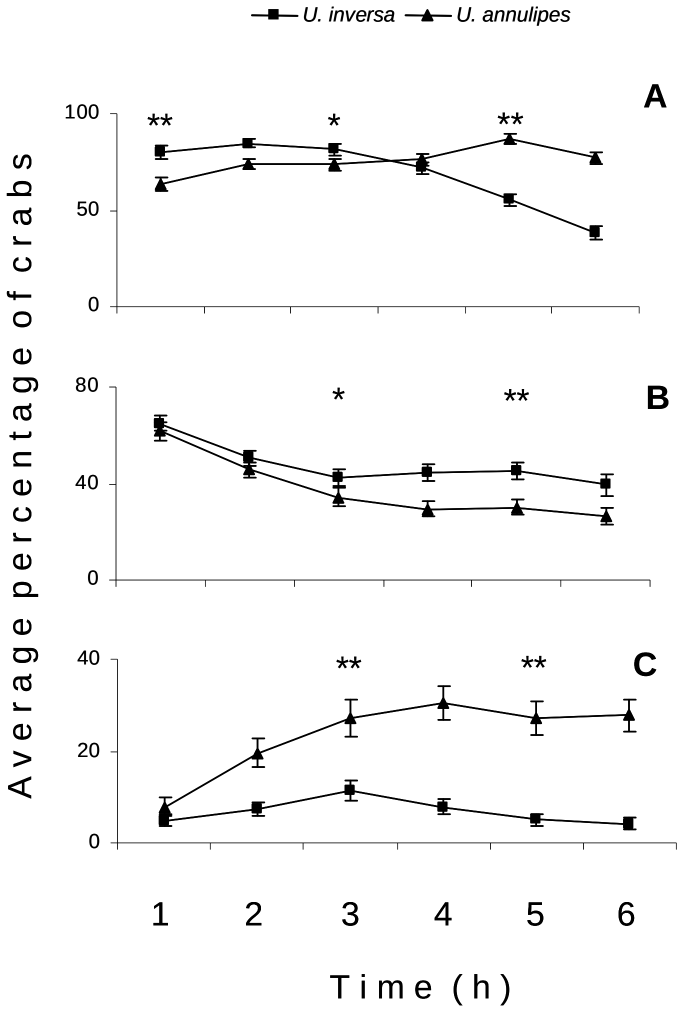

Figure 3. Comparison between activity patterns of *U. annulipes* and *U. inversa*. (A) Observed active crabs (normalized as percentage of the maximum number observed), averaged over sewage and vegetation treatments, at each of the six hours. Feeding (B) and waving (C) rates of the two species averaged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

3.3 Effect of vegetation type on crab behaviour

The vegetation treatment (Figure 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.

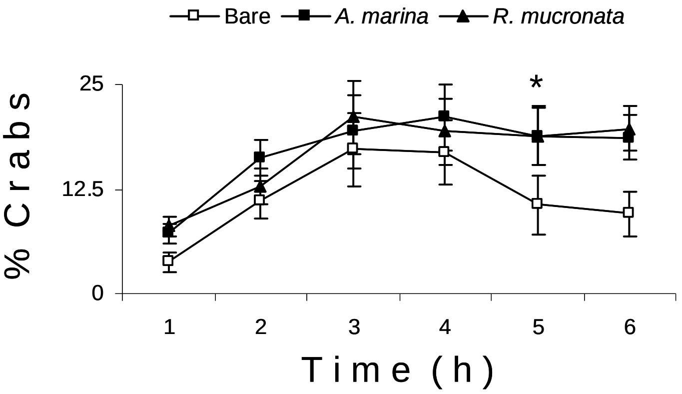

Figure 4. Waving activity for the three vegetation types, averaged over species factor (*U. annulipes* and *U. inversa*) and sewage treatment, during the entire low tide activity period. *: $0.01<p<0.05$; **: $p<0.01$. Error bars indicate SE (n=4).

3.4 Effect of sewage load on crab behaviour

Examining the trend of the feeding ratio among different sewage loadings throughout the entire six-hour period (Figure 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 (Figure 5), estimated considering only males, had low values at the beginning of the emersion period, increasing till the 3rd of 4th hour and then slightly decreasing approaching the sunset. The waving activity period was longer with increasing sewage concentrations throughout the entire sampling period, however, significant differences were only observed between 0% and 60% conditions at the 3rd hour of the emersion period (Table 3, SNK test).

The crabs wandered mostly in the central part of the low tide interval (2nd, 3rd, 4th hours) but interestingly this kind of restlessness was almost never present in the control plots (Figure 5). During the 3rd 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).

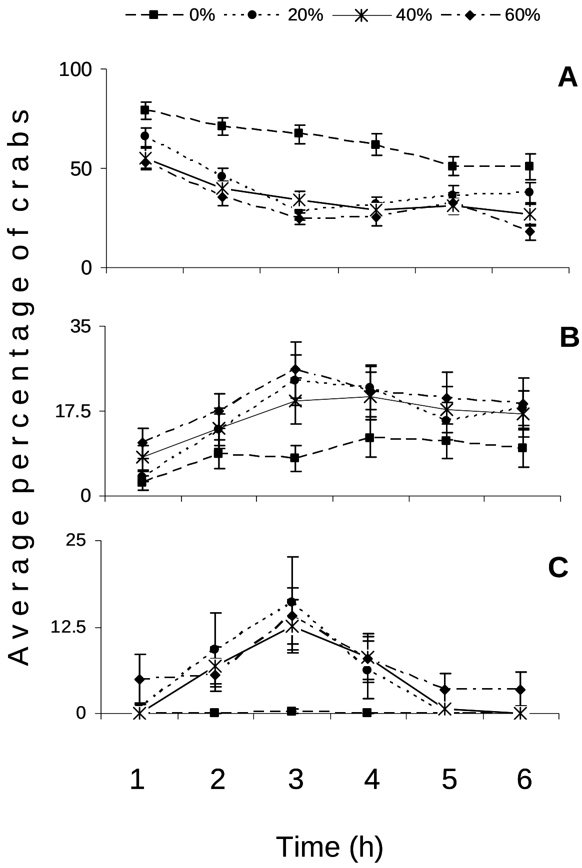

Figure 5. Effect of sewage concentrations on the behaviour of crabs, throughout the six-hour activity period, averaged over species factor (*U. inversa* and *U. annulipes*) and vegetation treatment. (A) Feeding; (B) Waving; (C) Walking. Error bars indicate SE (n=4).

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 (12h: Welch t-test for unbalanced variances; t=-2.13, df=10, p<0.05; 15h: t-test for unbalanced variances; t=-3.12, df=11, p<0.01; 18h: Student t-test; t=-3.87, df=6, p<0.01), independently of the hour of the day (Figure 6), although such difference was less pronounced at 12:00, namely soon after the emptying of the plots.

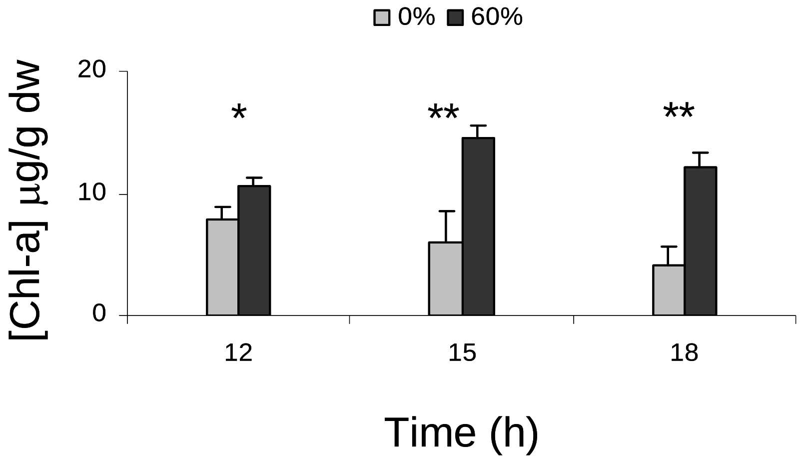

Figure 6. Chlorophyll-a concentrations on the top 1 cm sediment layer in treated plots (60% sewage concentration) compared to non contaminated ones, throughout 6 hours. Error bars indicate SE.

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	1st hour			3rd hour			5th 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									

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 (in press). These authors found similar mortality rates as well as similar levels of top sediment reworking between the two species exposed to similar sewage loadings, 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 *U. 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 Chl-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 explanation 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 recognized 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. (in press), 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 (Murai et al., 1983; Gherardi et al., 2002), 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 (in press). 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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Chapter 6

Ecosystem engineering potential of the gastropod *Terebralia palustris* (Linnaeus, 1767) in mangrove wastewater wetlands - a controlled mesocosm experiment

Gil Penha-Lopes[a, b*], Fabrizio Bartolini[c], Samwel Limbu[d], Stefano Cannicci[c], Yunus Mgaya[d], Erik Kristensen[e] and José Paula[a]

[a] Centro de Oceanografia - Laboratório Marítimo da Guia,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211 / Fax (351) 214869720
[b] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[c] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy
[d] University of Dar es Salaam, Department of Aquatic Sciences and Fisheries, P.O. Box 35064, Dar es Salaam, Tanzania
[e] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark

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Gil Penha-Lopes[a, b*], Fabrizio Bartolini[c], Samwel Limbu[d], Stefano Cannicci[c], Yunus Mgaya[d], Erik Kristensen[e] and José Paula[a]

[a] Centro de Oceanografia - Laboratório Marítimo da Guia,
Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa
Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211 / Fax (351) 214869720

[b] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[c] Dipartimento di Biologia Evoluzionistica, Università degli Studi di Firenze, via Romana 17, I-50125, Firenze, Italy
[d] University of Dar es Salaam, Department of Aquatic Sciences and Fisheries, P.O. Box 35064, Dar es Salaam, Tanzania
[e] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark

Abstract

The effect of different sewage concentrations (0, 20, 60 and 100%), vegetation (Bare, *Avicennia marina* or *Rhizophora mucronata*) and immersion periods (immersion/emersion period of 12/12h or 3/3 days just for 100%) conditions were studied for 6 months on survival and growth rates of *Terebralia palustris* (Linnaeus, 1767). Gastropods´ activity and ecosystem engineering preformed at bare and *A. marina* planted cells and 3 sewage conditions (0, 20 and 60%) were determined. Survival rates were higher than 70% in all treatments. Growth rate decreased significantly with increasing sewage concentrations (mainly at unplanted conditions) and longer immersion periods. A complete shift (from immersion to emersion periods) and a significant decrease in mobility and consequently its engineer potential, due to sewage contamination, lead to a 3-4 fold decrease in the amount of sediment disturbed. Sewage contamination, primary producers' abundance and environmental conditions may have influenced the gastropods survival, growth and its ecosystem engineering potential.

Keywords: *Terebralia palustris*, survival, ecosystem engineering, pollution, mangrove

1. Introduction

Mangrove forests are known to provide important ecosystem goods and services such as nursery areas for several important aquatic species, and a variety of food, timber and chemicals for local communities (Duke et al. 2007). These systems have recently shown a potential as natural wastewater treatment facilities, thus preventing coastal pollution (Wong et al., 1997). Field trials have shown that sediments of these ecosystems are very efficient in removing nutrients from sewage (Tam and Wong, 1995; 1996), without apparent impacts on mangrove trees (Wong et al., 1997) or significant effect on the benthic invertebrate communities (Yu et al., 1997).

Sewage filtering of constructed mangrove wetlands is now addressed worldwide, mainly due to low running cost and high efficiency potential (PUMPSEA, 2008; Yang et al., 2008), although it was shown that wastewater loadings above the system capacity and under weak hydrodynamic conditions usually lead to eutrophication and consequently hypoxic conditions (Gray et al., 2002). Low oxygen levels may decrease faunal diversity and biomass due to emigration of mobile species or high mortality of less mobile species in natural mangroves (Diaz and Rosenberg, 1995) or lower survival rates in constructed wetlands (Penha-Lopes et al., accepted). This could significantly affect ecosystem functioning (Biles et al., 2002; Le Hir et al., 2007), and coupled with a potential decrease in activity and behaviour of the more resistant species (Diaz and Rosenberg, 1995) potentially reduce the mangrove health and filtration efficiency.

Most studies on sewage filtration have focussed on the role of plants and sediment with associated microbes and microalgae (e.g., Wong et al., 1995; Wong et al., 1997; Tam, 1998) and only few have dealt with macrofaunal performance under severe conditions. Mangrove fiddler crabs maintained survival rates near 50% and efficient bioturbation via feeding and burrowing for 6 months in severe organic contaminated mangrove mesocosms (Penha-Lopes et al., accepted). Most infauna species such as crabs and polychaetes are key players in healthy ecosystems by increasing mineralization processes and nutrient cycling through burrowing and irrigation activities (Kristensen and Kostka, 2005), mainly in organic-rich systems (Hansen and Kristensen, 1998; Nielsen et al., 2003; Kinoshita et al., 2008; McHenga and Tsuchiya, 2008; Lindqvist et al., 2009).

Among all benthic species, gastropods are considered very resistant to low oxygen levels (Gray et al., 2002). They generally maintain high survival during hypoxic events (Stickle et al., 1989; Das and Stickle, 1993; Sagasti et al., 2001) and are among the first colonizers of previous anoxic environments (Gamenick et al., 1996). Nevertheless, they respond to low oxygen concentrations by decreasing their crawling and feeding activities, and consequently diminish metabolic rate and growth (Stickle et al., 1989; Das and Stickle, 1993; Cheung et al., 2008; Riedel et al., 2008). Gastropods play an important ecological role in natural systems through organic matter consumption (Slim et al., 1997; Fratini et al., 2004), bioturbation effects on nutrient cycling (Biles et al., 2002), regulation of meiofauna and microphytobenthos biomass (Carlén and Ólafsson, 2002; Pape et al., 2008), changes in the dynamics of suspended material (Kamimura and Tsuchiya, 2004) and effects on sediment stability (Orvain et al., 2003; 2004; 2006).

The mudwhelk *Terebralia palustris* (Linnaeus, 1767) is a key epifaunal species in East African mangrove forests. Studies have shown that it is important for the nutrient cycling by consuming large amounts of *Avicennia marina* (Forsk.) and *Rhizophora mucronata* Lam. litter It can also regulate microphytobenthic primary productivity through feeding and crawling activities (see Cannicci et al., 2008; Lee, 2008). However, this species disappeared completely following organic contamination of mangrove areas in Mozambique, Kenya and Zanzibar (Cannicci et al., in press) suggesting an upper tolerance limit to the conditions present in those areas.

It is therefore fundamental to investigate the direct and indirect effects of severe sewage contamination on species like *Terebralia palustris* to comprehend the effect of organic discharges on natural mangrove forests as well as to develop a sustainable and more efficient mangrove wastewater wetland. The major goal of this study was to determine survival and growth of *Terebralia palustris* under different domestic sewage loading, immersion periodicity and vegetation conditions. Further, its behaviour and bioturbation activities, and thus ecosystem engineering potential, were evaluated at different sewage concentrations in the presence or absence of mangrove trees.

2. Materials and methods

2.1 Experimental setup

A mesocosm system consisting of 27 cells (9 m^2 each) was constructed at the upper *Avicennia marina* (Forsk.) belt of the Kunduchi mangrove forest (Jangwani Beach) located at longitude 39^o 12’-39^o 13’ E and latitude 6^o 39’-6^o 41’ S, about 18 km from Dar es Salaam City Centre, Tanzania (see system description in Penha-Lopes et al., accepted). The 27 cells were divided as follows: “vegetation” treatment, with nine unvegetated cells, nine cells planted with *Avicennia marina*, and nine with *Rhizophora mucronata* Lam. saplings. Sediment was laid and saplings planted (at a density of 2.8 m^{-2}) on selected plots in early February 2006.

The system was initially inundated exclusively with seawater and sewage discharge was initiated in early October 2006. For each vegetation treatment, three replicate cells were exposed to sewage loading of 0, 20 and 60 % in seawater for the first 6 months period (October 2006 to April 2007). A diurnal tidal rhythm was simulated with 12 hour immersion to 0.1 m depth starting at 23:00. The sewage concentration was increased to 100% during the second 6 months period (April 2007 – October 2007) with 12/12 hours (100_{12h}) and 3/3 days (100_{3d}) immersion/emersion periods for all vegetation conditions (only 2 replicates). The basic chemical and biological characteristics of sewage-seawater mixtures are presented in Tables 1 and 2 (adapted from PUMPSEA, 2008). *Avicennia marina* and *R. mucronata* were 50.30±1.17 and 55.3±0.5 cm (±SE) tall, respectively, and litter fall from the young trees was absent when sewage discharge was initiated. They grew to 223.9±18.0 and 133.0±13.2 with litter fall of 1.17±0.20 and 2.8±0.7 g m^{-2} day^{-1} (for *A. marina* and *R. mucronata*, respectively) in October 2007 (PUMPSEA, 2008).

Terebralia palustris individuals were introduced to each cell in August 2006 together with individuals of abundant fiddler crab species found in East African mangrove forests, *Uca annulipes* (H. Milne Edwards, 1837) and *U. inversa* (Hoffmann, 1874). All fauna was randomly collected from the Jangwani mangrove forest near the mesocosm system. The chosen abundance of *T. palustris* (5 ind m^{-2}) was in the lower range of natural densities (Cannicci et al., in press). Fauna adapted to the system for almost two months before sewage discharge was initiated. At that time the gastropods had an average size (±SE) of 62.49±0.31 mm.

2.2 Gastropod Survival and Growth

Terebralia palustris survival and size increment (also denominated as growth from now on) in 0, 20 and 60% sewage concentrations was followed for the first 6 months. At this time, survival and growth rates were calculated based on the difference of the average value obtained for each cell with initial conditions. The survival and growth rates in 100% sewage (both 100_{12h} and 100_{3d}) were calculated from the fraction of individuals that stayed alive during the second 6 months period. For gastropod survival and size analysis (shell height), all individuals in each mesocosm were collected, checked for life activity, counted and measured (maximum linear dimension of the shell from the apex to the anterior edge of the lip) at each sampling period (immediately before, and 6 months and 12 months after starting sewage discharge), and then returned to the respective cell.

2.3 Gastropod Behaviour and Bioturbation

This set of experiments was done during an intensive campaign from March to April 2007. Due to similar survival and growth results between both planted cells, 3 replicates of

0%, 20% and 60% sewage in bare and *A. marina* cells were used. Temporal replication was conducted during half (except for 20%) and full moon, which coincides with neap and spring tides in the surroundings.

Environmental conditions: Top-sediment temperature, porewater content and dissolved oxygen (DO) of sewage mixtures were measured on one occasion in all cells receiving 0% and 60% sewage. Temperature was measured every 2 hours (except at night, 02.00 to 07:00) in 5 replicates per cell. Sediment for porewater content (determined as weight loss after drying sediment samples at 100°C for at least 12 hours) was sampled in 5 replicates per cell every 2 hours during the emersion period (12:00 to 22:00). Dissolved oxygen was measured right after immersion (24:00), before sunrise (6:00) and just before flushing out (11:00) twice in every cell using a dissolved oxygen probe (WTW Oxi 330i with an associated WTW CellOx 325 electrode).

Behaviour: Due to the considerable differences in behaviour between 0% and 60% sewage treatments during the first temporal replication, it was decided to include 20% sewage treated cells during the second replication period. At each period, the position of 8 marked gastropods of different sizes in each examined cell was noted every 2 hours over a 24 h cycle. The total daily movement of each individual was estimated as the sum of all 2 hour events, which were measured as the straight line between the initial and final position. Whenever possible (N=66, approximately 10% of all 2 hours events), the real path of each individual was measured from tracks in the surface sediment to obtain a correction of the straight line estimate. Individuals were located and identified by a buoy with a specific colour code attached to the shell during immersion and a specific colour code painted on their shell during emersion. Flags with the same colour codes were used to mark the 2-h positions.

Bioturbation: The amount of sediment displaced by different sized *T. palustris* individuals during movement was determined in the laboratory and extrapolated to the mesocosm results. Sediment displaced by the track area of individuals in different sediment types (i.e. porewater content) was measured and calculated under flooded, wet and dry conditions. Wild animals were collected from the same location as those used in the mesocom experiment, while sediment was collected from cells receiving no sewage. Sediment was transferred to 40x40x10 cm plastic boxes to a depth of 4 cm and seawater was added in excess. To simulate flooded periods 4 cm of water column were maintained, for wet conditions (21.9±0.4% of porewater) excess of water was removed only, and for dry conditions (6.9±0.9% of porewater) sediment was in addition placed at the sun for 1 hour. Gastropods with fresh weights ranging from 2.2 to 42.3 g were used. Individuals were placed on the sediment at flood, wet and dry conditions and allowed to move at least 30 cm. The displaced sediment was estimated on 5 cm subsections of the 30 cm track by assuming that, the cross section of tracks approximated half of an ellipse area ($A_{section}=1/4*\pi*a*b$). Measured track width is equivalent to a and maximum depth represents b. Using these laboratory based results, the total sediment displaced by individuals of known weight in the mesocosms during a 24 h cycle was estimated.

2.4 Statistical analysis

Survival and growth experiments: A two-way ANOVA was used to compare *Terebralia palustris* survivorship (using ArcSin transformed data) and average height increment for the two 6 months periods, under different vegetation conditions and sewage loading (0, 20 and 60% for the first period, and 100_{12h} and 100_{3d} for the second period). Initial population structure from each cell was compared with a Chi Square test. Because no differences were obtained in October 2006 (initial conditions), population data was pooled in order to compare the 6 month population structure to only one initial data set. The goal of this analysis was to understand if there is a size-dependent differential mortality. Prior to analyses, the homogeneity of variances was assessed using Cochran's test and data were transformed to $x' = \sqrt{(x + 1)}$ to remove heteroscedascity.

Behaviour and Bioturbation Experiment: A two-way ANOVA was used to compare total daily distance traveled and total weight of sediment displaced by gastropods under different vegetation conditions and sewage loading. To compare gastropod average velocity during emersion and immersion periods between sewage and vegetation treatments a three-way ANOVA was used. Due to the similar results obtained between temporal replicates for 0% and 60%, for the daily distance travel, total weight of sediment displaced and average velocity ANOVA analyses, both temporal replicates of 0% and 60% sewage loading treatments were averaged per plot, allowing only one balanced statistical analysis including 20% sewage loading. Prior to analyses, the homogeneity of variances was assessed using Cochran's test and data were transformed to $x' = \log (x + 1)$ to remove heteroscedasticity.

When appropriate, Student–Newman–Keuls (SNK) tests were used for multiple comparisons of the means. ANOVAs were performed using GMAV 5 software (University of Sydney, Australia). Prism 6.0 software was used to compare average individual distance per hour (velocity) along *T. palustris* size range correlations among different sewage and vegetation treatments, as well as sediment area disturbed as a function of *T. palustris* weight under different sediment conditions (wet and flooded).

3. RESULTS

3.1 Environmental conditions

Generally, nutrient concentration increased with increasing sewage loading (Table 1) while salinity and dissolved oxygen decreased. However, during the second 6 months period, although organic loading was similar, leading to lower DO during day and night periods, nutrient concentration was slightly lower, due to changes in the sewage quality coming from the hotel. Chlorophyll *a* concentration (as a measure of microalgal biomass) in the top 2 cm sediment layer was highest in unvegetated cells and tended to increase with sewage dosing (Table 2). Although chlorophyll was not measured in the 100% cells, the sediment surface was dark green indicating a higher concentration of microalgae when compared with all other treatments.

Table 1 - A) Chemical characteristics and oxygen uptake of the sewage-sea water mixtures used in the experimental mesocosms from October 2006 to April 2007 for 0 to 60% and from April 2007 to October 2007 for 100% sewage loading. (DO: Dissolved Oxygen) (N = 45). Values are given as averages ± SE (adapted from PUMPSEA, 2008).

Sewage loading	**Salinity (‰)**	**DO_{day} (µM)**	**DO_{night} (µM)**	**NH_4^+ (µM)**	**NO_3^- (µM)**	**PO_4^{3-} (µM)**
0%	39.1 ± 0.6	308 ± 133	197 ± 17	26 ± 2	3.6 ± 1.4	42 ± 4
20%	24.5 ± 0.9	312 ± 227	10 ± 5	123 ± 9	7.1 ± 2.9	109 ± 6
60%	18.9 ± 1.4	225 ± 256	9 ± 1	194 ± 17	5.0 ± 1.4	206 ± 14
100%$_{12h\ and\ 3d}$	1.87± 0.47	102 ± 98	4 ± 1	91 ± 11	6.0 ± 1.7	173 ±48

Table 2. Sediment surface chlorophyll a concentration at different vegetation and sewage concentration treatments in April 2007 is shown (N = 12). Values are given as averages ± SE (adapted from PUMPSEA, 2008).

	Chl a (µg.g^{-1})		
	0%	**20%**	**60%**
Bare	13.9±3.9	12.5±6.1	23.1±9.4
A. marina	5.4±1.1	17.1±12.0	14.0±3.5
R. mucronata	8.1±2.7	10.1±4.0	14.7±7.0

During the March-April 2007 campaign, the diurnal variation in top sediment temperature was similar in all treatments (Figure 1). The temperature was constant at around 27ºC at night and started to increase at dawn (7:00) reaching a maximum of about 37ºC around noon (13:00) followed by a gradual decrease back to the night time level. Dissolved oxygen (DO) in 0% cells decreased slightly from 200 µM in seawater entering at night (23:00) to 90-150 µM at sunrise and increased steeply during the day to values of 300- 400 µM just before emptying the cells (11:00) (Figure 1). The sewage mixture entering 60% cells contained almost no DO initially (23:00) and during the immersion period DO increased after sunrise in these cells, only slightly in planted cells (to 7-50 µM) and considerably in unvegetated cells (to more than 500 µM). Porewater content of the sediment was similar among treatments and decreased gradually during emersion from 25-27% at noon to about 22% at 23:00.

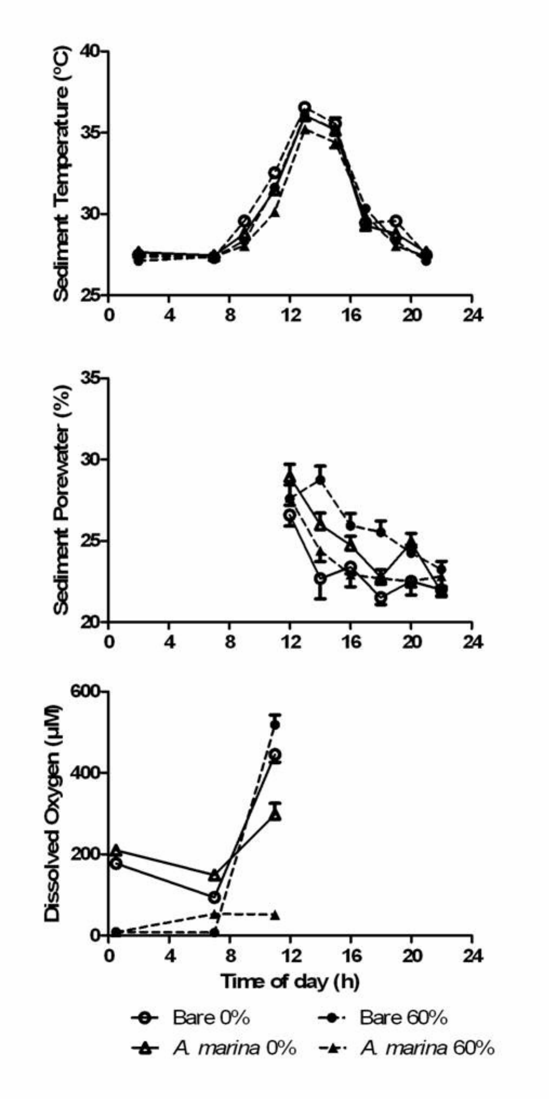

Figure 1 – Overlying water dissolved oxygen as well as sediment temperature and porewater content in cells with different vegetation and sewage treatments at April 2007. Values are given as average (±SE). n = 6 – 15.

