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Methylmercury, Trace Metals, Organotins and Their Effects on the Qatari Mangrove Shrimp, *Palaemon khori*

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Abstract: The Qatari mangroves of Al-Khor are being increasingly exposed to a wide variety of anthropogenic pollutants due to land reclamation and urban expansion. In this study, we evaluated the lethal and genotoxic effects of methylmercury, trace metals, and organotins, assessing mortality and aneuploidy levels (abnormal number of chromosomes) in the endemic shrimp *Palaemon khori* under laboratory conditions. In the experimental design, two different concentrations were used for each family of contaminant (single or combined): an environmental concentration equivalent to the maximum value reported in the environment and a value ten times higher, for a period of eight weeks. Survival decreased significantly when pollutants were administrated in combination, even at environmental concentrations (as shown by Cox proportional hazards ratios): similar levels of mortality would be reached by individual type of pollutants only at ten times the environmental concentration. This critical result, under controlled lab conditions, highlights the importance of monitoring mixtures of contaminant types over single ones in the marine environment. Aneuploidy was reported in all treatments and control ranging from 5% to 19% at week four and from 7% to 21% at week eight. All treatments presented significantly higher aneuploidy levels when compared to the control. However, no significant difference was observed between the two time periods, even though 30% of the treatments could not be assessed at week eight, as not enough animals were still alive. In conclusion, the use of endemic species should be considered a valuable tool to determine local perturbations, representing a regional bioindicator of multiple environmental stressors from the initial stages of contamination.

Keywords: aneuploidy; environmental pollution levels; genotoxicity; methylmercury; organotins; *Palaemon khori*; survival; trace metals

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1. Introduction

Anthropogenic activities constitute an increasing source of pollution to water and sediment in coastal areas worldwide [1,2]. The Arabian Gulf (also known as the Persian Gulf) is a particularly impacted area [1,3], with Qatar facing substantial threats to its marine ecosystem from industrial pollution and large coastal modifications to accommodate industrial facilities and urban housing [4–6]. A significant percentage of the contaminants introduced into the marine environment nowadays can be potentially genotoxic, mutagenic or carcinogenic [7,8]. These compounds can alter the integrity and functionality of genetic material at non-cytotoxic and non-lethal concentrations [9] and can lead to delayed detrimental effects at the individual and the population level [10]. Presence of genotoxic contaminants, such as methylmercury, trace metals and organotins have been previously reported in the

Arabian Gulf [5,11] and coastal surrounding countries, including Kuwait [12], Iran [13] and Qatar [5,14,15].

Methylmercury (MeHg) enters the marine environment through various pathways, but mainly through atmospheric deposition [16]. Its toxicity arises from its persistence and its tendency to bioaccumulate and bio-transfer in the food chain with potentially high concentrations in invertebrates and carnivorous fish [17,18]. In the Arabian Gulf, values of MeHg ranging from 0.112 to 0.865 mg/kg have been reported in tissues of various crustacean species in different locations [19], sometimes exceeding the maximum recommended level of 0.5 mg/kg wet weight [20]. In Qatar, concentrations of MeHg in local commercial fish species, such as *Lethrinus nebulosus*, were found to range from 0.064 to 0.871 mg/kg [21]. Methylmercury may complex with other chemical groups within the cells leading to DNA damage through free radical formation [22] and also produce chromosomal aberrations [23]: it has indeed been previously recognized as a genotoxic contaminant in aquatic organisms (e.g., [24]). Within marine animals, there has been genotoxicity studies on dolphins and fish [25,26] among others, with less data available on invertebrates. Recently, Leitão and colleagues [27] assessed the effect of some selected contaminants on the pearl oyster *Pinctada radiata* and reported Hg as being highly positively correlated to aneuploidy (abnormal number of chromosomes).

