Aquatic Toxicology
January 2020, Volume 218, Pages 105358 (10p.)
https://doi.org/10.1016/j.aquatox.2019.105358
https://archimer.ifremer.fr/doc/00592/70449/

Wastewater bioremediation by mangrove ecosystems impacts crab ecophysiology: in-situ caging experiment

Theuerkauff Dimitri [1, 2, *], Rivera-Ingraham Georgina A. [1], Lambert Sophia [2], Mercky Yann [1, 2], Lejeune Mathilde [1, 2], Lignot Jehan-Hervé [1], Sucré Elliott [1, 2]

[1] UMR MARBEC (University of Montpellier, CNRS, IFREMER, IRD), Montpellier, France
[2] Centre Universitaire de Mayotte, Route Nationale 3, BP 53, 97660 Dembeni, Mayotte, France

* Corresponding author : Dimitri Theuerkauff, email address : dimitri.theuerkauff@univ-mayotte.fr

Abstract :

Mangroves are tidal wetlands that are often under strong anthropogenic pressures, despite the numerous ecosystem services they provide. Pollution from urban runoffs is one such threats, yet some mangroves are used as a bioremediation tool for wastewater (WW) treatment. This practice can impact mangrove crabs, which are key engineer species of the ecosystem. Using an experimental area with controlled WW releases, this study aimed to determine from an ecological and ecotoxicological perspective, the effects of WW on the red mangrove crab Neosarmatium africanum. Burrow density and salinity levels (used as a proxy of WW dispersion) were recorded, and a 3-week caging experiment was performed. Hemolymph osmolality, gill Na+/K+-ATPase (NKA) activity and gill redox balance were assessed in anterior and posterior gills of N. africanum. Burrow density decreased according to salinity decreases around the discharged area. Crabs from the impacted area had a lower osmoregulatory capacity despite gill NKA activity remaining undisturbed. The decrease of the superoxide dismutase activity indicates changes in redox metabolism. However, both catalase activity and oxidative damage remained unchanged in both areas but were higher in posterior gills. These results indicate that WW release may induce osmoregulatory and redox imbalances, potentially explaining the decrease in crab density. Based on these results we conclude that WW release should be carefully monitored as crabs are key players involved in the bioremediation process.

Highlights

► Mangroves are suggested as biofiltering systems of domestic effluent. ► Mangrove crabs are involved through their bioturbation activities. ► Wastewater release impacts crab burrow density. ► Mangrove crabs are physiologically impacted by wastewaters.

Keywords : Mangrove, Bioturbation, Wastewate, r Ecophysiology, Oxidative stress, Osmoregulation

1 Introduction

Mangroves are critically threatened by human activities worldwide (Duke et al., 2007; Polidoro et al., 2010) although their ecological and socioeconomic importance are now well documented (Lee et al., 2014). They cover 137,760 km^2 in 118 tropical and subtropical countries and territories (Giri et al., 2011) and are characterized by being a unique coastal forest between land and sea.

Among the different possible threats, mangroves receive nutrients and pollutants from urban runoffs (Fusi et al., 2016; Lesirma, 2016; MacDonnell et al., 2017). However, mangrove trees are capable of absorbing excess in nutrient load (Reef et al., 2010) and are thus used in many tropical countries as a bioremediation tool for wastewater treatment (Ouyang and Guo, 2018). Whether natural or constructed mangrove wetlands (mesocosm), they are used for treating WW from aquaculture, sewage or others sources (Leung et al., 2016; Ouyang and Guo, 2016). WW is often a complex mixture of many pollutants, the most notable of which are the excess of organic matter and nutrients (mainly ammonium and phosphorus) but may also contain pharmaceutical residues, pesticides, heavy metals, etc. Moreover, recent studies in China showed that natural mangroves are the optimal paradigm under three scenarios of municipal WW treatment (Ouyang and Guo, 2018). In addition to eliminating excess nutrient and organic load, mangrove plants may bioaccumulate metals (Analuddin et al., 2017), and even immobilize some wastewater-borne pollutants, such as polycyclic aromatic hydrocarbons and polybrominated diphenyl ethers in the shape of iron plaques formed on their roots (Pi et al., 2017). They can also even mitigate different antibiotics (Li et al., 2016a; Liu et al., 2016). While most of these studies focus on plant or microbial communities, previous studies looking at the impact of WW on mangrove macrofauna showed contradictory effects (Bartolini et al., 2009; Cannicci et al., 2009; Capdeville et al., 2018; Fusi et al., 2016; Penha-Lopes et al., 2009b; Yu

et al., 1997). Thus, further focusing on the specific physiological consequences for macrofauna is still needed.

Among the macrofauna, burrowing mangrove crabs such as *Neosarmatium africanum* (Sesarmidae, de Man, 1887), previously known as *Neosarmatium meinerti* in Mayotte (Theuerkauff et al., 2018a), are engineer species playing a key role in the mangrove ecosystem: their bioturbation and organic matter degradation activities are crucial in element transfer within the matter cycle and allow the maintenance of sediment biochemical heterogeneity (Emmerson and McGwynne, 1992; Kristensen, 2008; Lee, 1998). They are also involved in the bioremediation process since their burrows allow WW to enter the sediment and to be absorbed by mangrove trees. Crab burrows also increase the air-sediment layer up to 400% and thus, impact microbial and geochemical processes (Kristensen and Kostka, 2013).

In the context of mangrove use for biofiltering, it is therefore crucial, to determine if these crabs are impacted by WW discharge. If this is the case, it could induce in the long term profound and global modifications of the ecological functioning of the mangrove ecosystem. Regular wastewater discharges may decrease crab abundance as shown for juvenile blue crabs *Portunus pelagicus* along a pollution gradient from the outfall of a secondary treated effluent (Wear and Tanner, 2007). Moreover, previous studies showed that WW may also impact crab community structures (Capdeville et al., 2018) or even their bioturbation activities (Bartolini et al., 2011). Previous results obtained by our group under laboratory conditions showed that WW exposure induces a burst of crab oxygen consumption as well as osmotic and redox imbalances (Theuerkauff et al., 2018a). This study aims to compare the aforementioned laboratory results (in which crabs were directly exposed to domestic WW for 5h) with field experimentation using the same WW discharge. To do this, we here merge the use of ecological markers (e.g. crab burrow density) with physiological indicators of cell homeostasis (e.g. osmoregulation and

energy-redox parameters) carried out in encaged crabs during 3 weeks to adequately evaluate the efficiency and challenges of using mangroves as biofilters.

2 Materials and methods

2.1 Study area

To assess the impact of WW on mangrove crabs, two areas were selected in the Malamani mangrove (Boueni's bay, Mayotte island, 12°55′1″S, 45°9′23″E) located in the Comoros Archipelago (Fig. 1A, B, C). This is an experimental site which has been used since 2008 to evaluate the capacities of the mangrove ecosystem for natural WW treatment (Herteman et al., 2011). Briefly, the WW produced by the nearby village of Malamani (400 population equivalent), is collected, stored and decanted in a pre-treatment (Imhoff) tank. About 10 m^3 of this pretreated WW is discharged daily 1h before low tide approximately every 24h (i.e. a low tide on two) through pierced pipes (Fig. 1E) on three 45m x 15m plots. In this study, only the plot dominated by *Ceriops tagal* and oriented perpendicular to the main coastline was considered (Fig. 1B,C). This area was designated as the "impacted area". A second area that was not subjected to WW discharge was used as a control site (Fig. 1 C). This area is located in the same mangrove belt and is also dominated by *Ceriops tagal* and was chosen as it did not differ from the impacted area before the start of WW discharge (Herteman, 2010). The chemical composition of the pretreated WW, surface water and porewater from impacted and control areas are reported in Table 1. More *in-situ* physicochemical parameters are reported in Capdeville (2018).

