Effects of pesticides and metals on penaeid shrimps in Maputo Bay, Mozambique – A field study

Marine Pollution Bulletin 173 (2021) 112964

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Effects of pesticides and metals on penaeid shrimps in Maputo Bay, Mozambique – A field study

Joachim Sturve [a,*], Mikael Gustavsson [b], Per-Olav Moksnes [c], Daniela C. de Abreu [d]

[a] Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[b] Department of Mathematical Sciences, Chalmers University of Technology and University of Gothenburg, Gothenburg, Sweden
[c] Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden
[d] Department of Biological Sciences, Eduardo Mondlane University, C.P. 257, Maputo, Mozambique

ARTICLE INFO

Keywords:
Mozambique
Decapods
Pesticide mixtures
Acetylcholine esterase
Metals

ABSTRACT

Estuaries are important nursery areas for many species and these habitats are often affected by anthropogenic activities. We investigate possible negative effects of pesticides and metals on penaeid shrimps in Maputo Bay, Mozambique. Shrimps and water samples were collected in three estuaries and one coastal area for biomarker and chemical analysis. Acetylcholinesterase (AChE) and glutathione-*S* transferase activities were analysed as biomarkers for pollutants. 37 different pesticides were analysed in water samples and shrimp muscle tissue was analysed for 10 metals. Risk assessment showed that the environmental thresholds were exceeded for several herbicides in three of four of the assessed nursery areas. Lower AChE activities were detected in shrimps captured close to an agriculture area and this location had the lowest shrimp densities. Metal analysis in shrimp showed low levels. Despite localized effects, results highlight the need to improve the regulation of pollutants in the Espírito Santo estuary in Maputo bay.

1. Introduction

Worldwide, anthropogenic activities affect estuarine and tropical shallow water systems such as mangrove forests. These impacts range from direct changes in species composition by harvesting and introduction of exotic species, to physical changes resulting from activities like harbour dredging, streams channelization and land reclamation. An additional important impact in these systems is eutrophication due to nutrient enrichment caused by agricultural runoff and addition of excessive level of organic matter (Day et al., 2013). Agricultural runoff and urban effluents discharged together with shipping activities also contribute with toxic compounds (Khan et al., 2014) including, among others, a wide range of pesticides, polyaromatic hydrocarbons and metals.

Mixtures of chemicals can be especially diverse in estuarine areas where aquatic organisms simultaneously can be exposed to chemicals from land-based and marine based activities. Meanwhile, due to the great variability of many physicochemical parameters, the estuarine environment imposes stressful conditions which the organisms inhabiting it have been adapted to. However, the ecological variability can affect the sensitivity to additional stressors, such as metals and other pollutants (Monserrat et al., 2007). The toxicity of metals towards aquatic organisms, including invertebrates, is well documented (Marsden and Rainbow, 2004; Chiarelli and Roccheri, 2014). Pesticides are mainly categorized into insecticides, herbicides and fungicides, with the insecticide group having the largest direct impact on aquatic invertebrates. Currently the most commonly used insecticides worldwide are organophosphates (OP), carbamates and synthetic pyrethroid substances (Grube et al., 2011).

OPs are neurotoxins which affect nerve signalling by inhibiting acetylcholineesterase (AChE) activity which catalyses the hydrolysis of the neurotransmitter acetylcholine (Galgani et al., 1992; Bocquené and Galgani, 1998). OPs are relatively non-persistent in aquatic systems, but since they have a low target-species specificity and a high toxicity they are considered to be a threat to many non-target organisms, such as fish, insects and crustaceans (Fulton and Key, 2001). Inhibition of AChE activity is a well-established biomarker for OP exposure and has been used as endpoint in monitoring studies for decades (van der Oost et al., 2003; Jordaan et al., 2013). However, most of these studies have been performed on fish and few studies have focused on invertebrates.

Glutathione *S*-transferases (GST) are enzymes often used as biomarker for compounds generally found in harbour areas, such as

* Corresponding author.
E-mail address: joachim.sturve@bioenv.gu.se (J. Sturve).

https://doi.org/10.1016/j.marpolbul.2021.112964
Received 19 April 2021; Received in revised form 9 September 2021; Accepted 12 September 2021
Available online 23 September 2021

polyaromatic hydrocarbons (PAHs) and pro-oxidants (van der Oost et al., 2003; Turja et al., 2020). GST belong to a family of phase II detoxification enzymes that are involved in a broad spectrum of detoxification processes such as conjugation and antioxidant defence (van der Oost et al., 2003). GST activities are commonly measured with an enzymatic assay using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, an assay that measures activity of all GST isoforms simultaneously and thus gives a broad overview of responses (Halliwell and Gutteridge, 1999).

Chemical monitoring is a complement to biomarker analysis and enables a chemical risk assessment. The risk assessment is performed by comparing measured chemical concentrations to relevant hazard estimates such as NOEC's, EC50's or other environmental thresholds. The risk assessment can indicate which chemical that is likely responsible for a biomarker response (Forbes and Calow, 2002). However, multiple studies have shown that the combined effect of chemical mixtures is often larger than explained by any individual component of the mixture. Therefore, the risk assessment of environmental samples must consider all compounds present within the mixture. Risk assessments of mixtures are regularly carried out using the concentration addition (CA) model (Kortenkamp et al., 2009; Bopp et al., 2016), a model which has been shown to closely predict the combined effect of pesticide mixtures (Belden et al., 2007; Rodney et al., 2013). Although synergistic effects have been shown for shrimps exposed to pesticides (Key et al., 2007) and the fact that CA assumes additivity among the mixture components it has also been shown that even when synergistic effects occur the model typically performs well (typically within a factor 2) and that synergistic effects seems to be rare at environmentally relevant concentrations (Cedergreen, 2014; Rodney et al., 2013). In both single compound and mixture risk assessment non-detects, i.e. compounds that are below their limit of detection, presents a conceptual problem. Non-detects can in theory be present at any concentration between zero (best-case) and the limit of detection (worst case). A chemical risk assessment must therefore also distinguish between situations which are proven to be safe, proven to be problematic or which are inconclusive (Gustavsson et al., 2017).

Penaeid shrimps are one of the most valuable fishery resources world-wide and are an important part of the economy of Mozambique (FAO, 2007; Silva and Masquine, 2014). Maputo Bay is the second largest shrimp fishing ground in Mozambique and supports the artisanal and semi-industrial shrimp fishery which together land about 250 tons per year (National Fishery Research Institute, IIP, 2013). As most of the penaeid shrimp life cycles depend on estuaries as nursery areas, their populations are threatened by anthropogenic activities in these systems. Previous studies in Maputo Bay have shown elevated levels of pesticides in the Infulene River in the northern part of Espírito Santo estuary and decreased AChE activity in fish and shrimp in adjacent mangrove habitats, indicating a negative impact from pesticides on the aquatic organisms (Sturve et al., 2016). Analyses of metals in the sediment from the same area have also showed elevated levels of Copper (Cu), Nickel (Ni) and cadmium (Cd) (Scarlet, 2015). However, little is known regarding contamination from pesticides and metals in the other two estuaries in Maputo Bay (the Incomati and Maputo River estuaries), whether the contamination in Espírito Santo is localized only to the northern part of the estuary, or if the levels of contaminants correlate to densities of shrimp in the nursery areas.