Most coastal areas and seas do contain either a high or limited amount of naturally occurring trace metals (TM), especially in the sediments [5]. Nevertheless, even at low concentrations their cationic forms are dangerous to living organisms because of their capacity to bind with short carbon chains and to bioaccumulate in protein-rich tissues [5,28]. Trace metals may indeed interfere with cellular metabolic functions causing harmful side effects [28,29]: their toxicity arises not only from the level of contamination but also from the biochemical role they play in the metabolic processes, as well as the extent to which they are absorbed and excreted [30]. Depending on the metal, these contaminants might not exert a strong impact individually, but when combined, their negative effects may be magnified [31,32], but also may be reduced (as in the case of the antagonistic effects of silver and copper in jellyfish [33]). The genotoxic effect of TM on marine invertebrates has been previously reported. Studies on the effect of TM on the cell aneuploidy in the mussels *Anodonta cygnea*, *Unio tumidus* and the gastropod *Viviparus viviparus* showed high levels of polyploid cells (42%) resulting from contamination by Cd, V, Cr, Ni, Cu, Mn, Zn, Fe, Sr and Hg [34]. Similarly, studies on the consequences of TM contamination on the oyster *Crassostrea angulata* using genotoxic endpoints revealed that marine contamination by Cd, Fe and Zn was linked to higher genetic abnormalities [35]. More recently, the genotoxic effect of trace metals on the Manila clam *Ruditapes philippinarum* was investigated and results revealed a strong correlation between aneuploidy and TM sediment contamination [36]. Other organisms for which such a correlation was observed include the bivalve *Mytilus edulis* [8], the pearl oyster *P. radiata* [37] and the barnacle *Balanus improvisus* [27].

Maritime activities have, in the past, also lead to the introduction of organotins (OT) into the marine environment until their ban in the 1980s (89/677/CEE). Organotins were used as wood protecting paints, disinfectants and biocides (especially in marine anti-fouling paints [38]), thus spreading worldwide from coastal zones to the open seas. This group of contaminants has a slow breakdown rate and, similar to MeHg and TM, also persists in the marine environment and bioaccumulate [39,40]. Organotin compounds, such as tributyltin (TBT) and its degraded products monobutyltin (MBT) and dibutyltin (DBT), can cause DNA damage, double-strand breaks, base damage and intra-strand crosslinks [41–43]. Their genotoxic effect has previously been reported in marine invertebrates, e.g., in the marine worm *Platynereis dumerilii*, and has been found to be dose-dependent with exposure [44]. Organotins have a high selectivity regarding their mode of action and have been classified as having neuro-, cyto- and genotoxic effects on several biological models such as the tunicate *Styela plicata* [45,46]. Specific reports on their genotoxicity are, however, rare compared to the reports on their other effects. The effect of TBT and triphenyltin (TPT) has

been investigated in the model fish *Danio rerio* revealing that chronic exposure to low levels of TBT, TPT and binary mixtures of TBT are genotoxic to zebrafish [47].

There is also now a scientific consensus that the assessment of marine ecosystems' health and the design of measures to enhance environmental quality should be performed using a combined approach, incorporating both chemical and biological measurements, in key sentinel species (e.g., [48,49]). Cytogenetic parameters and atypical cytogenetic features, such as aneuploidy, have shown their relevance as endpoint indicators for assessing marine environmental genotoxicity in different marine and invertebrate species, e.g., mussels *M. edulis* [50], the pearl oyster *P. imbricata radiata* [37] and the Manila clam *R. philippinarum* [36]. Moreover, in the Pacific oyster, *Magallana gigas* aneuploidy has been well documented [51,52], together with the negative relationship between aneuploidy and growth rate [53] and the persistence of aneuploidy levels, within and between generations [51,53,54].

A wide variety of marine organisms have been used as biomarkers to assess the state of the environment in which they reside [48]. The utilization of endemic species as biomarkers for local environments can offer a regional specific indicator of environmental stressors exclusive to that site [37,55]. Moreover, analyzing the impact of various pollutants that can biologically affect endemic species can give a better understanding and offer more effective means of monitoring and maintaining the integrity of local mangroves [56]. The objective of this study was to evaluate the mortality rate and the genotoxic impact (at the chromosomal level, i.e., aneuploidy) of MeHg, TM (Cr, Mn, Cd, V and Pb) and OT compounds (MBT, DBT and TBT) on the endemic shrimp *P. khori*, treated with different concentrations (environmental and ten times higher), individually (same type of contaminant) or in combination (more than one type of contaminant), over a time period of eight weeks.