2.2 Salinity and crab burrow mapping

A field survey was conducted in March 2015 to collect data on WW dispersion and crab burrow density in the Malamani mangrove. Four 80 m-long transects covering the impacted area as well as the control zone were established parallel to the shore. For each transect, 8 plots (1 m^2, located approximately 10 m from each other) were monitored after one day without rain. Thirty two plots were divided in 3 areas: one subjected to WW release and two control areas (one northern and one southern area, located respectively on each side of the impacted area). These plots were in all cases established on flat bottoms only, since it is where WW flows and infiltrates the sediment through the crab burrows. The geographical coordinates of these plots were recorded using a GPS system (Garmin GPSMAR®78S). Osmolality variation (used as a measure of salinity) around the WW discharge was used as a proxy of WW dispersion (as WW salinity is close to freshwater), ensuring that control areas were not impacted by wastewater release. Salinity was measured from 1.5 ml samples collected with a 3 ml plastic pipette from the interstitial water in burrows. The osmotic pressure (OP) of these samples was measured in duplicate by freezing point depression osmometry using an Advanced ™ Micro-Osmometer, model 3300 (Advanced Instruments, Inc.) using 20 µl per sample. The number of crab burrows in a 1 m^2 quadrat was also counted for each plot and used as a an estimation of the crab density (Mouton and Felder, 1996).

2.3 Animal collection, caging and sampling

Twenty adult *N. africanum* (Fig. 2) in intermolt stage C3 (as confirmed through epipodite examination) were collected in September 2015 from an undisturbed area of the Malamani mangrove at low tide. Specimens identified before as *N. meinerti* in Mayotte belong in fact to *N. africanum* species (Ragionieri et al., 2012), as recently confirmed by DNA sequencing (Ragionieri, pers. com.). Immediately after collection, ten crabs were individually introduced in artificial burrows (Fig. 1D) in the impacted and control area (Fig. 1C). We chose this caging

approach due to the fact that long-term tagging of individuals is not possible in crustaceans due to molting. Each artificial burrow consisted of a closed grid cylinder of 6 cm wide and 50 cm long inserted into a perforated PVC pipe ensuring adequate water flow. These artificial burrows were partially buried in the sediment with an angle of 45° (Fig. 1D). After 3 weeks, in October 2015, the crab and some water from each burrow were collected during low tide outside of the WW discharge period and was individually taken to the field laboratory located about 350 m (4-5 minutes walk) from the experimental site. For each crab, a hemolymph sample (without any anticoagulant) was obtained using a 0.5-ml syringe by inserting the needle between the cephalothorax and the first pereiopod. This sample was directly used for OP determination using 20 µl per sample in duplicate as described above. Crabs were then weighed and euthanized on ice for gill sampling. Gill pairs were divided in 2 groups according to their function (Neufeld et al., 1980; Theuerkauff et al., 2018b): anterior pairs (1 to 4) being respiratory gills, and posterior pairs (5 to 8) with both a respiratory and osmoregulatory function. Anterior and posterior gills were flash frozen in liquid nitrogen, stored separately into a container filled with liquid nitrogen (Voyageur 5, Air liquide). After sampling completion, frozen samples were transported to the University Center of Mayotte and then preserved at -80 ^{o}C until further analyses (see details in sections 2.4 and 2.5). Crab molting stages were re-checked *post-mortem* to avoid handling-associated stress and only animals in intermolt stage (C3) were considered.

2.4 Assessments of antioxidant defenses and oxidative damage in gills

For each crab, antioxidant defenses were quantified as the activities of superoxide dismutase (SOD) and catalase (CAT) and measured spectrophotometrically in anterior and posterior gills (right-sided gills 3 and 5). All frozen samples were diluted in a 50 mmol l^{-1} KPi buffer with 120 mmol l^{-1} KCl (pH=7.0) to 1:40 and 1:10 (w:v) for SOD and CAT measurements, respectively.

Tissue homogenization was achieved using 3 steel balls (Retsch, n°. FR0120) in a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany; 30s at 30 beats s^{-1}). SOD activity was determined in supernatants after centrifugation (3min, 13000 rpm, 4°C) using the protocol of McCord and Fridovich (1969) and modified by Livingstone et al. (1992). CAT activity was measured in supernatants following the decomposition of H_2O_2 in a 50 mmol l^{-1} KPi buffer according to the assay developed by Aebi (1984). All measurements were carried out at least in triplicate using a microplate reader (Tecan Infinite M200, TECAN, Männendorf, Switzerland). Samples were preserved on ice at all times and results were expressed per mg of proteins and body weight. Protein were quantified in triplicates according to Bradford (1976) with BSA (Sigma-Aldrich, St Louis, MO, USA) as standard. Oxidative damage was estimated as the concentration of malondialdehyde (MDA), a product resulting from lipid peroxidation, in right-sided anterior and posterior gills (gills 4 and 6, respectively). MDA was quantified through measurements of thiobarbituric acid reactive substances (TBARS) using the protocol originally described by Uchiyama and Mihara (1978) and further modified by Abele et al. (2002).

2.5 Assessment of Na^+/K^+-ATPase (NKA) activity

NKA activity was determined on right-sided anterior and posterior gills (2 and 7, respectively) by homogenizing tissues separately as described above in a buffer composed of 250 mM sucrose, 5 mM $MgCl_2$ (pH= 7.4) in a 1:6 (w:v) ratio. After centrifugation (3min, 13000 rpm, 4°C), protein concentration in supernatants was determined as described above, and all samples were diluted to achieve equal protein concentrations. The specific NKA ouabain-sensitive activity in these supernatants, was measured using a protocol originally by Flik et al. (1983) and adapted from Lorin-Nebel et al. (2013) and Tsai and Lin (2007). Briefly, specific NKA activity was measured in triplicate as the difference of ATP hydrolysis in two different media: both were composed of 100 mM NaCl, 30 mM imidazole, 0,1 mM Na_2EDTA and 5 mM $MgCl_2$,

3 mM Na-ATP and pH 7.4 but while one contained 14 mM KCl, the second contained 2.8 mM ouabain. After a 30 min incubation period at 37°C, the reaction was stopped by adding an ice-cold solution composed of TCA 5%, 0.33 mM $FeSO_4$, 0.66 M H_2SO_4 and 9.2 mM ammonium molybdate. The amount of inorganic phosphates released in presence or absence of oubain was colorimetrically measured after 10 min and using Na_2HPO_4 as standard (Sigma, France). The enzyme specific activity was expressed in µmol Pi mg protein^{-1} h^{-1}.

2.6 Statistics

All statistical analyses were performed in R version 3.3.2 (R Core Team, 2015) with RStudio Version 0.99.891 (RStudio, Inc). The Shapiro-Wilk test was used to test normality and the Bartlett test was used to test the homogeneity of variances. If data did not meet the assumptions for parametric statistical techniques, they were log transformed. A one-way ANOVA was performed on crab density and a Kruskal-Wallis test followed by a Nemenyi *post hoc* comparison test on surface water salinity. The relationship between crab burrow salinity and crab burrow density was determined using a linear regression. A two-way ANOVA was performed on crab body weight (sex and treatment), oxidative damage, antioxidant defenses and NKA activities according to gill position and treatment. All factors were considered as orthogonal and fixed. All ANOVA tests were followed by a Tukey's HSD *post hoc* comparison test and significance level was $p < 0.05$. Hemolymph osmotic pressures are reported with the osmotic curve previously published for *N. africanum* (Theuerkauff et al., 2018b). The residuals between hemolymph osmotic pressures and those given by the osmotic curve are compared by a Student t-test.