3.2 Gastropod Survival and Growth

The 6 months survival of *Terebralia palustris* was high and ranged from 72 to 91%. Significant differences were observed irregularly among some vegetation treatments: *A. marina* 0% versus *R. mucronata* 0% ($p<0.05$), Bare 20% versus *A. marina* 20% ($p<0.05$) and Bare 60% versus *R. mucronata* 60% ($p<0.01$) (Figure 2 and Table 3-A). Sewage dosing only affected survival significantly in the bare cells where more individuals survived in 0% ($p<0.05$) and 20% ($p<0.01$) than the 60% treatment.

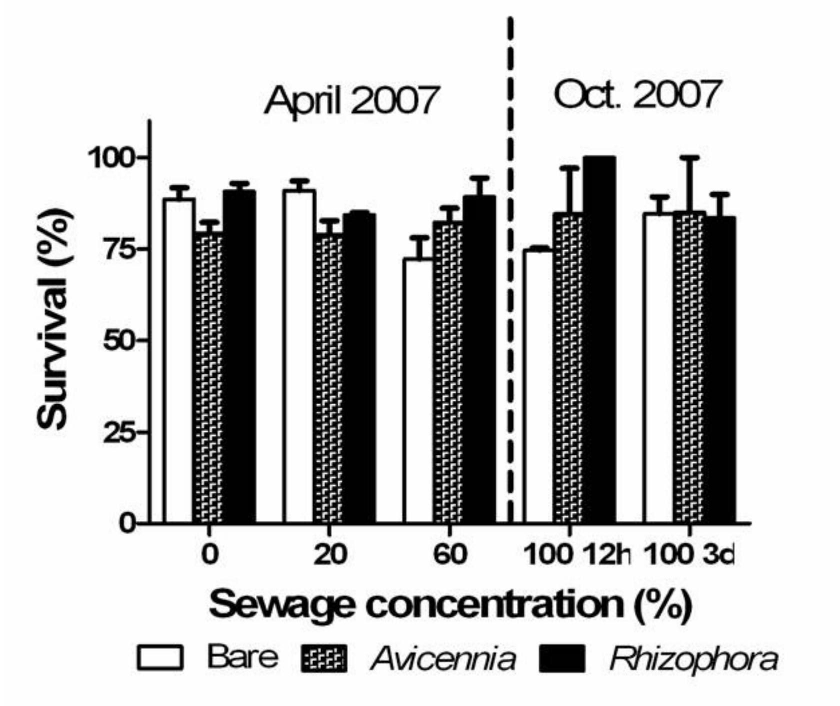

Figure 2 - Survival rates of *T. palustris* after 6 months (April 07) for all vegetation factors and 0%, 20% and 60% sewage concentration treatments. For the 100% sewage concentration plots, survival rates at Oct 07 (6 months after April 07) are calculated based on the April 07 survival. Values are given as average (±SE). n = 2-3.

Also the 100% treatments showed survival above 80% with no significant impact of vegetation and immersion-emersion treatments (Figure 2 and Table 3-B).

During the first 6 months, gastropods tended to increase height at a rate of 3 to 4 mm month^{-1} when reared at pristine conditions and at a rate of 2 mm month^{-1} when stressful situations were present. During this period *T. palustris* size increment (Figure 3 and Table 3-A) at pristine conditions (0%) was slower ($p<0.05$) in unplanted (~10 mm increase) than planted mesocosms (~16 and 19 mm for *A. marina* and *R. mucronata*, respectively). However, while growth remained constant with increasing sewage concentration in bare sediment, a significant decline of 23% and 50% ($p<0.05$) was detected in *A. marina* and *R. mucronata* treatments, respectively (Figure 3). The average height increment in 100% sewage during the second 6 month period was significantly lower than for 60% in the previous period, but within the range obtained during the second period for 0 to 60% (just indication). Furthermore, growth of 100_{3d} individuals (±0.2 mm month^{-1}) was reduced by 50% compared with 100_{12h} individuals (Figure 3 and Table 3-B).

Table 3 – A - Results of the three 2-way ANOVA on *Terebralia palustris* Survival Rates (Oct 06 to April 07) (transformation Arcsen), Average Size at initial conditions (Oct 06) and Average Size Increment (Oct 06 to April 07) just for 0, 20 and 60% sewage loading experiments. B - Results of the three 2-way ANOVA on *T. palustris* Survival Rates (April 07 to Oct 07)) (transformation Arcsen) and Average Size Increment (April 07 to Oct 07) just for 100% sewage loading experiments. Factors: Vegetation (fixed and orthogonal) and Sewage / Immersion Period (fixed and orthogonal). The degrees of freedom, DF, Variance, MS, F ratio and p-value (p) are showed for each of the tests.

A	Survival Rate (Oct 06 – April 07)			Average Size (Oct 06)			Average Size Increment (Oct 06 – April 07)		
	Df	MS	F	Df	MS	F	Df	MS	F
Vegetation	2.0	0.05	**4.84**[a]	2.0	63.50	0.43	2	3.52	1.70
Sewage	2.0	0.01	0.50	2.0	53.71	0.36	2	23.21	**11.21**[b]
Interaction	4.0	0.07	**6.22**[b]	4.0	87.45	0.59	4	12.34	**5.96**[b]
Residual	18.0	0.01		1341.0	147.80		18	2.07	
Total	26.0			1349.0			26		

B	Survival Rate (April 07 – Oct 07)			Average Size Increment (April 07 – Oct 07)		
	Df	MS	F	Df	MS	F
Vegetation	2.0	0.13	1.53	2.0	11.23	1.73
Immersion Period	1.0	0.03	0.35	1.0	47.15	**7.27**[a]
Interaction	2.0	0.17	2.06	2.0	0.17	0.03
Residual	6.0	0.08		6.0	6.49	
Total	11.0			11.0		

([a] = $p < 0.05$; [b] = $p < 0.01$)

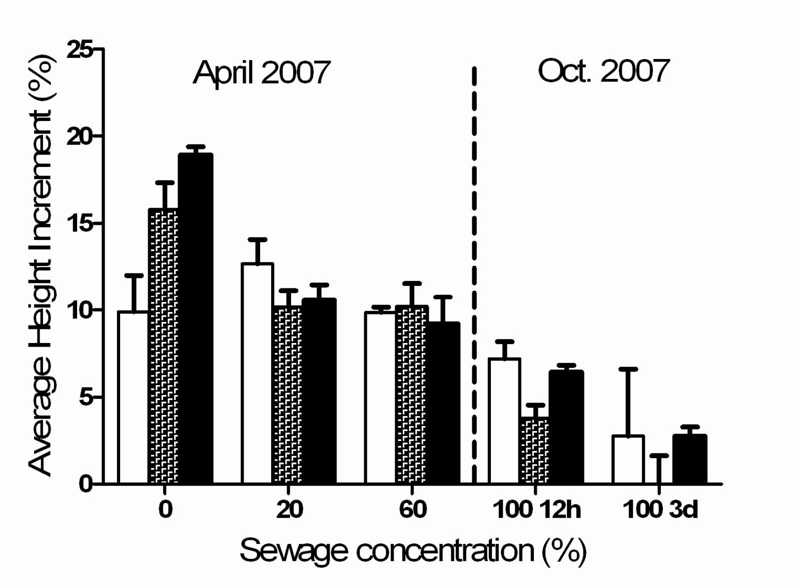

Figure 3 - Average height increment (± SD) of *T. palustris* after 6 months (April 07) for all vegetation factors and 0%, 20% and 60% sewage loading treatments. For the 100% sewage loading plots (12h and 3days), average height increment at Oct 07 (6 months after April 07) are calculated based on the April 07 gastropod average size. Values are given as average (±SE). n = 2-3.

Table 4 – A) Results of the 2-way ANOVA on: (1) distance accomplished (expressed as meters) log (x+1) transformed for total distance per individual in different conditions of vegetation (bare and planted) and sewage (0, 20 and 60%) averaging the two temporal replications; (2) sediment weigh (expressed as DW) log (x+1) transformed for total daily sediment disturbed per individual in different conditions of vegetation (bare and planted) and sewage (0%, 20% and 60%) averaging the two temporal replications. Factors: vegetation (fixed and orthogonal) and sewage (fixed and orthogonal). B) Results of the 3-way ANOVA on individual average velocity (expressed as m h^{-1}) after log (x+1) transformation in different conditions of vegetation (bare and planted) and sewage (0%, 20% and 60%) averaging the two temporal replications. Factors: vegetation (fixed and orthogonal), sewage (fixed and orthogonal) and immersion/emersion periods (fixed and orthogonal). The degrees of freedom, DF, Variance, MS, and value of F ratio are showed for each of the tests.

A)	**(2) Daily distance (0%, 20% and 60%)**			**(3) Sediment disturbed (0%, 20% and 60%)**		
Source	DF	MS	**F**	DF	MS	**F**
Vegetation	1	0.15	0.75	1	0.39	2.10
Sewage	2	11.94	**60.00** *	2	147.24	**739.91** *
Interaction	2	0.33	1.69	2	2.14	**10.98** *
RES	138	0.20		138	0.20	
TOT	143			143		

B)	**Average Velocity (0%, 20% and 60%)**		
Source	DF	MS	F
Vegetation – ve	1	0.01	0.02
Sewage – se	2	25.26	**75.38** *
Immersion/Emersion - im	1	51.97	**155.09** *
ve X se	2	1.7	**5.14** *
ve X im	1	0.89	2.64
se X im	2	112.28	**335.08** *
ve X se X im	2	0.84	2.52
RES	132	0.34	
TOT	143		

(* = $p < 0.01$)

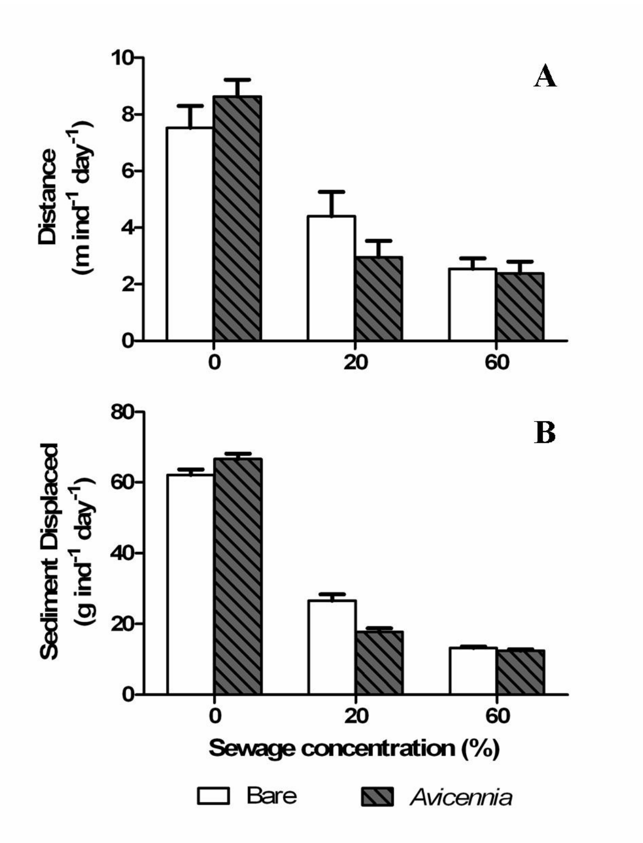

Figure 4 – A) Daily distance accomplished by *T. palustris* and B) Weight of sediment displaced per individual at different sewage loading and vegetation treatments. Values are given as average (±SE). n = 24.

3.3 Gastropod Behaviour and Bioturbation

Behaviour: The daily crawling distance of *T. palustris* decreased significantly ($p<0.01$) by more than 40% from pristine to sewage contaminated cells, while vegetation had no significant role (Figure 4-A and Table 4-A). Travel distances were always independent of individual size, exhibiting relationships with slopes that were not significantly different from zero. However, individuals inhabiting non-contaminated mesocosms (pristine) travelled longer daily distances than those inhabiting 60% cells (data now shown).

The response of *T. palustris* to different sewage loading and immersion is evident from the average individual crawling velocity over a 24 h cycle (Figure 5 and Table 4-B). Gastropods inhabiting pristine cells (0%) showed higher crawling activity ($p<0.01$) during immersion (30-80 cm h^{-1}) than during emersion (0-20 cm h^{-1}) regardless of the vegetation cover. Conversely, the gastropods did not move at all during immersion under contaminated conditions (15-80 cm h^{-1}), while they exhibited crawling during emersion ($p<0.01$), even in the afternoon sun peak.

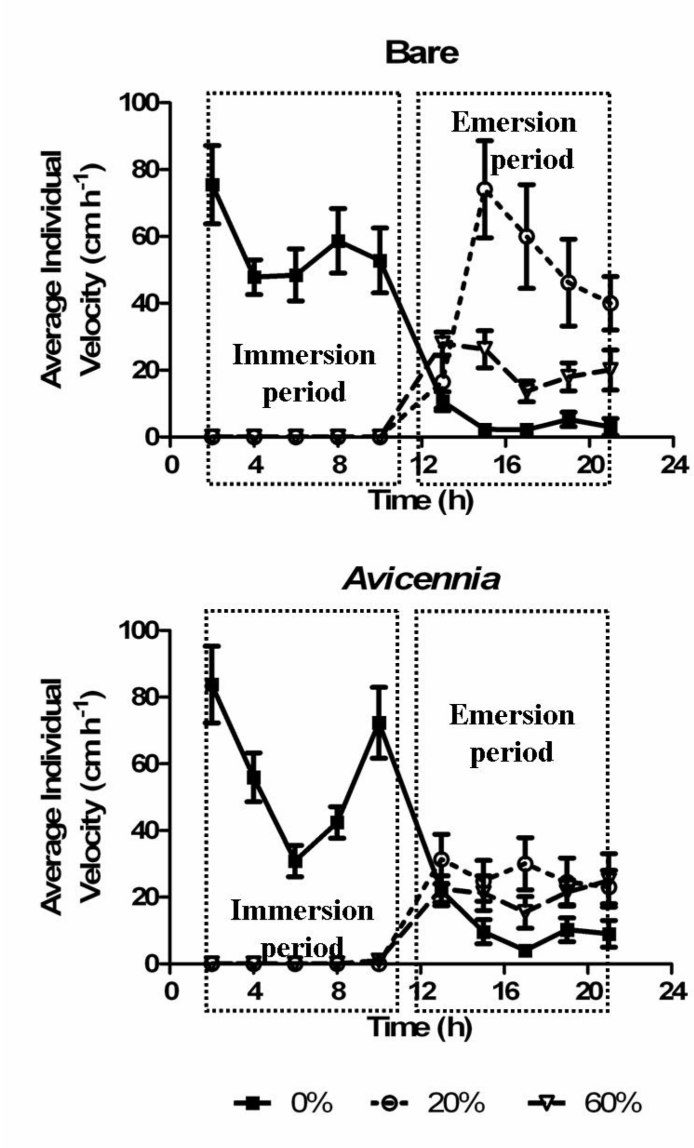

Figure 5 – Crawling velocity by *T. palustris* individuals at different sewage loading and vegetation treatments during immersion and emersion periods. Values are given as average (±SE). n=8

Bioturbation: Laboratory experiments on sediment displacement by gastropods as a function of body weight showed significant linear positive relationships for individuals kept under flooded and wet conditions (Figure 6). Those kept under dry conditions (6.9±0.9%) left no tracks, suggesting that no sediment was reworked. Slopes of the relationships were similar between wet and flooded conditions (P=0.228; pooled slope = 0.006), but the intercept was not (P<0.0001). This indicates that gastropods under flooded conditions displaced more sediment (by a factor of 1.5 to 2) than under wet conditions.

The sediment water content obtained for flooded and wet conditions in the laboratory agrees well with that found during immersion and emersion in the mesocosm cells (see Figure 1). The section area of gastropod tracks was greater during immersion than emersion, indicating that the amount of sediment displaced was not only related with the distance crawled, but also the flood condition.

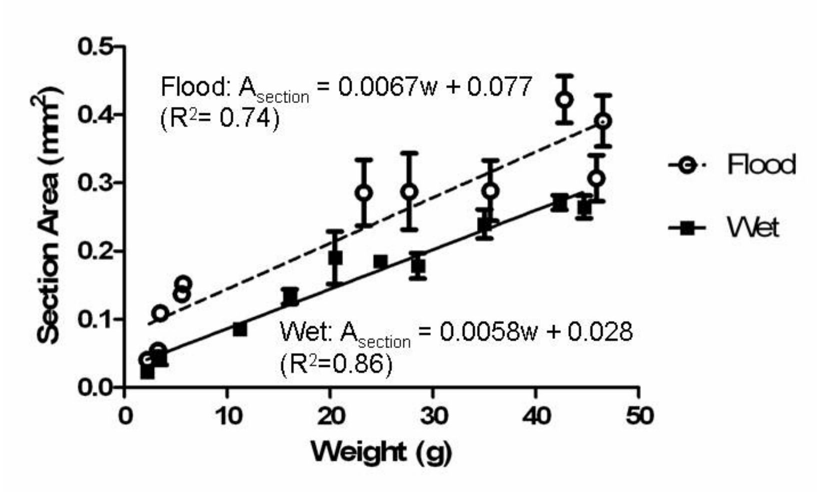

Figure 6 – Sediment area disturbed by crawling of *T. palustris* individuals as a function of displaced sediment weight under flooded and exposed, but wet sediment conditions. Linear regression equations are shown. Values are given as average (±SE). n=10

The amount of sediment disturbed decreased significantly with increasing sewage loading ($p<0.05$ for planted 20% Vs planted 60%, and $p<0.001$ for all other combinations). The daily amount of sediment disturbed per individual reached more than 60 g in pristine cells (at both vegetation conditions), while under contaminated conditions sediment displacement did not exceed 30 g (Figure 4-B). Individuals disturbed slightly less sediment (6%) in unplanted than planted pristine cells ($p<0.05$). The opposite was observed for 20% sewage exposure ($p<0.01$) with 33% higher displacement in unplanted cells.

The real and straight distances crawled by each individual showed a significant linear relationship with a slope close to unity, but an intercept towards the real distance ($D_{real}=1.12*D_{straight} + 12.1$, $R^2=0.84$). Consequently, the straight distance underestimated the real distance by 50% for short and about 25% for long distances. The amount of displaced sediment is therefore underestimated when derived from the straight distances, which must be taken into consideration.

4. Discussion

4.1 Survival and Growth

Although most animals may not survive in chronic stable hypoxia (defined as DO <64 µM), they have physiological and behavioural mechanisms to survive intermittent hypoxic periods for days (Diaz and Rosenberg, 1995). The immersion/emersion periodicity in our mesocosms may explain the high survival rates (>60%) registered even at high sewage loading (100%) and long immersion periods (3 d). Some gastropods crawled the cell walls above the water level during immersion with sewage mixtures apparently to escape from the “toxic” environment (also observed by Sagasti et al., 2001; Riedel et al., 2008 for other gastropod species). This sub-lethal behaviour may have also helped gastropods facing long periods of severe hypoxia conditions (< 10 μM) in contaminated cells during night. The remaining individuals stayed dormant and immobile, but alive on the sediment surface (see below). High tolerance to prolonged periods (days to weeks) of hypoxia or even anoxia has been shown for other gastropods (see Kapper & Stickle 1987 in Stickle et al., 1989; Das and Stickle, 1993; Sagasti et al., 2001; Cheung et al., 2008). These authors suggest that most gastropods are oxy-conformers, with oxygen consumption decreasing in proportion with the oxygen availability that can survive extended periods of hypoxia or anoxia. Also, metabolic rate depression is possible in molluscs because, instead of the classical and less efficient glycosis system, they switch to relatively more efficient succinate and propionate pathways during exposure to hypoxia and anoxia (Gade, 1983; Wu, 2002).

Survival of *T. palustris* was lowest (22%) in unvegetated treatments with 60% sewage. The lack of shading by trees during the sunny emersion periods may have exposed snails, suffering from hypoxia during night, to heat and stress by desiccation (Slim et al., 1997; Pape et al., 2008) causing excess mortality as shown for other molluscs (Stickle et al., 1989). Otherwise, sewage did not affect survival of small and larger gastropods differently, indicating similar tolerance to low oxygen by all sizes used in the present study (25 to 114 mm).

The average height increments of *T. palustris* registered in this study are within the range (~2 mm month^{-1} for 15 mm individuals) observed for this species in natural habitats (e.g., Soemodihardjo and Kastoro, 1977). The fast growth observed in pristine planted cells is probably supported by fallen litter as a complementary food source, because benthic microalgal biomass was low in pristine cells. Conversely, microalgae and probably bacteria are known to be major food sources for the conspecific *T. sulcata* in organic contaminated mangrove areas (Meziane and Tsuchiya, 2002). Despite the higher abundance of benthic microalgae and the presence of leaves in sewage treated planted cells, the gastropods height increments were similar to those obtained in pristine unvegetated cells. This indicates that at contaminated conditions the higher availability and quantity of food do not provide gastropods with a greater amount of energy for growth. If we assume that gastropods are food limited under pristine unvegetated conditions and that animals express maximum growth in planted pristine cells, a decline in oxygen levels may decrease the metabolic rate and thus feeding activity, assimilation or energy flow to growth. As a consequence we will observe reduced shell height increment as found for *Stramonita haemastoma* (Das and Stickle, 1993) and *Nassarius festivus* (Cheung et al., 2008). Furthermore, as *Terebralia palustris* is known to prefer salinities of 15-35 (Houbrik, 1991). The low salinity at high sewage loading could also have been a stress factor affecting physiology and consequently growth, especially when coupled with hypoxic conditions (Stickle et al., 1989).

The 100% sewage loading reduced shell size increment considerably compared with the more diluted sewage treatments. The impact was more pronounced in the 100_{3d} than the 100_{12h} treatment, although the total immersion/emersion time was identical over a 6 days period in both treatments. However, the longer immersion periods in the 100_{3d} treatment with low oxygen and salinity may affect the gastropod metabolism so severely that they cannot recover feeding activity and somatic growth fully during the extended emersion periods. Nevertheless, other factors should be considered when comparing the lower growth rates obtained at 100% compared with the 0 to 60% during the previous 6 months which could include: 1) the natural growth rate decrease as the gastropods grow (e.g., Chatzinikolaou and Richardson, 2008); 2) the decrease in feeding activity (or food assimilation) due to an increase in chronic stress possibly due to the artificial system "environment" and/or hypoxic conditions; 3) as well as the colder temperatures that are typical from April to October (cold season) (Sallema and Mgaya, 2004), known to decrease snails metabolism and consequently growth (e.g., Chatzinikolaou and Richardson, 2008).

4.2 Behaviour and Bioturbation

The daily activity of *Terebralia palustris* changed according to environmental conditions. Tidal simulations in the experimental mesocosms functioned similarly to tides in natural mangroves, where animals are covered with seawater once (or twice) a day. The crawling distance achieved at 60% sewage loading is similar (~3 m d^{-1}) to that registered by Wells and Lalli (2003) in Australian mangrove forests. Under less contaminated or even pristine conditions these gastropods moved much faster, reaching crawling distances higher than 8 m d^{-1} as reported for Kenyan mangrove forests (Vannini et al., 2008).

Reduced locomotory activity of *T. palustris* in the wild can be caused by obstacles (i.e. high density of pneumatophores) and excess availability of food (i.e. high abundance of litter). A gastropod feeding on leaf litter will decrease its motile activity and attract other individuals to feed on the same litter (Fratini et al., 2001). It is therefore expected that the crawling distance in the planted mesocosms cells is lower than in the unplanted cells. However, this pattern was only observed in the 20% sewage treated cells, probably due to the low biomass of benthic microalgae.

Although many gastropods showed little or no mortality under low oxygen conditions, hypoxia induced sub-lethal behavioural changes (Sagasti et al., 2001; Riedel et al., 2008, and present study). Gastropods showed a distinct behavioural change in sewage impacted cells when compared with pristine ones. *T. palustris* in pristine cells behave similarly to that reported for natural environments (Fratini et al., 2004; Vannini et al., 2008). Conversely, the inactive behaviour during immersion periods in cells subjected to 20% or 60% sewage loading is probably due to low DO and associated toxic metabolites (e.g, sulphide) release from the sediment. A similar response has been shown for the gastropod *Hexaplex trunculus*, which showed significantly reduced activity during severe hypoxic events (Riedel et al., 2008).

Since individuals in contaminated cells do not feed during immersion, they compensate with high activity levels during emersion periods. Other invertebrates (e.g., crabs) may also increase foraging activity after periods of hypoxia (Sagasti et al., 2001). However, the animals need not to move much during emersion in contaminated treatments because of the higher biomass of benthic microalgae. However, the reported lower growth rates under contaminated planted conditions, suggests that the low animal activity during emersion periods (at 20 and 60% sewage loading) may therefore not only be caused by the high food availability, but rather by other less obvious factors. One such factor could be to avoid desiccation by decreased activity during hot and dry periods (Slim et al., 1997; Pape et al., 2008), which was exactly the condition during the first 5 hours (almost 50% of the time) of emersion periods in our system. Total daily activity was not different in small and large snails, that responded similarly to increase of sewage loading, demonstrating that larger animals process bigger amount of food, mainly microalgae and bacteria due to the low abundance of litter available, during the same travel distance.

The amount of sediment displaced by *T. palustris* depended significantly on the gastropod size (weight), environmental conditions (sediment characteristics and availability of different food types) and especially on anthropogenic influence (sewage), which altered the intensity and periodicity of animal activity. The estimated sediment displaced by gastropods was, as expected, positively correlated to their weight. During movement, these organisms displace (e.g., by pushing directly into the frontal and surrounding sediment) sediment in proportion to their volumetric size (Gilbert et al., 2007). Also, a decrease in locomotion and foraging activity due to depressed metabolism under hypoxic or hot and dry conditions will result in lower particle reworking and redistribution. Especially, if gastropod activity is shifted to periods (emersion) where more stable (drier) sediments conditions diminish bioturbation potential, as occurred in the contaminated cells.

4.3 Implications for constructed mangrove wetland functioning

The high tolerance of *T. palustris* to severe sewage loading demonstrated its high potential for use in constructed mangrove wetlands. However, the low growth rates and changes in behaviour and activity even at relatively low sewage concentrations (e.g., 20%) demonstrated a certain degree of stress. Besides, hypoxic conditions cause a decrease in the amount of energy allocated by gastropods to reproduction (Sagasti et al., 2001; Cheung et al., 2008), and hamper hatching success and larval development (Chan et al., 2008). As a consequence, *T. palustris* populations will eventually decline due to lack of recruitment. This may explain the complete absence of this species from organically enriched mangrove areas as reported by Cannicci et al (in press) and Machiwa and Hallberg (1995).

Ecosystem engineers have recently been addressed as powerful agents to restore ecological systems when managed well (Byers et al., 2006). By limiting microalgal biomass directly by grazing or indirectly by subduction below the sediment surface (Carlén and Ólafsson, 2002; Pape et al., 2008), *T. palustris* suppresses the development of dense algal mats, and consequently avoid the development of near surface anoxic zones (Kristensen and Alongi, 2006; Marsden and Bressington, 2009). Microphytobenthic primary production can potentially be enhanced when the biomass is grazed below the carrying capacity by deposit-feeding invertebrates (Blanchard et al., 2001). These invertebrates may also stimulate benthic bacterial biomass and activity through faecal pellet production and mucus secretion (Solan and Wigham, 2005). Furthermore, the continuous mixing of the top layer by epifauna will enhance carbon mineralization and nutrient cycling by improving sediment aeration and drainage (Aller, 1994), as well as by increasing surface areas for microbial activity (Solan and Wigham, 2005). Consequently, faunal grazing and bioturbation may increase sediment capacity to degrade organic matter. Nevertheless, the high tolerance of *T. palustris* to survive long periods of hypoxia is counteracted by lowered, growth rates due to decreasing foraging activity. This will inevitably diminish the significance of *T. palustris* as an epibenthic microalgal grazer, sediment bioturbator and consequently its role for improving the functioning of mangrove wastewater treatment facilities.