2. Materials and Methods

2.1. Sample Collection

Live specimens (n = 750) of the shrimp *P. khori* (average size: 300 mm total length; Figure 1) were collected in early April 2016 at low tide from Al-Khor mangroves (25.690020° N, 51.55572° E; Figure 2), using fine nets in shallow water to facilitate the collection of specimens. Water temperature at the site varied slightly from 27 °C to 29 °C. Salinity values showed little variation between 42 ppt to 43 ppt and the pH was steady at 7.9. Immediately after collection, specimens were stored in ice boxes filled with aerated fresh seawater from the sampling sites and brought to the Environmental Science Center (ESC), Qatar University, where they were transferred to a large tank containing aerated seawater.

2.2. Experimental Design

Twenty-five small tanks (5 L) were prepared, each filled with 3 L of seawater, gently aerated, constantly filtered by activated carbon filters and maintained at a constant temperature of 22 °C and pH 8.01 (these physicochemical parameters were checked every three days). Each tank was dosed with a selected type of contaminant/combination of multiple types of contaminants (©Sigma Aldrich, Rockville, MD, USA) belonging to the three main group of aquatic pollutants (MeHg, TM and OT) known to be present in the area [49] at set concentrations (µg/L; Table 1): the highest concentration found in seawater obtained from the literature [Env] and a concentration ten times higher [10*Env]. To ensure that the concentration within the tank was constant (not affected by the activated carbon filters and evaporation), each tank was tested every three days to monitor the concentration of the pollutants. The concentration only needed to be adjusted to maintain the initial dosage after six weeks from the beginning of the experiment. There were two replicate tanks for each treatment (referred to as experiment 1 and experiment 2). Only one tank with natural seawater was used as control, in the attempt to reduce the number of animals removed from their natural environment, following ethical practice in the use of animals for research and assuming natural mortality in this tank would have been lower than in the treatments. After eight days of acclimation, 30 shrimp were placed in each 5 L tank and fed

with goldfish flakes (ca. 500 mg) every three days for the full duration of the experiment. The experiment was run for a total of eight weeks to mimic a long-term exposure to the different treatments. The exposure time of eight weeks was chosen from the literature regarding aneuploidy [57,58]

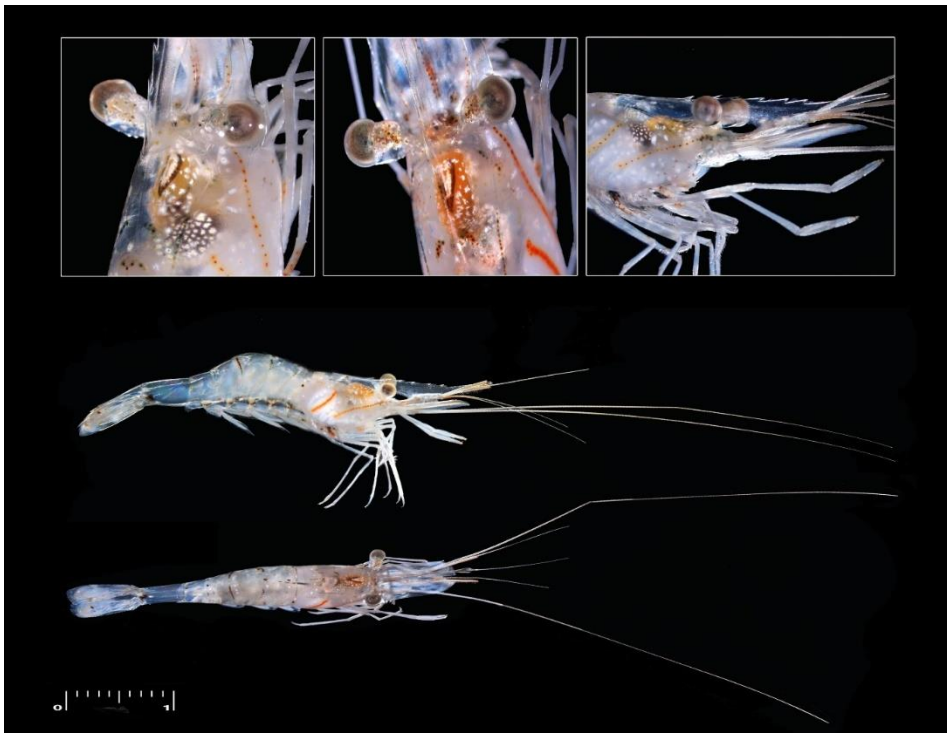


Figure 1. The endemic palaemonid shrimp *Palaemon khori* (Picture courtesy of the Environmental Science Center photographic section, University of Qatar). Scale: 1 cm.