3 Results

3.1 Surface water salinity and crab burrow density

Salinity values of the crab burrow residual water ranged from 64 mOsm·kg^{-1} to 1360 mOsm·kg^{-1}. Due to the effect of the WW effluent, salinity values significantly decreased in the impacted area (Kruskal-Wallis test, $p < 0.001$) (Fig. 3A). The crab burrow density demonstrates a similar pattern since lower density was found in the impacted area (Fig. 3B; one-way ANOVA; df error = 29; F = 45.5; $p < 0.001$) and the variations in crab density are correlated (Fig. 3C) with the variations in salinity ($r^2 = 0.58$, $p < 0.001$).

3.2 Crab size

Crab body weight was significantly different between males and females (47.4g ± 8.9g and 31.1g ± 2.6g, respectively) but not between treatments (two-way ANOVA; df error = 16; sex F = 34.2; df = 1; $p < 0.001$; treatment F = 1.3; df = 1; interaction: F = 2.9; df = 1). Except for weight, no sex-related differences were recorded and thus, only pooled results are shown. No mortality was recorded throughout the experiment.

3.3 Osmotic pressure and NKA activity

Hemolymph OP of crabs collected from the impacted area was significantly lower compared to the OP collected from crabs of the control area (Fig. 4A, Student t-test, $p < 0.01$). OP of the water collected from the artificial burrows was also lower in the impacted area (Student t-test, $p < 0.01$). However, when considering the residuals (Fig.3 B) between hemolymph OP observed on the field and hemolymph OP (predicted at the same salinity using the osmotic curve published by Theuerkauff et al., 2018), a significant decrease is observed for crabs collected from the impacted area (one-sided Student t-test, $p < 0.01$). However, differences were not significant for those crabs caged in the control area (2-sided Student t-test, $p = 0.94$) showing that there is not only an effect of decreased salinity but also of WW exposure. Gill NKA activity

(Fig. 4C) showed no significant difference between crabs of the two areas but NKA activity was 2.8 higher in posterior gills compared to anterior gills (two-way ANOVA, table 2A).

3.4 Oxidative stress

SOD activity (Fig. 5A) did not differ between anterior and posterior gills (two-way ANOVA, table 2B) unlike CAT activity (Fig. 5B, two-way ANOVA, table 2C). However, for both gill types, SOD activity was significantly lower in crabs collected from the impacted area compared to crabs from the control area. Branchial MDA concentration (Fig. 5C) was the same for impacted and control crabs but differed according to gill type (two-way ANOVA, table 2D). For both impacted and control animals, the MDA content of posterior gills was 1.9 times higher compared to the anterior gills (two -way ANOVA, table 2D).

4 Discussion

To our knowledge, this study corresponds to the first *in-situ* ecotoxicological assessment of the effects of regular and controlled urban WW discharges on crab physiology in a natural mangrove. This new approach using a caging experimental protocol in a natural mangrove forest is complementary to previous studies that focused on artificial mesocosms (Bartolini et al., 2009; Penha-Lopes et al., 2012, 2009a) or to *in-situ* studies using sampled crabs in polluted mangroves (Amaral et al., 2009; Penha-Lopes et al., 2009b). As already discussed in Capdeville (2018), the response of the crab community and the individual physiological response may be linked to WW discharge but also to the induced environmental modifications. Indeed, different feedback effects are possible since WW discharges induce major changes in mangrove plants (Herteman et al., 2011) and microbial communities (Bouchez et al., 2013), which are the food source for most of the mangrove crab species.

4.1 Decrease of crab burrow density as a consequence of WW release

As already demonstrated, eutrophication occurs in the impacted area (Bouchez et al., 2013; Capdeville et al., 2018; Herteman et al., 2011). However, this increase in nutrient concentration is highly variable spatially and is mainly due to the unequal dispersion of the WW in the impacted area (different microrelief conditions). WW flows on the soil according to surface roughness and infiltrates the sediment through the crab burrows. Therefore, flat bottoms are highly exposed to WW runoffs, while mounds surrounding mangrove trees are likely to be preserved from direct exposure to the effluent water. Consequently, in this study, visual countings only considered the flat bottom areas. This methodological choice may explain why a clear decrease in crab density was observed in the impacted area, differing from previous results provided by Capdeville (2018). In their study, they did not record differences in crab abundance between impacted and control areas in 2012 and 2014, but crab burrows were counted from 1 m^2 areas containing 50% of flat bottoms and 50% tree mounds (Capdeville, pers. com.). Even if crabs could take refuge on the tree mounds, the decrease in crab burrow density reported for flat bottoms should limit WW infiltration and alter the bioremediation processes as well as their engineering role. This decrease in burrow density strongly correlates with the salinity decrease and is consistent with previous observations (Herteman, 2010) showing that WW dispersion induces a decrease in salinity around the impacted area. Our results contrast with those obtained in other similar mangrove forests in Kenya and Mozambique by Cannicci et al. (2009) and Bartolini et al. (2011). In these studies, an increase in sesarmid and fiddler crab biomass was observed in a peri-urban mangrove receiving WW. However, these authors also found a negative effect of the effluent on ecosystem functionality marked by a decrease in crab bioturbation activity, which is a cryptic ecological degradation according to Dahdouh-Guebas et al. (2005). Nevertheless, it is difficult to compare these

different studies since WW composition is highly variable both in time and space (local dispersion and WW origin).

4.2 Osmoregulatory disruption

In natural conditions, *N. africanum* is a hypo-osmoregulator in SW but the osmotic pressure of the hemolymph slightly decreases with decreased salinity (Theuerkauff et al., 2018b; this work). These values were significantly lower in caged crabs in the impacted area, thus experiencing a decreased osmoregulatory capacity even if gill NKA activities remained unmodified. This has already been observed to occur in other mangrove crab species maintained in laboratory conditions and exposed for 5h to the same WW effluent (Theuerkauff et al., 2018a). This decrease in osmoregulatory capacity is representative of the physiological condition of the animal (Lignot et al., 2000) and could be due to histological damage in the gill epithelia (Theuerkauff et al., 2018a). Such morphological damage has already been described when considering the toxic effect of ammonia (Leone et al., 2017), which can reach up to 3800 µM in the WW discharged in our study area (Capdeville, 2018). Given that NKA pumps are also involved in the excretion of ammonia-N, as already discussed in Theuerkauff et al. (2018a), this pattern may also reveal a trade-off between osmoregulation and ammonia excretion since NH_4^+ can substitute and compete with K^+ in NKA and other K^+ channels (Weihrauch and Donnell, 2017). Moreover, other pollutants present in the WW with a potential additive or synergic effect (e.g. with salinity variation) may have also contributed to this osmoregulatory disturbance. This could be the case of high nitrite or nitrate concentrations (Romano and Zeng, 2013), which can reach up to 3.7 and 22 µM in the wastewater from Malamani (Herteman, 2010). Since WW composition is highly variable, it is important to note that these potential additive, synergic or antagonist responses to stressors may also vary. Therefore, WW bioremediation in natural mangroves may require specific field impact studies and case-by-case

follow-ups by the competent authorities. It would also be very interesting to study the resilience of the ecosystem and especially if crabs may recover physiological health when exposure stops.