The objective of the present study was to investigate possible negative effects of pesticides and metals on shrimps in tropical estuaries/coastal waters. Two penaeid shrimp species, *Penaeus indicus* and *Metapenaeus monoceros*, were collected at four different nursery areas (3 estuaries and one coastal area) within Maputo Bay, Mozambique. Acetylcholinesterase (AChE) and glutathione *S*-transferase (GST) activities were analysed as biomarkers for pollutants. Pesticides were analysed in water samples and metals in shrimp muscle. The risk from the pesticide mixtures detected was determined using concentration addition for a best and worst-case scenario. In addition, shrimp densities were determined to discuss possible impacts on shrimp populations.

2. Materials and methods

2.1. Chemicals and reagents

Acetylthiocholine (ACTC) iodine, butyrylthiocholine (BuCTC) and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma Aldrich (St Louis, United States). Bovine serum albumin (BSA), Folin-Ciocalteu's phenol reagent, KH_2PO_4, Na_2HPO_4*$12H_2O$, NaH_2PO_4*H_2O, Na_2CO_3 were obtained from Merck (Darmstadt, Germany). NaOH was obtained from KEBO Lab (Spånga, Sweden). All chemicals were of analytical grade.

2.2. Field sampling

2.2.1. Shrimp sampling

Two species of shrimp, *P. indicus* and *M. monoceros* were sampled at four potential nursery areas for penaeid shrimp in Maputo Bay: Espírito Santo estuary, Maputo River estuary, Incomati River estuary and Bembe coastal area in the south-eastern part of the Bay (see map, Fig. 1). The Bembe coastal area has very little freshwater input from rivers, and has limited anthropogenic activities and was therefore regarded as a reference site for effects of pollutants. Maputo and Incomati rivers estuaries are mainly affected by agricultural runoff from upstream Mozambican agricultural activities, as well as from neighbouring countries, being international rivers. Espírito Santo estuary, is considered as being more affected by pollutants than other areas in Maputo Bay, due to agricultural runoff, industrial and urban effluents and harbour and shipping activities (Scarlet and Bandeira, 2014; Scarlet, 2015; Sturve et al., 2016). Within this estuary, samples were collected from two separate locations. The first being the mouth of Matola River in the less developed south-western part of the estuary, mainly affected by industrial activities. The second at the mouth of the Infulene River in the highly exploited and populated north-eastern part of the estuary, affected by heavily agricultural runoff and industrial and urban effluents (Scarlet and Bandeira, 2014) (see map, Fig. 1).

Shrimps were sampled in March and October, times representing seasonal difference regarding rain burden, with March being the end of the wet and warm season and October, the end of the dry and cold, with different agricultural practices and pesticide uses. The shrimp species were selected based on their high commercial value and importance for Mozambican fisheries (Samucidine et al., 2015). Shrimp samples were obtained by trawling at all above-mentioned locations and immediately after capture, shrimp were killed and stored frozen on dry ice. The samples were transported on dry ice to Sweden where sample preparation was performed.

In Espírito Santo estuary, additional trawls were carried out in March at five different sites to assess the distribution of the shrimp species within the nursery area. Besides Matola and Infulene sites also the locations; Maputo west (MW) located along the more industrial northern side of the estuary (mainly affected by industrial and harbour activity), and Catembe (C) and Tembe (T) located at the less developed areas along southern side (see map Fig. 1) (see de Abreu, 2017 for sampling methods).

2.2.2. Water sampling for pesticide analyses

Water grab samples were collected in both sampling months at the same sites as the shrimps (one grab sample per site) in 3-l glass jars and stored in coolers with ice in the field and refrigerators upon arrival to the laboratory. Water samples were run through solid phase extraction columns within 24 h. In October, additional samples were taken from two locations inside the Infulene River in the Espírito Santo estuary. These freshwater samples were taken upstream and downstream of the effluent discharge from Maputo's sewage treatment plant. All sampling equipment was washed with ethanol before use. In the field the glass jars

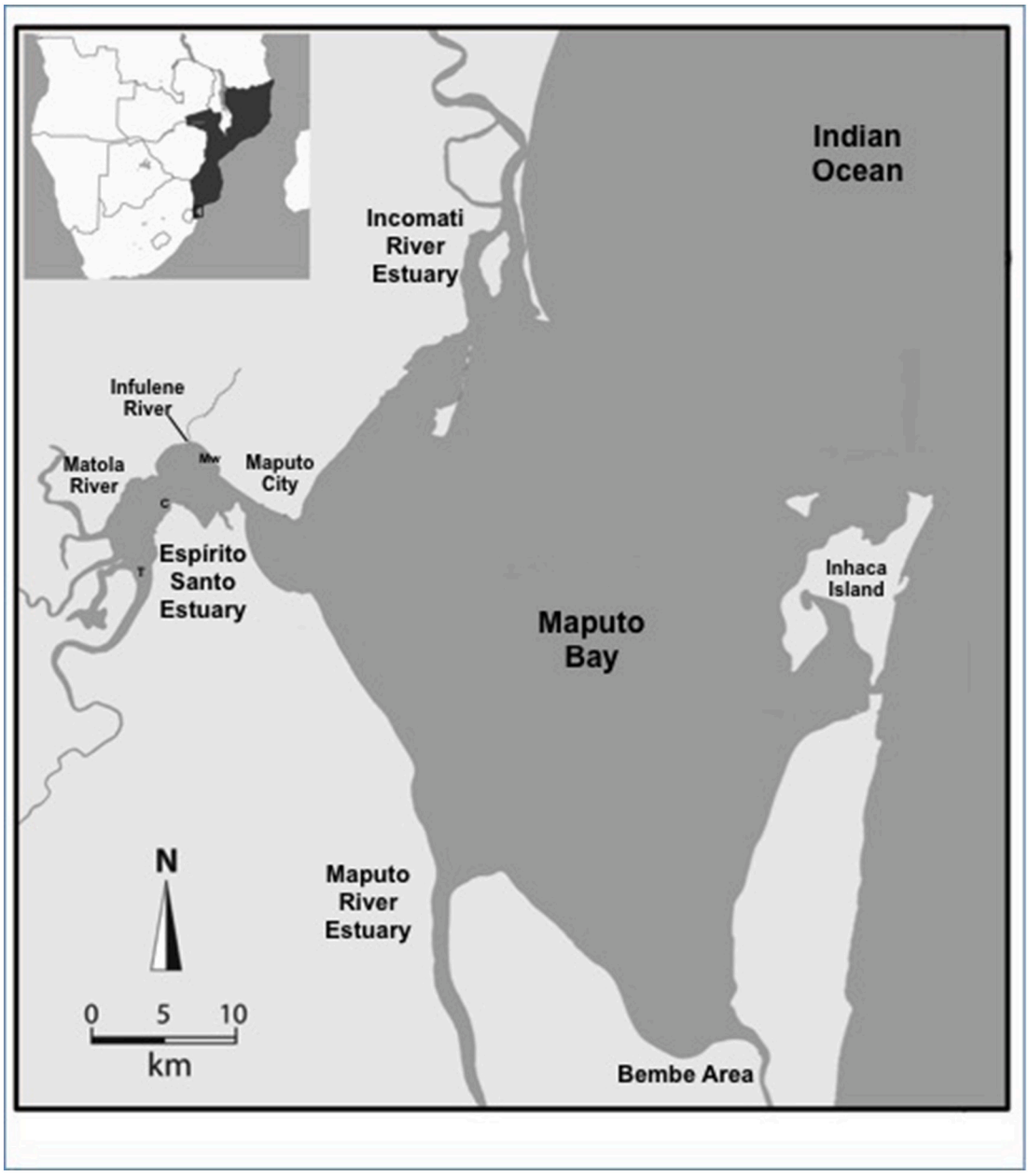

Fig. 1. Map of Maputo Bay showing the location of the four nursery areas sampled (adapted from Scarlet and Bandeira, 2014). The sampling areas within Espírito Santo Estuary includes Infulene, Matola and Tembe (T) rivers, Catembe (C) and Maputo west area (to the west of Maputo City).

were subsequently rinsed with estuarine water from the site before the actual sample was collected.