The decrease in individual-specific activity at high contamination levels can be compensated by increasing the population size sufficiently to maintain the desired bioturbation activity. These gastropods may achieve much higher densities ($>100.m^{-2}$) and larger sizes (>> 10 cm) in natural mangroves (Houbrik, 1991; Pape et al., 2008) than used in our mesocosm system. By increasing the mangrove wetlands gastropod density to values of 100 m^{-2} will allow a complete top sediment (3 mm deep) reworking every 10 days based on the results obtained in the present study. The high survival under sewage exposure justifies the use of an increased gastropod density and average size in the wetlands. However, due to lack of recruitment, the sewage exposed populations must continuously be supplemented with individuals from pristine areas. Nevertheless, further studies are needed to better understand the consequences of increasing fauna density on biotic (e.g., microalgae growth) and abiotic (e.g., sediment biogeochemistry and oxygen concentrations during immersion periods), as well as develop low energy cost methodologies to improve environmental conditions for *Terebralia palustris*, or introduction of other mangrove species (such as sesarmid crabs) able to cope with the severe conditions at contaminated mesocosms, as was found for fiddler crab species (Penha-Lopes et al., accepted).

The use of *T. palustris* to improve mangrove wetland functioning also faces other positive consequences. A large proportion of the large litter fall in mature wetlands can be processed by these gastropods (~0.65g h^{-1}) (Fratini et al., 2004). As a consequence, large accumulations of decaying litter are avoided and ecosystem functioning is improved. However, a shift in feeding behaviour from microphytobenthos to litter may significantly change the sediment bioturbation potential and probably counteract the improved functioning by processing litter. Another important consequence of gastropod bioturbation is the construction of “fluffy” (less consolidated) surface sediment and increasing the roughness of the sediment-water interface (Orvain et al., 2003), thus enhancing the sediment erosion and transport (Le Hir et al., 2007; Orvain et al., 2007). Conversely, a decrease in gastropod bioturbation will result in higher benthic microalgal biomass (Carlén and Ólafsson, 2002; Pape et al., 2008), which reduces erosion and transport of sediment (Sutherland et al., 1998; Quaresma et al., 2004). Thus, decreased bioturbation due to discharge of nutrient-rich sewage may reduce sediment transport and affect nutrient cycling and dynamics in mangrove forests and adjacent systems (Kristensen et al., 2008).

Most coastal marine areas are today strongly affected by human activities (Howarth et al., 2000; Halpern et al., 2008), which lead Teal et al. (2008) to speculate: “how much the world’s bioturbation has already been reduced and what effect any further loss may have on the function of the marine ecosystem”. In this study, we addressed this problem in mangrove ecosystems where such studies are scarce (Teal et al., 2008), and concluded that even small contamination levels may strongly decrease the activities and consequently bioturbation of key species in mangrove habitats.

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Chapter 7

Population structure, density and food sources of *Terebralia palustris* (Potamididae: Gastropoda) in a low intertidal *Avicennia marina* mangrove stand (Inhaca Island, Mozambique)

Gil Penha-Lopes[a,b*], Steven Bouillon[b,c], Perrine Mangion[b], Adriano Macia[d], and José Paula[a]

a. Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Avenida Nossa Senhora do Cabo, 939, 2750-642 Cascais, Portugal
Gil Penha-Lopes (*corresponding author): gil.penha-lopes@fc.ul.pt;
Tel: (+351) 214 869 211
Fax+351.214869720
b. Department of Analytical and Environmental Chemistry
Vrije Universiteit Brussel, Brussels
c. Katholieke Universiteit Leuven, Dept. of Earth & Environenmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.
d. Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C.P. 257 Maputo, Moçambique

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Gil Penha-Lopes[a,b*], Steven Bouillon[b,c], Perrine Mangion[b], Adriano Macia[d], and José Paula[a]

a. Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Avenida Nossa Senhora do Cabo, 939, 2750-642 Cascais, Portugal
Gil Penha-Lopes (*corresponding author): gil.penha-lopes@fc.ul.pt;
Tel: (+351) 214 869 211
Fax+351.214869720
b. Department of Analytical and Environmental Chemistry
Vrije Universiteit Brussel, Brussels
c. Katholieke Universiteit Leuven, Dept. of Earth & Environenmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.
d. Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C.P. 257 Maputo, Moçambique

Abstract

Population structure and distribution of *Terebralia palustris* were compared with environmental parameters within microhabitats in a monospecific stand of *Avicennia marina* in southern Mozambique. Stable carbon and nitrogen isotope analysis of *T. palustris* and potential food sources (leaves, pneumatophore epiphytes, and surface sediments) were examined to establish the feeding preferences of *T. palustris*. Stable isotope signatures of individuals of different size classes and from different microhabitats were compared with local food sources. Samples of surface sediments 2.5 to 10 m apart showed some variation (-21.2‰ to -23.0‰) in $\delta^{13}C$, probably due to different contributions from seagrasses, microalgae and mangrove leaves, while $\delta^{15}N$ values varied between 8.7‰ to 15.8‰, indicating its very high variability within a small-scale microcosm. Stable isotope signatures differed significantly between the *T. palustris* size classes and between individuals of the same size class, collected in different microhabitats. Results also suggested that smaller individuals feed on sediment, selecting mainly benthic microalgae, while larger individuals feed on sediment, epiphytes and mangrove leaves. Correlations were found between environmental parameters and gastropod population structure and distribution versus the feeding preferences of individuals of different size classes and in different microhabitats.

While organic content and the abundance of leaves were parameters that correlated best with the total density of gastropods (>85%), the abundance of pneumatophores and leaves, as well as grain size, correlated better with the gastropod size distribution (>65%). Young individuals (height <3cm) occur predominantly in microhabitats characterized by a low density of leaf litter and pneumatophores, reduced organic matter and larger grain size, these being characteristic of lower intertidal open areas that favour benthic microalgal growth. With increasing shell height, *T. palustris* individuals start occupying microhabitats nearer the mangrove trees characterized by large densities of pneumatophores and litter, as well as sediments of smaller grain size, leading to higher organic matter availability in the sediment.

Keywords: *Terebralia palustris*, population structure, distribution, feeding, stable isotopes, mangroves.

1. Introduction

Mangrove systems represent complex and highly dynamic environments in which faunal assemblages typically occupy distinct horizontal or vertical zones, and manifest complex temporal patterns in their activities (Robertson and Alongi, 1992; Hogarth, 2007). Although biological, chemical and physical properties are expected to be reflected in strong correlations between benthic fauna and sedimentary properties, most studies have shown these relationships to be relatively weak (e.g., Chapman and Tolhurst, 2007). The crucial role of marine invertebrates in the food web, nutrient cycling and overall energy flux in Indo-Pacific mangrove ecosystems has become a standard paradigm in ecological research on these tidal forests (Bouillon et al., 2008). Recently, evidence is emerging that many invertebrates have a small home-range and derive most of their diet from locally available food sources (Guest et al., 2006). Small-scale changes in their stable isotope signatures (derived from the carbon and nitrogen sources of their diet) may be a useful tool to track and explain invertebrate microdistribution patterns (Guest and Connolly, 2004; Guest et al., 2004).

During the last decade, one of the most common and abundant mangrove gastropods, *Terebralia palustris* (Linnaeus), has been the subject of several ecological studies due to the significant quantities of leaf litter that it consumes and processes, and it has become a recognized link in nutrient cycling in mangrove forests (Slim et al., 1997; Fratini et al., 2004). It is found in substrata that range from mud to sand, and extends from the upper *Avicennia marina* belt down to the lower fringe stands (Rambabu et al., 1987; Fratini et al., 2004). *Terebralia palustris* is known to be omnivorous, feeding on detritus, leaf litter, mangrove propagules, carrion, sediment particles, benthic diatoms and bacteria (Nishihira, 1983; Rambabu et al., 1987; Dahdouh-Guebas et al., 1998; Fratini et al., 2000).

A spatial separation between young and older individuals was first observed by Soemodihardjo and Kastoro (1977). Due to anatomical differences in the structure of the radula, only large (shell height >5 cm) *T. palustris* actively feed on fallen mangrove leaves, propagules and fruits, while small individuals (shell height <5 cm) are usually detritivorous or deposit feeders (Nishihira, 1983; Houbrik, 1991; Slim et al., 1997; Dahdouh-Guebas et al., 1998; Fratini et al., 2004; Pape et al., 2008). This different feeding strategy has been used to explain the spatial segregation between juveniles (more common in tidal channels and pools) and adults (common in the forest) reported by various authors for Jakarta (Soemodihardjo and Kastoro, 1977), Western Australia (Wells, 1980) and Gazi Bay, Kenya (Slim et al., 1997; Pape et al., 2008). In contrast, some other studies have shown no spatial separation between juveniles and adults (e.g., Fratini et al., 2004 for Gazi Bay, Kenya). Only Pape et al. (2008) have attempted to elucidate the population structure or distribution of *T. palustris* relative to environmental variables and stable isotopic composition. Their study was undertaken along two transects from the landward mudflat zone to the seaward seagrass zone (passing through the mangrove forest), with sampling intervals of 8 m.

The present study aimed to elucidate *T. palustris* microdistribution, as well as its feeding strategies, at spatial scales of 1.25 to 2.50 m. Its spatial distribution and population structure was compared with important abiotic and biotic parameters, such as organic matter content, sediment granulometry, leaf litter and pneumatophore abundance. Together with the environmental parameters, foot muscle and potential food sources of *T. palustris* were subjected to stable isotope analysis (carbon and nitrogen) to better elucidate the relationship between gastropod feeding strategy and distribution. The variability in stable isotope signatures of the sediments and different size classes of *T. palustris* were also documented in each microhabitat.

2. Materials and methods

2.1 Study site

Inhaca is a small island (42 km^2) situated 32 km off Maputo in southern Mozambique, East Africa (26ºS 33ºE). The east coast is exposed to the Indian Ocean, whereas the western and southern coasts face Maputo bay. The island is positioned in a transitional region of tropical to warm subtropical conditions and constitutes a barrier between Maputo Bay and the Indian Ocean. The climate of Inhaca island is characterized by hot and wet (September – March) and warm and dry (April-September) seasons (Bandeira, 1995).

In August 2006, this study was conducted in the low intertidal *Avicennia marina* belt in the Saco mangrove during a spring low tide. The Saco bay is located at the southern end of Inhaca Island, occupies an area of 204 ha, and is bordered by mangroves. While *Ceriops tagal*, *Bruguiera gymnorrhiza* and *Rhizophora mucronata* are found along the channels and in the most densely forest areas, *A. marina* covers the upper and lower limits of the mangrove (Kalk, 1995; De Boer, 2000). The subtidal zone is characterized by patches of seagrasses (Kalk, 1995). At the seaward edge, the *A. marina* trees are large, often more than 5 m high, and their pneumatophores can extend to several meters around the trunk (Kalk, 1995). In this lower *A. marina* belt, three areas were randomly selected and, in each of these, four pneumatophore zones were studied (Fig. 1):

Lower - lower half of the pneumatophore zone (over half a pneumatophore zone away from the *A. marina* canopy)

Mid – central pneumatophore zone.

Upper - upper half of the pneumatophore zone (the latter two zones comprising equal divisions of under half a pneumatophore zone from the *A. marina* canopy).

Canopy – under the *A. marina* canopy.

Each of the pneumatophore zones (excluding the Canopy) comprised a belt that was between 5 and 10 m long and an average of 5 m wide (see Fig. 1 for clarification). An Inter-pneumatophore zone was similarly delineated between pneumatophore zones corresponding to the area adjacent to the Upper and Canopy regions of the pneumatophore zones (see Fig.1).

2.2 Population structure

Four quadrats of 0.25 m² were randomly allocated in each of the pneumatophore and inter-pneumatophore zones. All *T. palustris* specimens inside the quadrats were collected (including those with more than 50% of the body inside the quadrat) and stored in a cool box. In the laboratory, they were counted, weighed (wet weight) and their height (shell height – SH - maximum linear dimension of the shell from the apex to the anterior edge of the lip) was measured.

2.3 Environmental data

While some biogeochemistry parameters are known to be relatively constant throughout the year, or oscillate seasonally or with important events (e.g., heavy rains or winds), some

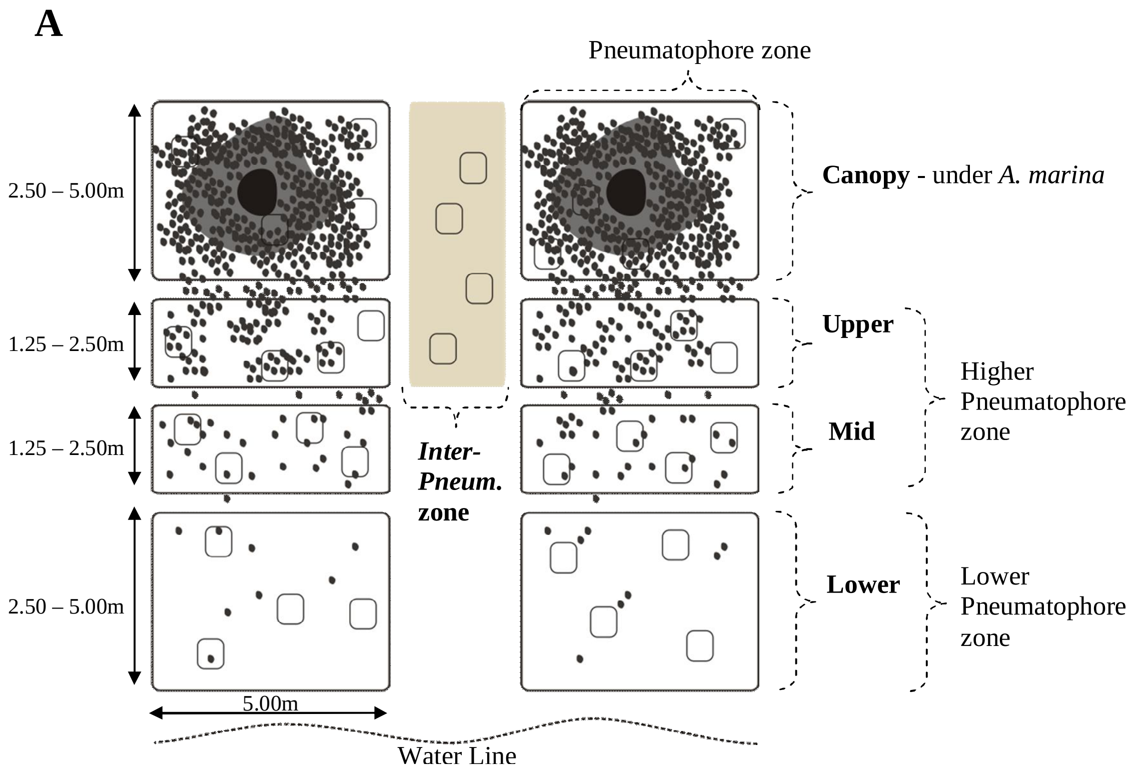

Fig. 1 – Zonation in the lower *Avicennia marina* belt in the Saco, Inhaca Island, southern Mozambique, during low tide. **A** –top view of two pneumatophores zones, where Canopy, Upper, Mid *and* Lower sampling zones are shown as rectangular areas 1.25 - 5.00 m long and 5 m wide. An Inter-pneumatophore zone (grey zone) is also shown. Small black dots designate pneumatophores (●) and small squares sample quadrats of 0.25 m^{-2} (▢). **B** – lateral view of a pneumatophore zone.

fluctuate significantly during a single tidal cycle (e.g., temperature, salinity) (e.g., Chapman and Tolhurst, 2007). Only parameters more or less consistent within a tidal cycle were thus measured in this study, providing a realistic explanation of the observed population structure and distribution patterns.

2.3.1 Pneumatophore and leaf density

The number of pneumatophores (protruding >0.5 cm above the sediment) and leaves were counted in each quadrat.

2.3.2 Granulometry

Two sediment cores (3 cm Ø and 2 cm depth) were extracted per quadrat. Each core was stored in a plastic vial and stored in a cool box during transport to the laboratory. There the sediment samples were oven-dried at 105 °C to constant weight. The different sediment particle sizes were separated on a mechanical shaker using a series of sieves ranging from 2 mm upwards and graded following the Wentworth scale. The residues in each sieve were weighed and assigned to a textural group according to Folk (1954). The median grain size of each sample and the textural group in each zone (derived from 24 replicates from each of the three study areas) was determined using Gradistat 5 software (Blott and Pye (2001).

2.3.3 Organic content

Each sediment core was stored in a cool box during transport to the laboratory. There the samples were dried at 105 ºC to a constant weight, ignited at 550 ºC for three hours and cooled in a desiccator. The loss on ignition (LOI) was measured and the organic content expressed as a percentage of the dry weight (Heiri et al., 2001).

2.4 Stable isotopes

2.4.1 *Terebralia palustris*

Whenever possible, five (or more) individuals of each size class (SH<3, 3<SH<5 and SH>5 cm) per zone were washed with distilled water and part of the foot muscle was removed and dried at 60°C for 72 h. After drying, the samples were frozen until further processing for stable isotope analysis.

2.4.2 Sediment, leaves and pneumatophore epiphytes

Two sediment cores (3 cm Ø and 2 cm depth) were collected in each zone. As the isotopic signature of mangrove leaves generally does not change significantly with decomposition (Zieman et al., 1984; Dehairs et al., 2000), at least five mangrove leaves of all the species present (*A. marina*, *R. mucronata* and *C. tagal*) were randomly picked from different trees in or just around the study area. Pneumatophore epiphytes were gently scraped off with surgical blades. These scrapings were randomly taken from within the range occupied by *T. palustris*. Sediment, leaves and epiphytes were transport to the laboratory in a cool box. Leaves and epiphytes were subsequently washed with distilled water, and dried at 60°C for 72 h, while the sediment samples were freeze-dried.

2.4.3 Analytical procedures

All samples were ground to a fine powder and subsamples of the sediment and epiphytes were acidified with dilute (5 %) HCl before analysis to remove carbonates. δ^{13}C and δ^{15}N analyses were performed with a ThermoFinnigan Flash1112 elemental analyzer, coupled to a ThermoFinnigan delta +XL via a Conflo III interface, with a typical reproducibility of ± 0.15 ‰ for both $\delta\ ^{13}$C and $\delta\ ^{15}$N. All stable isotope ratios are expressed relative to the conventional standards (VPDB limestone for carbon and atmospheric N_2 for nitrogen) as δ values, defined as:

$$\delta^{13}C = \frac{X_{sample} - X_{s\tan dard}}{X_{s\tan dard}} * 10^3 \qquad [‰]$$

where X = ^{13}C/^{12}C or ^{15}N/^{14}N in the case of δ^{15}N.

2.5 Statistical analyses

Stable isotope signatures of all samples (sediment, leaves and epiphytes) and between *Terebralia palustris* of different size classes and zones were compared using non-parametric tests (Kruskal–Wallis), followed by a Dunn multicomparisons test whenever statistical significances were found. Principal component analysis (PCA) was used to reduce the multidimensional environmental data sets to lower dimensions. The variables used in the PCA were percent organic matter, granulometry, number of leaves and pneumatophores per quadrat. Analysis of similarities (ANOSIM) has been widely used for testing hypotheses on spatial differences and temporal changes in biological assemblages (Clarke and Gorley, 2006). This test was used to separate zones by analysing each of the following data sets separately: 1) population data (*T. palustris* density and average height); 2) environmental data (% organic matter, granulometry, number of leaves and pneumatophores per quadrat). The link between environmental variables and *T. palustris*' distribution and abundance was analysed using the BEST analysis. This test maximises the rank correlation between the resemblance matrices of the environmental (percent organic matter, abundance of leaves and pneumatophores, and average grain size) and community variables (average shell length and average density), where all permutations of the variables are tried in the BIOENV algorithms (Clarke and Gorley, 2006). Prior to the PCA, ANOSIM and BEST analyses, environmental data were log(x+1)-transformed (for PCA and ANOSIM) or log(x)-transformed (for BEST) and normalized, while *T. palustris* population data were square root-transformed. Although ANOSIM compares zones with all variables, for a more detailed analysis of each environmental or population variable, non-parametric tests (Kruskal-Wallis) were used followed by Dunn's test whenever differences were detected using the Kruskal-Wallis test. Kruskal-Wallis and Dunn tests were performed using GraphPad Instat, while PCA, ANOSIM and BEST analyses were performed using Primer 6 software.

3. Results

3.1 Environmental factors

Environmental variables were significantly different between zones (Table 1). When analysed with ANOSIM (Table 2), there was a clear separation between all the five zones, defined *a priori* (global R= 0.65). All the pairwise tests between the zones manifested significant differences between them, although some zones were found to overlap (Lower vs Mid, and Mid vs Upper). With the help of PCA (Fig. 2), it was possible to reduce all the environmental data sets to two principal components, which explained more than 77% of the cumulative percentage variation (PC1, 50% and PC2, 27%).

Table 1 – Average (± SD) pneumatophore and leaf density, sediment median grain size, percentage of sediment organic matter (LOI), *Terebralia palustris* height, density and total biomass registered in each of the 5 zones. Different letters indicate a significant difference ($p<0.05$) between the 5 zones for each variable. (number in parenthesis indicate number of replicates)

	Local Properties				***Terebralia palustris***		
Zone	**Pneumatophore density (m^{-2})**	**Leaf density (m^{-2})**	**Median grain size (mm)**	**LOI (% Org. Mat)**	**Height (cm)**	**Density (ind m^{-2})**	**Biomass (wet weight) (g m^{-2})**
Inter- pneumatophore	2.3±5.5^{a} (12)	23. 7±68.5^{a} (12)	1.3±0.2^{a,b} (12)	2.7±2.5^{a,b} (8)	2.3±0.8^{a} (551)	182. 7±224.5^{b,c} (12)	44.05 ± 58.44^{b} (12)
Lower	64. 7±28.5^{b} (12)	2. 7±3.9^{a} (12)	1.4±0.4^{a} (12)	1.6±0.4^{a} (8)	2.8±1.3^{a,b,c} (7)	2.3±4.7^{a} (12)	0.85 ± 1.56^{a} (12)
Mid	85.0±37.3^{b,c} (12)	8, 7±7.2^{a,b} (12)	1.4±0.3 ^{a} (12)	3.4±3.1^{a,b} (8)	2.6±0.8^{b} (582)	193. 7±159.3^{c} (12)	62.42 ± 39.65^{b} (12)
Upper	256.3±77.6^{c,d} (12)	37.3±27.4^{b,c} (12)	0.9±0.4^{b,c} (12)	3.1 ±1.5^{a,b} (8)	3.8±1.1^{c} (383)	127.7±81.0^{c} (12)	114.26 ± 46.96^{c} (12)
Canopy	398.0±142.0^{d} (12)	140. 7±99.4^{c} (12)	0.8±0.2^{c} (12)	5.0±1.3^{b} (8)	4.7±0.6^{d} (86)	29.0±39.9^{a,b} (12)	46.42 ± 62.36^{a,b} (12)

Table 2 - Results from an ANOSIM global and pair-wise tests using Bray–Curtis similarity (for population data) or Euclidean distance (for environmental), when comparing: 1) population data (*T. palustris* density and average height); 2) environmental data (% organic matter content; median grain size; number of leaves and pneumatophores per quadrat. R-values >0.75 were interpreted as well separated; R>0.5 as overlapping but clearly different, and R<0.25 as barely separable at all. p-values also provide the significance of the results obtained.

Pair-wise	**Environmental data Global*R*=0.65 (*p*= 0.001)**	**Population data Global*R*=0.26 (*p*= 0.001)**
Lower Vs Mid	*R*=0.176 (*p*= 0.009)	*R*=0.077 (*p*= 0.002)
Lower Vs Upper	*R*=0.822 (*p*= 0.001)	*R*=0.768 (*p*= 0.001)
Lower Vs Canopy	*R*=0.997 (*p*= 0.001)	*R*=0.203 (*p*= 0.041)
Lower Vs Inter-pneumatophore	*R*=0.453 (*p*= 0.002)	*R*=0.542 (*p*= 0.002)
Mid Vs Upper	*R*=0.218 (*p*= 0.012)	*R*=0.007 (*p*= 0.847)
Mid Vs Canopy	*R*=0.800 (*p*= 0.001)	*R*=0.017 (*p*= 0.017)
Mid Vs *Inter-Pneum*atophore	*R*=0.500 (*p*= 0.001)	*R*=0.041 (*p*= 0.701)
Upper Vs Canopy	*R*=0.902 (*p*= 0.001)	*R*=0.131 (*p*= 0.043)
Upper Vs *Inter-Pneum*.	*R*=0.830 (*p*= 0.001)	*R*=0.022 (*p*= 0.517)
Canopy Vs *Inter-Pneum*.	*R*=0.936 (*p*= 0.001)	*R*=0.058 (*p*= 0.142)

The Canopy and Upper zone data are found more frequently on the negative side of the PC1 axis, where the number of leaves, pneumatophores and sediment organic matter are higher and the mean grain size is smaller. The Lower and Inter-pneumatophore zones are clearly located at the positive side of the axis, while the Mid zone attained both negative and positive values. In the PC2, negative values are related to an increase in medium grain size and the number of pneumatophores, and a decrease in sediment organic matter. This axis clearly separates the Inter-pneumatophore zone from all the other zones (Fig. 2). Using Gradistat 5, the Lower and Mid zones were classified as “sandy gravel”, the Upper and Inter-pneumatophore zones as “muddy sand” and the Canopy zone as “gravelly mud”.

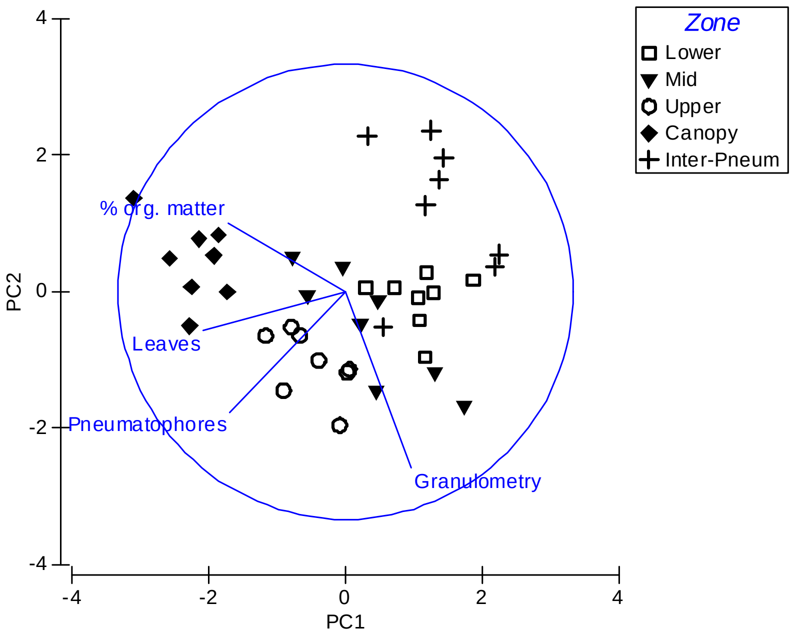

Fig. 2 – Two-dimensional scatter plot of the First and Second Canonical axes for all environmental variables measured in each study zone. Variable eigenvectors for PC1 (x-axis) and PC2 (y-axis) are respectively: leaves (-0.626; -0.173), pneumatophores (-0.510; -0.528), organic content (-0.516; 0.304), and average grain size (0.285;-0.774) (see text for further explanation).