4.3 Oxidative stress modulation

As previously observed in laboratory conditions, posterior gills maintain higher CAT activities and MDA levels compared to anterior gills (Theuerkauff et al., 2018a). This pattern also occurs in *Carcinus aestuarii* (Rivera-Ingraham et al., 2016) and is probably linked to the osmoregulatory function of these gills (Rivera-Ingraham and Lignot, 2017). Osmoregulation is an energy-demanding process as ATPase pumps are key players for active ion transport. Therefore, in decapod crabs, posterior gills exhibit numerous mitochondria and a high abundance and activity of NKA (Copeland and Fitzjarrell, 1968; Lignot and Charmantier, 2015; Pequeux, 1995). This is also the case for *N. africanum* (Theuerkauff et al., 2018b; this work). These pumps are located in specialized ionocites (aka mitochondria-rich cells), and mitochondria are considered as the major ROS producers in aquatic animals (Abele et al., 2007). This may explain the higher MDA levels recorded in the posterior gills. Also, compared to anterior gills, their high CAT activities may be an adaptation to maintain redox homeostasis in these gills (Rivera-Ingraham et al., 2016). Long-term exposure under field conditions did not induce oxidative damage in either anterior or posterior gills but a marked decrease in SOD activity is observed in both tissues. This redox imbalance may be explained by a wide variety of different stressor and/or molecules contained in WW (see reviews by Abele et al., 2012; Lushchak, 2011). For example, such decrease has already been reported after exposure to heavy metals (Jasinska et al., 2015) or even infections (Neves et al., 2000; Rameshthangam and Ramasamy, 2006). Moreover, such changes may work in synergy with environmental factors, such as temperature, as observed for the Cape River crab after exposure to silver nanoparticles AgNP (Walters et al., 2016) or low levels of oxygen (Li et al., 2016b).

4.4 Oxidative stress and gender

Gender-related variability in the oxidative stress response has already been reported in the shore crab *Carcinus maenas* (Pereira et al., 2009) and other invertebrates (Correia et al., 2003; Espinosa and Rivera-Ingraham, 2016; Radhika et al., 1998), even if most papers on aquatic organisms lack information about gender (Abele et al., 2012). Previous laboratory conditions reported a gender difference in response to WW exposure (Theuerkauff et al., 2018a), which could potentially lead to sex-dependent tolerance to a pathological environment (Fanjul-Moles and Gonsebatt, 2012). However, these differences were not reported in this field study probably because waters in the field are temporally and spatially diluted. Nevertheless, these gender-related differences should be considered as an important factor when studying other species or stress intensities.

5 Conclusion

This study demonstrates that mangrove crab abundance is significantly decreased in WW-impacted areas, and encaged crabs forced to remain in such areas show both osmoregulatory and redox disturbances. Many different compounds present in the WW, such as ammonium or nitrite, could be responsible for the aforementioned results, and these compounds may furthermore have potential additive, synergic or antagonist effects. Therefore, WW bioremediation by natural mangroves should require specific field impact studies and case-by-case follow-ups by competent authorities. This work emphasizes the need for a proper WW management based on results from both laboratory and field analyses. Moreover, if the observed effects on osmoregulation and oxidative stress could explain the decrease in crab burrow density, crabs may also escape and/or avoid the discharged area. More behavioral studies on crabs under WW discharge are so needed. Further studies should also look at the

digestive tract and especially the hepatopancreas which is involved in absorption and nutrient storage, enzyme synthesis, lipid and carbohydrate metabolism, detoxification and absorption processes (Wang et al., 2014). Finally, this study clearly indicates that mangrove crabs are affected by WW exposure and are most likely impacted by uncontrolled WW discharges that occur in mangrove systems in tropical countries across the world.

Acknowledgements

The authors are thankful to the *Syndicat Intercommunal d'Eau et d'Assainissement de Mayotte* (SIEAM) and especially Kissimati Abdallah for her help on the field. This study was partly funded through the Marie Curie Actions EU grant FP7-PEOPLE-2013-IEF (grant number 622087-"IAS-Life") awarded to GRI and by the French National Agency for Water (ONEMA), which is now part of the French Agency for Biodiversity (AFB).

References

Abele, D., Heise, K., Pörtner, H.O., Puntarulo, S., 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J. Exp. Biol. 205, 1831–1841. https://doi.org/10.1016/j.ecolind.2011.04.007

Abele, D., Philipp, E., Gonzalez, P.M., Puntarulo, S., 2007. Marine invertebrate mitochondria and oxidative stress. Front. Biosci. 12, 933–946. https://doi.org/10.2741/2115

Abele, D., Vázquez-Medina, J.P., Zenteno-Savín, T., 2012. Oxidative Stress in Aquatic Ecosystems, Oxidative Stress in Aquatic Ecosystems. Wiley-Blackwell. https://doi.org/10.1002/9781444345988

Aebi, H., 1984. Catalase in Vitro. Methods Enzymol. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Amaral, V., Penha-Lopes, G., Paula, J., 2009. RNA/DNA ratio of crabs as an indicator of mangrove habitat quality. Aquat. Conserv. Mar. Freshw. Ecosyst. 19, S56–S62. https://doi.org/10.1002/aqc

Analuddin, K., Sharma, S., Jamili, X., Septiana, A., Sahidin, I., Rianse, U., Nadaoka, K., 2017. Heavy metal bioaccumulation in mangrove ecosystem at the coral triangle ecoregion, Southeast Sulawesi, Indonesia. Mar. Pollut. Bull. 0–1.

https://doi.org/10.1016/j.marpolbul.2017.07.065

Bartolini, F., Cimò, F., Fusi, M., Dahdouh-Guebas, F., Lopes, G.P., Cannicci, S., 2011. The effect of sewage discharge on the ecosystem engineering activities of two East African fiddler crab species: Consequences for mangrove ecosystem functioning. Mar. Environ. Res. 71, 53–61. https://doi.org/10.1016/j.marenvres.2010.10.002

Bartolini, F., Penha-Lopes, G., Limbu, S., Paula, J., Cannicci, S., 2009. Behavioural responses of the mangrove fiddler crabs (*Uca annulipes* and *U . inversa*) to urban sewage loadings : Results of a mesocosm approach. Mar. Pollut. Bull. 58, 1860–1867. https://doi.org/10.1016/j.marpolbul.2009.07.019

Bouchez, A., Pascault, N., Chardon, C., Bouvy, M., Cecchi, P., Lambs, L., Herteman, M., Fromard, F., Got, P., Leboulanger, C., 2013. Mangrove microbial diversity and the impact of trophic contamination. Mar. Pollut. Bull. 66, 39–46. https://doi.org/10.1016/j.marpolbul.2012.11.015

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cannicci, S., Bartolini, F., Dahdouh-Guebas, F., Fratini, S., Litulo, C., Macia, A., Mrabu, E.J., Penha-Lopes, G., Paula, J., 2009. Effects of urban wastewater on crab and mollusc assemblages in equatorial and subtropical mangroves of East Africa. Estuar. Coast. Shelf Sci. 84, 305–317. https://doi.org/10.1016/j.ecss.2009.04.021

Capdeville, C., 2018. Évaluation des capacités de résistance et de résilience de l'écosystème mangrove en réponse à des apports d ' eaux usées domestiques prétraitées. Université de Toulouse.