2.3. Pesticide analyses of water samples

2.3.1. SPE extraction

The solid phase extraction (SPE) procedure of water was a modified version of a method previously described by Jeannot et al. (2000) and performed at the Marine and Aquatic Ecology Lab from the Department of Biological Sciences from Eduardo Mondlane University. Previous to the application of the water sample to the SPE-column (500 mg/6 mL cartridge, ISOLUTE C18, Biotage, Sweden) the column was rinsed with 6 mL methanol followed by 6 mL acetone and conditioned with 6 mL distilled water. Thereafter, the water sample was applied to the column and pressure applied with vacuum suction. When the entire sample had passed through the SPE-column the cartridge was rinsed with 6 mL distilled water and dried with an airflow. The SPE columns were wrapped in aluminium foil and stored at -20 °C until analysis. The analytes were eluted from the SPE cartridges using 6 mL methanol followed by 6 mL acetone under vacuum. The eluate was evaporated to dryness under nitrogen at 40 °C. The sample was reconstituted in 0.5 mL methanol:water (1:1) and centrifuged at 10,000 rpm in 10 min. The supernatant was transferred to vials for final determination on a high performance liquid chromatography-triple quadrupole mass spectrometry (HPLC-MS/MS).

2.3.2. Instrumental

The determination of pesticide residues in the samples were performed on a binary liquid chromatography (UFLC) system equipped with an auto injector (Shimadzu, Kyoto, Japan) coupled to an API 4000 triple quadrupole (MS/MS) (Applied Biosystems, Foster City, USA) with an electrospray ionization interface (ESI) performed in both positive and negative mode. The chromatographic separation was carried out using gradient elution on a Xbridge (Waters Corporation, Milford, USA) C18

reversed phase column (50 × 3 mm, 5-micron particle size) at 35 °C and a flow rate of 0.3 mL/min. The mobile phase consisted of 10 mM acetic acid in water (mobile phase A) and methanol (mobile phase B). The gradient was initiated with 100% of mobile phase A and 0% of mobile phase B. The percentage of mobile phase B was increased linearly to 95% in 11 min and maintained at 95% for 5 min. Thereafter the mobile phase composition was returned to the initial composition in 1 min and maintained for 4 min before the next injection. The total sample runtime was 21 min.

2.3.3. Method validation

Three blank samples were made on each sampling occasion. The blank sample consisted of 500 mL distilled water and was treated the same way as the 'real' water samples. In addition, at the time of the analysis three SPE-cartridges were rinsed and conditioned as previously described for the real samples and subsequently spiked with 100 μL of a standard mixture of the investigated pesticides at a concentration of 1 μg/mL methanol. The results from the analyses of the three cartridges containing the standard mixture were used to compensate for loss in recovery during sample preparation and ion-suppression during instrumental analysis.

2.4. Metal analyses in shrimp muscle tissue

Muscle tissues for metal content analyses were oven dried at 70 °C for 7 days. Ten metals (arsenic, cadmium, cobalt, chromium, copper, manganese, nickel, lead, vanadium, zinc) were analysed using a standard temperature program based on the method EN 13805:2014, 0.3 g to 0.5 g of shrimp dried muscles were digested in a mixture of 7 mL nitric acid (67% w/v Normatron) and 1 mL hydrogen peroxide (30%, PA, Merck) in a microwave digester (Milestone Start-D SK-10). All digestions were performed in 100 mL Teflon digestion vessels. After cooling to room temperature the digested samples were diluted with high purity water (18.2 MΩ) from a Milli-Q system (Millipore) to a total volume of 50 mL. The concentrations of the elements were determined by inductively coupled plasma mass spectrometry (ICP-MS), using a Thermo Scientific ICAP Qc equipped with a hexapole collision/reaction systems and a autosampler CETAC ASX 520. LOD were calculated from 3 times the standard deviation from digested blanks and LOQ were calculated from 10 times the standard deviation from digested blanks. Tort-3 was used as certified reference material (see Table 6 in Supplementary material) (methodology based on SS-EN ISO 17294-2:2016 and SS-EN 14902:2005).

2.5. Mixture risk assessment using concentration addition

The risk from the detected mixtures was estimated using the CA concept (Kortenkamp et al., 2009). For each sample the risk quotient (RQ) was calculated according to:

$$RQ_{CA} = \sum_{i}^{n} \frac{MEC_i}{Threshold_i}$$

where 'MEC' is the measured environmental concentration and 'Threshold' is the hazardous concentration for each detected pesticide (i): Four different effect thresholds were applied. One overall environmental thresholds (ET) that is protective of all species groups and one set of threshold each for the species groups algae, aquatic invertebrates and fish (Backhaus and Faust, 2012).

To generate the ETs data was gathered from reports on active substances published by the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA). ETs (predicted no effect concentrations) are present in the reports published by ECHA while ETs from the EFSA reports were determined by taking the most sensitive bioassay and dividing the result with a trigger value (10 for algal and macrophyte EC50 data, 100 for fish and aquatic invertebrate EC50 data and 10 for fish and aquatic invertebrate NOEC data). These trigger-values are used to cover the differences between individual lab studies and a field situation (EFSA, 2013). It was assumed that all data reported to EFSA and ECHA was equally relevant from a risk assessment perspective. ETs were also gathered from individual substance the data sheets which support the European Water Framework Directive (CIRCABC, 2017), see Tables S2 and S3 in the S.I. for all collected data.

Individual 'species group' thresholds were determined by, 1) collecting all individual species data listed in the ECHA and EFSA reports, 2) excluding all data on formulated products, 3) excluding all data reported as "greater than" or "smaller than", 4) determine the average sensitivity for each species and compound, 5) determine the geometric mean toxicity of each individual compound for each species group. Geometric means were determined independently for EC50 and NOEC data and NOEC data were used for aquatic invertebrates and fish if available while only EC50 data were used for algae. 6) Finally, the same trigger values as used to determine the ETs were used. The procedure follows the current guidance for near field exposure (EFSA, 2013).

Limit data were used if no precise data could be obtained (6 out of 111 species group thresholds) and QSAR data from ECOSAR v2.0 was used for one compound (Fenobucarb), see Tables S2 and S3 in S.I. for full details.

The two freshwater samples collected in Infulene River were analysed using the original thresholds, while freshwater-specific thresholds were adjusted by dividing it with an additional factor of 10 when assessing the risk in samples collected in the estuarine environment. This is done to compensate for the greater biodiversity in the marine environment (ECHA, 2008). We calculated the Maximum Cumulative Ratio (MCR) for each mixture in accordance to:

$$MCR = \sum_{i}^{n} \frac{RQ_{CA}}{max\left(\frac{MEC_i}{Threshold_i}\right)}$$

The MCR is used as a measure of the evenness of contribution to the total risk from mixture components (Price and Han, 2011).

2.6. Biochemical analyses

Shrimp were thawed and hepatopancreas, gill and muscle tissues were dissected and homogenized (glas/Teflon) in ice-cold homogenization buffer (0.1 M Na^+/K^+-phosphate buffer + 0.15 M KCl, pH 7.4). The homogenates were centrifuged at 10,000*g* for 20 min. The supernatants from each sample were aliquoted and stored at -80 °C until analysis. All steps were performed at 0-4 °C.