3.2 Stable isotope analysis

3.2.1 Primary producers

The mean carbon stable isotope ratio of *Avicennia marina* (-28.8±0.5‰) was significantly lower than the values obtained for *Rhizophora mucronata* (-27.5±0.5‰) and *Ceriops tagal* (-27.2±0.3‰) (Fig. 3). The nitrogen stable isotope ratios manifested the opposite trend with much higher values in *A. marina* (7.1±1.2‰) compared to *R. mucronata* and *C. tagal* (0.9±0.3 and 1.4±0.5‰, respectively) (Fig. 3). The carbon stable isotope ratios of epiphytes (-24.1±1.1‰) scraped off the pneumatophores were more enriched than those of the mangrove leaves and their δ^{15}N signatures were rather low (1.3±0.3‰) (Fig. 3).

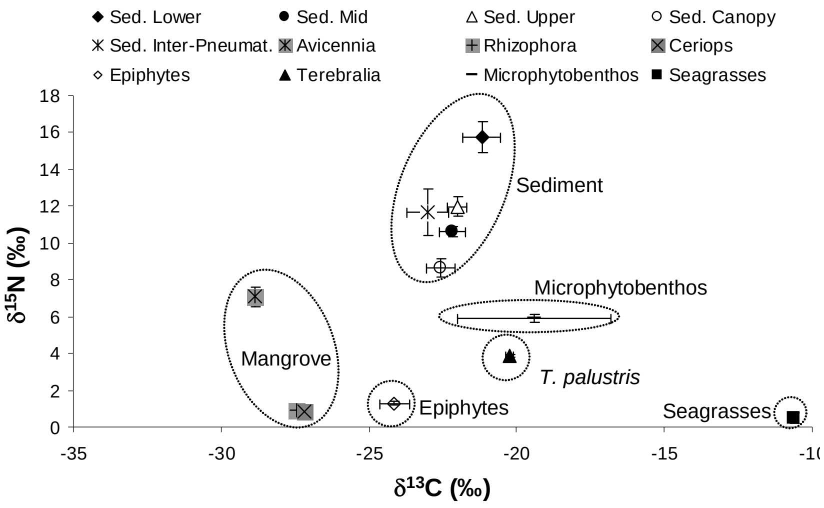

Fig. 3 – Plot of average δ^{13}C and δ^{15}N values in *T. palustris* foot muscle, sediment and primary producers in the study area. Microphytobenthos data were obtained from previous studies in Saco Bay, Inhaca, Mozambique (Abreu et al., in press). Error bars indicate SE (n= 5-60).

3.2.2 Sediment

The sediment $\delta^{13}C$ signatures (-22.2±0.7‰) were generally enriched compared to mangrove leaf tissue (~-28‰) (Fig 5). However, sediment from the different zones manifested some significant differences regarding their $\delta^{15}N$ and C/N ratios (Table 3). From the canopy down to the lower pneumatophore zone, there was a slight increase in the sediment $\delta^{13}C$ signatures (going from -22.6 to -21.2 ‰), whereas the C/N ratios remained more or less constant along this transect (~14±0.5, Table 3). In contrast, sediment from the inter-pneumatophore zone differed from that in the pneumatophore zone, having $\delta^{13}C$ even more depleted than in the canopy zone and a greater C/N ratio. Although sedimentary $\delta^{15}N$ values became enriched from the Canopy towards the Lower zone (from 8.7‰ to 15.8‰), the high variability masked any clear trend. Average $\delta^{13}C$ and $\delta^{15}N$ values in the Inter-pneumatophore zone were similar to those found in Mid and Upper zones.

Table 3 – Average (±SD) $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) and C/N ratios obtained for sediment samples from different zones. Different letters indicate significant difference - $p<0.05$ - between the 5 zones. (N=6)

	Stable Isotopes		**C/N ratio**
Zone	δ^{15}**N**	δ^{13}**C**	
Inter-pneumatophore	11.7±2.9^{a,b}	-23.0±1.6	18.8±2.0^{a}
Lower	15.8±2.0^{a}	-21.2±1.6	13.6±1.3^{b}
Mid	10.6±0.7^{b}	-22.2±1.1	14.5±1.0^{b}
Upper	12.0±1.2^{a,b}	-22.0±0.8	14.5±0.8^{b}
Canopy	8.7±1.3^{b}	-22.6±1.2	13.7±1.2^{b}

3.2.3 *Terebralia palustris*

Stable isotope signatures in *T. palustris* individuals changed according to individual size, as well as to the zone they inhabited. Irrespective of the sampling zone, the overall picture was that, with increasing animal size, $\delta^{13}C$ values tended to decrease but the $\delta^{15}N$ values were more irregular (Table 3, Fig. 5). The $\delta^{13}C$ signatures of individuals larger than 5 cm was significantly different ($p<0.001$) from smaller size classes (-20.1±0.8 ‰, -19.9±0.8 ‰ and -21.3±0.9 ‰ for size classes <3, 3-5 and >5 cm, respectively). Small individuals (<3cm) collected in the Mid zone were significantly ($p<0.001$) depleted in $\delta^{13}C$ when compared to the ones collected in the Upper and Inter-pneumatophore zone, while individuals 3 to 5 cm in height were significantly ($p<0.001$) enriched in the Inter-pneumatophore zone relative to those found in the Mid and Upper zones. The $\delta^{15}N$ signature of small *T. palustris* was significantly enriched in the Upper zone when compared to the individuals collected in the Mid ($p<0.05$) and Inter-pneumatophore ($p<0.001$) zones, while mid-size individuals inhabiting the Inter-pneumatophore zone were significantly enriched relative to those found in the Canopy zone ($p<0.05$).

Table 4 – Average (±SD) $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of foot muscle of *T. palustris* for different size classes and zones. Different letters indicate significant difference - $p<0.05$ - between the zones. Number in parenthesis indicates number of replicates. (n.a. – not available)

	$\delta^{13}C$ Stable Isotope (‰)				$\delta^{15}N$ Stable Isotope (‰)			
Size classes (cm)	Inter-pneumatophore	Mid	Upper	Canopy	Inter-pneumatophore	Mid	Upper	Canopy
x < 3	-19.4±0.6^{a} (8)	-20.9±0.4^{b} (7)	-20.0±0.5^{a} (8)	n.a.	3.9±0.2^{a} (8)	3.7±0.2^{a} (7)	2.9±0.2^{b} (8)	n.a.
3 < x < 5	-18.9±0.7^{a} (8)	-20.3±0.6^{b} (9)	-20.5±0.8^{b} (5)	-19.9±0.3^{a,b} (4)	4.6±0.4^{a} (8)	4.3±0.3^{a,b} (9)	4.1±0.2^{a,b} (5)	4.0±0.1^{b} (4)
x > 5	-21.2 (1)	-21.1±0.8 (2)	-21.0±1.0 (7)	-22.0±0.8 (4)	4.0 (1)	3.9±0.2 (2)	3.7±0.3 (7)	3.7±0.5 (4)

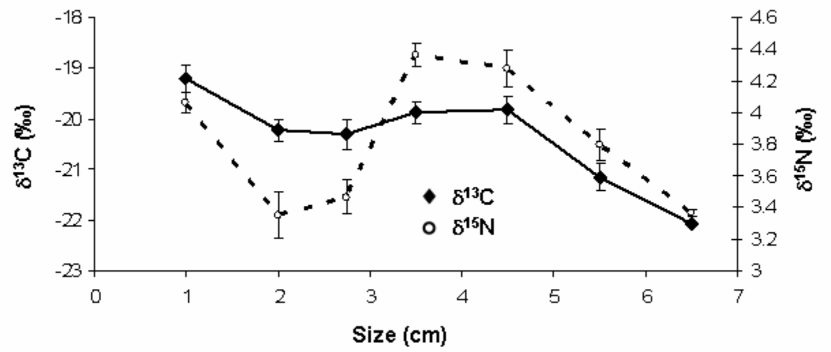

Fig. 4– Changes in $\delta^{15}N$ and $\delta^{13}C$ (Avg±SE) in *T. palustris* foot muscle with increasing shell height in the study area.

3.3 Population distribution and structure

A clear pattern was found in *T. palustris* microscale distribution (Fig. 5). In the Lower zone, none or very few individuals were found (Table 1). The smallest animals occurred predominantly in the Inter-pneumatophore and Mid zones, where the highest densities of *T. palustris* were observed. Small to large individuals were collected in the Upper zone, although the latter at much higher densities, while only larger individuals were observed in the Canopy zone. There was a significant increase in size in *T. palustris* from the Mid zone towards the Canopy zone (Table 1). Although the mean biomass (wet weight) mirrored the average density, the former is strongly influenced by the average size, resulting in a very low biomass in the Lower zone and very high in the Upper zone (Table 1).

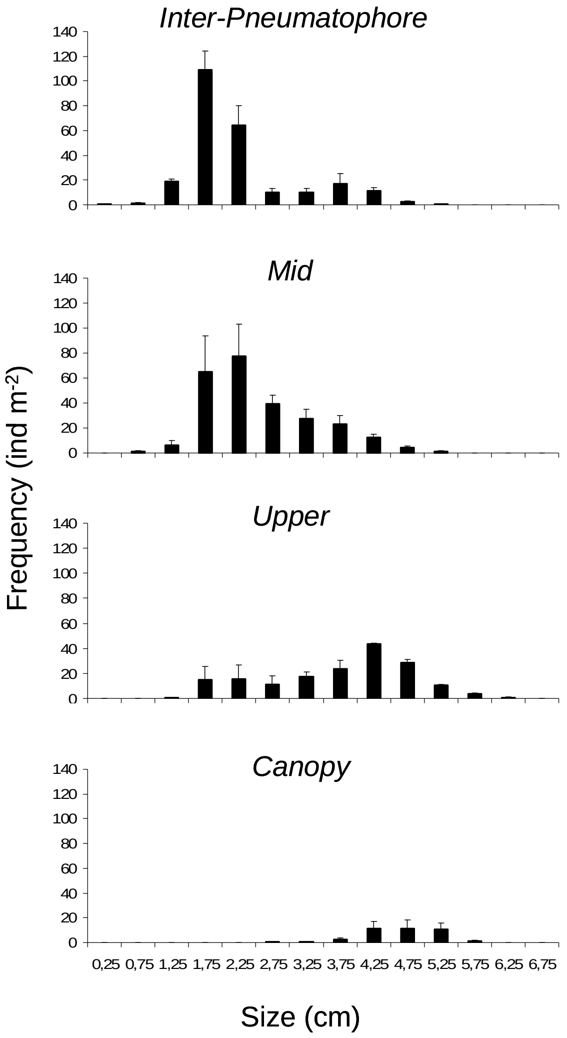

Fig. 5 – Size frequency distribution of *T. palustris* (Avg±SE) in the four (Mid, Upper, Canopy and Inter-pneumatophore) *Avicennia marina* zones. The Lower zone is not shown due the very low density of individuals encountered.

ANOSIM analysis (Table 2) indicated that the zones were barely separable with regard to both *T. palustris* density and average size (Global $R = 0.26$). However, the pairwise tests indicated that the Lower zone was significantly different from all the other zones ($p < 0.041$), as was the Canopy zone from the Mid ($p < 0.041$) and Upper ($p < 0.041$) zones, but they were barely separable ($R < 0.25$). All the other pairwise comparisons overlapped and were not significantly different from each other (Table 2).

BEST analysis indicated that 85.5% of the *T. palustris* abundance can be explained by only two environmental variables: sediment organic content and the abundance of leaves, with the former accounting for ~80% of the variation. Granulometry and the density of leaves and pneumatophores account for ~65% of the gastropod size distribution, with the pneumatophore density explaining slightly more than 45% of this distribution.

4. Discussion

4.1 Microhabitat characterization

The environmental variables covered in this study were found to gradually change from Lower and Inter-pneumatophore zone towards the Canopy. The more congested the mangrove root systems (towards the Canopy) the more effective they become as litter traps, increasing the organic content of the soil, and also trap smaller sediment particles, creating a more muddy sediment (Robertson and Alongi, 1992; Hogarth, 2007). This further increases the organic content as finer particles have a larger surface area for the adsorption of organic matter (Gray, 1974). The Inter-pneumatophore zone had almost no pneumatophores and very sandy sediments. However, the leaf density was also as high as the Mid zone, where pneumatophore density was much higher. This may be due to the fact that the Inter-pneumatophore zones is located between the Upper and Canopy zones, both of these generating considerable leaf litter, and the Inter-pneumatophore zone is a passive corridor that exports mangrove litter (supported by the depleted δ^{13}C values measured in sediment in this zone).

With regard to the stable isotopic analyses, all the mangrove leaves had similar C and N isotopic signatures when compared to previous studies undertaken in the Saco mangrove (e.g., Macia, 2004), except for the δ^{15}N values obtained for *Avicennia marina* leaves, which were much higher. Results obtained in the surface sediments yielded average δ^{13}C values that were similar to other studies, although the δ^{15}N was, on average, much higher (8.7‰ to 15.8‰) than that found in previous studies (1.0‰ to 4.0‰) (e.g., Bouillon et al., 2002; 2004; Macia, 2004). Previous literature has already recorded high δ^{15}N signatures for *A. marina* leaves as well as for mangrove surface sediment (for a review see Bouillon et al., 2008). The high values and variability of the δ^{15}N signatures are likely to be due to a difference in nitrogen processes in the sediments of the different zones. Also, during sampling, runoff water from a nearby village was regularly observed, which could be a source of isotopically enriched nitrogen (Bouillon et al., 2008).

Due to difficulties in sampling, δ^{13}C data for seagrasses and benthic microalgae were obtained from previous studies (Abreu et al., in press). These data provide a clear distinction between the δ^{13}C signatures of mangrove tissues (~ -28 ‰) compared to the other carbon sources available in the area, i.e. epiphytes (-23.7‰), benthic microalgae (-19.4‰) and seagrass (-11.9‰) detritus.

4.2 Population structure microdistribution and feeding preferences

The spatial segregation between juveniles and adults of *T. palustris* reported by various authors for Jakarta (Soemodihardjo and Kastoro, 1977), Western Australia (Wells, 1980) and Gazi Bay, Kenya (Slim et al., 1997; Pape et al., 2008) was also encountered in the present study. Small *T. palustris* were more common on lower, open sandflats, while larger individuals tended to reside inside the mangrove forest. Both previous studies (Slim et al., 1997; Pape et al., 2008) suggested that 5 cm was the critical size at which δ^{13}C decreased, indicating an increase in leaf consumption, most likely caused by an ontogenetic change in diet (Houbrik, 1991). However, it should be stressed that in the Saco, the *T. palustris* population attained a maximum height of 6.25 cm. The clear decrease in δ^{13}C values in individuals measuring more than 5 cm in height is in agreement with previous research undertaken in *Ceriops tagal* (Slim et al., 1997) and *Sonneratia alba* (Pape et al., 2008) mangrove forests where gastropods reach much larger shell sizes.

Small *T. palustris* were characterized by an average stable carbon isotope value of -20.1‰, which was 2.1‰ enriched relative to their suggested main food source (sediment, with a δ^{13}C average value of -22.2 ‰). Although a general increase of 0 – 1 ‰ could partially explain the observed discrepancy (Bouillon et al., 2008), selective assimilation of microbenthic algae could be taking place, as suggested by Pape et al (2008). Microbenthic algae in the Saco displayed an average δ^{13}C value of -19.4 ‰ (Abreu et al., in press), a value similar to the one we obtained for smaller *T. palustris*. The high nutritional value of microalgae compared to mangrove tissue may also explain the higher densities of smaller gastropods in the lower intertidal zones, since the microalgal biomass in the upper zones is probably lower due to the lower light intensity under the dense canopy and inhibition caused by soluble tannins released by mangrove leaves (Alongi and Sasekumar, 1992). On the other hand, δ^{13}C signatures of larger *T. palustris* all fell in a narrow range between -21 and -22 ‰, which is still ~6.0 ‰ enriched relative to their apparent food source, namely *A. marina* leaves (δ^{13}C value of -28.8 ‰), which accounted for more than 90% of the total number of leaves found in litter in the study area (personal observation).

The large difference in carbon isotope signatures between larger snails and *A. marina* mangrove leaves suggests that other food sources contributed the bulk of their diet. In this study, larger individuals were also observed to actively feed on pneumatophore epiphytes and graze the surface sediments. Both these carbon sources would provide the more enriched values of δ^{13}C observed. These findings thus suggest that leaves may not be the most important diet of these gastropods and, although they are known to consume a large amount of leaf material (Fratini et al., 2004), other food sources merit consideration in global carbon budgets in mangrove habitats. This is especially true of small gastropods that may represent a significant percentage of the *T. palustris* biomass, as in the present study. Despite the potentially large-scale movement of organic matter, the significant differences found between gastropods inhabiting the different zones indicate that they obtain their nutrition variably from the local sediment, leaves and epiphytes. This has been illustrated by Guest et al. (2004) for crabs and other gastropod species inhabiting the perimeter of saltmarshes and mangrove forests. However, it must also be recognised that the δ^{13}C and δ^{15}N in the animals they studied is not only a reflection of their food source at the time of sampling, but also results from the diet consumed during the preceding period (McCutchan et al., 2003).

Previous studies have demonstrated that the density of these gastropods is strongly correlated to grain size and organic content (e.g., Wells, 1980; Rambabu et al., 1987; Fratini et al., 2004), while others have found no such relationship (Pape et al., 2008). Results of our study indicate that the environmental parameter that best explained *T. palustris* distribution and density (higher than 85%) is the sediment organic matter content, followed by the abundance of leaves. This correlation seems reasonable since a higher availability of food resources usually positively affects the invertebrate biomass inhabiting a particular microhabitat.

The environmental variables that better explained *T. palustris* population size structure distribution (more than 65%) were grain size, and the number of leaves and pneumatophores, with the latter (pneumatophores) accounting for more than 45% of this distribution. The higher the density of pneumatophores, the finer the sediment and the greater the abundance of leaves, leads to a larger animal size in any particular zone. According to our stable isotope analyses, a higher concentration of organic detritus and microalgae would promote higher densities of smaller individuals, while a high density of mangrove leaves would lead to a greater abundance of larger individuals. However, if we focused only on organic matter content and leaf density, we would be unable to explain more than 40% of the *T. palustris* population structure microdistribution.

The absence of juvenile gastropods from muddier sediments in the mangrove forest was observed in earlier studies, and was attributed to the finer sediment and the associated biogeochemical properties, such as high salinity (Pape et al., 2008). It is also known that smaller individuals have a larger surface/volume ratio, decreasing their resistance to desiccation, and may occupy lower zones that provide a longer immersion period, as well as benefiting them with more or less continuous run-off from the upper zones during low tide (personal observation). Although this seems to indicate the existence of an “ontogenic migration” from the Inter-pneumatophore zone to the Mid pneumatophore zone, after which the animals migrate towards the Canopy zone, this was not proven in this study and other reasons may also merit consideration. Differences in recruitment, growth, mortality and predation, as well as intra-specific competition for space or resources, may also be contributors in this regard.

5. Conclusion

Stable isotope signatures suggest that smaller *T. palustris* feed on sediment and benthic microalgae, while larger individuals feed on sediment, epiphytes and mangrove leaves. This further suggests that the population structure and distribution of *T. palustris* are correlated with environmental factors within the different microhabitats. While the organic content seems to better correlate with the density of *T. palustris*, gastropod size distribution correlates best with the number of pneumatophores. Young *T. palustris* (height <3 cm) occur predominantly in lower intertidal microhabitats characterized by a reduced number of leaves and pneumatophores, reduced organic matter content and large sediment grain sizes. As they grow larger, *T. palustris* are found closer to the mangrove tree microhabitats characterized by a higher density of pneumatophores and litter, as well as smaller sediment grain sizes, providing richer organic matter.

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Chapter 8

Stable isotopes as indicators of sewage inputs and trophic changes in constructed mangrove wastewater wetlands

Gil Penha-Lopes[a,b*], Perrine Mangion[b], Samwael Limbu[c], Erik Kristensen[d], Frank Dehairs[b], Steven Bouillon[b,e] and José Paula[a]

[a] Centro de Oceanografia, Laboratório Marítimo da Guia - Faculdade de Ciências da Universidade de Lisboa, Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211
[b] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[c] University of Dar es Salaam, Faculty of Aquatic Sciences and Technology, P.O. Box 35064, Dar es Salaam, Tanzania
[d] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark
[e] Katholieke Universiteit Leuven, Dept. of Earth & Environmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.

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Gil Penha-Lopes[a,b*], Perrine Mangion[b], Samwael Limbu[c], Erik Kristensen[d], Frank Dehairs[b], Steven Bouillon[b, e] and José Paula[a]

[a] Centro de Oceanografia, Laboratório Marítimo da Guia - Faculdade de Ciências da Universidade de Lisboa, Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal

*Corresponding author: gil.penha-lopes@fc.ul.pt

Phone: (+351) 214 869 211

[b] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels

[c] University of Dar es Salaam, Faculty of Aquatic Sciences and Technology, P.O. Box 35064, Dar es Salaam, Tanzania

[d] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark

[e] Katholieke Universiteit Leuven, Dept. of Earth & Environmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.

Abstract

The effect of 60% organic-rich secondary treatment sewage concentration (diluted in seawater) and presence of mangrove trees on the benthic food web structure of mangrove constructed wetlands (MCW) was assessed. Stable isotopes (SI) proved to be a reliable tool to better understand MCW food web structure and to measure anthropogenic impact in different ecosystem compartments. In plots receiving secondary sewage, δ^{15}N signatures of microalgae and sediments were elevated, while those of mangrove leaves remained similar as in the control plots. A significant increase in δ^{15}N signatures was also observed in higher trophic levels exposed to sewage (the fiddler crab *Uca annulipes* and the gastropod *Terebralia palustris*). These results suggest that in mangrove sewage treatment facilities, the introduction of macrofauna may be essential to maintain and control microphytobenthos biomass and productivity, as well as mangrove litter consumption and degradation in sewage treatment facilities, reducing these systems running and maintenance cost.

Keywords: constructed wetlands, mangroves, stable isotopes, pollution, ecological indicators

1. Introduction

Mangrove forests have shown the potential to act as filters for anthropogenic wastes, thus preventing coastal pollution (Wong et al., 1997; Valiela and Cole, 2002), and the efficiency of constructed mangrove wetlands (CMW) has recently been addressed (Yang et al., 2008). These systems do not mimic the ecosystem structure and dynamics of natural mangrove environments. They often have specific sediment, hydrological and geochemical properties that tend to improve system efficiency (Crites et al., 2006). Sewage loading can lead to higher primary (Hillebrand et al., 2000; Savage et al., 2004) and secondary productivity (deBruyn et al., 2003; Cross et al., 2006), but also to a decrease in fauna and flora diversity, due to the low oxygen levels and high concentrations of toxins, such as sulfide (Wu, 2002; Hargrave et al., 2008). The food web structure and dynamics within constructed mesocosms systems are often impoverished as observed in contaminated natural ecosystems (Cross et al., 2006; Singer and Battin, 2007).

Stable isotope (SI) signatures have been successfully used to trace organic matter and nutrient transformations within mangrove food webs, due to the distinct signal from different food sources (Bouillon et al., 2008), and several studies have focussed on the role of primary producers and microbial communities for the diet of invertebrates within these forests (e.g., Bouillon et al., 2002; 2004b; Penha-Lopes et al., in press). The limited (0–1‰) or substantial (2.6 to 3.4‰) fractionation of carbon and nitrogen SI, respectively, between an organism and its dietary sources is the key to understand food web dynamics. While analysis of δ^{13}C can elucidate the origin of the diet of consumers, δ^{15}N signatures can identify the trophic level of consumers (McCutchan et al., 2003; Bouillon et al., 2008).

Recently, carbon and nitrogen SI signatures have also been proposed as a versatile approach for assessing and monitoring anthropogenic contamination within ecosystems (Cabana and Rasmussen, 1996; Costanzo et al., 2001; Vizzini et al., 2005; Cole et al., 2006). Although δ^{13}C may track sewage-sludge contamination (depleted in ^{13}C) (Gearing et al., 1991), δ^{15}N are considered the most robust and reliable indicator of sewage and animal waste contamination (Van Dover et al., 1992; Heikoop et al., 2000; Vizzini and Mazzola, 2006). The dissolved inorganic N pool in sewage has a significantly higher δ^{15}N than marine nitrogen as a result of microbial activity (nitrification and denitrification) and volatilization of gaseous NH_3 before sewage discharge (Michener and Lajtha, 2007). δ^{15}N signatures of sediment, primary producers and invertebrates have shown their high potential as early indicators of anthropogenic pollution, even at low sewage concentrations (McClelland et al., 1997; McClelland and Valiela, 1998; Struck et al., 2000; Cole et al., 2004; Cole et al., 2005). Anthropogenic pollution enters the food web through primary producers assimilation of sewage-derived N, and is as such transferred to higher trophic levels, although some consumers can also directly exploit sewage organic matter (Michener and Lajtha, 2007).

Stable isotope studies from natural mangrove ecosystems contaminated with sewage or agricultural wastes are scarce. Only three studies have identified nitrogen SI derived from sewage in mangrove tree tissues (Fry et al., 2000; Costanzo, 2003; Pitt et al., 2009) and associated crab species (Pitt et al., 2009). No studies have, to our knowledge, yet applied SI as a tool to track sewage derived carbon and nitrogen in CMW food webs.

The present study aims to validate the effects of anthropogenic sewage on the SI of different CMW compartments (sediment, microphytobenthos, mangrove trees and fauna species) as indicators of sewage exposure. We also use SI to examine potential diet shift of two key macrofauna species (*Uca annulipes* and *Terebralia palustris*) under different domestic sewage loadings (i.e. 0 and 60% of sewage with BOD of 150 mg L^{-1} diluted in seawater) and vegetation conditions (i.e. *Avicennia marina*, *Rhizophora mucronata*, or unvegetated). Our ultimate goal is to understand the importance of these macrofauna species on CMW functioning. Both these species already demonstrated to play an important ecological role in natural and CMW through organic matter consumption, regulation of meiofauna and microphytobenthos biomass, affect sediment turnover and stability through bioturbation activities, and increase organic matter degradation and gas exchange mainly during emersion periods (Cannicci et al., 2008; Kristensen, 2008; 2008a; Kristensen et al., 2008b; Lee, 2008; accepted-a; accepted-b). In parallel studies, both these species survival was evaluated in pristine and contaminated conditions, and survival rates nearly 50 and 80% for crabs and gastropods were observed, respectively, regardless of sewage concentration (Penha-Lopes et al., accepted-a; accepted-b).