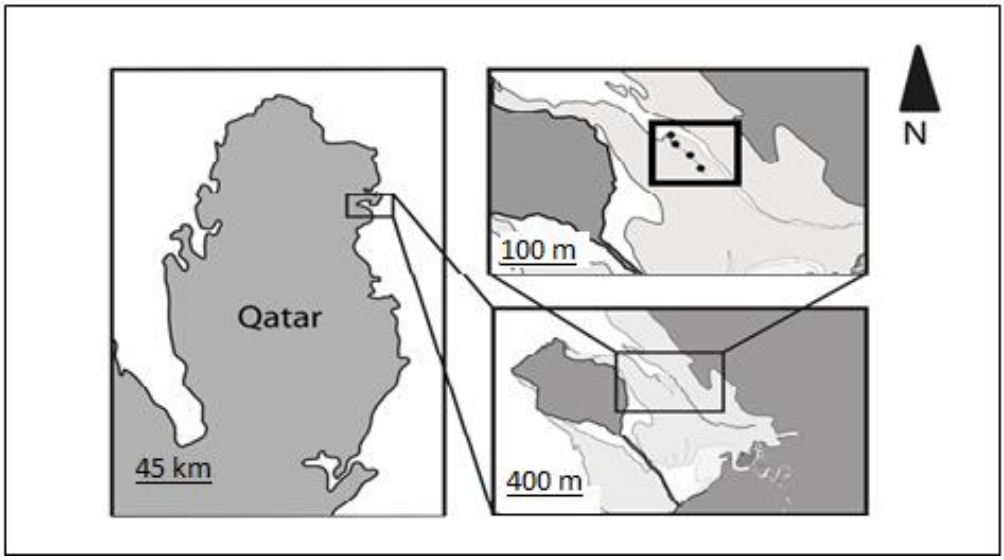


Figure 2. Sampling site: Al-Khor, Qatar. Dark grey areas indicate land, light grey areas mangroves and white areas water. The thick rectangular box represents the sampling area, with dark circles indicating the sampling locations.

2.3. Mortality and Aneuploidy Scoring

Mortality was checked daily in all tanks, and any dead shrimp were removed. Ten individual shrimp (when available) were sampled from each tank, at four (T_4) and eight

weeks (T_8) after initial dosing for aneuploidy evaluation. The specimens were submerged for 4 h in an aerated 0.09% solution of colchicine in seawater and kept at room temperature (23 °C–26 °C). The gonads were then dissected and subjected to a hypotonic treatment for 45 min in 0.9% solution of sodium citrate and fixed in a freshly prepared mixture of absolute ethanol and glacial acetic acid (3:1), following an established procedure [59]. Slides were prepared for each individual sample, according to the air drying technique of Thiriot-Quiévreux and Ayraud [60], and stained with Giemsa (4%, pH 6.8) for 15 min. Chromosome counts were made on apparently intact metaphases, by microscope observation (Nikon Eclipse E400 with incorporated Nikon DS-Fi1 image acquisition camera). The same observer to reduce the subjectivity associated with different observers performed the chromosome counting. *Palaemon khori* normally present 48 chromosomal pairs ($2n = 96$), 26 metacentric, 7 sub metacentric, 12 sub telocentric and 3 telocentric [59]. The level of aneuploidy was estimated by counting the total number of aneuploid metaphases over the total number of metaphases scored per tank.

Table 1. Concentration (µg/L) of contaminants added to each tank. MeHg = Methylmercury; OT = Organotins (MBT = Monobutyltin; DBT = Dibutyltin; TBT = Tributyltin); TM = Trace metals. Env = environmental concentration; 10*Env = 10 times environmental concentration. Single = single type of contaminant; Combined = combination of different types of contaminants, – = no contaminants added.