Capdeville, C., Abdallah, K., Buffan-Dubau, E., Lin, C., Azemar, F., Lambs, L., Fromard, F., Rols, J.-L., Leflaive, J., 2018. Limited impact of several years of pretreated wastewater discharge on fauna and vegetation in a mangrove ecosystem. Mar. Pollut. Bull. 129, 379–391. https://doi.org/10.1016/j.marpolbul.2018.02.035

Copeland, D.E., Fitzjarrell, A.T., 1968. The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus* rathbun) with notes on modified mitochondria. Zeitschrift für Zellforsch. 92, 1–22. https://doi.org/10.1007/BF00339398

Correia, A.D., Costa, M.H., Luis, O.J., Livingstone, D.R., 2003. Age-related changes in antioxidant enzyme activities, fatty acid composition and lipid peroxidation in whole body *Gammarus locusta* (Crustacea: Amphipoda). J. Exp. Mar. Bio. Ecol. 289, 83–101. https://doi.org/10.1016/S0022-0981(03)00040-6

Dahdouh-Guebas, F., Hettiarachchi, S., Lo Seen, D., Batelaan, O., Sooriyarachchi, S., Jayatissa, L.P., Koedam, N., 2005. Transitions in ancient inland freshwater resource management in Sri Lanka affect biota and human populations in and around coastal lagoons. Curr. Biol. 15, 579–586. https://doi.org/10.1016/j.cub.2005.01.053

Duke, N.C., Meynecke, J.-O., Dittmann, S., Ellison, A.M., Anger, K., Berger, U., Cannicci, S., Diele, K., Ewel, K.C., Field, C.D., Koedam, N., Lee, S.Y., Marchand, C., Nordhaus, I., Dahdouh-Guebas, F., 2007. A World Without Mangroves? Science (80-.). 317, 41–42. https://doi.org/10.1126/science.317.5834.41b

Emmerson, W.D., McGwynne, L.E., 1992. Feeding and assimilation of mangrove leaves by

the crab *Sesarma meinerti* de Man in relation to leaf-litter production in Mgazana, a warm-temperate southern African mangrove swamp. J. Exp. Mar. Bio. Ecol. 157, 41–53. https://doi.org/10.1016/0022-0981(92)90073-J

Espinosa, F., Rivera-Ingraham, G.A., 2016. Subcellular evidences of redox imbalance in well-established populations of an endangered limpet. Reasons for alarm? Mar. Pollut. Bull. 109, 72–80. https://doi.org/10.1016/j.marpolbul.2016.06.019

Fanjul-Moles, M.L., Gonsebatt, M.E., 2012. Oxidative Stress and Antioxidant Systems In Crustacean Life Cycles, in: Abele, D., Vazquez-Medina, J.P., Zenteno-Savin, T. (Eds.), Oxidative Stress in Aquatic Ecosytems. Abele, Doris, Oxford, pp. 208–223.

Flik, G., Wendelaar Bonga, S.E., Fenwick, J.C., 1983. Ca^{2+}-dependent phosphatase and ATPase activities in eel gill plasma membranes-I. Identification of Ca^{2+}-activated ATPase activities with non-specific phosphatase activities. Comp. Biochem. Physiol. B 76, 745–754. https://doi.org/10.1016/0305-0491(83)90388-7

Fusi, M., Beone, G.M., Suciu, N.A., Sacchi, A., Trevisan, M., Capri, E., Daffonchio, D., Din, N., Dahdouh-Guebas, F., Cannicci, S., 2016. Ecological status and sources of anthropogenic contaminants in mangroves of the Wouri River Estuary (Cameroon). Mar. Pollut. Bull. 109, 723–733. https://doi.org/10.1016/j.marpolbul.2016.06.104

Giri, C., Ochieng, E., Tieszen, L.L., Zhu, Z., Singh, A., Loveland, T., Masek, J., Duke, N., 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Glob. Ecol. Biogeogr. 20, 154–159. https://doi.org/10.1111/j.1466-8238.2010.00584.x

Herteman, M., 2010. Evaluation des capacités bioremédiatrices d'une mangrove impactée par des eaux usées domestiques. Application au site pilote de Malamani, Mayotte. Université Paul Sabatier - Toulouse III.

Herteman, M., Fromard, F., Lambs, L., 2011. Effects of pretreated domestic wastewater supplies on leaf pigment content, photosynthesis rate and growth of mangrove trees: A field study from Mayotte Island, SW Indian Ocean. Ecol. Eng. 37, 1283–1291. https://doi.org/10.1016/j.ecoleng.2011.03.027

Jasinska, E.J., Goss, G.G., Gillis, P.L., Kraak, G.J. Van Der, Matsumoto, J., Souza, A.A. De, Giacomin, M., Moon, T.W., Massarsky, A., Gagné, F., Servos, M.R., Wilson, J., Sultana, T., Metcalfe, C.D., 2015. Assessment of biomarkers for contaminants of emerging concern on aquatic organisms downstream of a municipal wastewater discharge. Sci. Total Environ. 530–531, 140–153. https://doi.org/10.1016/j.scitotenv.2015.05.080

Kristensen, E., 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. J. Sea Res. 59, 30–43. https://doi.org/10.1016/j.seares.2007.05.004

Kristensen, E., Kostka, J.E., 2013. Macrofaunal Burrows and Irrigation in Marine Sediment: Microbiological and Biogeochemical Interactions, in: Interactions Between Macro- and Microorganisms in Marine Sediments. American Geophysical Union, pp. 125–157. https://doi.org/10.1029/CE060p0125

Lee, S.Y., 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. Mar. Freshw. Res. 49, 335–343. https://doi.org/10.1071/MF99078

Lee, S.Y., Primavera, J.H., Dahdouh-Guebas, F., Mckee, K., Bosire, J.O., Cannicci, S., Diele, K., Fromard, F., Koedam, N., Marchand, C., Mendelssohn, I., Mukherjee, N., Record, S.,

2014. Ecological role and services of tropical mangrove ecosystems: A reassessment. Glob. Ecol. Biogeogr. 23, 726–743. https://doi.org/10.1111/geb.12155

Leone, F.A., Lucena, M.N., Garçon, D.P., Pinto, M.R., Mcnamara, J.C., 2017. Gill Ion Transport ATPases and Ammonia Excretion in Aquatic Crustaceans, in: Weihrauch, D., Donnell, M.O. (Eds.), Acid-Base Balance and Nitrogen Excretion in Invertebrates. Springer, pp. 61–107. https://doi.org/10.1007/978-3-319-39617-0

Lesirma, S., 2016. Environment Impact Assessments in developing economies. Int. J. Environ. Sci. 1, 41–53.

Leung, J.Y.S., Cai, Q., Tam, N.F.Y., 2016. Comparing subsurface flow constructed wetlands with mangrove plants and freshwater wetland plants for removing nutrients and toxic pollutants. Ecol. Eng. 95, 129–137. https://doi.org/10.1016/j.ecoleng.2016.06.016

Li, Y., Li, Q., Zhou, K., Sun, X.L., Zhao, L.R., Zhang, Y. Bin, 2016a. Occurrence and distribution of the environmental pollutant antibiotics in Gaoqiao mangrove area, China. Chemosphere 147, 25–35. https://doi.org/10.1016/j.chemosphere.2015.12.107

Li, Y., Wei, L., Cao, J., Qiu, L., Jiang, X., Li, P., Song, Q., Zhou, H., Han, Q., Diao, X., 2016b. Oxidative stress, DNA damage and antioxidant enzyme activities in the pacific white shrimp (*Litopenaeus vannamei*) when exposed to hypoxia and reoxygenation. Chemosphere 144, 234–240. https://doi.org/10.1016/j.chemosphere.2015.08.051

Lignot, J.-H., Charmantier, G., 2015. Osmoregulation and excretion, in: Chang, E.S., Thiel, M. (Eds.), The Natural History of the Crustacea. Vol 4: Physiology. Oxford University Press, New York, pp. 249–283.