2. Materials and methods

2.1 Experimental setup

A mesocosm system consisting of 18 cells (9 m^2 each) was constructed at the upper *Avicennia marina* (Forsk.) belt of the Jangwani mangrove forest near Dar es Salaam, Tanzania (see system description at Penha-Lopes et al., accepted-b). Six cells were either kept as unvegetated controls (bare), planted with *Rhizophora mucronata* Lam. or *A. marina* (Forsk.) mangrove trees. Sediment was established and saplings planted (at a density of 2.8 m^{-2}) on selected cells in early February 2006. Epifauna was introduced to all cells in late August 2006. Males and females of the two most abundant fiddler crab species in the area (*Uca annulipes* and *U. inversa*) were introduced at a total density of 18 crabs m^{-2}, while the common mangrove gastropod, *Terebralia palustris* (Linnaeus, 1767) was established at a density of 5 ind. m^{-2}. The fauna was randomly collected from the Kunduchi mangrove forest immediately prior to introduction. The system was initially inundated exclusively with seawater and sewage loading was initiated in early October 2006. Sewage was mixed with natural seawater to obtain a loading of 60%. A diurnal tidal rhythm was simulated with 12 hour inundation to 0.1 m depth starting at 23:00. The basic chemical and biological characteristics of sewage-seawater mixtures are presented in Tables 1 and 2 (adapted from PUMPSEA, 2008). *A. marina* and *R. mucronata* were 50.30±1.17 and 55.3±0.5 cm (±SE) tall, respectively, and litter fall from the young trees was absent when sewage discharge was initiated. They grew to 223.9±18.0 and 133.0±13.2 cm, respectively, with litter fall rates of 1.2 ± 0.2 and 2.8 ± 0.7 g DW m^{-2} day^{-1} (for *A. marina* and *R. mucronata*, respectively) in October 2007 (PUMPSEA, 2008)

Table 1 - Chemical characteristics and oxygen uptake of the sewage-sea water mixtures used in the experimental mesocosms. (DO: Dissolved Oxygen) (n = 45). Values are given as averages ± SE (adapted from PUMPSEA, 2008).

Sewage loading	**Salinity (‰)**	**DO_{day} (μM)**	**DO_{night} (μM)**	**NH_4^+ (μM)**	**NO_3^- (μM)**	**PO_4^{3-} (μM)**
0%	39.1 ± 0.6	308 ± 133	197 ± 17	26 ± 2	3.6 ± 1.4	42 ± 4
60%	18.9 ± 1.4	225 ± 256	9 ± 1	194 ± 17	5.0 ± 1.4	206 ± 14

Table 2. Sediment surface chlorophyll-a concentration at different vegetation and sewage concentration treatments in April 2007 is shown (n = 12). Values are given as averages ± SE (adapted from PUMPSEA, 2008).

	Chl a (μg g^{-1})	
	0%	**60%**
Bare	13.9±3.9	23.1±9.4
A. marina	5.4±1.1	14.0±3.5
R. mucronata	8.1±2.7	14.7±7.0

2.2 Stable isotopes

2.2.1 Fauna

Fauna was sampled before sewage loading was initiated and again after six and twelve months (October 2006, April 2007 and October 2007). At each date, two to four male crabs of *U. annulipes* and *T. palustris* individuals were collected from each cell. To discriminate the effects of sewage in the mesocosms, five to ten specimens were collected from uncontaminated sites in the Kunduchi mangrove forest concurrently to the samplings in the plots. After collection, the animals were immediately transported in cool boxes to the laboratory where the tissues from the largest claw of each crab and part of the foot muscle of each gastropod was removed and immediately freeze-dried and stored at -30 ºC until stable isotope analysis.

2.2.2 Sewage, sediment and primary producers

Three sediment cores (3 cm Ø and 2 cm depth) were collected in each cell at October 2006 and March and October 2007. A single sample of sewage particulate organic matter (SPOM) was obtained from the primary wastewater pond before sewage was discharged into the mesocosms. Six mangrove leaves (3 old and 3 new) were randomly picked from different trees in each of the planted cells at the 3 sampling periods. Benthic microalgae (BMA) were gently scraped off the sediment with surgical blades in March 2007. Sediment, leaves and BMA were transport to the laboratory in a cool box. Leaves were subsequently washed with distilled water, and dried at 60°C for 72 h, while the sediment and benthic microalgae samples were freeze-dried.

2.2.3 Analytical procedures

All samples were powdered by grinding before analysis. Subsamples of the sediment were furthermore acidified with dilute (5 %) HCl to remove carbonates. Concentrations of organic carbon, total nitrogen and C/N ratios were determined by combusting pre-weighted subsamples in a ThermoScientific Flash1112 elemental analyzer, using acetanilide for calibration. $\delta^{13}C$ and $\delta^{15}N$ analyses of fauna samples were performed on the same subsamples after combustion gases were transferred to a ThermoFinnigan delta V via a Conflo III interface. All stable isotope ratios are expressed relative to the conventional standards (VPDB limestone for carbon and atmospheric N_2 for nitrogen) as:

$$\delta X = \left[\frac{R_{\mathrm{sample}} - R_{\mathrm{s\,tan\,dard}}}{R_{\mathrm{s\,tan\,dard}}}\right] \times 10^{3} \qquad [‰]$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and where X = ^{13}C or ^{15}N .

IAEA-CH-6 and IAEA N1reference materials were used for normalizing our $\delta^{13}C$ and $\delta^{15}N$ data, respectively, using the single-point anchoring method.

2.2.4 Statistical analysis

Fauna specimens were collected in October 2006 and March and October 2007, while sediment and leaf samples were obtained in October 2006 and October 2007, and microalgae at October 2007, due to logistical limitations or absence of sufficient material during those campaigns. Slightly different analyses were done, therefore, on each variable. As the system is considered to be in a steady state in October 2007, carbon and nitrogen stable isotopes differences in different ecological compartments (sediment, leaves and BMA) were analysed using a 2-way ANOVA, with sewage as one factor and compartment-vegetation as another factor. Statistical analysis between new and old leaves of both mangrove species was done using a 3-way ANOVA. The factors were Time x Sewage (3 levels – October 2006, October 2007 0% and October 2007 60%), mangrove species (2 levels) and leave age (2 levels). Stable isotope variation in biota was analysed using 3-way ANOVA. The factors were time (3 levels), vegetation (3 levels) and sewage (2 levels). All factors were fixed and orthogonal. Prior to analyses, the assumption of homogeneity of variances was tested using Cochran's test. When significant differences were detected, post-hoc SNK tests were used to identify differences.

3. Results

Nutrient concentrations in the water column increased with increasing sewage loading, while salinity and dissolved oxygen decreased (Table 1). Chlorophyll-a concentration (as an indicator of microalgae biomass) in surface sediment increased with sewage dosing and decreased with vegetation cover (Table 2). Sewage discharge resulted in an increase of $\delta^{15}N$ in almost all mangrove compartments (sediment, MBA, as well as macrofauna) except for leaf tissues, while it had little effect on $\delta^{13}C$ signatures (Figure 1).

3.1 Primary producers and sediment

Sewage SPOM obtained from the primary wastewater pond showed a $\delta^{13}C$ signature of -22.6 ‰, and was relatively depleted in ^{15}N (5.0 ‰). No significant changes in $\delta^{13}C$ values of mangrove leaf tissues were observed during the 12 months sewage exposure from October 2006 to October 2007 compared with seawater exposure, but old leaves slightly enriched in ^{13}C (-27.2±0.3 and -26.3±0.9‰, respectively) compared to new leaves (-28.0±0.3 and -28.0±0.5‰, respectively) (Table 3). Sediment TOC and TN (Table 4-A) showed a striking difference at the beginning and end of the experiment, with values on average 1.8 and 2.3 times higher after 12 months of experiment (at 0% and 60% sewage concentrations, respectively for both carbon and nitrogen). Although an increase in TOC and TN was clearly observed mainly in cells subjected to sewage discharge, the C/N ratios remained similar with large variability.

Table 3. –Results of 3-way ANOVA of $\delta^{13}C$ and $\delta^{15}N$ leaf signatures among campaigns vs Sewage (initial, 12 months 0% and 12 months 60%), mangrove species (*A. marina* and *R. mucronata*) and leaf age (old and new) conditions. DF = degrees of freedom; MS = mean square; F = F statistic. Cochran's C = 0.08 ($\delta^{13}C$) and C = 0.11 ($\delta^{15}N$). n = 3

	$\delta^{13}C$			$\delta^{15}N$		
Source	DF	MS	F	DF	MS	F
Time - Sewage – Ti-Se	2	0.4391	0.52	2	106.372	**9.19****
Vegetation – Ve	1	0.0400	0.05	1	1422	**122.86****
Age – Ag	1	101.124	**11.96****	1	52.212	**4.51***
Ti-Se X Ve	2	11.750	1.39	2	28.261	2.44
Ti-Se X Ag	2	0.3547	0.42	2	41.769	**3.61***
Ve X Ag	1	14.884	1.76	1	96.410	**8.33****
Ti-Se X Ve X Ag	2	0.1643	0.19	2	58.772	**5.08***
RES	24	0.8458		24	11.575	
TOT	35			35		

* $p < 0.05$
** $p < 0.01$

The $\delta^{13}C$ signature of sediment POC exposed to seawater, regardless of vegetation condition, decreased slightly during the 12 months and exhibited a value of -18.7‰ in October 2007, which was similar to that of BMA (-18.5‰) and more enriched (p<0.001) than leaf tissue signatures of both mangrove species (Figure 1). However, sediment $\delta^{13}C$ signatures under sewage exposure exhibited lower values in planted (-19.4 ‰ and -21.4 ‰ for *R. mucronata* and *A. marina* respectively) than bare cells (-17.9 ‰) due to litter fall in the planted cells, although differences were not statistically significant (Table 5).

There was considerable variability in $\delta^{15}N$ signatures of mangrove leaves, with a significant effect of both time-sewage exposure, mangrove species and leaf age (Table 3). Post-hoc SNK tests revealed that $\delta^{15}N$ of *A. marina* (8.9±0.5‰ as an average for both new and old leaves) was significantly higher than that obtained for *R. mucronata* (6.1±0.5 and 3.2±0.8‰ for new and old leaves, respectively). $\delta^{15}N$ tended to decrease with time, particularly in cells loaded with 60% sewage, although no significant differences were observed (Figure 1).

The mean $\delta^{15}N$ signatures of microalgae under sewage exposure (10-12.6 ‰) was significantly higher (p<0.001) than those obtained under seawater exposure (~0.7 ‰). Sediment $\delta^{15}N$ signatures (Table 4-A) followed sewage POM, microalgae and leaf composition. Sediment δ^{15}N increased significantly (Table 5) with time and sewage concentration.

Table 4 – Sediment carbon and nitrogen stable isotopes, TOC, TN and C/N ratio at different campaigns (0, 6 and 12 months), vegetation (Bare, *A. Marina, R. mucronata*) and sewage (0 and 60%) conditions. Values are given as averages ± SE (n=3)

	0%			**60%**		
	Bare	***A. marina***	***R. mucronata***	**Bare**	***A. marina***	***R. mucronata***
δ^{13}C (‰)						
0	-16.5 ± 1.1	-16.5 ± 1.1	-16.5 ± 1.1	-16.5 ± 1.1	-16.5 ± 1.1	-16.5 ± 1.1
6	-18.2 ± 0.2	-19.2 ± 1.4	-18.5 ± 0.2	-17.5 ± 0.0	-17.5 ± 0.6	-17.5 ± 0.1
12	-18.4 ± 0.2	-19.3 ± 0.3	-18.3 ± 0.5	-17.9 ± 0.4	-21.4 ± 0.9	-19.4 ± 1.2
δ^{15}N (‰)						
0	4.8 ± 1.2	4.8 ± 1.2	4.8 ± 1.2	4.8 ± 1.2	4.8 ± 1.2	4.8 ± 1.2
6	4.2 ± 0.6	4.7 ± 1.7	4.8 ± 0.5	7.7 ± 0.6	8.1 ± 0.6	8.1 ± 0.1
12	8.5 ± 1.2	8.7 ± 0.4	7.3 ± 0.5	10.1 ± 0.2	9.3 ± 0.8	10.2 ± 1.0
TOC (%)						
0	0.30 ± 0.16	0.30 ± 0.16	0.31 ± 0.17	0.31 ± 0.17	0.31 ± 0.17	0.31 ± 0.17
6	0.23 ± 0.01	0.20 ± 0.03	0.25 ± 0.01	0.31 ± 0.03	0.34 ± 0.00	0.30 ± 0.01
12	0.49 ± 0.04	0.49 ± 0.10	0.65 ± 0.09	0.67 ± 0.09	0.62 ± 0.04	0.78 ± 0.20
TN (%)						
0	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
6	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
12	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.09 ± 0.02
C/N (mol/mol)						
0	10.3 ± 1.3	10.3 ± 1.3	10.3 ± 1.3	10.3 ± 1.3	10.3 ± 1.3	10.3 ± 1.3
6	10.8 ± 1.0	10.8 ± 2.4	10.0 ± 1.1	9.4 ± 0.1	10.5 ± 1.0	9.9 ± 0.6
12	11.0 ± 0.2	11.2 ± 0.4	10.6 ± 0.2	9.3 ± 0.3	11.4 ± 0.3	10.7 ± 0.3

3.2 Primary consumers

SI signatures of crabs and gastropods in the Kunduchi mangrove forest bordering the Jangwani mesocosm system (δ^{13}C of -16.0±1.3‰ and -22.5±0.7‰, and δ^{15}N of 5.6±0.6‰ and 4.8±0.2‰, respectively) showed no significant variation throughout the year (Figure 1). Fiddler crabs in cells exposed to seawater showed similar SI signatures to those living in the wild (-16.2±0.8‰ and 6.8±1.4‰ for δ^{13}C and δ^{15}N, respectively) regardless of vegetation conditions, except in October 2007 where δ^{15}N was significantly ($p<0.05$) higher (8.7±0.7‰) (Table 4-B).

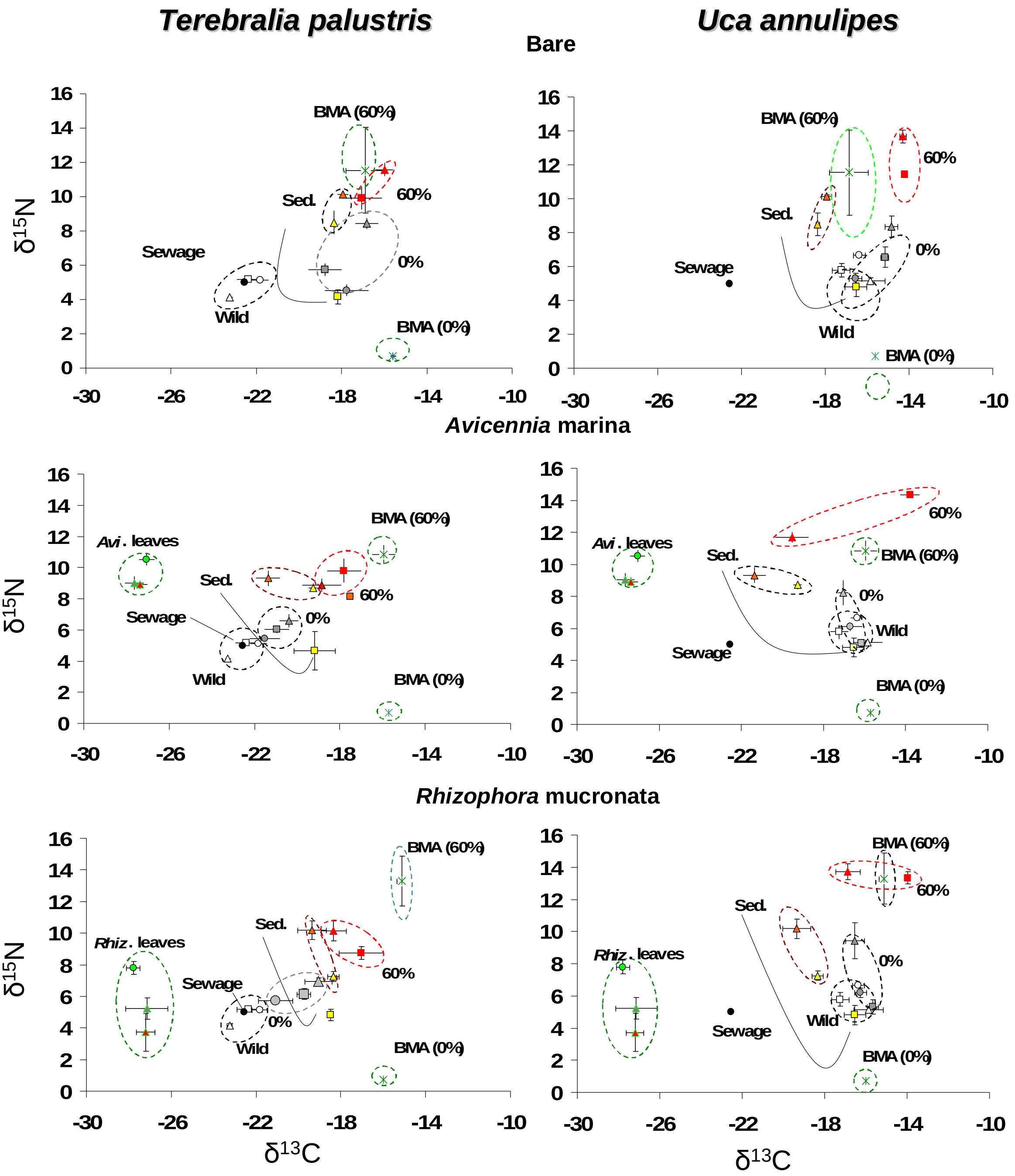

Figure 1 - Plot of δ^{13}C and δ^{15}N of sediments, leaves, benthic microalgae (BMA), *Terebralia palustris* (foot muscle) and *Uca annulipes* (claw muscle) under different vegetation and sewage concentration conditions. Values are given as mean ± S.E (n = 3-9). Red colour mean samples from 60% sewage concentration plots, while grey from pristine plots (0%). Fauna and flora samples are indicated as "O" at time zero, "□" at time 6 months and "Δ" at time 12 months. Sediment data is given for time zero (□ – indicated with a line) and 12 months (Δ).

Table 5 – Summary of 3-way ANOVAs for A) Sediment, B) crab and C) gastropods $\delta^{13}C$ and $\delta^{15}N$ signatures among campaigns (0, 6 and 12 months), vegetation (Bare, *A. Marina*, *R. mucronata*) and sewage (0 and 60%) conditions. DF = degrees of freedom; MS = mean square; F = F statistic. A) Cochran's C = 0.35 ($\delta^{13}C$) and C = 0.14 ($\delta^{15}N$); B) Cochran's C = 0.13 ($\delta^{13}C$) and C = 0.15 ($\delta^{15}N$); C) Cochran's C = 0.08 ($\delta^{13}C$) and C = 0.11 ($\delta^{15}N$). n = 3

A	$\delta^{13}C$			$\delta^{15}N$		
Source	DF	MS	F	DF	MS	F
Time - Ti	2	312117.0	**44.59****	2	824.817	**96.41****
Vegetation – Ve	2	38117.0	**5.45****	2	0.0217	0.03
Sewage – se	1	0.0817	0.12	1	390.150	**45.60****
Ti X Ve	4	20817.0	**2.97***	4	0.4442	0.52
Ti X Se	2	46717.0	**6.67****	2	130.050	**15.20****
Ve X Se	2	0.3617	0.52	2	0.6050	0.71
Ti X Ve X Se	4	13067.0	1.87	4	0.7025	0.82
RES	36	0.7000		36	0.8556	
TOT	53			53		

B	$\delta^{13}C$			$\delta^{15}N$		
Source	DF	MS	F	DF	MS	F
Time - Ti	2	8.82	**15.27****	2	1.65	2.36
Vegetation – Ve	2	17.00	**29.43****	2	117.26	**167.52****
Sewage – se	1	1.04	1.80	1	206.50	**295.01****
Ti X Ve	4	6.13	**10.62****	4	2.33	**3.34***
Ti X Se	2	0.40	0.70	2	0.90	1.29
Ve X Se	2	6.92	**11.98**	2	61.75	**88.22****
Ti X Ve X Se	4	1.94	**3.37**	4	3.85	**5.5****
RES	36	0.57		36	0.7	
TOT	53			53		

C	$\delta^{13}C$			$\delta^{15}N$		
Source	DF	MS	F	DF	MS	F
Time - Ti	2	230017.0	**18.32****	2	15.650	2.52
Vegetation – Ve	2	192267.0	**15.31****	2	646.550	**103.91****
Sewage – se	1	187267.0	**14.92****	1	558.150	**89.70****
Ti X Ve	4	16992.0	1.35	4	37.525	**6.03****
Ti X Se	2	47017.0	**3.74***	2	10.850	1.74
Ve X Se	2	76467.0	**6.09****	2	188.750	**30.33****
Ti X Ve X Se	4	17292.0	1.38	4	0.4525	0.73
RES	36	12556.0		36	0.6222	
TOT	53			53		

* $p < 0.05$ ** $p < 0.01$

Gastropods in seawater cells showed slightly (-17.8±0.9‰) or distinctly (-20.0±0.7‰) higher δ^{13}C values under planted or bare conditions, respectively, compared with individuals from the wild. The δ^{15}N signature of gastropods increased significantly from October 2006 (4.5±0.3‰) to October 2007 (8.4±0.3‰) under bare conditions, while the increment was low (from 5.5 to 6.7‰) under planted condition (Table 4-C).

Fiddler crabs and gastropods kept under sewage exposure for 6 and 12 months were substantially enriched in ^{15}N compared to those obtained from seawater exposure or in the wild. δ^{15}N of *T. palustris* ranged from 8.7 to 12.0‰ and were always slightly lower than for *U. annulipes* (11.4 to 14.3‰). While in most treatments both species tended to increase their δ^{15}N signature with time, at *A. marina* mesocosms, a slight decrease was observed for gastropods (from 9.8 to 8.9‰) and crabs (from 14.3 to 11.7‰). Planted mesocosms exposed to sewage only showed a decrease in δ^{13}C after 6 months for both species. The change was more pronounced for fiddler crabs (-13.8 to -19.5‰) than for gastropods (-17.8 to -18.8‰).

4. Discussion

4.1 Primary producers and sediment

Mangrove plants, such as benthic microalgae and mangrove trees, have the potential to strip nutrients efficiently from wastewater (e.g., Tam and Wong, 1989; Lau et al., 1997; Wu et al., 2008). Microalgae are particularly interesting in this context because they assimilate DIN directly from water column, which makes them reliable indicators of nutrient removal (Cole et al., 2004; Cole et al., 2005). Furthermore, due to the rapid turnover of microalgal biomass, their elemental composition reflects any changes in wastewater composition swiftly. BMA incorporate carbon into their tissues by assimilating bicarbonate (Michener and Lajtha, 2007). Although SPOM was enriched in ^{13}C, the δ^{13}C signature of BMA was not influenced by sewage exposure in our study and maintained values commonly found in natural mangrove areas (approximately ~ 17‰, Bouillon et al. 2008). Conversely, sewage exposure resulted in a significant increase of the BMA δ^{15}N signature, reaching values above 10‰. Values between 10 and 25‰ are commonly observed for microalgae subjected to sewage-derived nitrogen (Costanzo et al., 2001; Cole et al., 2004; Pitt et al., 2009). Although in the present study only sewage SPOM data is available, it is predicted that sewage DIN δ^{15}N signature would be much higher due to the intensive nitrification and denitrification processes occurring in the wastewater pond where sewage is kept for nearly 12h before being discharged into the mesocosms (Michener and Lajtha, 2007).

Mangrove trees, on the other hand, have slower biomass turnover and the δ^{15}N signature of tissues represents N acquired over time scales of growing seasons (Fry et al., 2000). Furthermore, mangrove trees may to a large extent obtain DIN from ground water and porewater deep in sediments via their extended root system (Boto et al., 1985; Alongi, 1996). The use of mangrove tree stable isotope signatures as environmental markers have therefore provided mixed results (Fry et al., 2000; Cole et al., 2004; Pitt et al., 2009, and present study). The $\delta^{15}N$ signatures of mangrove tissues at initial conditions in our study are at the higher range (-22 to 20‰) found in natural and uncontaminated mangrove areas (France, 1998; Bouillon et al., 2002; Bouillon et al., 2008). Low concentration of nutrients in the sediment during initial conditions might have increased $\delta^{15}N$ due to root stimulation of nitrification and denitrification and consequently ^{15}N fractionation (Fry et al., 2000; McKee et al., 2002). In any case, a slight difference in the $\delta^{15}N$ signature (nearly 3‰) was found between old and new leaves of *R. mucronata*, which could indicate assimilation of sewage-derived nitrogen by the new leaves. However, since mangrove trees may resorb up to 64% of N from senescing leaves prior to abscission (Rao et al., 1994), foliar N cannot be used as a short-term proxy of the source of tree N (Kolb and Evans, 2002).

Sediments may act as a sink of N derived from primary treated sewage through the burial of microalgae and diffusion of dissolved organic and inorganic nitrogen. However, these sources are apparently of limited importance in our mesocosm system as only a small increase in sediment $\delta^{15}N$ (up to 5‰) was observed in sewage exposed cells. The inventory of refractory organic matter in these sediments is sufficiently large to prevent a marked changes in $\delta^{15}N$ within the 12 months time scale

4.2 Primary consumers

Mangrove fiddler crabs and gastropods feed on microphytobenthos, bacteria and mangrove detritus (France, 1998; Meziane and Tsuchiya, 2002; Bouillon et al., 2004a; Penha-Lopes et al., in press), but these gastropods are also capable of feeding on mangrove leaves (e.g., Fratini et al., 2004; Penha-Lopes et al., in press). Similar SI signatures of *Uca annulipes* and *Terebralia palustris* collected in the wild and in planted mesocosms indicate that both species had similar diets irrespective of captivity or sewage treatment as also found by previous studies (e.g., France, 1998). *Terebralia palustris* individuals in bare cells were enriched in ^{13}C ($\delta^{13}C$ of ~-18‰) due to a shift in diet towards a larger contribution of microphytobenthos ($\delta^{13}C$ of ~-16‰), given the absence of mangrove litter ($\delta^{13}C$ of ~-27‰) in these plots. The ^{13}C depletion of *T. palustris* in planted cells ($\delta^{13}C$ of ~ -20‰) indicates that litter is indeed consumed, but the $\delta^{13}C$ signatures still do not reach those observed in the wild ($\delta^{13}C$ of ~-22‰), which is probably a consequence of the young age and low biomass of trees.

The $\delta^{15}N$ signature of both consumers was significantly higher in sewage exposed cells, corroborating that mangrove fauna have a potential as ecological indicators of sewage impacted environments (Pitt et al., 2009). Primary producers in sewage impacted aquatic systems assimilate nitrogen enriched in ^{15}N, which subsequently lead to higher $\delta^{15}N$ in consumers (McClelland and Valiela, 1998; Cole et al., 2004; Pitt et al., 2009). The higher consumer $\delta^{13}C$ in sewage exposed cells during March 2007 indicates a preferential consumption and assimilation of microalgae. The subsequent decrease in the $\delta^{13}C$ signature of gastropods and crabs in planted cells in October 2007 is a consequence of the increase in tree biomass (i.e. litter fall) and canopy shading (mainly in *A. marina* cells). Despite the low $\delta^{13}C$, it is unlikely that fiddler crabs fed directly on mangrove detritus. They probably gained ^{13}C from microorganisms that have colonized decaying litter (France, 1998).

The unexpected slight increase in $\delta^{15}N$ of fauna in the cells that were considered isolated from sewage (most evident for *T. palustris* and *U. annulipes* in bare and *R. mucronata* mesocosms) indicates a possible contamination. This has subsequently been confirmed by the engineers to be due to temporary logistical problems with the piping. However, this unintended methodological fault allow us to confirm that $\delta^{15}N$ signatures of the fauna are sensitive for detecting more subtle sewage loadings.

The highly sensitive SI signatures of these organisms to pollution may provide a tool to get early warnings of an incipient sewage problem before more dramatic effects are evident (McClelland et al., 1997; McClelland and Valiela, 1998). Furthermore, fiddler crabs (Cannicci et al., 1999) and gastropods (Vannini et al., 2008) show high fidelity to a specific area; increasing their competence as a local biomarker (Schlacher et al., 2005). However, care should be taken when using *T. palustris* for this purpose, because it is usually absent in anthropogenically impacted mangrove environments (Cannicci et al., 2009) and it may shift between diets depending on food source availability (Fratini et al., 2004; Fratini et al., 2008; Pape et al., 2008; Penha-Lopes et al., in press). Fiddlers crabs, on the other hand, seem to be a better and more reliable choice as they are abundant in peri-urban mangrove forests (Cannicci et al., 2009) and are loyal to their food source (i.e. BMA).