Tank	Treatment	Contaminants [µg/L]	Code
1	Control	–	Control
2 & 14	[MeHg]	MeHg [5.55] [60]	EnvSingle
3 & 15	[10*MeHg]	MeHg [55.50]	10*EnvSingle
4 & 16	[OT]	(MBT [2.75] + DBT [2.75] + TBT [2.75]) [61]	EnvSingle
5 & 17	[10*OT]	MBT [27.50] + DBT [27.50] + TBT [27.50]	10*EnvSingle
6 & 18	[TM]	(Cr [333] + Mn [316] + Cd [170] + V [8] + Pb [10]) [62–65]	EnvSingle
7 & 19	[10*TM]	Cr [3330] + Mn [3160] + Cd [1700] + V [80] + Pb [100]	10*EnvSingle
8 & 20	[TM + MeHg]	Cr [333] + Mn [316] + Cd [170] + V [8] + Pb [10] + MeHg [5.55]	EnvCombined
9 & 21	[10*TM + MeHg]	Cr [3330] + Mn [3160] + Cd [1700] + V [80] + Pb [100] + MeHg [55.50]	10*EnvCombined
10 & 22	[TM + OT]	Cr [333] + Mn [316] + Cd [170] + V [8] + Pb [10] + MBT [2.75] + DBT [2.75] + TBT [2.75]	EnvCombined
11 & 23	[10*TM + OT]	Cr [3330] + Mn [3160] + Cd [1700] + V [80] + Pb [100] + MBT [2.75] + DBT [2.75] + TBT [2.75]	10*EnvCombined
12 & 24	[MeHg + OT]	MeHg [5.55] + MBT [2.75] + DBT [2.75] + TBT [2.75]	EnvCombined
13 & 25	[10*MeHg + OT]	MeHg [55.5] + MBT [2.75] + DBT [2.75] + TBT [2.75]	10*EnvCombined

2.4. Statistical Analysis

All analyses were performed using R 3.6.3 created by R Core Team, Pen. State [61]. Shrimp survival data from two replicated experiments with different treatments (Table 1) were compared with Kaplan–Meier curves [62,63], using log-rank statistics, pairwise tests and Cox proportional hazards ratios (survival package; survminer package [64]). The latter were based on the Akaike information criterion (AIC) and Concordance Index (from 0.5, random prediction to 1.0, perfect concordance) applied to different levels (control; EnvSingle: single type of contaminant at environmental concentration; 10*EnvSingle: single type of contaminant at ten times the environmental concentration; EnvCombined: two combined contaminants at environmental concentration; 10*EnvCombined: two combined contaminants at ten times the environmental concentration). Hazard ratios were visualized with forest plots. We used a censored model, as we removed ten animals at T_4, which we do not know if they would have survived, and we stopped the experiment at T_8 (surviving individuals were not followed any longer).

Average aneuploid levels could not be calculated for [10*TM], [TM + MeHg], [10*TM + MeHg] and [10*TM + OT] as not enough animals remained alive at T_8 to allow for sampling. Aneuploidy ratios (rounded to percentage) were tested for significant differences among treatments (including the control) and time (T_4 vs. T_8) for the remaining treatments fitting a Generalized Linear Models (GLM; [61]) with Poisson distribution and log link function (null deviance/df = 34.48/33 = 1.04).