2.6.1. Acetylcholinesterase activity

AChE activity was measured according to a modification of the spectrophotometric method described by Ellman et al. (1961) adapted to a microplate reader (Sturve et al., 2016). Final concentration in the reaction mixture was 0.5 mmol L^{-1} DTNB in 0.1 mol L^{-1} Na-phosphate buffer. After 5 min, incubation of the reaction buffer with 10 μL of the sample (room temperature in the dark) the reaction was started with the addition of acetylthiocholine iodine (2.6 mmol L^{-1} final concentration) (total volume 300 μL). Change in absorbance at 412 nm was followed spectrophotometrically and the activity calculated using the extinction coefficient of TNB $\varepsilon = 13{,}600\ M^{-1}\ cm^{-1}$ (Ellman et al., 1961).

2.6.2. Glutathione S-transferase activity

GST activity was measured according to Habig et al. (1974) adapted to a microplate reader according to Stephensen et al. (2000). Reaction mixture contained 2 mM CDNB, 1 mM GSH in 0.1 M Na phosphate buffer (pH 7.5). Change in absorbance was monitored at 340 nm and the extinction coefficient for glutathione-DNB adduct $\varepsilon = 9600\ M^{-1}\ cm^{-1}$ was used to calculate enzymatic activity.

2.6.3. Protein content

Protein content in the analysed tissue samples was determined using a modified version of the method described by Lowry et al. (1951) adapted to a microplate reader. Bovine serum albumin (BSA) was used for protein standard.

2.7. Statistical analyses

Results from the biochemical analyses were analysed for normality and the Levene's test was performed for homogeneity of variances. This was followed by a 1-way ANOVA and in the case where ANOVA showed significance ($p < 0.05$) the test was followed by Student-Newman-Keuls test (homogeneous variance) or Dunnett's T3 for samples with heterogenic variance. If demand for normal distribution was not met, Kruskal-Wallis H test was performed followed by Mann-Whitney *U* test when there was a significant difference between the samples. Data are shown as mean ± standard error. All statistical analyses were performed in SPSS 11.0.

Differences in juvenile shrimp densities (<25 mm carapace length, CL) between areas in March were tested in one-factor ANOVA-models for each species, using site as independent variable, and densities of shrimp (no. 100 m^2) as dependent variable.

3. Results

3.1. Pesticide analyses

The sampling campaign in the estuaries covered five different sampling sites and two time-points, March and October. Within the 10 samples 9 of the 37 analysed pesticides were detected at least once (Table 1, for a complete list of pesticides analysed see Table S1 in the S.I.). Six of the detected pesticides were herbicides, two fungicides and one, is a herbicide/algaecide. Generally, results show a temporal difference with higher total concentrations of pesticides detected in samples taken in March as compared to October at four out of five sites. A spatial difference was also observed with the highest levels of most pesticides detected in samples taken at the two sites within the Espírito Santo estuary, at Matola and Infulene rivers. However, levels of Trifluralin were high also in Bembe in March. Bembe was originally selected as a reference site based on the low levels of anthropogenic activities in the area, but results suggest that also this area is subjected to pesticide pollution. Nine pesticides were identified in the samples taken in October in the Infulene River, 5 of which overlapped with the pesticides identified in the marine samples (Table 2). The compounds only found in Infulene River were thiabendazole, dicamba, MCPA and malathion.

Of the 48 RQ_{CA} calculated (12 samples with one RQ each for ET, algae, aquatic invertebrate and fish for each) only 11 samples had an MCR larger than or equal to 2 (Table S4 in S.I.). Thus, in the majority of cases a single compound contributed more than half of the total mixture risk.

In March, four out of five sampled nursery areas showed pesticide detections above the environmental threshold (Table 1; Table S1 in the S.I.). At Incomati estuary, Matola and Bembe Trifluralin exceeded its environmental threshold (30 ng L^{-1}), while at Infulene Irgarol was detected above its threshold (2.5 ng L^{-1}). None of these five sites showed any exceedances in October (Table 1; Table S1 in the S.I.). At the two freshwater sites within Infulene River, where samples were only taken in October, the Malathion concentration exceeded its thresholds at the downstream site, and the Alachlor concentration exceeded its threshold at upstream site (thresholds 6 and 250 ng L^{-1} respectively) (Table 2; Table S1 in the S.I.). Of the six threshold exceedances five were in the range of a factor 1-7, while Malathion exceeded its threshold with a factor 568.

Considering the risk from the full mixtures detected in the samples taken during March at the sites Incomati, E. Santo (M) and Bembe the risk is primarily towards fish, while at E. Santo (I) algae is most at risk (Table 3). During October only the two freshwater sites showed concentrations exceeding the respective thresholds with algae being at risk at Influene Downstream the risk being towards the aquatic invertebrates at Influene upstream.

It should be noted that in the samples taken in March at E. Santo (M) and Bembe the threshold for algae are exceeded and the RQ for aquatic invertebrates is close to one. Irrespective of the species group the largest risk-contributor in both these samples is Trifluralin (Waterfall figures, S.I.).

3.2. Metal analyses

Metal analyses of pooled shrimp samples collected in March in the four nursery areas show that the metal levels are relatively low in shrimp muscle and that there are no large spatial or species differences

Table 2
Levels of pesticides in grab samples from two fresh water sites in Infulene River taken in October. Levels are shown as ng L^{-1}. [<X indicates concentrations below the LoD where X is the LoD.] (28 additional pesticides were analysed but not detected, see S.I. Table 3.)

Substance ng L^{-1}	Pesticide type	Infulene Upstream	Infulene Downstream
Alachlor	Herbicide	361	66
Atrazin	Herbicide	21	66
Carbendazim	Fungicide	7.9	17
Metoxuron	Herbicide	12	23
Thiabendazole	Fungicide/parasiticide	6.9	11
Terbuthylazine	Herbicide	4.4	<2
Dicamba	Herbicide	<5	65
MCPA	Herbicide	<1	6.9
Malathion	Insecticide	<498	3405

Table 1
Levels of detected pesticides in grab samples from five different estuaries in Maputo Bay shown as ng L^{-1}. Samples were taken in March (Mar) and October (Oct). [<X indicates concentrations below the LoD where X is the LoD.] (28 additional pesticides were analysed but not detected, see S.I. Table 3.)

		Espírito Santo				Incomati		Maputo		Bembe	
Substance ng L^{-1}	Pesticide type	Matola		Infulene							
		Mar	Oct	Mar	Oct	Mar	Oct	Mar	Oct	Mar	Oct
Alachlor	Herbicide	<1	3.0	<1	3.7	3.6	<1	3.5	<1	<1	<1
Atrazin	Herbicide	39	9.7	19	6.9	4.2	4.7	12	16	3.2	4.1
Carbendazim	Fungicide	<1	<1	<1	<1	<1	<1	1.2	<1	<1	<1
Irgarol	Herbicide/algaecide	1.7	<1	3.3	1.1	<1	<1	<1	<1	<1	<1
Metoxuron	Herbicide	22	<10	38	<10	<10	<10	<10	<10	<10	<10
Propamocarb	Fungicide	1	40	15	48	31	43	8.2	42	11	33
Trifluralin	Herbicide	184	<19	<19	<19	73	<19	<19	<19	172	<19
Terbuthylazine	Herbicide	<2	<2	<2	<2	<2	<2	7.2	13	<2	<2
Diuron	Herbicide	6.6	2.0	4.8	2.5	16	8.3	1.8	3.2	1.4	<1

Table 3
RQ determined using the ET (most sensitive data divided by trigger value) as well as independently for the three different organism groups: algae, aquatic invertebrates and fish. (E. Santo – Espírito Santo; I – Infulene; M – Matola; Down – Downstream; Up - Upstream). The MCR can be found in S.I. Table S4.