However, the fact that *T. palustris* chose also to feed on mangrove leaves in planted contaminated mesocosms, with high microalgae biomass, is of a great importance to the functioning of these constructed wetlands. A large proportion of refractory material (litter) in mature wetlands can be processed by these gastropods, known to consume an average of 0.65g leaves h^{-1} (Fratini et al., 2004), and are either assimilated or release in the form of more labile substrates through faeces. As a consequence, large accumulations of decaying litter are avoided and ecosystem functioning is improved.

A decrease in feeding activity due to luxury growth of microalgae in these mesocosms contaminated sediments was observed for fiddler crabs (Bartolini et al., accepted; Penha-Lopes et al., accepted-b), which may potentially lead to establishment of thick “algal mats” (Kristensen and Alongi, 2006), causing extended anoxia and sulfidic conditions in near-surface sediment (Kristensen and Alongi, 2006; Marsden and Bressington, 2009).

5. Conclusion

Our results confirm the potential use of stable isotopes (SI) as a useful tool to better understand food web structure in mangrove constructed wetlands, and provide experimental support for the use of $\delta^{15}N$ signatures as a robust indicator of anthropogenic nutrient inputs in different ecosystem compartments. The application of secondary treated sewage increased the $\delta^{15}N$ signatures of both microalgae and sediment organic matter, which was subsequently ingested and assimilated by both species of primary consumers. Mangrove leaves, in contrast, showed no significant shifts in $\delta^{15}N$ over the time frame considered here. These results suggest that in mangrove sewage treatment facilities, the introduction of macrofauna may be important to maintain the natural food web dynamics, thus reducing these systems running and maintenance cost.

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Chapter 9

The role of biogenic structures on the biogeochemical functioning of mangrove constructed wetlands sediments – a mesocosm approach

Gil Penha-Lopes[a,b*], Erik Kristensen[c], Mogens Flindt[c], Perrine Mangion[b], Steven Bouillon[d] and José Paula[a]

[a]Centro de Oceanografia, Laboratório Marítimo da Guia - Faculdade de Ciências da Universidade de Lisboa, Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211
[b] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark
[c] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[d] Katholieke Universiteit Leuven, Dept. of Earth & Environenmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.
c. Katholieke Universiteit Leuven, Dept. of Earth & Environenmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.
d. Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C.P. 257 Maputo, Moçambique

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Gil Penha-Lopes[a,b*], Erik Kristensen[c], Mogens Flindt[c], Perrine Mangion[b], Steven Bouillon[d] and José Paula[a]

[a]Centro de Oceanografia, Laboratório Marítimo da Guia - Faculdade de Ciências da Universidade de Lisboa, Avenida Nª Senhora do Cabo, 939, 2750-374 Cascais, Portugal
*Corresponding author: gil.penha-lopes@fc.ul.pt
Phone: (+351) 214 869 211
[b] Institute of Biology, University of Southern Denmark, DK-5230 Odense M, Denmark
[c] Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Brussels
[d] Katholieke Universiteit Leuven, Dept. of Earth & Environenmental Sciences, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.

Abstract

Carbon dioxide and methane production and carbon oxidation pathways were evaluated in 4 mangrove mesocosms subjected daily (for 12 h) to seawater mixed with 0% (pristine) or 60% sewage (contaminated) in the absence or presence of mangrove trees and biogenic structures (pneumatophores and crab burrows). Total CO_2 and methane emissions increased significantly in sewage contaminated mesocosms, as well as benthic primary production. Biogenic structures increased low tide carbon gas emissions at contaminated (30%) and particularly pristine conditions (60%). FeR was substituted by SRR when sewage was loaded into the cells under unvegetated and planted conditions, while the contribution of aerobic respiration to total metabolism remained above 50%. Our results clearly show impacts of sewage on the partitioning of electron acceptors in mangrove sediment and confirm the importance of biogenic structures for biogeochemical functioning.

Keywords: carbon mineralization, microbial pathways, constructed wetlands, mangrove, domestic sewage, biogenic structures

1. Introduction

Mangrove forests represent a productive ecosystem that play a key role for carbon and nutrient cycling along many tropical coasts (Duarte et al., 2005; Kristensen et al., 2008a). They are sources or sinks of atmospheric CO_2 depending on the balance of primary production, accretion and permanent storage of organic matter (OM) in sediments, OM exported by tides or consumed by both terrestrial and marine species, as well as carbon mineralization in sediments and creek waters (Bouillon et al., 2008b; Kristensen et al., 2008a; Nagelkerken et al., 2008).

Carbon oxidation in sediments is controlled by the availability of labile OM and electron acceptors (Canfield et al., 2005). Aerobic respiration and anaerobic sulfate reduction (SR) are usually the major decomposition pathways in mangrove sediments, while manganese and iron respiration, denitrification and methanogenesis have traditionally been considered unimportant (Kristensen et al., 2008a; Alongi, 2009). Low oxygen penetration into reactive marine sediments typically favours high importance of SR (Canfield et al., 2005), but other anaerobic processes may predominate in mangrove environments depending on factors such as sediment grain size, tidal inundation, forest type, organic content, and bioturbation intensity (Kristensen, 2000; Alongi, 2009).

Mangrove sediments are characterized by an abundance of biogenic structures, such as tree pneumatophores and infauna burrows. These structures change the biogeochemical balance and increase the exchange of gases and solutes several fold, making them important conduits that influence carbon dynamics in mangrove forest (Holmer et al., 1999; Kitaya et al., 2002; Kristensen et al., 2008b). Pneumatophores have open lenticels during air exposure, allowing rapid diffusion of gases into (e.g. O_2) and from (e.g. CO_2 and CH_4) deep sediments via the air-filled aerenchyma tissue to the atmosphere (Purnobasuki & Suzuki 2004, 2005), but can also stimulate SR through root exudates (Alongi, 1998; Kristensen and Alongi, 2006). Infaunal burrows, on the other hand, enhance the exchange of gases and nutrients through increasing the area of sediment and air/water interfaces as well as transport of labile detritus to subsurface layer during bioturbation activities (Kristensen, 1988; 2008; 2008b).

This way biogenic structures may increase the importance of aerobic and suboxic, such as Fe reduction (Nielsen et al., 2003; Kristensen and Alongi, 2006) and denitrification (Hansen and Kristensen, 1998; Heilskov and Holmer, 2001; Purvaja et al., 2004) pathways for carbon mineralization in subsurface sediments ay the same time that fuel anaerobic carbon degradation deep in the sediment (Kristensen, 2000; Ferreira et al., 2007). Also, both denitrifying bacteria (Corredor and Morell, 1994; Rivera-Monroy and Twilley, 1996) and methanogens (Purvaja and Ramesh, 2001; Hegde et al., 2003; Alongi et al., 2005) can increase significantly their activity at high nutrient and organic loading, coexisting with highly productive sulphate reducers (Holmer and Kristensen, 1994a; Lyimo et al., 2002).

The use of wetlands for processing nutrient and organic-rich wastewater has proved effective to prevent coastal pollution in developing countries (Crites et al., 2006). However, possible harmful effects due to toxic materials and pathogens in wastewater (Al-Sayed et al., 2005) and anthropogenic degradation of natural wetlands have forced managers to use constructed wetlands (Kivaisi, 2001). In many cases constructed mangrove wetlands have proven to be efficient in nutrient filtration (Wu et al., 2008a; Yang et al., 2008), with high survival and growth rates of mangrove flora (Wong et al., 1997b; Yang et al., 2008) and associated macrofauna (Penha-Lopes et al., accepted-a; Penha-Lopes et al., accepted-b). However, a continuous loading of organic rich wastewater at a level above the system capacity usually leads to severely reduced O_2 penetration (Gray et al., 2002) and OM accumulation in sediments (Holmer and Kristensen, a; b; Valdemarsen et al., in press-a) which may induce negative effects on sediment associated flora and fauna due to stimulated SR and toxic sulfide accumulation (Hargrave et al., 2008). Studies on nutrient filtration efficiency of constructed wetlands for wastewater treatment have so far only focused on the behaviour of plants and/or microorganisms for periods shorter than 2 years (Wong et al., 1995; Stottmeister et al., 2003; Wu et al., 2008b), while macrofauna and associated biogeochemical functioning have been ignored.

Knowledge on the ecological response of mangrove sediments to urban sewage emissions is urgently needed and this study aims to improve the understanding of direct and indirect effects of severe sewage contamination. This manuscript focuses on carbon gas (i.e. CO_2 and CH_4) emissions under immersion and emersion periods, with special emphasis on the importance of biogenic structures (pneumatophores and crab burrows), as well on the most important carbon oxidation pathways used. Both these processes are important to fully comprehend the effects of organic discharge on mangrove forests and to provide basic knowledge for future development of sustainable mangrove wastewater wetlands. We also provide rough net budgets of carbon gas emission in constructed mangrove wetlands under different sewage and vegetation treatments and discuss the long-term implications for the ecosystem functioning and health.

2. Materials and methods

2.1 Experimental setup

A mesocosm system consisting of 4 cells (9 m^2 each) was constructed at the upper *Avicennia marina* (Forsk.) belt of the Jangwani mangrove forest near Dar es Salaam,Tanzania (see system description in Penha-Lopes et al., accepted-a). The 4 cells were divided according to “vegetation” treatment, with 2 unvegetated cells and 2 cells planted with *A. marina* saplings. Sediment was laid and saplings planted (at a density of 2.8 m^{-2}) on selected plots in early February 2006. The system was initially inundated exclusively with seawater and secondary sewage loading was initiated in early October 2006. One replicate was assigned per sewage loading for each vegetation treatment. Secondary sewage was mixed with natural seawater to obtain loadings of 60%. A diurnal tidal rhythm was simulated with 12 hour inundation to 0.1 m depth starting at 23:00. The basic chemical characteristics of sewage-seawater mixtures are presented in Table 1. *Avicennia marina* trees were 50.3±1.2 cm (±SE) tall and litter fall from the young trees was absent when sewage discharge was initiated. They grew to 107.8±7.8 cm with litter fall of 1.17±0.20 g m^{-2} day^{-1} in April 2007 (PUMPSEA, 2008).

2.2. Epifauna and biogenic structures

Mangrove epifauna was introduced to each cell in August 2006. Two of the most abundant crab species found in Indo-Pacific mangrove forests, *Uca annulipes* (H. Milne Edwards, 1837) and *U. inversa* (Hoffmann, 1874) populated the mesocosms (approximately 20 crabs m^{-2}). Fauna density, abundance of biogenic structures (pneumatophores and burrows) and burrow morphology at the time of the present measurements were obtained (Table 2) from parallel studies (PUMPSEA, 2008; Penha-Lopes et al., accepted-b). Although other macro (e.g., gastropods, polychaetes and pligochaetes) and meiofauna were registered at the mesocosms, no differences on biomass and diversity were found between mesocosms at different sewage concentrations and vegetated conditions (Penha-Lopes et al, unpublished data).

2.3 Sediment Properties

Triplicate sediment cores (5 cm i.d.) from each mesocosm cell were sliced into 0–2, 4–6, 8-10 cm depth intervals and analyzed for organic content (loss on ignition, LOI) in February 2007. Simultaneously, 9 cores for determination of sediment density and water content was sliced into 0-1, 1-2, 2-3, 3-4, 4-6, 8-10, 12-14 cm depth intervals. Water content was determined from water loss upon drying of sediment subsamples at 100°C for 24 h. Wet density was measured as the weight of a known sediment volume. Water content and density is presented as average porosity of all examined cells due to limited variability. Organic content was determined as weight loss of dried sediment after combustion for 6 h at 520°C. Chlorophyll-a (Chl *a*) content was analyzed only in the surface sediment (0-2 cm) by the standard spectrophotometric method (Lorenzen, 1967). Solid phase Fe was determine for the same cells in a previous study (Penha-Lopes et al., accepted-b) following the methodology described in Kristensen et al (submitted).

2.4. Sulfate reduction assay

Rates of sulfate reduction were measured by the core injection technique of Jørgensen (1978). Three 16-cm-long sediment cores were retrieved from each mesocosm cell using 20-cm long and 2.6-cm i.d. core tubes with silicone-filled injection ports. Carrier-free $^{35}S\text{-}SO_4^{2-}$ was injected at 1-cm intervals to 13 cm depth, and the cores were incubated at 25ºC with dry surface in darkness for 4–6 h. Subsequently, each core was sliced at 2-cm intervals and fixed in 20% zinc acetate. Samples were stored frozen until distillaiton by the one step total reduced sulfide extraction described in Fossing and Jørgensen (1989). The ^{35}S activity in sediment and distillate was determined by liquid scintillation analysis, and sulfate reduction rates were determined from the relative activity of ^{35}S recovered in the distillate.

2.5. Anoxic sediment incubations

The vertical distribution of total anaerobic microbial CO_2 production (TACP) and respiratory Fe(III) reduction was determined in sediment from all treatments. Three sediment cores (8 cm i.d.) were sliced into 0-2, 2-4, 8-10, 12-14 cm depth intervals. Sediment from each depth was rapidly homogenized and transferred into 8 glass scintillation vials (jars), which were capped with no headspace, taped to prevent oxygen intrusion and incubated in the dark at 28°C. Every 7 days two vials from each depth were sacrificed for determination of changes in dissolved CO_2 and reactive Fe pools. Sediment was transferred to double centrifuge tubes and porewater was obtained by centrifugation (10 min, 1500 rpm). Samples for CO_2 were preserved with $HgCl_2$ and analyzed as soon as possible using the flow injection/diffusion cell technique of Hall & Aller (1992). Furthermore, solid phase particulate iron pools were determined on jar sediment as described above. Reaction rates in jars were calculated as the slope of the linear fit of concentration versus time.

2.6. Denitrification assay.

Rates of denitrification during immersion were measured in April 2006 using the ^{15}N

isotope pairing technique on intact sediment cores (Nielsen, 1992). Ten undisturbed sediment cores from each cell were adjusted to ~10 cm height and placed in groups of 5 into 2 tanks. After stabilization overnight, one tank was exposed to light and the other was kept in darkness. The denitrification assay was initiated by amending the water of each tank with $^{15}NO_3^-$ (99.2% ^{15}N, Sigma-Aldrich) to a final concentration of ~60µM. The water above the cores was mixed manually to ensure homogeneous distribution of tracer. About 15 min later, the core tubes were sealed gas tight either with rubber stoppers or transparent lids for dark and light incubations, respectively. After incubation for 3 to 5 h (O_2 did not change more than 20% from air saturation), sediment and water column in each core were mixed completely with a PVC rod. Duplicate 20 ml samples of the resulting slurry were transferred to gas-tight vials (12 ml Extainers, Labco), preserved with formaldehyde (100 µL, 38%) and sealed ensuring no entrapped air. ^{15}N content of N_2 was analyzed using combined gas chromatography/mass spectrometry (Robo-Prep-G1 in line with Tracermass, Europa Scientific) as described by Risgaard-Petersen and Rysgaard (1995). Denitrification rates were calculated from $^{29}N_2$ and $^{30}N_2$ production rates as described by Nielsen (1992).

2.7. CO_2 and CH_4 exchange across the sediment-water/air interface

Exchange of carbon gases across the sediment-water interface (TCO_2) and over air exposed bare (without biogenic structures) sediments (TCO_2 and CH_4) were determined in mesocosm cells following the techniques described in detailed by Kristensen et al (2008b). Three of the cores were left exposed to sunlight to determine net primary production (NPP) and three cores were darkened with aluminium foil to determine respiration (RSP) at pristine and contaminated sediment at both planted and unplanted conditions. The CO_2 fluxes were also determined in deployments on exposed sediments (emersion) with one crab burrow and sediment with 3 pneumatophores of *Avicennia marina*. Occasionally, sediment–air methane emissions were tested simultaneously with the in situ CO_2 exchange measurements in selected mesocosms. In this study we designate the carbon release from sediment at emersion periods as CO_2, while wet flux and jar accumulation of dissolved inorganic carbon is considered as TCO_2 and TACP, respectively.

2.8. Epifauna respiration

The respiratory CO_2 release by fiddler crabs (both *Uca* species) and gastropods were determined from laboratory incubations at in situ temperature using the LI-6400 equipment and technique described in Kristensen et al (2008b). A group of 30 different sized (0.4 to 8 g wet weight) fiddler crabs of each species and sex, and 30 gastropods (2 to 45 g wet weight) were caught randomly at the Kunduchi mangrove forest and immediately brought to the laboratory.

2.9. Statistical analysis

Gas fluxes were tested using 2-way ANOVA, except for denitrification where a 3-way ANOVA was used. When relevant, the ANOVA's were followed by a Bonferroni test or SNK to find differences between treatments. A significance level of $\alpha = 0.05$ was used in all ANOVA tests, unless otherwise stated.

3. Results

3.1 Water and sediment characteristics

Nutrient concentrations increased with increasing sewage loading, while salinity and dissolved oxygen decreased (Table 1). The daily variation in top sediment temperature was similar in all treatments. The temperature was constant at around 27ºC at night and increased at dawn (7:00) to reach a maximum of ~37ºC at noon (13:00) followed by a slow but gradual decrease back to the night time level. In all cells porosity ranged from 0.35 to 0.47, except for high porosity of 0.66 in the 12-14 cm depth interval (Figure 1). Organic content was constant with depth in the upper 10 cm of the sediment in all treatments and ranged from 0.9 to 1.9 % (Figure 1). In non-contaminated cells Chlorophyll-a concentration in the top sediment was 13.9 ± 3.9 µg g^{-1} in unvegetated and 5.4 ± 1.1 µg g^{-1} in *A. marina* vegetated treatments, and in the 60% sewage treated cells the corresponding values were 23.1 ± 9.4 and 14.0 ± 3.5 µg g^{-1}, respectively, increasing then with sewage dosing and decreasing with vegetation cover.

Solid phase iron profiles differed among treatments (Figure 2). Total iron differed according to vegetation, and when integrated to 14 cm depth total iron was ~250 and 100-140 µmol cm^{-3} in vegetated and non-vegetated sediment, respectively, irrespective of contamination level. Fe(II) always dominated in contaminated cells occasionally exceeding 20 µmol cm^{-3}, decreasing with depth at unvegetated but remained constant below 5 cm at planted treatments, while Fe(III) never exceeded 10 µmol cm^{-3} and was almost absent below 2 cm depth irrespective of vegetation. In non-contaminated cells, however, the distribution of Fe(II) and Fe(III) appeared constant with depth, and a much higher importance of Fe(III) was observed (Fe(III) was on average 90 and 40 µmol cm^{-3} non-vegetated and vegetated cells, respectively).

Table 1 - Chemical characteristics of the sewage-sea water mixtures used in the experimental mesocosms from October 2006 to April 2007. (DO: Dissolved Oxygen) (N = 45). Values are given is averages ± SE (adapted from PUMPSEA, 2008).

Sewage loading	**Salinity (‰)**	**DO_{day} (µM)**	**DO_{night} (µM)**	**NH_4^+ (µM)**	**NO_3^- (µM)**	**PO_4^{3-} (µM)**
0%	39.1 ± 0.6	308 ± 133	197 ± 17	26 ± 2	3.6 ± 1.4	42 ± 4
60%	18.9 ± 1.4	225 ± 256	9 ± 1	194 ± 17	5.0 ± 1.4	206 ± 14

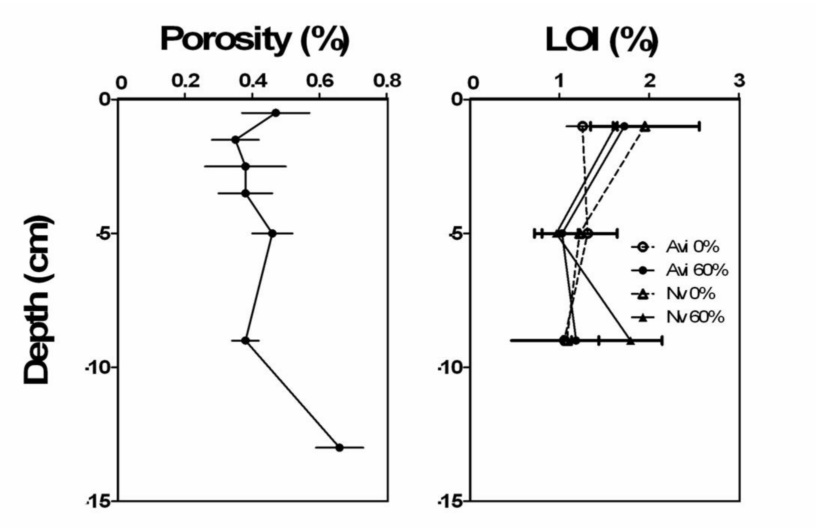

Figure 1 – Vertical profiles of A. initial sediment porosity in mesocosms (October 2006) and B. organic matter (loss on ignition, LOI) under both vegetation (*A. marina* vegetated (Av) and non vegetated (Nv)) and sewage loading conditions (February 2007). Values are given as mean ± S.E (n = 3). Adapted from Penha-Lopes et al (accepted-b)

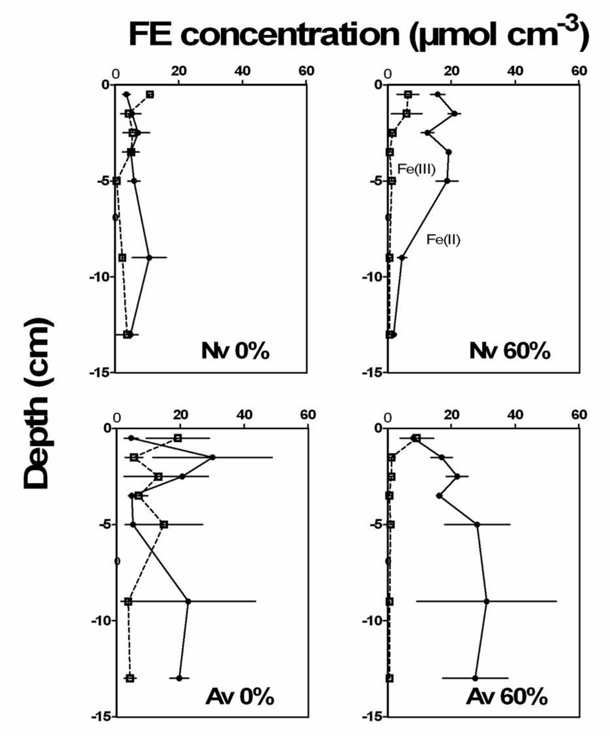

Figure 2 – Vertical profiles of solid phase iron under both vegetation (*A. marina* vegetated (Av) and non vegetated (Nv)) and sewage loading conditions. Values are given as mean ± range (n = 2).

3.1 Carbon mineralization pathways

Anaerobic respiration processes, sulfate reduction (SRR) and iron reduction (FeR), were determined independently and total anaerobic CO_2 production (TACP) and FeR was assessed from the same jar incubations. TACP was clearly affected by sewage amendment (Figure 3 - upper). TACP ranged from 117-667 nmol cm^{-3} d^{-1} in non-contaminated sediment and was 386-939 nmol cm^{-3} d^{-1} in contaminated sediment. On average sewage amendment increased TACP by 58 to 330% in unvegetated sediment, mainly in the 12-14 cm depth interval, while the corresponding TACP enhancement of 87 to 150% in vegetated sediment was primarily observed in the uppermost sediment layers (1-2cm).

SRR also showed a clear and significant response to sewage loading, mainly in the upper 5 cm of the sediment (Figure 3 - mid). SRR was < 30 nmol cm^{-3} d^{-1} in non-contaminated sediments, but was generally stimulated to >> 100 nmol cm^{-3} d^{-1} in the top 4 cm of contaminated sediments. Below 4 cm depth SRR resembled non-contaminated sediment (~25 nmol cm^{-3} d^{-1}, vegetated cells) or was significantly higher (>75 nmol cm^{-3} d^{-1}, non-vegetated cells).

The trend was opposite for FeR (Figure 3 - lower) where non-contaminated sediments showed highest rates of FeR in the upper 10 cm (0.8 and 0.45 µmol cm^{-3} d^{-1} in unvegetated and vegetated sediment), roughly twice as high as FeR in the corresponding contaminated sediment (0.5 and 0.2 in unvegetated and vegetated sediment). Below 10 cm depth FeR rates were similar in all treatments. The very low value at 3 cm depth in contaminated unvegetated sediment is considered an outlier. Overall, the presence of trees appeared to decrease FeR in both non-contaminated (up to 92%) and contaminated treatments (by 32 to 75%).

Denitrification was strongly affected by the interaction of vegetation, sewage and light exposure ($p<0.001$). In contaminated sediments denitrification rates in the dark (400 to 550 µmol m^{-2} d^{-1}) were stimulated nearly 10 times when compared to non-contaminated sediments (30 to 50 µmol m^{-2} d^{-1}), for non-vegetated and vegetated sediments, respectively (Figure 4). Light exposure positively stimulated denitrification in all treatments, leading to denitrification rates of ~2800 and 1900 µmol m^{-2} d^{-1} in non-vegetated and vegetated contaminated sediments, respectively. The corresponding rates in non-contaminated cells were more than one order of magnitude lower, and similar values were obtained between light and darkness and both vegetation conditions (ranging from 33 to 88 µmol m^{-2} d^{-1}).

Depth profiles of TACP was generally higher than the estimated sum of SRR, FeR and denitrification (assumed to occur only in the upper 2 cm) when converted to carbon units (SRR x 2 = CO_2 production; FeR/4 = CO_2 production; Den = 4/5 CO_2) (Table 2). TACP was 20 to 2000% higher than the sum of individual anaerobic processes, except for unvegetated non-contaminated sediment where TACP was 20 and 50% lower at 8 and14 cm depth, respectively. In any case, FeR was the most important measured contributor to TACP in non-contaminated sediments, while SRR dominated TACP in contaminated sediments.