3. Results

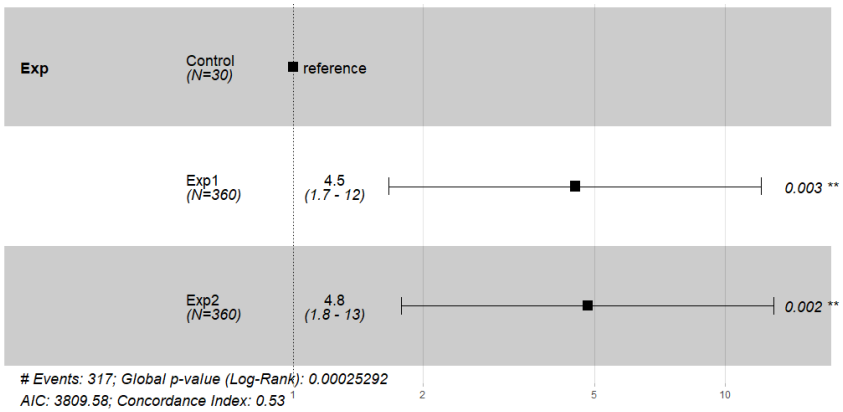
The effects of the different treatments on the shrimp survival varied across contaminant combinations, but as no statistical differences were found between the two replicated experiments (Figure 3A), replicates were combined; separate analyses for experiment 1 and experiment 2 are presented in the Supporting Information section (Figures S1–S4). Overall, survival probability did not change significantly when the shrimp were dosed with a single type of contaminant at the concentration found in the environment (log-rank: $p = 0.11$; Figure 4A). This was sustained by analyzing each specific treatment individually (Figure 3B) or grouping all treatments as a category of a single type of contaminants (EnvSingle, Cox proportional hazards ratios: $p = 0.181$; Figure 3C). Single types of contaminants adversely affected survival when applied at ten times the recorded environmental concentration (log-rank: $p = 0.00015$; Figure 4B; Cox proportional hazards ratios: $p = 0.005$; Figure 3C), with the exception of MeHg (Cox proportional hazards ratios: $p = 0.127$; Figure 3B). A combination of more than one type of contaminant instead significantly reduced survival even at environmental concentration (log-rank: $p = 0.00087$; Figure 3B; Figure 4C; Cox proportional hazards ratios: $p = 0.001$; Figure 3C) and even more so at ten times that concentration (log-rank: $p < 0.0001$; Figure 3B; Figure 4D; Cox proportional hazards ratios: $p < 0.001$; Figure 3C). Pairwise comparisons among treatments on survival, using log-rank tests, are available in the Supporting Information section (Tables S1–S3).

Aneuploid metaphases were observed in all treatments (Table S4/Figure 5) ranging from 5% to 19% at T4 and from 7% to 21% at T8, with the lowest aneuploid levels observed in the control tanks. Please note that in some cases it was not possible to sample ten alive animals at T8 time for aneuploidy analyses (presented as "na" in Table S4). No statistically significant difference was detected in aneuploidy levels between T_4 and T_8 for the same contaminant/contaminant combination (Table S4). All treatments presented significantly higher levels of aneuploidy than the control (Table S4; Figure 4).

Table 2. Estimated parameter, standard error (SE), z-value and *p*-value of the generalized linear model (GLM) with Poisson distribution for aneuploid levels (in percentage) by treatment (see text for details) and duration of the experiment (T_4 vs. T_8). Please note that the GLM was run only with treatments where average level of aneuploidy was calculated in all tanks (thus excluding the ones with not enough individuals surviving at T_8). Asterisks denote different levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	Estimate	SE	z-Value	*p*-Value	
Intercept	1.766	0.292	6.045	1.49×10^{-9}	***
[MeHg]	0.965	0.315	3.064	0.00218	**
[10*MeHg]	1.139	0.311	3.665	0.00025	***
[OT]	0.693	0.323	2.148	0.03174	*
[10*OT]	1.027	0.313	3.275	0.00106	**
[TM]	0.714	0.322	2.216	0.02669	*
[TM+OT]	0.811	0.319	2.541	0.01106	*
[MeHg+OT]	1.112	0.312	3.571	0.00036	***
[10*MeHg+OT]	1.153	0.311	3.711	0.00021	***
T_8	0.050	0.088	0.572	0.56754	

(**A**)



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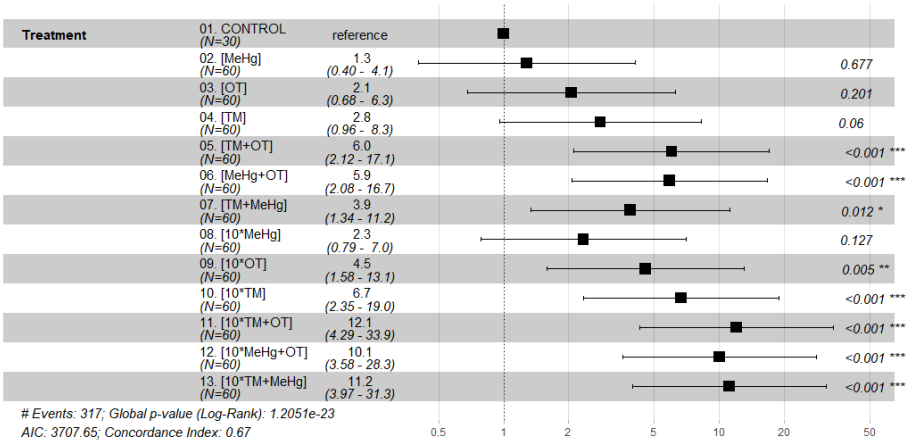