		ET	Algae	Aqua. inv.	Fish
Incomati	March	2.54	0.74	0.40	6.25
Incomati	October	0.05	0.13	0.02	0.01
E. Santo (I)	March	1.44	0.71	0.02	0.03
E. Santo (I)	October	0.48	0.39	0.01	0.01
E. Santo (M)	March	6.95	1.92	0.95	15.76
E. Santo (M)	October	0.04	0.30	0.01	0.01
Maputo River	March	0.18	0.38	0.13	0.06
Maputo River	October	0.15	0.33	0.08	0.03
Bembe	March	5.75	1.03	0.87	14.70
Bembe	October	0.01	0.07	0.00	0.00
Infulene Down	October	568.00	0.40	567.62	0.58
Infulene Up	October	1.54	1.58	0.07	0.05

(Table 4). Of the 10 metals analysed, 8 were detectable in muscle tissue of both shrimp species while both chromium and lead levels were below limits of detection in all samples.

3.3. Biomarker analyses

Enzymatic activities of AChE and GST were measured in hepatopancreas, gill and muscle tissues of both shrimp species collected in March and October. No differences between sites in AChE and GST activities were observed in muscle or gill tissue (data not shown). In March, AChE activities were significantly lower in hepatopancreas of both shrimp species collected at the Infulene site in Espírito Santo estuary, and for *P. indicus* collected at Incomati River compared to the reference site Bembe (Table 5; Fig. 2). No differences between sites were detected in GST activities in hepatopancreas (Table 5). In October, densities of the two shrimp species were very low, precluding analyses at several sites, and the results showed no significant difference between sites in any of the measured biomarkers (Table 5).

3.4. Shrimp abundance in the Espírito Santo estuary

Densities of *M. monoceros* in Espírito Santo estuary in March were consistently low in Infulene and West Maputo in the northern part of the estuary (1.6 and 2.5 shrimp 100 m^{-2}, respectively) in comparison to the other three sites in the less developed southern part of the estuary (9.0-40 shrimp 100 m^{-2}), but no significant differences were found between the sites. A similar, non-significant pattern was found for *P. indicus*, with very low densities in Infulene and West Maputo (0.6 and 5.2 shrimp 100 m^{-2}, respectively) in comparison to the other sites (8.7-17.9 shrimp 100 m^{-2}) (see Fig. 3).

4. Discussion

The overall aim of the present study was to investigate possible negative effects of pesticide mixtures and metals on shrimps in a tropical estuary, selecting the Maputo bay area in Mozambique as a study site. Concentrations of most pesticides were low in all estuaries analysed and the mixture risk was typically dominated by one or two compounds (S.I. Waterfall figures). However, in six out of nine samples the detected pesticides exceeded recommended thresholds indicating that there is a risk of negative effects. It should be noted that all thresholds, which the detected pesticide concentrations are compared to, originated within a European Union regulatory context. But, as there is no clear evidence for tropical species being more or less sensitive than temperate species (Kwok et al., 2007; Lopes et al., 2007; Daam and Van Den Brink, 2010; Sanchez-Bayo and Hyne, 2011) these thresholds were used for

Table 4
Levels of metals in muscle tissue of *M. monoceros* and *P. indicus* collected at five different sites in the Maputo Bay area. Levels are expressed as μg/g dry tissue. Values are displayed as mean ± SE, n = 2 in all sites except Incomati (E. Santo – Espírito Santo). [<X indicates concentrations below the LoD where X is the LoD.]

	Metapenaeus monoceros				*Penaeus indicus*			
	Bembe	Maputo	Incomati	E. Santo	Bembe	Maputo	Incomati	E. Santo
Vanadium	0.088 ± 0.006	0.090 ± 0.060	0.028	0.084 ± 0.025	0.084 ± 0.011	0.085 ± 0.015	0.035	0.092 ± 0.038
Chromium	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
Manganese	3.2 ± 1.5	2.4 ± 0.2	1.7	2.4 ± 0.3	2.15 ± 0.05	1.55 ± 0.55	1.8	2.35 ± 1.05
Cobalt	0.12 ± 0.03	0.16 ± 0.08	0.034	0.11 ± 0.01	0.085 ± 0.024	0.07 ± 0.025	0.039	0.081 ± 0.015
Nickel	0.19 ± 0.02	0.29	<0.25	0.76 ± 0.24	<0.25	0.34	0.83	0.43 ± 0.16
Copper	33 ± 4	35 ± 10	24	30.5 ± 10	27.5 ± 1.5	23.5 ± 2.5	33	25 ± 6
Zink	43.5 ± 1.5	44 ± 9	34	45.5 ± 2.5	40 ± 3	29 ± 2	43	34.5 ± 3.5
Arsenic	9.9 ± 3.1	5.8 ± 2.9	4.3	13.5 ± 5.4	6.6 ± 0.2	6.3 ± 0.9	4.3	4.9 ± 0.8
Cadmium	0.065 ± 0.02	0.04 ± 0.29	0.004	0.021 ± 0.014	0.027 ± 0.004	0.025 ± 0.003	0.007	0.015 ± 0.002
Lead	<0.085	<0.085	<0.085	<0.085	<0.085	<0.085	<0.085	<0.085

Table 5
Activities of the biomarkers acetylcholinesterase (AChE) (expressed as μmol min^{-1} mg $protein^{-1}$) and glutathione S transferase (GST; expressed as mmol min^{-1} mg $protein^{-1}$) in hepatopancreas from *P. indicus* and *M. monoceros* collected in Maputo Bay in March and October. Results are displayed as mean ± SE. (E. Santo – Espírito Santo; n.s. = not sampled.)

Month	Site		*Penaeus indicus*		*Metapenaeus monoceros*	
			AChE	GST	AChE	GST
March	Bembe		11.18 ± 1.60	0.37 ± 0.06	7.34 ± 0.45	0.81 ± 0.06
	Maputo		9.08 ± 0.71	0.34 ± 0.07	6.57 ± 1.16	0.86 ± 0.09
	Incomati		10.41 ± 1.78	0.19 ± 0.03	4.48 ± 0.55*	0.44 ± 0.07
	E. Santo	Infulene	6.36 ± 0.52*	0.18 ± 0.02	4.84 ± 0.41*	0.56 ± 0.09
		Matola	n.s.	n.s.	6.99 ± 0.61	0.62 ± 0.09
October	Bembe		6.88 ± 0.81	0.07 ± 0.01	5.13 ± 1.01	0.12 ± 0.01
	Maputo		n.s.	n.s.	6.34 ± 1.06	0.14 ± 0.01
	Incomati		n.s.	n.s.	2.95 ± 0.79	0.11 ± 0.03
	E. Santo	Infulene	15.21 ± 4.38	0.11 ± 0.04	6.20 ± 0.91	0.12 ± 0.02
		Matola	n.s.	n.s.	n.s.	n.s.

* Significantly different from reference site Bembe, $p < 0,05$.