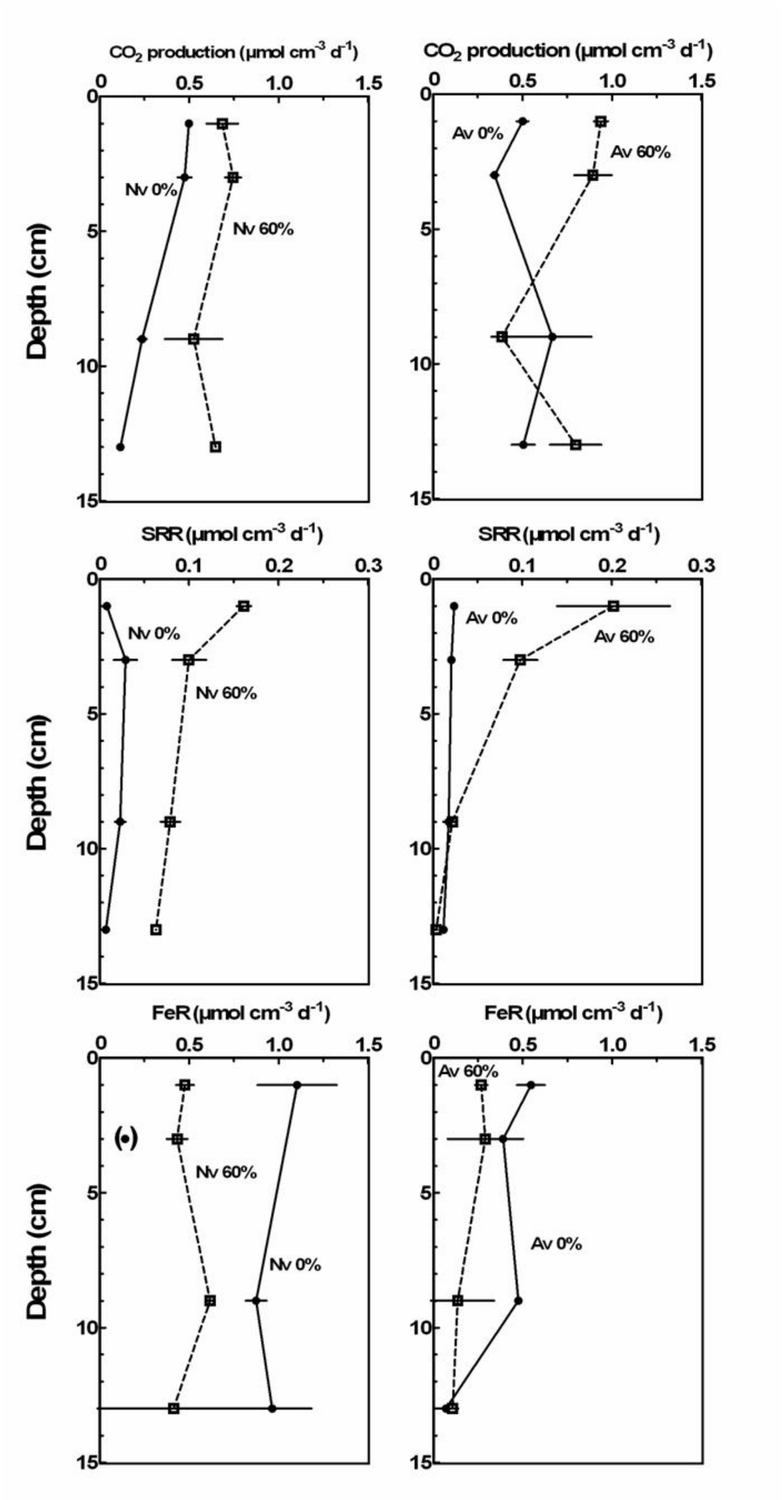

Figure 3 - Vertical profiles of anaerobic CO$_2$ production (upper); sulfate reduction (SRR, mid); and iron reduction (FeR, lower) in 0 and 60% sewage treated sediments. Results are shown for sediment from *A. marina* vegetated (Av) and non vegetated (Nv) cells. Values are given as mean ± SE (n=3). (•) indicates an outlier.

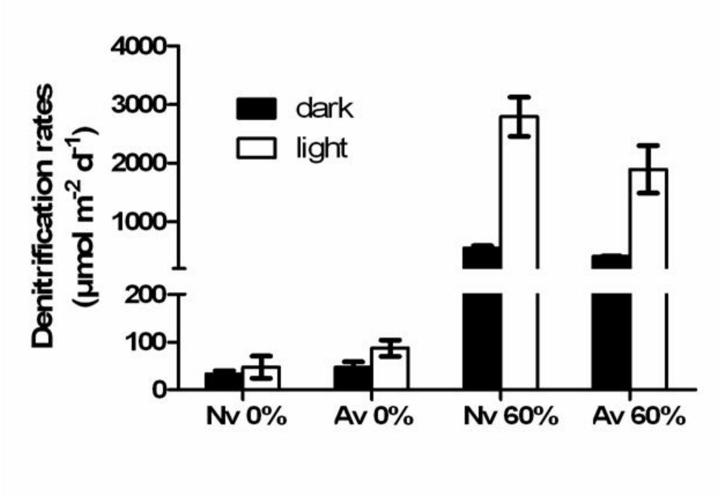

Figure 4 - Denitrification by the sediment in 0 and 60% treatments with different vegetation types (Av – *A. marina*; B – non-vegetated). Rates are given for light and dark conditions. Values are given as mean ± SE (n=2-3).

3.2 CO_2 exchange across the sediment-water/air interface

The sediment-water exchange of TCO_2 was not influenced by plants but was significantly enhanced by sewage (Figure 5 - upper). Sewage exposure increased the dark TCO_2 efflux (respiration: RSP) by a factor of four ($p<0.05$) from 25 to 104 mmol m^{-2} d^{-1} in non-vegetated cells and a factor of two (although statistically insignificant) from 33 to 62 mmol m^{-2} d^{-1} in vegetated cells. Benthic net primary production (NPP) during inundation was not influenced by the presence of plants, since CO_2 uptake rate in the light ranged from 11 to 34 mmol m^{-2} d^{-1} under pristine conditions and increased significantly ($p<0.01$) for contaminated sediments to 136 to 301 mmol m^{-2} d^{-1} in non-vegetated and vegetated cells, respectively.

Table 2 - Depth integrated (0-14 cm) total anaerobic CO_2 production (mmol m^{-2} d^{-1}) in jar-experiments as well as depth integrated FeR and SRR based CO_2 production. “Other” represents unaccounted CO_2 from jars, which supposedly is aerobic respiration. Values represent a 24 h cycle emission (emersion / immersion during dark period). Numbers in parenthesis indicate percent contribution to total CO_2 production. (For integrated calculations average values from 4-6 accounted for 4-7cm, 8-10 for 7-11cm, and 12-14 for 11-14cm depth)

Process	Unvegetated		*Vegetated*	
	0%	60%	0%	60%
Total Jar CO_2 prod	44	89	72	97
FeR CO_2 prod	25 (57)	18 (20)	13 (18)	7 (7)
SRR CO_2 prod	5 (11)	26 (29)	5 (7)	18 (19)
Other (aerobic!?)	13 (30)	47 (53)	54 (75)	69 (71)
CO_2 flux	31	167	48	103
CO_2 jar / CO_2 flux	1.41	0.53	1.5	0.94

Gross primary production (GPP = RSP – NPP) was similar in planted and unplanted mesocosm cells. However, under pristine conditions GPP ranged from 45 to 60 mmol m^{-2} d^{-1} while under 60% sewage treatment the rates were 4.3 to 6.7 times higher ($p<0.05$) for unvegetated and planted conditions, respectively.

CO_2 emissions from exposed and darkened sediment to the atmosphere (RSP) were greatly increased ($p<0.05$) by sewage exposure from 6-14 to 42-62 mmol m^{-2} d^{-1}, while vegetation had no significant effect (Figure 5 - lower). RSP was nearly 10 times higher in contaminated (62 ± 14 mmol m^{-2} d^{-1}) than non-contaminated (6±1 mmol m^{-2} d^{-1}) cells for unvegetated mesocosms ($p<0.01$). In vegetated cells CO_2 emmissions were not significantly stimulated by sewage amendment, although the average CO_2 emission was ~3 times higher in contaminated cells (14 ± 6 and 42 ± 3 mmol m^{-2} d^{-1} in non-contaminated and contaminated sediment, respectively). RSP of air-exposed sediment was similar in rates and pattern to that of inundated sediment. Benthic net primary production (NPP) in exposed non-contaminated sediment was similar regardless of vegetation (~0.5 ± 1.5 mmol m^{-2} d^{-1}). NPP in non vegetated contaminated sediment was significantly stimulated ($p<0.001$) by sewage amendment and was 61 ± 10 mmol m^{-2} d^{-1}. However, in vegetated contaminated cells, a surprising light CO_2 emission was observed (3.9 ± 1.4 mmol m^{-2} d^{-1}), which was only different ($p<0.001$) from that measured in unvegetated 60% cells. Furthermore, in exposed sediment there was significant interaction between vegetation and sewage contamination ($p<0.001$). Gross primary production was greatly enhanced ($p<0.05$) in unvegetated contaminated cells (~123 mmol m^{-2} d^{-1}) when compared with the other treatments (6 to 38 mmol m^{-2} d^{-1}).

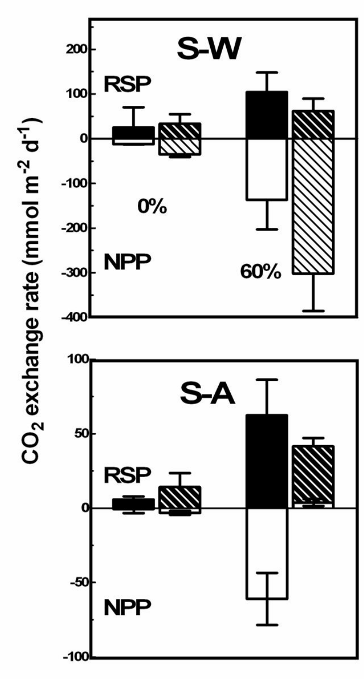

Figure 5 – Sediment-Water (S-W) and Sediment-Air (S-A) fluxes of CO_2 in unvegetated (solid and open bars) and *Avicennia marina* vegetated (hatched bars) cells exposed to 0 and 60% sewage loading in the dark (RSP – black background) and in light (NPP – white background). Notice the difference in scaling between upper and lower panels. Values are given as mean ± SE (n=3).

CH_4 emissions were generally 2-3 orders of magnitude lower than CO_2 emissions. CH_4 emission during air exposure was similar in unvegetated and vegetated non-contaminated sediments (34.8 to 53.7 µmol m^{-2} d^{-1}) but increased significantly ($p<0.05$) 8 to 10 times in contaminated sediments (Figure 6). Furthermore, one test deployment showed dramatically increased CH_4 emissions up to 6000 µmol m^{-2} d^{-1}.in sediments with biogenic structures (*A. marina* pneumatophores, ~400 m^{-2}).

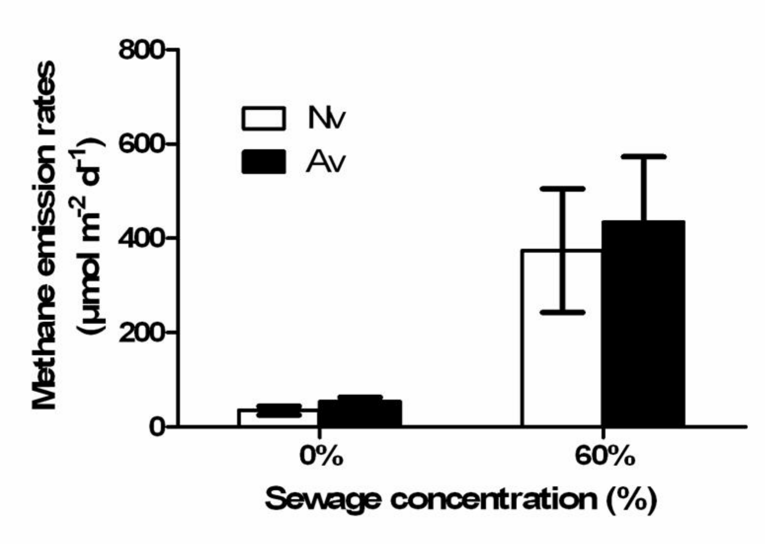

Figure 6 - Emission of CH_4 from unvegetated (Nv - open bars) and *Avicennia marina* vegetated (Av - solid bars) sediment exposed to 0 and 60% sewage loading in the darks. Values are given as mean ± SE (n=3)

3.3 CO_2 emissions by pneumatophores, burrows, crabs and gastropods

Emission of CO_2 was enhanced considerably by the presence of pneumatophores and burrows (Figure 7). The measured rates of CO_2 release from chamber deployments with *Avicennia marina* pneumatophores in pristine and contaminated cells were 17 to 5.7 times higher, respectively, than across the bare sediment surface ($p<0.001$). CO_2 emission in deployments with burrows was increased 5.6 in non-contaminated sediment ($p<0.01$) and 2.9 in contaminated sediment ($p<0.001$). It is important to note, however, that the number of pneumatophores and burrows trapped inside the measuring chamber (1 burrow equivalent to 200 m^{-2} and 3 pneumatophores equivalent to 600 m^{-2}) was much higher than the abundance of biogenic structures in the cells (Table 2).

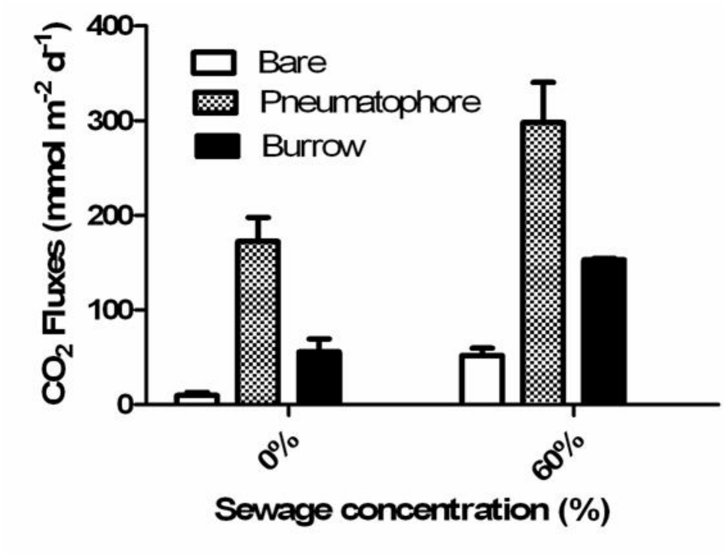

Figure 7 – Dark emission of CO$_2$ from air-exposed sediment in unvegetated and *Avicennia marina* vegetated sediment exposed to 0 and 60% sewage loading. Rates are shown for sediment with 600 *Avicennia marina* pneumatophores m^{-2} and sediment with 200 crab burrows m^{-2}. Values are given as mean ± SE (n=3-6)

Table 3 – Abundance of fiddler crabs, gastropods and pneumatophores in mesocosm cells with and without vegetation and sewage treatment. Burrow volume and wall surface area data are from (Penha-Lopes et al., accepted-a). Values are given as mean ±se (n=3). Na: not applicable

Vegetation	Sewage (%)	Crab density (m^{-2})	Burrow density (m^{-2})	Gastropod density (m^{-2})	Pneumatophore density (m^{-2})	Burrow volume (cm^{-3})	Burrow wall surface area (cm^{-2})
Unvegetated	0	12±1	16±1	4±0	n.a	37.91±4.14	27.48±2.17
	60	11±3	14±4	4±0	n.a	41.57±9.55	28.04±4.47
Vegetated	0	16±1	21±1	4±0	15±1	34.74±2.33	22.73±3.27
	60	10±2	13±3	4±0	25±2	47.52±6.57	28.97±2.92

The estimated CO$_2$ emission per pneumatophore was similar in pristine (0.27±0.08 mmol pneum.$^{-1}$ day^{-1}) and contaminated sediment (0.38±0.11 mmol pneum.$^{-1}$ day^{-1}). By multiplying with the average pneumatophore density observed in each treatment (Table 2), we obtain an estimate for the increase in CO$_2$ emission due to pheumatophores of 4.1 (29%) and 9.5 mmol m^{-2} d^{-1} (15%) in non-contaminated and contaminated cells, respectively. The average CO$_2$ emissions from individual crab burrows (including animal respiration) were within the same range than from single pneumatophores at non-contaminated and contaminated conditions (0.23±0.11 and 0.51±0.10 mmol m^{-2} d^{-1}, respectively). Most burrows contained one crab, but this could not be documented since crabs rapidly retreated to their burrows when disturbed. No attempts were made to recover the inhabitants, but in many cases, they were visible in the burrow opening. We therefore must rely on the estimate of

Skov et al, (2002) that 81% of all burrows were inhabited. Part of the excess CO_2 emission from burrows must therefore originate from crab respiration.

Since crabs inhabiting the sampled burrows were not identified, but average CO_2-production by crabs was described by a power function regardless of crab species and sex (Figure 8 - upper). Thus, when using the average weight of all sampled crabs (1.1 g wet wt) CO_2-production by crabs was 0.16 mmol CO_2 ind.$^{-1}$ d^{-1}. Taking into consideration the crab respiration inside the burrow and corrected (subtract) for RSP from a surface area corresponding to the burrow opening, CO_2 emissions per burrow increased ($p<0.001$) from 0.08±0.07 mmol burrow^{-1} day^{-1} to 0.39±0.01 mmol burrow^{-1} day^{-1} in non-contaminated and contaminated cells, respectively. Multiplying these values by the average burrow density in each treatment (Table 2), the area-specific burrow wall respiration increased ($p<0.05$) from 68.7±57.7 mmol m^{-2} day^{-1} in non-contaminated to 312.5±28.2 mmol m^{-2} day^{-1} in contaminated mesocosms. Dividing the burrow CO_2 release by the amount of gas released by an area of exposed sediment equivalent to the area of the burrow opening (BOA), a ratio of 44 is obtained at non-contaminated conditions. However, since the burrow total surface area (BTSA) is only 21 times larger than the BOA, the area-specific burrow wall CO_2 release appears 2.1 times greater than that of the surface sediment. In contaminated sediments the corresponding CO_2 flux ratio is 56, resulting in 2.7 times increased area-specific CO_2 release in the burrow lumen.

Terebralia palustris respiration also followed a power function when related to body weight (Figure 8 - Lower). The average *T. palustris* individual (22g wet wt) therefore respired 0.24 mmol CO_2 ind.$^{-1}$ d^{-1}.Thus when applying the abundance of *T. Palustris* in the mesocosm cells (4 m^{-2}), 0.97 mmol m^{-2} d^{-1} CO_2 production is attributable to gastropods..

The role of the different compartments for CO_2 emission in air exposed sediment during dark incubations, suggested that the bare sediment surface was responsible for >70% of CO_2 release in contaminated cells and < 60% in non-contaminated cells (Table 3). Thus fauna and crab burrows accounted for up to 40% of the CO_2 release in unvegetated cells, whereas pneumatophores increased the importance of biogenic structures for CO_2 emissions by 13 and 16% in planted non-contaminated and contaminated cells, respectively.

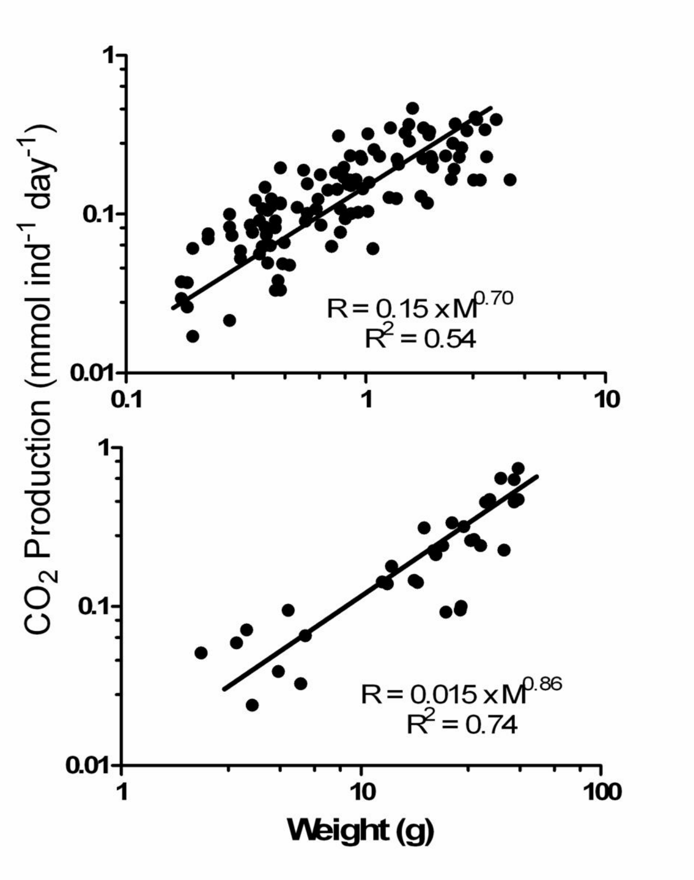

Figure 8 – Respiratory CO_2 production (R) by individual fiddler crabs (*Uca* spp.) and *Terebralia palustris* as a function of live body weight (M). Values are given as mean ± SE (n = 100 sequential 10 s measuring intervals). The fiddler crab data contains males and females. The regression equation is given.

Table 4 - CO_2 emission budget in the dark during emersion for both unvegetated and *Avicennia marina* planted sediment exposed to 0% and 60% sewage. Rates are partitioned into the contribution of bare sediment, pneumatophores, burrows, crabs and gastropods. *Terebralia palustris* respiration is based on an average animal size of 22 g (0.24 mmol CO_2 ind.$^{-1}$ d^{-1}), while crab respiration is based on an average size of 1.1 g (0.16 mmol CO_2 ind.$^{-1}$ d^{-1}). It is assumed that 0.81 crabs were present inside each burrow. Rates are given as mean ± SE (mmol m^{-2} d^{-1}). Numbers in parenthesis are the % contribution of each component. na: not applicable.

	Unvegetated		*Vegetated*	
	0%	60%	0%	60%
Bare sediment	6 ± 1 (60)	62 ± 14 (94)	14 ± 6 (47)	42 ± 3 (70)
Pneumatophores	na	Na	4 ± 1 (13)	10 ± 1 (16)
Burrows	1 ± 1 (10)	1 ± 1 (1.5)	8 ± 2 (27)	5 ± 1 (9)
Crabs	2 ± 0 (20)	2 ± 0 (3)	3 ± 0 (10)	2 ± 0 (3)
Gastropods	1 ± 0 (10)	1 ± 0 (1.5)	1 ± 0 (3)	1 ± 0 (2)
Total	10 ± 1	66 ± 14	30 ± 6	60 ± 3

4. Discussion

4.1 Carbon fluxes

The exchange of TCO_2 across mangrove sediment surfaces devoid of biogenic structures represents the net outcome of near surface heterotrophic carbon mineralization and carbon fixation by microphytobenthic primary producers (Kristensen & Alongi 2006). Benthic respiration (RSP) are comparable to the global average of 61 ± 46 mmol m^{-2} d^{-1} found for mangrove sediments (Bouillon et al., 2008a). While sewage amendment and inundation/emersion periods clearly affected RSP in our mesocosm system, there was no apparent effect of vegetation on benthic RSP. Even though the saplings of *A. marina* grew well during the 12 month period since February 06, their biomass was apparently insufficient to significantly affect microbial pathways and organic matter dynamics. This way the results obtained here from vegetated cells should therefore be considered with care with respect to the effects of vegetation, especially when trying to extrapolate to mature constructed wetlands and forests.

Alongi (2009) noted higher average CO_2 efflux when mangrove sediment are exposed to the atmosphere (69 ± 8 mmol CO_2 m^{-2} d^{-1}) than when they are immersed by tides (49 ± 6 mmol CO_2 m^{-2} d^{-1}), which may be explained by the combination of higher sediment temperatures during emersion, faster molecular diffusion in gases than fluids and increased surface area for aerobic respiration and chemical oxidation. In contrast, we found a tendency towards a ~2 times faster CO_2-efflux during immersion than emersion, which could be explained by the higher amount of dissolved labile organic matter (secondary treatment or seawater) available for decomposition when mesocosms were inundated with seawater - sewage mixture.

The consistently 2 to 4 times higher near-surface microheterotrophic activity observed in contaminated than non-contaminated cells, as indicated by higher CO_2 release in the dark during both inundation and air exposure (Figure 5), is caused by the regular amendment with organic matter and subsequent stimulation of microbial heterotrophic activity in these cells. Other aquatic systems subjected to massive and continuous organic loading, such as natural sediments impacted by fish farming (Holmer and Kristensen, 1992; Holmer and Heilskov, 2008) and constructed laboratory systems (Valdemarsen et al., in press-a; Valdemarsen et al., in press-b), show similar dramatic increases in mineralization rates (2 to 10 times). In this experiment, where organic matter content was similar in non-contaminated and contaminated mesocosms (Figure 1), it therefore appears that excess benthic metabolism in amended mesocosms is primarily driven by organic/nutrient input as opposed to indigenous organic matter. Furthermore, the lack of C-accumulation in sewage amended mesocosms indicate that sewage derived organic carbon was either totally or partially mineralized during inundation, or lost from system when the water phase was flushed during emersion periods. Also, the organic matter deposited or infiltrated in the sediment seems also to be rapidly mineralize hampering its accumulation in the sediment, as observed in previous studies (Valdemarsen et al., in press-a).

Stimulated nutrient driven benthic primary production during immersion also contributed significantly to the C-loading in contaminated cells, with values 10 times higher than at pristine conditions, although benthic Chla concentration was only 2 to 3 times higher. The lower primary productivity at inundated pristine mesocosms as well as during emersion period at both sewage concentration is probably caused by deficiency/exhaustion of nutrients and/or photoinhibition and dessication (Blanchard et al., 2001; Consalvey et al., 2004). We have no explanation for the surprising lack of NPP in contaminated planted cells during emersion, except for unknown methodological faults or coincidental selection of sites poor in microphytobenthos (Colijn and Dejonge, 1984). In any case, GPP was 4.3 to 6.7 times higher in contaminated than non-contaminated mesocosm cells. A similar difference was found between an anthropogenically impacted (Mtoni) and a pristine (Ras Dege) mangrove forest in Tanzania (Kristensen et al., 2008b).

Methane emission from sediment to the atmosphere in our non-contaminated mesocosms was hardly detectable, as observed for most pristine natural mangrove areas (Alongi 2009). The higher nutrient and organic loading in contaminated cells stimulated methane emissions 8-10 times, which is similar to other organic enriched mangrove sediments during oxygen depletion (Purvaja and Ramesh, 2001; Hegde et al., 2003; Alongi et al., 2005). However, since CH_4 emissions were 2 to 3 orders of magnitude lower than CO_2 emissions in our system, they contribute insignificantly to carbon budgets in sewage amended mesocosms (see below).

4.2 The role of biogenic structures

When measuring CO_2 exchange across the sediment-air interface at bare sites, measured rates may be underestimates due to the exclusion of biogenic structures (pneumatophores and crab burrows) (see Kristensen et al., 2008a). Transport of gases is then largely limited to diffusion across the horizontal sediment–water/air interface. This deficit is most pronounced during emersion when gas exchange from deep sediment is enhanced through open lenticels of pneumatophores (Allaway et al., 2001) and unplugged fiddler crab burrows (De la Iglesia et al., 1994). Under contaminated conditions, the effect of biogenic structures on CO_2 emissions is decreased due to the high carbon mineralization at the sediment surface (Table 5). In this experiment, pneumatophores increases (13 to 16%) CO_2 emission from air-exposed sediment during darkness (Table 3), but we cannot partition the relative contribution of root respiration (Kitaya et al., 2002) and CO_2 derived from heterotrophic CO_2 production in deep sediments (Scholander et al., 1955). Thus, our methods may overestimate the importance of pneumatophores for CO_2 exchange between sediment and air in exposed sediments. However, estimated CO_2 emission from *A. marina* pneumatophores in pristine and contaminated cells (0.27 to 0.38 mmol d^{-1}) was comparable to rates found by Kristensen et al. (2008b) for pristine and anthropogenic impacted mangroves in Tanzania, as well as for 50% taller pneumatophores measured in Japan by Kitaya et al. (2002).

Diffusive CO_2 transport from crab burrows to the atmosphere originates from carbon mineralization in the deep sediments that diffuses through the burrow walls (Pinder and Smits, 1993; Datta, 2005) as well as crab respiration. The contribution from burrows is augmented due to the large surface area of burrow walls and enhanced decomposition within the burrow lining (e.g., Botto et al., 2006). These processes not only occur during emersion, but also during inundation when fiddler crab burrows are plugged. Consequently CO_2 must accumulate inside inundated burrows, causing a burst of CO_2 emission once the crab burrows are opened, but this process is unknown and should be considered. In contrast to root respiration, crab and burrow respiration is an integrated part of C-budgets as they reflect heterotrophic CO_2 production (Kristensen & Alongi 2006). Although, crab burrows occurred in low abundance in this experiment, they may contributed significantly (2 to 27%) to the total CO_2 emission (Table 3).