Lignot, J.-H., Spanings-Pierrot, C., Charmantier, G., 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture 191, 209–245. https://doi.org/10.1016/S0044-8486(00)00429-4

Liu, X., Liu, Y., Xu, J.R., Ren, K.J., Meng, X.Z., 2016. Tracking aquaculture-derived fluoroquinolones in a mangrove wetland, South China. Environ. Pollut. 219, 916–923. https://doi.org/10.1016/j.envpol.2016.09.011

Livingstone, D.R., Lips, F., Garcia Martinez, P., Pipe, R.K., 1992. Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis* . Mar. Biol. 112, 265–276.

Lorin-Nebel, C., Felten, V., Blondeau-Bidet, E., Grousset, E., Amilhat, E., Simon, G., Biagianti, S., Charmantier, G., 2013. Individual and combined effects of copper and parasitism on osmoregulation in the European eel *Anguilla anguilla*. Aquat. Toxicol. 130–131, 41–50. https://doi.org/10.1016/j.aquatox.2012.11.018

Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006

MacDonnell, C.P., Zhang, L., Griffiths, L., Mitsch, W.J., 2017. Nutrient concentrations in tidal creeks as indicators of the water quality role of mangrove wetlands in Southwest Florida. Ecol. Indic. 80, 316–326. https://doi.org/10.1016/j.ecolind.2017.05.043

McCord, J.M., Fridovich, I., 1969. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.

Mouton, E.C., Felder, D.L., 1996. Burrow distributions and population estimates for the fiddler crabs *Uca spinicarpa* and *Uca longisignalis* in a Gulf of Mexico salt marsh.

Estuaries 19, 51–61. https://doi.org/10.1007/BF02690100

Neufeld, G.J., Holliday, C.W., Pritchard, J.B., 1980. Salinity adaptation of gill Na, K-ATPase in the blue crab *Callinectes sapidus* . J. Exp. Zool. 211, 215–224. https://doi.org/10.1002/jez.1402110210

Neves, C.A., Santos, E.A., Bainy, A.C.D., 2000. Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Palemonidae) infected by Probopyrus ringueleti (Isopoda, Bopyridae). Dis. Aquat. Organ. 39, 155–158. https://doi.org/10.3354/dao039155

Ouyang, X., Guo, F., 2018. Optimal selection of mangrove paradigms for municipal wastewater treatment : an intuitionistic fuzzy analytical hierarchical processes. Chemosphere 197, 634–642. https://doi.org/10.1016/j.chemosphere.2017.12.102

Ouyang, X., Guo, F., 2016. Paradigms of mangroves in treatment of anthropogenic wastewater pollution. Sci. Total Environ. 544, 971–979. https://doi.org/10.1016/j.scitotenv.2015.12.013

Penha-Lopes, G., Bartolini, F., Limbu, S., Cannicci, S., Kristensen, E., Paula, J.J., 2009a. Are fiddler crabs potentially useful ecosystem engineers in mangrove wastewater wetlands? Mar. Pollut. Bull. 58, 1694–1703. https://doi.org/10.1016/j.marpolbul.2009.06.015

Penha-Lopes, G., Flindt, M.R., Ommen, B., Kristensen, E., Garret, P., Paula, J., 2012. Organic carbon dynamics in a constructed mangrove wastewater wetland populated with benthic fauna: A modelling approach. Ecol. Modell. 232, 97–108. https://doi.org/10.1016/j.ecolmodel.2012.02.005

Penha-Lopes, G., Torres, P., Narciso, L., Cannicci, S., Paula, J., 2009b. 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. Bio. Ecol. 381, 25–32. https://doi.org/10.1016/j.jembe.2009.08.009

Pequeux, 1995. Osmotic Regulation in Crustaceans. J. Crustac. Biol. 15, 1–60. https://doi.org/10.1163/193724095X00578

Pereira, P., de Pablo, H., Dulce Subida, M., Vale, C., Pacheco, M., 2009. Biochemical responses of the shore crab (*Carcinus maenas*) in a eutrophic and metal-contaminated coastal system (Óbidos lagoon, Portugal). Ecotoxicol. Environ. Saf. 72, 1471–1480. https://doi.org/10.1016/j.ecoenv.2008.12.012

Pi, N., Wu, Y., Zhu, H.W., Wong, Y.S., Tam, N.F.Y., 2017. The uptake of mixed PAHs and PBDEs in wastewater by mangrove plants under different tidal flushing regimes. Environ. Pollut. 231, 104–114. https://doi.org/10.1016/j.envpol.2017.07.085

Polidoro, B.A., Carpenter, K.E., Collins, L., Duke, N.C., Ellison, A.M., Joanna, C., Farnsworth, E.J., Fernando, E.S., Kathiresan, K., Koedam, N.E., Livingstone, S.R., Miyagi, T., Moore, G.E., Nam, V.N., Ong, J.E., Ellison, J.C., Farnsworth, E.J., Fernando, E.S., Kathiresan, K., Koedam, N.E., Livingstone, S.R., Miyagi, T., Moore, G.E., Nam, V.N., Ong, J.E., Primavera, J.H., Salmo, S.G., Sanciangco, J.C., Sukardjo, S., Wang, Y., Yong, J.W.H., 2010. The loss of species: Mangrove extinction risk and geographic areas of global concern. PLoS One 5. https://doi.org/10.1371/journal.pone.0010095

Radhika, M., Abdul Nazar, A.K., Munuswamy, N., Nellaiappan, K., 1998. Sex-linked

differences in phenol oxidase in the fairy shrimp *Streptocephalus dichotomus* Baird and their possible role (Crustacea: Anostraca). Hydrobiologia 377, 161–164. https://doi.org/10.1023/A:1003244318887

Ragionieri, L., Fratini, S., Schubart, C.D., 2012. Revision of the *Neosarmatium meinerti* species complex (Decapoda: Brachyura: Sesarmidae), with descriptions of three pseudocryptic Indo–West Pacific species. Raffles Bull. Zool. 60, 71–87.

Rameshthangam, P., Ramasamy, P., 2006. Antioxidant and membrane bound enzymes activity in WSSV-infected *Penaeus monodon* Fabricius. Aquaculture 254, 32–39. https://doi.org/10.1016/j.aquaculture.2005.10.011

Reef, R., Feller, I.C., Lovelock, C.E., 2010. Nutrition of mangroves. Tree Physiol. 30, 1148–1160. https://doi.org/10.1093/treephys/tpq048

Rivera-Ingraham, G.A., Barri, K., Boe l, M., Farcy, E., Charles, A.-L., Geny, B., Lignot, J.-H., 2016. Osmoregulation and salinity-induced oxidative stress: is oxidative adaptation determined by gill function? J. Exp. Biol. 219, 80–89. https://doi.org/10.1242/jeb.128595

Rivera-Ingraham, G.A., Lignot, J.-H., 2017. Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. J. Exp. Biol. 220, 1749–1760. https://doi.org/10.1242/jeb.135624

Romano, N., Zeng, C., 2013. Toxic Effects of Ammonia, Nitrite, and Nitrate to Decapod Crustaceans: A Review on Factors Influencing their Toxicity, Physiological Consequences, and Coping Mechanisms. Rev. Fish. Sci. 21, 1–21. https://doi.org/10.1080/10641262.2012.753404

Theuerkauff, D., Rivera-Ingraham, G.A., Mercky, Y., Lejeune, M., Lignot, J.H., Sucré, E., 2018a. Effects of domestic effluent discharges on mangrove crab physiology: Integrated energetic, osmoregulatory and redox balances of a key engineer species. Aquat. Toxicol. 196, 90–103. https://doi.org/10.1016/j.aquatox.2018.01.003