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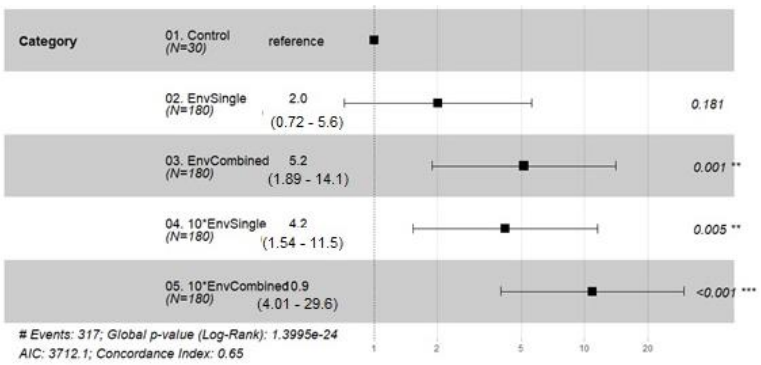

Figure 3. Forest plots of hazard ratios: (**A**) experiments (control, experiment 1 and experiment 2); (**B**) individual treatments for experiment 1 and 2 combined (for actual concentration please refer to Table 1); (**C**) treatments for experiment 1 and 2 combined, grouped in categories (control; EnvSingle: single type of contaminant at environmental concentration; EnvCombined: two combined types of contaminants at environmental concentration; 10*EnvSingle: single type of contaminant at ten times the environmental concentration; 10*EnvCombined: two combined types of contaminant at ten times the environmental concentration). Number of events, global *p*-values and AIC and Concordance Index can be found below each plot. Asterisks denote different levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