The CO_2 emission rates from single burrows are within the range reported in Kristensen et al (2008b) in Tanzanian for ocypodid or grapsid mangrove crabs (0.2 to 0.5 mmol $bur.^{-1}$ d^{-1}). However, the presence of sewage increased CO_2 emission from individual burrows by a factor of 5 times due to stimulated activity along the burrow walls of microbial communities known to inhabit these systems (Kristensen et al., 2005). However, the area specific CO_2 emission by burrow walls was 2 to 3 times larger when compared with sediment surface under both pristine and impacted situations. Higher burrow emission values are expected if other mangrove crabs are present, such as sesarmids, known to have much larger burrows (Berti et al., 2008).

4.3 Carbon mineralization pathways

Organic loading and biogenic structures also affected major pathways for organic C mineralization between treatments and with depth in the sediments (Table 4). The high rates of anaerobic CO_2 production in contaminated sediment are typical for environments with a continuous input of organic matter (Kristensen et al., 2000). However, there is no consistent depth pattern, which may be due to the low depth resolution, considering that the majority of the sewage organic matter as well as primary and secondary productivity is mineralized explicitly at the sediment surface. Also, is more likely that organic matter lability is independent of sediment depth and that the contamination signal reaches to more than 10 cm depth. Since only unvegetated sediment with 0% sewage showed a depth dependent decrease in CO_2 production, tree roots in the vegetated sediment must drive high metabolic activity at depth through dissolved carbon exudation (Kristensen and Alongi, 2006). Rates of CO_2 jar production in our mesocosms are within the lower range observed by Kristensen et al (submitted) for pristine and polluted mature mangroves.

Apart from aerobic respiration (OxR), all other major microbial pathways, such as Fe(III) reduction (FeR) and sulfate reduction (SRR) were quantified in this experiment, Since total anaerobic CO_2 production by jar incubations is considered a measure of total carbon oxidation (TACP), carbon oxidation by aerobic respiration should in principle be: OxR = TACP – FeR/4 – 2SRR, where FeR and SRR are converted to carbon units using standard stoichiometries (Canfield et al. 2005). However, total anaerobic CO_2 production in jars was much higher than the sum of SRR and FeR, mainly in vegetated mesocosms (Table 4). This must be due to importance of other respiration pathways not quantified in jar experiments. However, while denitrification showed to be minor in the present study, manganese and methanogenesis have traditionally been considered unimportant in mangrove sediments (Kristensen et al., 2008a; Alongi, 2009), leading us to consider that in situ these discrepancies could be due aerobic respiration (Table 4). Although these rates must be overestimated, although no good explanations can be given at the present moment. it has been shown that aerobic respiration is considered one of the most important respiration processes with a share of 40 to 50% (see review by Kristensen et al., 2008a).

FeR was substituted by SRR in contaminated sediment due to the almost complete absence of Fe(III) (Figure 2 and 3). Thus, SRR increased particularly in the upper 5 cm of contaminated cells, as observed in other sediments where labile organic matter is continuously supplied to the sediment surface (Valdemarsen et al 2009). Pneumatophores appeared to increase OxR by stimulating O_2 diffusion, via the roots, into otherwise anoxic sediment (Table 4). This process appears crucial for maintaining Fe(III) for FeR, especially in vegetated non-contaminated cells.

Table 5 - Calculation overview of daily (24 h) total system carbon gas emission budgets. The table is separated into measured input parameters and calculated budget components. Basic assumptions: (a) 1 d has 12 h light and 12 h dark periods; (b) sediment is covered by water (immersion period) during half of each 12 h period; (d) (d) abundance of pneumatophores (pneum.) and crab burrows is equal to a crude median of all examined cells: 20 and 16 m^{-2}, respectively; (e) burrows contain on average 0.81 crab inhabitants (Skov et al. 2002). Input parameter: time = time of day; budget component: time = timing per day

	Acronym	Calculation/ Upscaling	Unit	Time	Tidal Level	Data Location
Input parameters						
Sediment emission (immersion)	Se-im	Measured	mmol m^{-2} h^{-1}	Light/Dark	immersion	Fig. 3
Sediment emission (emersion)	Se-em	Measured	mmol m^{-2} h^{-1}	Light/Dark	emersion	Fig. 4
Sediment emission (em) + 3 pneum.	PSe	Measured	mmol m^{-2} h^{-1}	Light/Dark	immersion	Fig. 5
Sediment emission (em) + 1 burrow	PBe	Measured	mmol m^{-2} h^{-1}	Light/	immersion	Fig. 5
Pneumatophore emission	Pe	(PSe – Se-em)/3	mmol ind.$^{-1}$ h^{-1}	Dark	immersion	Text
Total Burrow emission	TBe	PBe – Se-em	mmol bur.$^{-1}$ h^{-1}	Dark	immersion	Text
Crab emission	Ce	Measured	mmol ind.$^{-1}$ h^{-1}	Dark	immersion	Text
Burrow emission	Be	TBe – 0.81Ce	mmol bur.$^{-1}$ h^{-1}	Dark	immersion	Text
Budget component						
Sediment (im)	DSe (im)	Se x 9 m^{-2}	mmol 12^{-1}	6 h light + 6 h dark	immersion	
Sediment (em) + Be	DSe (em)	(Se + 0.5*16 Be) x 9 m^{-2}	mmol 12^{-1}	6 h light + 6 h dark	emersion	
Sediment (em) + Be + Pe	DSe (em)	(Se + 20Pe + 0.5*16 Be) x 9 m^{-2}	mmol 12^{-1}	6 h light + 6 h dark	emersion	

Roots may also stimulate heterotrophic processes by excreting labile organic exudates into the surrounding sediment (Andersen and Kristensen, 1988; Aller, 1994; Kristensen, 2000; Kristensen and Alongi, 2006), and this process may explain the slightly higher mineralization rates observed at depth in vegetated non-contaminated sediment when compared to bare non-vegetated sediment.

The ratio of total anaerobic CO_2 production (14cm depth integrated) and gas emission from sediments during a 24h cycle was 1.5 in non-contaminated cells (Table 4), which might indicate CO_2 consumption during authigenic mineral formation and losses via chemotrophy by sulphide oxidizers (Bouillon et al. 2008a, Kristensen et al. 2008a), or CO_2 is being released and not measured (e.g., burst CO2 from crab burrows). For unvegetated contaminated sediments, the ratio is <1, indicating that CO_2 production via SRR must occur below 14 cm sediment depth, (Alongi et al., 2005).

Denitrification in mangrove ecosystems, is primarily regulated by nitrate availability, temperature, salinity and organic matter availability (Alongi, 2009). The rates obtained in our non-contaminated experimental cells are within the low range (27 to 470 µmol m^{-2} d^{-1}) of the ones found in East African (e.g., Kristensen et al., submitted) and Thailand mangroves (Kristensen et al., 1998). The high rates obtained in contaminated sediment must be driven by high ammonium availability and consequently coupled nitrification-denitrification and increased O_2 availability due to high benthic NPP (Canfield et al., 2005). Other studies have also recorded high denitrification at rates of more than 15000 µmol m^{-2} d^{-1} in contaminated mangrove sediments (Corredor and Morell, 1994; Rivera-Monroy et al., 1999), and it has been proposed that high denitrification rates in contaminated mangroves is governed by increased N-availability and higher nutrient driven 0_2 production by benthic microalgae (e.g., Risgaard-Petersen et al., 1994). The present data confirm the potential of mangrove sediments for nitrate depuration of secondary sewage as suggested by Corredor and Morell (1994).

4.3 Wetland functioning and environmental implications

Estimates of total inorganic carbon in the different mangrove mesocosms can be provided by taking into consideration the contribution by various mangrove compartments (Table 5). The estimated total CO_2 emission to the atmosphere in the mangrove mesocosms in February 2007 showed that more than 50% is in most cases released from the sediment during emersion periods, especially in vegetated cells where pneumatophores are present (Table 6).

Fauna and biogenic structures accounted for up to 60% of the total CO_2 emission in non-contaminated mesocosms, increasing by 2-3 times the global average found for mangrove sediments (Bouillon et al., 2008a), while the corresponding contribution in contaminated cells was always below 30% (Table 3).

The role of fauna in organic matter degradation is known to decrease in anthropogenic impacted situations, such as in sediments bellow fish farms (Holmer and Heilskov, 2008). However, the biogenic contributions in our mesocosms must be lower than in mature wetlands, where mangrove trees are typically larger with more well developed pneumatophores (e.g., Dahdouh-Guebas et al., 2007) and the abundance and diversity of crabs is much higher (Hartnoll et al., 2002; Skov et al., 2002). For instance, if the density of biogenic structures is raised to a level corresponding to that commonly observed in mature mangrove environments (e.g. 300 burrows m^{-2} and 70 pneumatophores m^{-2}, Kristensen et al. (2008b)) their contribution to CO_2 emissions during emersion increase considerably, to account for at least 16% and up to 180% for unvegetated and vegetated conditions, respectively, by increasing OM decay rate and consequently decrease OM sediment pool (Kristensen, 2000).

Table 6 – Total emission of CO_2 (mmol $12h^{-1}$) to the atmosphere in the mangrove mesocosms at both inundation and emersion periods and different vegetation and sewage conditions. The emissions at the unvegetated plots are assumed to be devoid of pneumatophores but not burrows. At plots conditions, burrows and pneumatophores density are shown in Table 3 while natural densities conditions 300 burrows m^{-2} and 70 pneumatophores m^{-2} are considered. See Table 5 for further explanation. The rates were calculated for 12 h low tides and 12 h high tides d^{-1}. Error values are SE.

A		Immersion CO_2	Emersion CO_2 (plots)	Total CO_2 (plots)
Unvegetated	0%	87.3 ± 14	27.9 ± 16.1	115.2 ± 21.0
	60%	164.0 ± 13.0	146.5 ± 15.6	310.5 ± 20.3
Vegetated	0%	72.9 ± 13.0	93.4 ± 15.6	166.3 ± 20.3
	60%	-399.6 ± 13.0	250.2 ± 15.6	-149.4 ± 20.3

B		Immersion CO_2	Emersion CO_2 (natural)	Total CO_2 (natural)
Unvegetated	0%	87.3 ± 14	79.8 ± 16.1	167.1 ± 21.0
	60%	164.0 ± 13.0	197.0 ± 15.6	361.0 ± 20.3
Vegetated	0%	72.9 ± 13.0	406.1 ± 15.6	479.0 ± 20.3
	60%	-399.6 ± 13.0	610.4 ± 15.6	210.8 ± 20.3

A higher abundance of biogenic structures (roots, pneumatophores and burrows) will also have profound effects on sediment biogeochemistry through simultaneously enhanced translocation of oxygen and organic matter to deep sediment layers. As a consequence, both FeR and SRR may be stimulated by organic enrichment (Alongi, 1998), and whereas FeR may dominate C-mineralization at low to intermediate organic loading, SRR is typically the dominating mineralization pathway in heavily impacted sediments. Below a certain maximum metabolic threshold, accumulation of generated sulfides may be sufficiently hampered by continuous O_2-driven reoxiditation processes and precipitation of Fe-sulfides (Valdemarsen et al., in press-a). Conversely, if the maximum metabolic threshold is exceeded and Fe available for precipitation is depleted, free sulfide may accumulate with deleterious consequences for flora and fauna (Hargrave et al., 2008). The threshold for sewage delivery was not exceeded in our mesocosm study and it is at present not known. The exact threshold must depend on sediment properties (e.g. iron content and permeability), the type and age of the vegetation as well as the abundance of burrowing infauna. Although the organic loading rate in the present study was below the maximum metabolic threshold, the oxidation/reduction balance had shifted in favour of SRR, which in combination with the low salinity of discharged sewage also favoured methanogenesis as observed in (Purvaja and Ramesh, 2001). Furthermore, it appeared that the presence of pneumatophores and burrows facilitated CH_4 transport and emission from the sediment to the atmosphere (Purvaja et al., 2004; Kristensen et al., 2008b), indicating that specific studies should address these processes to better understand the role biogenic structures in heavily impacted ecosystems on greenhouse gas emissions.

In conclusion, biogenic structures and bioturbation activities change sediment physical, chemical and biological structure and dynamics, leading not only to an increase in benthic mineralization but also to change the dominating microbial mineralization pathways. Our results confirm the importance of fauna and flora for the functioning of mangrove forests (Alongi, 2009) as well as their effective contribution to system mineralization and consequently filtration efficiency under organic-rich conditions (Canfield et al., 2005; Kristensen, 2008).

Furthermore, models on carbon dynamics in constructed wetlands exposed to sewage are strongly needed to improve our understanding of the system efficiency and metabolic threshold, including the reduction/oxidation processes involving sulphur and iron, as well as the role wetlands, including biogenic structures, for the emission of potential greenhouse gases (CO_2, CH_4 and N_2O), and consequences for global warming.

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SECTION III

GENERAL DISCUSSION

Ecosystem indicators

Until recently, the reliable fauna biomonitors found in mangrove ecosystems were few and mainly in developing countries (Table 1). Most biomonitor studies have shown that epimacrofauna tends to accumulate, with high efficiency, heavy metal contaminants in their tissues (Uma Devi and Prabhakara Rao, 1989a; b; MacFarlane et al., 2000; De Wolf et al., 2001; Mtanga and Machiwa, 2007), as well as anthropogenic derived organic matter characterized by high concentrations of ^{15}N (Pitt et al., 2009). In Brazil, different contaminants (from domestic sewage to sodium hydroxide) have shown to interfere with the biochemical and physiological conditions of mangrove oysters (Zanette et al., 2006).

Bioindicators are also not that common, and most studies have been done in developing countries (Table 1). Industrial pollution is known to affect growth and development of one of the most common fish species in mangrove habitats, the mudskipper (Kruitwagen et al., 2006; Kruitwagen et al., 2008). In mangrove ecosystems subjected to high discharges of organic matter and nutrients, increase in saprobic indexes (Chen et al., 2008), using ciliate species, as well as lower (bryozoan and gastropod communities) or higher (crab communities) diversity and abundance can be observed, potentially being dependent of the degree of contamination, system functioning and species involved (Machiwa and Hallberg, 1995; Yu et al., 1997; Linton and Warner, 2003; Cannicci et al., in press). Very recently, physiological changes in fiddler crabs inhabiting peri-urban and pristine mangroves indicated that lower RNA/DNA ratio could be a potential indicator of anthropogenic sewage contamination (Amaral et al., 2009).

The data collected and analysed during this thesis allowed to increase significantly the number of potential bioindicators of sewage pollution for East Africa, as well as validate biomonitors in controlled situations. Limited taxonomic expertise or resources are characteristic in developing countries, one of the main reasons why emphasis is placed on cost-effective techniques such as taxonomic minimalism. This technique already proved to effectively detect the effect of environmental or anthropogenic disturbance on meio and macrofauna communities at high taxonomic levels, from family to phylum (Thorne and Williams, 1997; Chapman, 1998; Kennedy and Jacoby, 1999; Savage et al., 2001; Gesteira et al., 2003). In this study, taxonomic minimalism targeting infauna major groups (chapter 1) was not a reliable tool to assess anthropogenic contamination mainly due to the fact that different systems and contaminants lead to completely different responses of infauna communities. Although differences between countries were observed, the multidimensional scaling analysis, as well as diversity indices, showed that identifying organisms at high taxonomic levels are indeed useful tools for environmental impact assessment to be used within the same region/country. The only exception was the Oligochaeta group that decreased in both peri-urban mangroves studied, being considered a potential bioindicator of disturbed ecosystems. Nevertheless, taxonomic groups should be identified to lower levels in order to provide a better understanding of community changes.

Although reproductive parameters of crustaceans have already been addressed as a proxy of heavy metal contamination in temperate countries (e.g., Elumalai et al., 2005), only recently the distinction between fecundity and fertility as a proxy of contamination was used and proved to be an effective indicator of industrial sewage contamination, and provided better information than analysing clutch size by pooling all embryo stages (Ford et al., 2003). This study (chapter 2 and 3) corroborated these findings, as in contaminated areas females of mangrove fiddler crabs and shrimps were found to carry higher number of eggs at early stages (fecundity), followed by higher brood loss and similar egg numbers before hatching (potential fertility). Other positive bioindicators observed in the same studies included longer reproductive periods and higher ovigerous female percentage, as well as better embryo quality. While sewage discharge could be favouring detritivorous feeding, and consequently gonad development and larval release, allowing females to bread continuously, Vergamini and Mantelattto (2008) have also found that another crustacean species (*Panopaeus americanus*) has changed to a continuous and effective reproduction as a strategy to establish and maintain a stable population living associated with stressful energy demanding conditions in a human-impacted mangrove in Brazil. However, it should not be exclude the possibility of other factors observed at Costa do Sol be exerting some influence in crustaceans reproductive dynamics, such as lower salinities, different hydrological regimes, other contaminants, as well as muddier sediment when compared to pristine locations (PUMPSEA, 2008; Cannicci et al., in press).

Negative indicators included higher level of parasitism by a Bopyridae isopod, *Pseudione elongata*, that lead to the physiological reproductive death of the host (*Palaemon concinnus*), indicating some degree of stress on the host shrimp at the peri-urban mangrove. Other negative indicators included decrease survival of fiddler crabs (chapter 4) and changes in crabs' behaviour (chapter 5) and growth rates of gastropods (chapter 6) in severe contaminated conditions. The high organic matter availability and high microbial metabolism in contaminated mesocosms may be responsible for low oxygen levels in the water column and sediment (Canfield et al., 2005). The associated higher microbial oxygen consumption in burrow walls (Gribsholt et al., 2003; Nielsen et al., 2003) may augment hypoxia inside burrows and coupled with release of sulfide increase stress and may cause crab death by asphyxiation or drowning (Diaz and Rosenberg, 1995). Both the immersion/emersion periodicity in mesocosms and the high tolerance to prolonged periods of hypoxia or even anoxia of gastropods (see Kapper & Stickle 1987 in Stickle et al., 1989; Das and Stickle, 1993; Sagasti et al., 2001; Cheung et al., 2008) may have explained the high survival rates (>60%) registered even at high sewage loading (100%) and long immersion periods (3 days). However, the low salinities at high sewage loading could have been a stress factor affecting physiology and consequently decreasing growth, especially when coupled with hypoxic conditions (Stickle et al., 1989).

Stable isotope studies from natural mangrove ecosystems contaminated with sewage or agricultural wastes are scarce, with only one study registering an increase in nitrogen stable isotope ratio on mangrove associated crab species (Pitt et al., 2009). In chapter 8, it was demonstrated for the first time the potential use of stable isotopes (SI) as a useful tool to prove the existence of a continuous anthropogenic impact, even at low concentrations. This study registered a significant increase in nitrogen isotope ratio in microphytobenthos and detritus found in sediment, then ingested and assimilated by both primary consumers, increasing δ^{15}N signature of fauna mangrove species' muscle tissues (fiddler crabs and gastropods). This study further revealed that in mangrove sewage treatment facilities, the introduction of macrofauna appears to be essential to maintain the natural food web dynamics, thus reducing these systems running and maintenance cost, mainly in mature systems where large accumulations of decaying litter are expected and consumed by *Terebralia palustris* (also demonstrated in Chapter 7).

Table 1 – Mangrove fauna biomonitors and bioindicators registered in (A) the literature, (B) as well as those identified during the present study.

A

Monitor/ Indicator	**Type of contaminant**	**Species**	**Location**	**Response**	**Reference**
Monitor	Heavy metals	Fiddler crabs	Visakhapatnam Harbor (India)	accumulation	Uma Devi and Prabhakara Rao, 1989a; b
		Littoraria scabra, gastropod	Dar es Salaam (Tanzânia)	Accumulation and morphology	De Wolf et al., 2001
		oysters, *Saccostrea cucullata*	Mzinga Creek Tanzânia	accumulation	Mtanga and Machiwa, 2007
		crab, Heloecius cordiformis	Sydney, Australia	accumulation	MacFarlane et al., 2000
	Domestic sewage, fertilizer, sodium hydroxide, chlorine	oyster *Crassostrea rhizophorae*	Brazil	biochemical and physiologycal	Zanette et al., 2006
	Organic matter and nutrients	Mangrove crabs	Moreton Bay, Australia	^{15}N enrichment in muscle tissues	Pitt et al., 2009
Indicator	Industrial pollution	mudskipper *Periophthalmus argentilineatus*	Tanzania	abnormal growth pattern and occurrence of unilateral anophthalmia	Kruitwagen et al., 2006; Kruitwagen et al., 2008
	Organic matter and nutrients	bryozoan communities	Kingston harbour, Jamaica	Lower diversity	Linton and Warner, 2003
		Uca annulipes	Maputo, Mozambique	Lower RNA/DNA ratio	Amaral et al., 2009
		Fiddler and sesarmid crabs	Kenya and Mozambique	Increase diversity and abundance	Cannicci et al., in press
		Terebralia palustris, gastropod	Kenya and Mozambique	Decrease abundance	Machiwa and Hallberg, 1995; Yu et al., 1997; Cannicci et al., in press
		Ciliates	constructed mangrove sewage treatment belts in Southern China	Saprobic índex increase	Chen et al., 2008

B

Monitor	Organic matter and nutrients	*Uca* spp and *Terebralia palustris*	constructed mangrove sewage treatment wetland in Tanzania	enrichment in ^{15}N in muscle tissues	Penha-Lopes et al., submitted-a
Indicator	Organic matter and nutrients	*Uca annulipes*	Maputo, Mozambique	Increase reproductive season and activity	Penha-Lopes et al., accepted-c
		Palaemon concinnus shrimp	Maputo, Mozambique	Increase in size, reproductive period and % of ovigerous females and % of shrimps with parasites	Penha-Lopes et al., submitted-b
		Uca spp and *Terebralia palustris*	constructed mangrove sewage treatment wetland in Tanzania	Change in behaviour and bioturbation activities, decrease in growth rates	Bartolini et al., accepted; Penha-Lopes et al., accepted-a; Penha-Lopes et al., accepted-b
		Infauna	Mozambique and Kenya	Decrease diversity indexes and abundance of Oligochaeta	Penha-Lopes et al., submitted-c

The role of ecosystem engineering

Mangrove forests represent a productive ecosystem that play a key role for carbon and nutrient cycling along many tropical coasts (Duarte et al., 2005; Kristensen et al., 2008). These systems have recently shown a potential as natural wastewater treatment facilities, thus preventing coastal pollution (Wong et al., 1997), with field trials showing that sediments of these ecosystems are very efficient in removing nutrients from sewage (Tam and Wong, 1995; 1996). However, most studies have focused on the behaviour of plants and/or microorganisms (Wong et al., 1995; Stottmeister et al., 2003; Wu et al., 2008b), while macrofauna and associated biogeochemical functioning have been ignored. In chapter 4, 5, and 6 the bioturbation potential of two key macrofauna groups fiddler crabs and gastropods indicated that although general behaviour was significantly changed, mainly by decreasing its feeding activity and consequently bioturbation on top sediment layers, the overall sediment manipulation was not severly affected in contaminated mangrove mesocosms. Higher MBA biomass will inevitably diminish the significance of benthic fauna as an epibenthic microalgal grazer, allowing higher primary productivity (Blanchard et al., 2001), sediment bioturbator and consequently its role for improving the functioning of natural mangroves as well as mangrove wastewater treatment facilities.

Also, crab galleries morphology only changed in unvegetated conditions, becoming shallower, probably due to hypoxic or anoxic subsurface sediments, although volume and surface area was still similar in constructed wetlands. In these systems, mangrove trees will be present and their roots will be able to counteract the elevated oxygen consumption due to the high organic matter mineralization (Kristensen and Alongi, 2006).

In chapter 9, for the first time, the carbon mineralization measured as carbon dioxide emission and microbial pathways were identified and quantified in different vegetated and sewage conditions, as well as in the presence or absence of biogenic structures in mangrove constructed wetlands. Increased sewage contamination lead to higher carbon mineralization and methane emissions in the constructed mesocosms, as well as benthic primary production. Biogenic structures increased low tide carbon gas emissions at contaminated 30% and particularly in pristine conditions 60%. FeR was substituted by SRR when sewage was loaded into the cells under unvegetated and planted conditions, while the contribution of aerobic respiration to total metabolism remained above 50%. These results clearly show impacts of sewage on the partitioning of electron acceptors in mangrove sediment which may induce negative effects on sediment associated flora and fauna due to stimulated SR and toxic sulfide accumulation (Hargrave et al., 2008). Also, it confirmed the importance of biogenic structures in constructed wetlands by improving significantly the organic matter mineralization, as was found to occur in natural systems (Kristensen, 2000) or control laboratory experiments (Heilskov and Holmer, 2001; Heilskov et al., 2006; Valdemarsen et al., in press-a; b).

Final remarks

Considering the above discussion, one might consider that the present study clearly increased the number of ecological indicators and biomonitors of sewage pollution, as well as focussed on new ways of analysing the effect of sewage on mangrove associated fauna. Although easy and inexpensive methods, such as taxonomic minimalist, tried to be applied to East Africa infauna communities with little effect, identification should be taken to lower levels in order to provide a better understanding of community changes. As was already used in other ecosystems, and as an indication of several types of contaminants, for the first time in mangrove ecosystems population structure, parasite infection, RNA/DNA ratio and a set of reproductive parameters, comprehending maturation curves, fecundity, potential fertility and embryo quality assessed through fatty acid analysis, as well as nitrogen SI ratios, were tested as bioindicators or biomonitores for habitat quality, with most of these being recognized as reliable indicators of domestic sewage pollution. Nevertheless, these bioindicators tested in natural ecosystems should be validated in controlled conditions. Future research should focus on other key mangrove species, such as sesarmid crabs, as well as to better understand these bioindicators' responses to different types and levels of stressors in order to better identify and monitor impacts on ecosystems.

Most of the world natural estuarine and coastal ecosystems are becoming extensively degraded, mainly due to increasing anthropogenic pollution and habitat destruction. The information compiled in this study bioindicators and biomonitores can thus be useful for sustainable management programmes of mangrove ecosystems and associated resources, as well as to be applied to other valuable ecosystems or other types of forcing functions acting on these sensitive ecosystems, such as climate change, and consequent sea-level rise, global trends in human activities, including habitat destruction and overexploitation of resources.

Ecosystem engineers have recently been addressed as powerful agents to restore ecological systems when managed well. Both key macrofauna species studied managed to keep high survival rates in extreme anoxic and contaminated conditions, although growth rates and daily activities were significantly affected. Nevertheless, by limiting microalgal biomass directly by grazing or indirectly by subduction below the sediment surface, both key mancrofauna species suppressed the development of dense algal mats, and consequently avoided the development of near surface anoxic zones even at high sewage concentrations. Also, the continuous mixing of the top layer by epifauna enhanced carbon mineralization and nutrient cycling by improving sediment aeration and drainage, and by increasing surface areas for microbial activity. Consequently, faunal grazing and bioturbation, as well as biogenic functioning, increased sediment capacity to degrade organic matter. Biogenic structures and bioturbation activities change sediment physical, chemical and biological structure and dynamics, leading not only to an increase in benthic mineralization but also to change the dominating microbial mineralization pathways to more efficient ones sub-oxic or aerobic respiration. Our results confirmed the importance of fauna and flora biogenic structures on the functioning of mangrove forests sediments as well as their effective contribution to system mineralization and consequently filtration efficiency under organic-rich conditions.

Notwithstanding, further research is necessary to address the processes and microbial communities inhabiting mangrove fauna galleries, especially at high sewage loadings, in order to explain the higher mineralization rates found in these biogenic structures. Also, research should focus on greenhouse gas emissions from these wetlands, knowing that most anaerobic respiration pathways lead to the release of gases much more powerful than carbon dioxide and consequently their effect on global warming is much more effective.

Also, the advantage of longer immersion periods versus sustainable fauna communities should be further investigated over long periods. Such zooremediation approach should be widespread to other functional constructed wetlands in order to better understand the potential in its efficiency and associated ecosystem sustainability.

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