Theuerkauff, D., Rivera-ingraham, G.A., Roques, J.A.C., Bertini, M., Lejeune, M., Farcy, E., Lignot, J., 2018b. Salinity Variation in a Mangrove Ecosystem : A Physiological Investigation to Assess Potential Consequences of Salinity Disturbances on Mangrove Crabs. Zool. Stud. 57, 1–16. https://doi.org/10.6620/ZS.2018.57-36

Tsai, J.-R., Lin, H.-C., 2007. V-type H^+-ATPase and Na^+,K^+-ATPase in the gills of 13 euryhaline crabs during salinity acclimation. J. Exp. Biol. 210, 620–627. https://doi.org/10.1242/jeb.02684

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271–278. https://doi.org/10.1016/0003-2697(78)90342-1

Walters, C.R., Cheng, P., Pool, E., Somerset, V., 2016. Effect of temperature on oxidative stress parameters and enzyme activity in tissues of Cape River crab (*Potamanautes perlatus*) following exposure to silver nanoparticles (AgNP). J. Toxicol. Environ. Heal. - Part A 79, 61–70. https://doi.org/10.1080/15287394.2015.1106357

Wang, W., Wu, X., Liu, Z., Zheng, H., Cheng, Y., 2014. Insights into hepatopancreatic functions for nutrition metabolism and ovarian development in the crab *Portunus trituberculatus*: Gene discovery in the comparative transcriptome of different hepatopancreas stages. PLoS One 9. https://doi.org/10.1371/journal.pone.0084921

Wear, R.J., Tanner, J.E., 2007. Spatio-temporal variability in faunal assemblages surrounding the discharge of secondary treated sewage. Estuar. Coast. Shelf Sci. 73, 630–638. https://doi.org/10.1016/j.ecss.2007.03.008

Weihrauch, D., Donnell, M.O., 2017. Acid-Base Balance and Nitrogen Excretion in Invertebrates. Springer. https://doi.org/10.1007/978-3-319-39617-0

Yu, R.Q., Chen, G.Z., Wong, Y.S., Tam, N.F.Y., Lan, C.Y., 1997. Benthic macrofauna of the mangrove swamp treated with municipal wastewater. Hydrobiologia 347, 127–137. https://doi.org/10.1023/A:1003027520750

Figure legends

Figure 1: Location of the Comoros archipelago and the study site in Mayotte (A); aerial view of the experimental setup in the mangrove of Malamani (B) and of the control and impacted areas (C); artificial burrow (D); pierced pipes discharging WW in the impacted area (E). *Neosarmatium africanum* as well as water samples used for chemical analyses were collected within the *Avicenia marina* and *Ceriops tagal* belts in non-impacted sites. In B, the experimental parcels 1 and 2 corresponds to the *Ceriops tagal* and *Avicenia marina* belts, respectively. 3 corresponds to the overflow discharging site. Only the parcel 1 was considered in this study. In C, artificial burrows (n=20) are indicated by red (impacted, n=10) and yellow (control north only, n=10) circles. In B and C, grey lines represent the WW transporting pipes and the white lines represent the WW discharging drains.

Figure 1

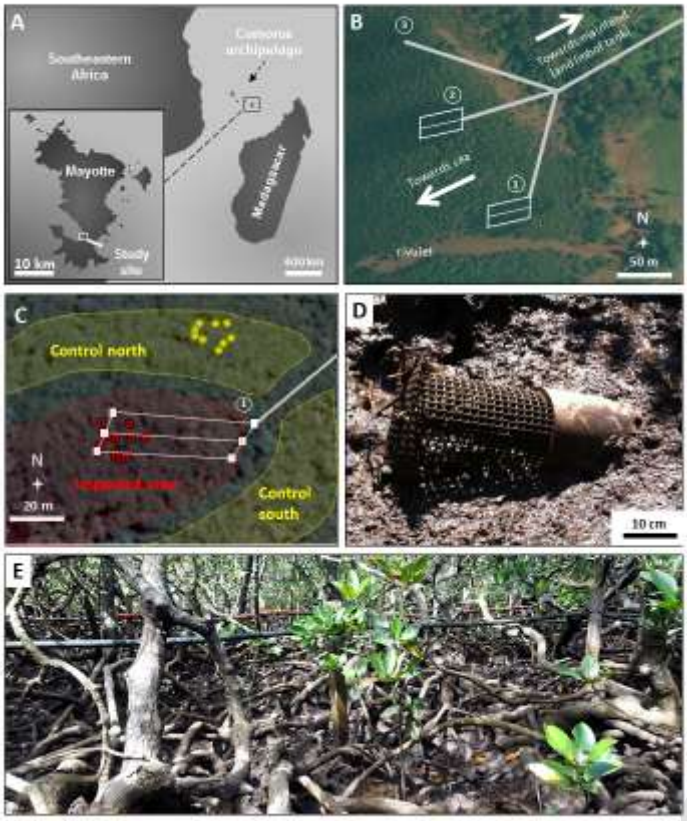

Figure 2: Dorsal (A, C) and ventral (B, D) view of *Neosarmatium africanum* female (A, B) and male (C, D). Scale bars: A = 2.6 cm; B = 1.7 cm; C = 2.3 cm and D = 2.5 cm.

Figure 2

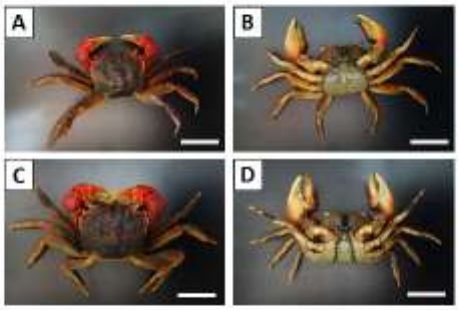

Figure 3: Surface water salinity (A), crab burrows density (B) and relationship between crab density and salinity (C). Control south (CS, green, n = 11), Impacted area (I, red, n = 10), Control north (CN, green, n = 11). Different letters represent statistically significant differences at $p < 0.05$ according to Kruskal-Wallis test followed by a Nemenyi post hoc comparison test (A) or a one-way ANOVA followed by Tukey's HSD test (B). Boxplots show medians (central crossbars), 25th and 75th percentiles (boxes) and whiskers which extent to the most extreme data point which is no more than 1.5 times the interquartile, data point outside this range are represented by an open circle.