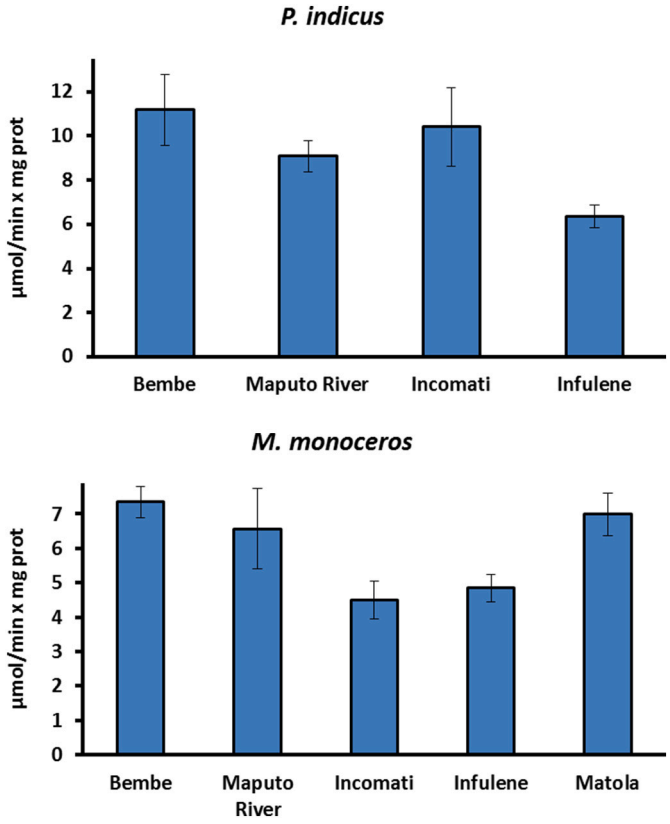

Fig. 2. Acetylcholine esterase activity (µmol min^{-1} mg $protein^{-1}$) in hepatopancreas from *Penaeus indicus* and *Metapenaeus monoceros* sampled at four different estuaries and the control area at Bembe in Maputo Bay. The sites Infulene and Matola are within the Espírito Santo estuary. Shrimps were sampled in March. Charts show mean ± SE, * indicates significantly different form reference site Bembe, $p < 0,05$.

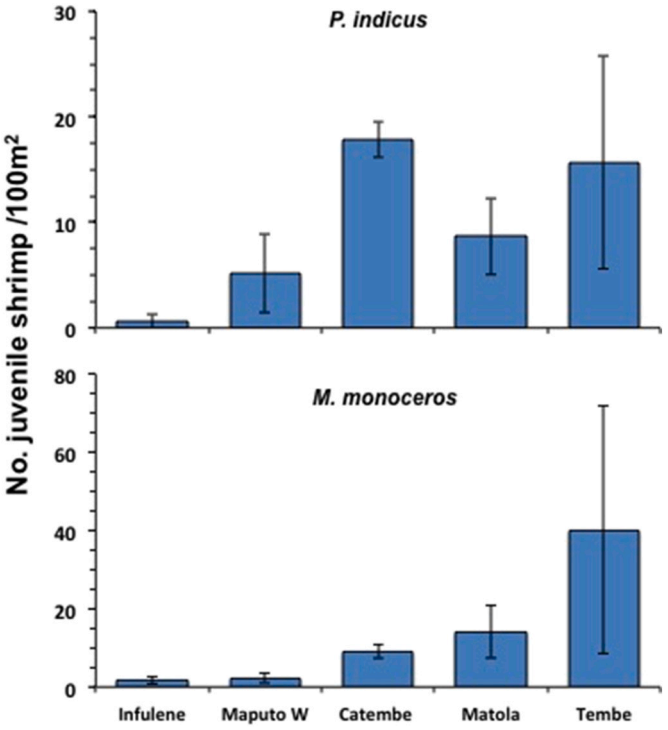

Fig. 3. Mean densities (n = 2-4) of *Penaeus indicus* and *Metapenaeus monoceros* (+SE) at 5 locations within the Espírito Santo estuary in March. Infulene and Maputo west (Maputo W) are located along the more industrial northern side of the estuary. Whereas the other sites are located in less developed areas along southern side (Catembe) and at the bottom of the estuary (Matola and Tembe rivers) (see Fig. 1 for location details).

estimating the risk also in Maputo Bay. In contrast to the detected concentrations (where concentrations were sometimes higher in October as compared to March) a strong seasonal variability in risk was demonstrated (Table 3). Not a single sample taken in October had a higher risk from its detected mixture as compared to the mixture from the same site in March. I addition, the environmental thresholds were exceeded at four out of five nursery areas in March, but at none of those five locations in October. This is most likely due to the application of the detected pesticides on crops prior to the wet season combined with higher water flows due to the heavy rain during the season. At the sites Infulene River and Matola River in Espírito Santo estuary, and in the Bembe area all exceedances were from herbicides, suggesting algae and macrophytes in the area may be adversely affected. Although the herbicides may not have a direct impact on penaeid shrimp, they may have indirect effects by affecting their food sources. Recent stable isotope studies of *P. indicus* and *M. monoceros* in Maputo Bay have shown that they mainly feed on the surface sediment, including benthic microalgae (de Abreu et al., 2017). It also shows that their densities show a positive correlation with microalgae in the sediment in several nursery areas (de Abreu et al., 2017) indicating a dependence on microalgae for food.

In contrast, one fresh water sample taken in the Infulene River had concentrations of the insecticide malathion that exceeded the threshold value more than 500 times. The threshold for malathion is based on a reproduction assay with *Daphnia magna* (NOEC = 60 ng L^{-1}; EFSA, 2009) while the detected concentration was 3405 ng L^{-1}. The currently suggested maximum environmental concentration in near field situations is 5000 ng L^{-1} (EFSA, 2009) as this should allow the aquatic invertebrate population to recover, even after repeated exposures. However, 34 different species, primarily crustaceans (19) and insects (11), but also fish (3) and one amphibian have reported LC50's below the detected concentrations (US EPA, 2017). In order to assess the risk specifically towards local penaeid shrimp a dedicated and specific laboratory assay would have to be performed.

The limit of detection of malathion, cypermethrin, pirimiphos-methyl and diazinon exceeds their marine environmental thresholds by a factor 830, 533, 10 and 1.25 respectively. These four compounds are thus not shown to be at safe concentration, even if they are not found. This is a common problem when performing chemical risk assessment on environmental samples, especially for chemicals which by design are bioactive and consequently have low environmental thresholds (Gustavsson, et al., 2017). This problem clearly demonstrates the strength of using several complementary approaches, such as a combination of chemical analysis and biomonitoring, when assessing the chemical risk in the environment.

Earlier studies have found elevated levels of metals in the sediment in the northern part of Espírito Santo estuary, close to Infulene River (Scarlet, 2015). However, in the present study we found no indication of elevated levels of metals in the muscle tissue of shrimp from this site, or from any of the other studied nursery area in Maputo Bay in March. The levels were relatively low compared to similar studies conducted in the Mediterranean Sea (Gokoglu et al., 2008). The risk that metals pose in the environment is difficult to determine from tissue residues, as metals can be stored in organisms in several non-toxic forms. Bioconcentration can therefore occur over prolonged periods of time with the metals being stored in for instance granules or metallothionein complexes (Luoma, 2008). The risk towards consumers is also hard to derive, as metals may be present in different oxidative states, as well as both in inorganic and organic forms (Bosch et al., 2016). Nevertheless, the European food safety authority has published thresholds for seven out of the ten metals analysed in shrimp tissue (Table S4 in S.I.). Out of these seven, only arsenic (As) would potentially exceed its given threshold under non-

excessive consumption. Subsequent studies where the speciation of As is determined in shrimp tissue would be needed to make a definitive risk assessment.