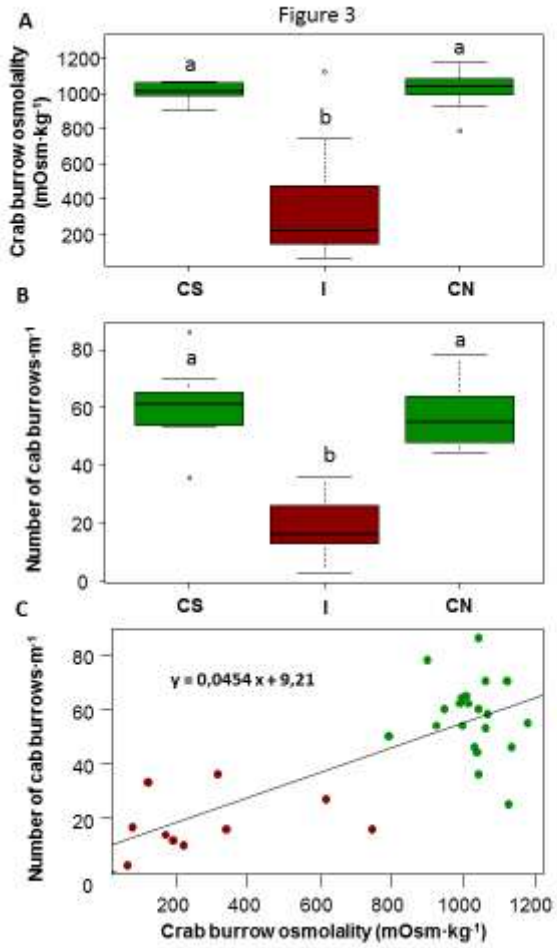

Figure 4: Hemolymph osmotic pressure according to water (from artificial burrow) osmotic pressure (A); OP residuals (B) and Na^+/K^+-ATPase activity in anterior (A, light green/red) and posterior (P, dark green/red) gills (C). A: Black symbols and dotted lines correspond to 95% confidence interval of the osmotic curve of *N. africanum* (see Theuerkauff et al., 2018). Different letters represent statistically significant differences at $p < 0.05$ from two-way ANOVA followed by Tukey's HSD test. Boxplots show medians (central crossbars), 25th and 75th percentiles (boxes) and whiskers which extent to the most extreme data point which is no more than 1.5 times the interquartile, data point outside this range are represented by an open circle.

Figure 4

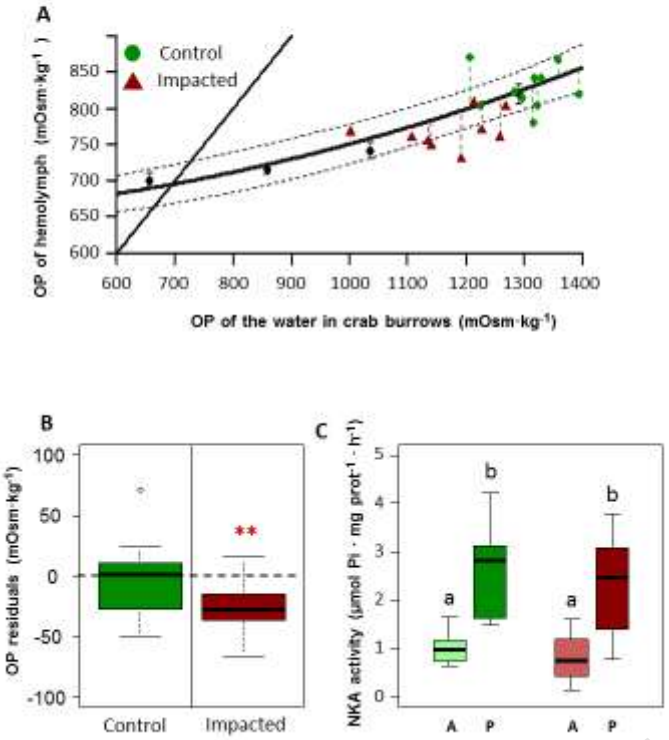

Figure 5: Redox parameters in anterior (A, light green/red) and posterior (P, dark green/red) gills of *N. africanum*: SOD activity (A); CAT activity (B) and oxidative damage (C) measured by TBARS concentration. Units (U). Different letters represent statistically significant differences at $p < 0.05$ according to a two-way ANOVA followed by Tukey's HSD test. Boxplots show medians (central crossbars), 25th and 75th percentiles (boxes) and whiskers which extent to the most extreme data point which is no more than 1.5 times the interquartile, data point outside this range are represented by an open circle.

Figure 5

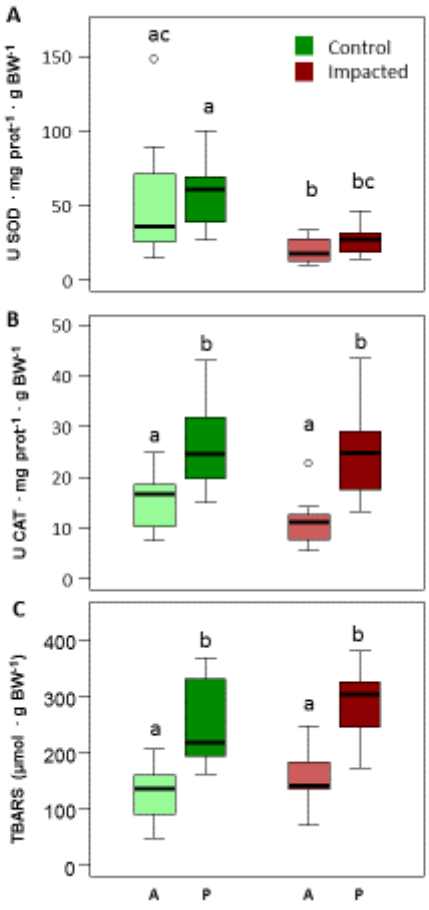

Table 1: Chemical characterization of the (undiluted) WW as well as the surface water and porewater for both the impacted and control sites (partly modified from Capdeville, 2018 and 2019). N.A. = data not available. For WW, mean values (± SE) were obtained from a total of 4 measurements made on two consecutive days. Surface water (residuals pools) and porewater (piezometers installed at approximately 1 m depth) data was collected at low tide and mean values (± SE) were obtained from two-day measurements under similar hydrological conditions, in the upper and lower parts of the areas (total of 4 measurements).

Table 1										
		Wastewater	Control area			Impacted area				
			Surface Water		Porewater		Surface Water		Porewater	
	NH_4-N (µm)	3834 ± 134	37.97 ± 20.28		0.38 ± 0.23		294 ± 147		0.14 ± 0.09	
	NO_3^- (µm)	0.46 ± 0.03	1.05 ± 0.21		0.35 ± 0.20		1.28 ± 0.64		0.39 ± 0.22	
	NO_2^- (µm)	0.64 ± 0.25	0.61 ± 0.11		0.06 ± 0.01		4.20 ± 0.08		0.07 ± 0.00	
	PO_4^{3-} (µm)	186 ± 0.1	0.83 ± 0.01		5.93 ± 1.28		0.37 ± 0.15		10.79 ± 2.1	
	Temperature (°C)	28.1	23.2 ± 1.2		24.8 ± 0.2		22.8 ± 0.2		25.0 ± 0.0	
	Salinity (psu)	0.39 ± 0.02	41.1 ± 1.3		39.5 ± 1.7		35.3 ± 2.0		45.8 ± 0.2	
	pH	7.23 ± 0.00	7.58 ± 0.01		n.a.		7.45 ± 0.01		n.a.	

Table 2: Results for the two-way ANOVAs on NKA activity (A), SOD activity (B), CAT activity (C) and TBARS concentration (D). Factors, df (degrees of freedom), MS (variance) and value of F ratio are shown. Statistically significant effects are indicated by asterisks: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

Table 2																
		A	**NKA activity**			**B**	**SOD activity**			**C**	**CAT activity**			**D**	**TBARS**	

	Factors	df	MS	F		df	MS	F		df	MS	F		df	MS	F
	Gill	1	25.3	**40.20 *****		1	0.86	3.48		1	1601	**27.5 *****		1	119780	**27.6 *****
	Treatment	1	0.71	1.13		1	6.15	**24.68 *****		1	54	0.94		1	7133	1.64
	Gill x Treatment	1	0.035	0.057		1	0.018	0.075		1	28	0.49		1	121	0.028
	Error	36	0.62			36	0.24			33	58			27	4330	
	df: degrees of freedom; MS: variance; Statistically significant effects are indicated by asterisks: *** (p< 0.001).															