Although no OP pesticides were detected in the water samples from the 4 nursery areas in Maputo Bay, significantly lower AChE activity was detected in both *P. indicus* and *M. monoceros* shrimp collected in March at the mouth of Infulene River in the northern part of Espírito Santo estuary. This suggests that chemicals with similar modes of action, probably OPs, are present in the water, either below limits of detection or not analysed, leading to the biological effect observed (Albendin et al., 2017). The fact that AChE inhibition was observed in both shrimp species studied strengthen this hypothesis. This once again shows the need of combining biological effect studies with chemical analysis for risk assessment. Unfortunately, no samples for chemical analysis were collected in Infulene River in March, however high levels of the OP malathion were detected in the Infulene River at the later sampling in October, indicating that OPs are used in the intensive agriculture surrounding the river. This result is consistent with an earlier study that showed lower AChE activities in freshwater fish, and mangrove fish, shrimps and clams in this area compared to reference. This coincided with the detection of several OPs in the water of the Infulene River (Sturve et al., 2016). AChE is a well-established biomarker for OP compounds and used for decades in monitoring programs (van der Oost et al., 2003) but little information has been available regarding AChE activities in tropical regions. In October, no trend of lower AChE activities were seen in shrimp at Infulene River compared to the less urbanized Bembe area in the present study, despite the fact that malathion was detected in the Infulene River at the same time. AChE activity is affected by OP compounds and it is likely that higher levels of OPs such as malathion would have been present during the wet season due to the higher transport of pesticides from the surrounding crop fields. It has also been shown that AChE activity can be inhibited by metals (Delalli et al., 2021). However, metal analysis in the shrimp showed low levels and it is not likely that they are causing the observed decrease in AChE activity in shrimps from the Infulene area.

Glutathione *S*-transferase has been proposed as a biomarker for polyaromatic hydrocarbons, a group of compounds often found in areas with high shipping activities, in both aquatic vertebrates (van der Oost et al., 2003; Sturve et al., 2005) as well as in invertebrates (Turja et al., 2020). Despite Espírito Santo estuary being affected by shipping activities, no differences were observed in GST activities in the shrimp from the different estuaries. This suggests that, despite the high shipping activities, levels of pollutant were not high enough to affect this biomarker.

Densities of juvenile shrimp were very low in the Infulene area in Espírito Santo in March, >25× lower than in Matola River area, where no inhibition in AChE activity was observed in the shrimp (Fig. 2). Although there could be many other reasons than pollutants for the low levels of shrimp in the highly exploited and heavily populated northern part of the estuary, this results warrants further studies to assess the potential impact of pesticides on the benthic community in this estuary. A recent study assessing natural factors affecting shrimp densities in Maputo Bay did not find any correlation between distance to the closest mangrove habitat and the densities of shrimp in Espírito Santo (de Abreu, 2017). This suggests that the low abundance of mangroves in the northern part of the estuary (de Boer, 2002) is not the direct explanation of low shrimp densities in this area. Even if earlier studies (Sturve et al., 2016) and the present results indicate that pesticides to some degree may contaminate the northern part of Espírito Santo estuary, and that the sediment in the same area is also polluted by metals (Scarlet, 2015), densities of shrimp were very high in other parts of the estuary. In fact, recent studies show that despite the apparent high level of anthropogenic impact, the Espírito Santo estuary has the highest densities of juvenile penaeid shrimp of all assessed estuaries in Maputo Bay, and constitutes an important nursery area for the commercial fishery of *P. indicus* and *M. monoceros* in the Bay (de Abreu, 2017). Thus, it is important not to view this estuary as a "lost cause" for conservation, but to improve the regulation of pollutants and the overall management of this highly productive ecosystem.

5. Conclusions

The present study investigates the levels of pesticides and metals, and possible effects of OPs on shrimp in four nursery areas in Maputo Bay, Mozambique. Analyses of metals in shrimp showed no indication of elevated levels in Maputo Bay, whereas chemical analyses show that levels for several herbicides in the water exceed environmental threshold in three of the four assessed nursery areas. This might have indirect effects on shrimp population by affecting their food sources. Biomarker results showed decreased AChE activities in shrimp from the northern part of Espírito Santo estuary during the wet season, indicating an impact from OP pesticides. The absence of detected OP pesticides in the water samples, despite finding decreased AChE activities, demonstrate the value of using both methods in a complementary fashion when trying to find the root causes of environmental impacts. Although the study only detected localized effects of contaminants on shrimp, the results highlight a need to improve the regulation and control of pollutants in the Espírito Santo estuary to maintain the high production of shrimp in this important nursery area.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112964.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was partially funded by the Swedish Cooperation Agency (SIDA) through the bilateral program with Eduardo Mondlane University (Mozambique), to which we are very grateful. The project was also funded by the SIDA Uforsk grant 2013-06279. We also acknowledge Emilia Sturve that helped with water extractions.

References

Albendin, G., Arellano, J.M., Manuel-Vez, M.P., Sarasquete, C., Arufe, M.I., 2017. Characterization and in vitro sensitivity of cholinesterases of gilthead seabream (Sparus aurata) to organophosphate pesticides. Fish Physiol. and Biochem. 43 (2), 455–464.

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. 46, 2564–2573.

Belden, J.B., Gilliom, R.J., Lydy, M.J., 2007. How well can we predict the toxicity of pesticide mixtures to aquatic life? Integr. Environ. Assess. Manag. 3, 364–372.

Bocquené, G., Galgani, F., 1998. Biological effects of contaminants: cholinesterase inhibition by organophosphate and carbamate compounds. ICES Tech. Mar. Environ. Sci. 22.

Bosch, A.C., O'Neill, B., Sigge, G.O., Kerwath, S.E., Hoffman, L.C., 2016. Heavy metals in marine fish meat and consumer health: a review. J. Sci. Food Agric. 96, 32–48.

Bopp, S.K., Kienzler, A., Linden, S.van der, Lamon, L., Paini, A., Parissis, N., Richarz, A. N., Triebe, J., Worth, A., 2016. Review of Case Studies on the Human and Environmental Risk Assessment of Chemical Mixtures. EUR 27968 EN (2016).

Cedergreen, N., 2014. Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS One 9 (5), e96580.

Chiarelli, R., Roccheri, M.C., 2014. Marine invertebrates as bioindicators of heavy metal pollution. Open J. Met. 4, 93–106.

CIRCABC, 2017. Communication and Information Resource Centre for Administrations, Businesses and Citizens. https://circabc.europa.eu/w/browse/b55f4c81-d664-43db-8b27-264b26a7424b.

Daam, M.A., Van Den Brink, P.J., 2010. Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. Ecotoxicology 19 (1), 24–37.

Day, J.W.D., Crump, B.C., Kemp, W.M., Yáñez-Arancibia, A., 2013. In: Estuarine Ecology, Second edition. Wiley-Blackwell, New Jersey USA, p. 550.

de Abreu, D.C., 2017. Evaluation of Suitable Nursery Areas for Penaeid Shrimps in Shallow Water Systems in Southern Mozambique. University of Gothenburg, Gothenburg, Sweden. PhD Thesis.

de Abreu, D.C., Paula, J., Macia, A., 2017. Tropical seascapes as feeding grounds for juvenile penaeid shrimps in southern Mozambique revealed using stable isotopes. estuarine. coastal and shelf science. Estuar Coast Shelf Sci. 198, 21–28.
de Boer, W.F., 2002. The rise and fall of the mangrove forests in Maputo Bay, Mozambique. Wetl. Ecol. Manag. 10, 313–322.
Dellali, M., Douggui, A., Harrath, A.H., Mansour, L., Alwasel, S., Beyrem, H., Gyedo-Ababio, T., Rohl-Lupher, M., Boufahja, F., 2021. Acute toxicity and biomarker responses in Gammarus locusta amphipods exposed to copper, cadmium, and the organochlorine insecticide dieldrin. Environ. Sci. Pollut. Res. 1–12.
ECHA, 2008. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.10: Characterization of Dose [Concentration]-Response for Environment.
EFSA, 2009. European food safety authority. Sci. Rep. 333, 1–118 (2009).
EFSA, 2013. Guidance on Tiered Risk Assessment for Edge of Field Surface Waters.
Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7 (2), 88–95.
FAO, 2007. Fishery and Aquaculture Country Profile – Statistic, Indicators and Narrative – Republic of Mozambique.
Forbes, V.E., Calow, P., 2002. Applying weight-of-evidence in retrospective ecological risk assessment when quantitative data are limited. Hum. Ecol. Risk Assess. 8 (7), 1625–1639.
Fulton, M.H., Key, P.B., 2001. Annual review, acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. Chem. 20 (1), 37–45.
Galgani, F., Bocquene, G., Cadiou, Y., 1992. Evidence of variation in cholinesterase activity in fish along a pollution gradient in the North Sea. Mar. Ecol. Prog. Ser. 91 (1–3), 77–82.
Gokoglu, N., Yerlikaya, P., Gokoglu, M., 2008. Trace elements in edible tissues of three shrimp species (Penaeus semisulcatus, Parapenaeus longirostris and paleomon serratus). J. Sci. Food Agric. 88 (2), 175–178.
Grube, A., Donaldson, D., Kiely, T., Wu, L., 2011. Pesticides Industry Sales and Usage 2006 and 2007 Market Estimates. U.S. Report for the Environmental Protection Agency, Washington, DC.
Gustavsson, M., Kreuger, J., Bundschuh, M., Backhaus, T., 2017. Pesticide mixtures in the swedish streams: environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation. Sci. Total Environ. 598, 973–983.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139.
Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, third edition. Oxford University Press, Oxford.
Jeannot, R., Sabik, H., Sauvard, E., Genin, E., 2000. Application of liquid chromatography with mass spectrometry combined with photodiode array detection and tandem mass spectrometry for monitoring pesticides in surface waters. J. Chromatogr. A 879, 51–71.
Jordaan, M.S., Reinecke, S.A., Reinecke, A.J., 2013. Biomarker responses and morphological effects in juvenile tilapia Oreochromis mossambicus following sequential exposure to the organophosphate azinphos-methyl. Aquat. Toxicol. 144, 133–140.
Key, P., Chung, K., Siewicki, T., 2007. Toxicity of three pesticides individually and in mixture to larval grass shrimp (*Palaemonetes pugio*). Ecotoxicol. Environ. Saf. 68 (2), 272–277.
Khan, M.B., Masiol, M., Hofer, A., Pavoni, B., 2014. Harmful elements in estuarine and coastal systems. In: Bini, C., Bech, J. (Eds.), PHEs, Environment and Human Health. Springer. https://doi.org/10.1007/978-94-017-8965-3_2.
Kortenkamp, A., Backhaus, T., Faust, M., 2009. State of the Art Report on Mixture Toxicity. Report Contract Number 070307/2007/485103/ETU/D.1.
Kwok, K.W., Leung, K.M., Lui, G.S., Chu, V.K., Lam, P.K., Morritt, D., Crane, M., 2007. Comparison of tropical and temperate freshwater animal species' acute sensitivities to chemicals: implications for deriving safe extrapolation factors. Integr. Environ. Assess. Manag. 3 (1), 49.
Lopes, I., Moreira-Santos, M., da Silva, E.M., Sousa, J.P., Guilhermino, L., Soares, A.M.V. M., Ribeiro, R., 2007. In situ assays with tropical cladocerans to evaluate edge-of-field pesticide runoff toxicity. Chemosphere 67 (11), 2250–2256.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.
Luoma, Rainbow, 2008. Metal Contamination in Aquatic Environments: Science and Lateral Management. ISBN-13: 978-0521279017.
Marsden, I.D., Rainbow, P.S., 2004. Does the accumulation of trace metals in crustaceans affect their ecology - the amphipod example? J. Exp. Mar. Biol. Ecol. 300 (1–2), 373–408.
Monserrat, J.M., Martínez, P.E., Geracitano, L.A., Amado, L.L., Martins, C.M.G., Pinho, G. L.L., Bianchini, A., 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 146 (1), 221–234.
National Fishery Research Institute, IIP, 2013. Relatório anual 2013. Maputo, Moçambique. IIP (Instituto Nacional de Investigação Pesqueira) – National Report in Portuguese.
Price, P.S., Han, X., 2011. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. Int. J. Environ. Res. Public Health 8 (6), 2212–2225.
Rodney, S.I., Teed, R.S., Moore, D.R.J., 2013. Estimating the toxicity of pesticide mixtures to aquatic organisms: a review. Hum. Ecol. Risk Assess. 19, 1557–1575.
Samucidine, K., Leong, E., Nrepo, M., Barros, P., 2015. Estado do stock de camarão e da pescaria na Baía de Maputo – Moçambique em 2015. Relatório Interno de Investigação Pesqueira, N°34. Instituto Nacional de Investigação Pesqueira, Maputo. Mozambique, 30 pp.
Sanchez-Bayo, F., Hyne, R.V., 2011. Comparison of environmental risks of pesticides between tropical and nontropical regions. Integr. Environ. Assess. Manag. 7 (4), 577–586.
Scarlet, M.P.J., 2015. Biomarkers for Assessing Benthic Pollution Impacts in a Subtropical Estuary, Mozambique. University of Gothenburg, Gothenburg, Sweden.
Scarlet, M.P., Bandeira, S., 2014. Pollution in Maputo Bay. In: Bandeira, S., Paula, J. (Eds.), The Maputo Bay Ecosystem. WIOMSA, Zanzibar Town, pp. 347–371.
Silva, E., Masquine, R.Z., 2014. Shallow-water shrimp fisheries in Maputo Bay. In: Bandeira, S., Paula, J. (Eds.), The Maputo Bay Ecosystem. WIOMSA, Zanzibar Town, pp. 277–283.
Stephensen, E., Svavarsson, J., Sturve, J., Ericson, G., Adolfsson-Erici, M., Förlin, L., 2000. Biochemical indicators of pollution exposure in shorthorn sculpin (Myoxocephalus scorpius), caught in four harbours on the southwest coast of Iceland. Aquat. Toxicol. 48 (4), 431–442.
Sturve, J., Berglund, Å., Balk, L., Broeg, K., Böhmert, B., Massey, S., Savva, D., Parkkonen, J., Stephensen, E., Koehler, A., Förlin, L., 2005. Effects of dredging in Göteborg harbor, Sweden, assessed by biomarkers in eelpout (Zoarces viviparus). Environ. Toxicol. Chem. 24 (8), 1951–1961.
Sturve, J., Scarlet, P., Halling, M., Kreuger, J., Macia, A., 2016. Environmental monitoring of pesticide exposure and effects on mangrove aquatic organisms of Mozambique. Mar. Environ. Res. 121, 9–19.
Turja, R., Sanni, S., Stankevičiūtė, M., Butrimavičienė, L., Devier, M.H., Budzinski, H., Lehtonen, K.K., 2020. Biomarker responses and accumulation of polycyclic aromatic hydrocarbons in Mytilus trossulus and Gammarus oceanicus during exposure to crude oil. Environ. Sci. Pollut. Res. Int. 27 (13), 15498.
US EPA, 2017. United States Environmental Protection Agency. Ecotoxicol. Knowledgebase. https://cfpub.epa.gov/ecotox/.
van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Phar. 13 (2), 57–149.