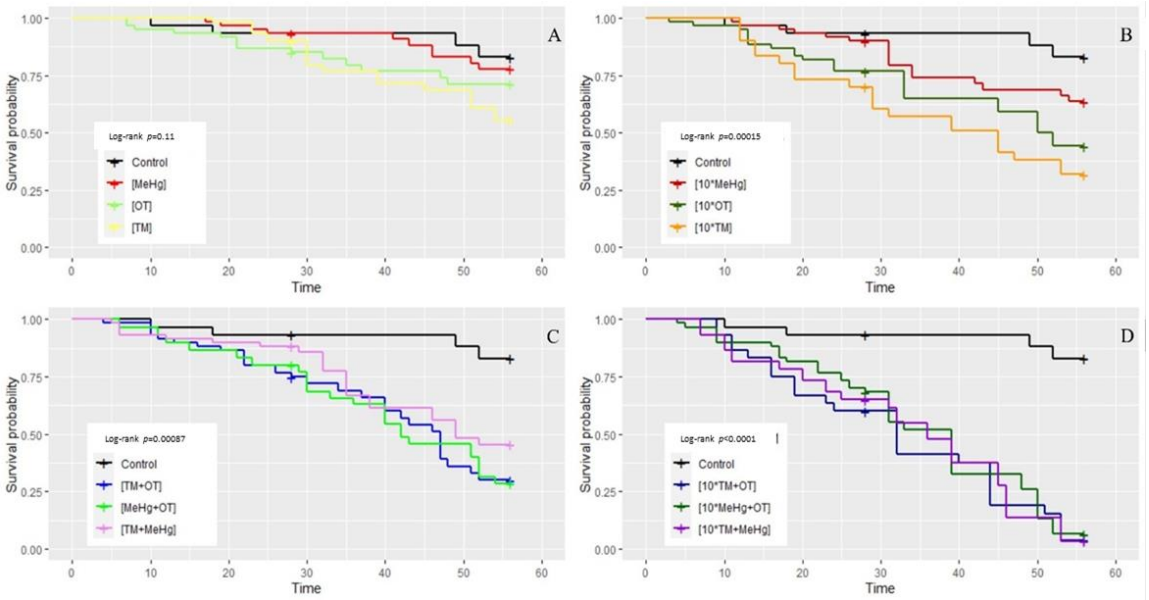

Figure 4. Survival curves of *Palaemon khori* for eight weeks of the study, under different treatments (experiment 1 and 2 combined). Curves are grouped by categories: (**A**) single type of contaminant at reported environmental concentrations; (**B**) single type of contaminant at ten times the reported environmental concentration; (**C**) combination of two types of contaminants at reported environmental concentrations; (**D**) combination of two types of contaminants at ten times the reported environmental concentrations. Refer to the legend for colors. *p* values (log-rank model) are reported in each panel. Values of concentrations can be found in Table 1.

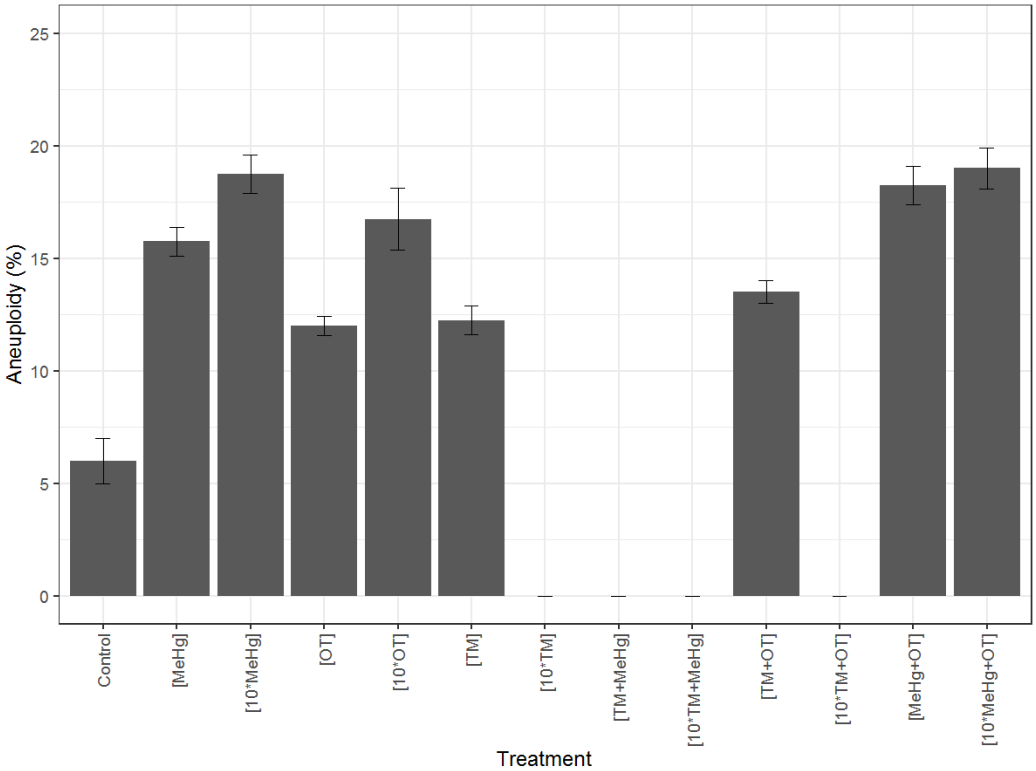

Figure 5. Average percentage of aneuploidy (in ten animals—treatments with not enough individuals are not graphed). These data are the ones analysed with the GLM (Table 2). See Table 1 for detailed information.

4. Discussion

Mangroves are one of the most endangered coastal ecosystems worldwide [48]. The grey or white mangrove Avicennia marina is the most established coastal tree found in Qatar [49]. Large areas of these ecologically important mangroves have recently been uprooted as part of a port development scheme and work is currently underway to restore this damage. These mangroves provide one of the most productive areas of vegetation in a region where the extreme environmental conditions constrain most vegetation growth. An endemic species of palaemonid shrimp, *Palaemon khori* [50], has been described in the *A. marina* mangrove forest at Al-Khor, Qatar. This species represents an important component of the trophic chain within the mangrove-associated faunal community. The use of endemic species can be a valuable tool to determine local perturbations and can offer a region-specific bioindicator of environmental stressors (e.g., [51,52]). Moreover, the analysis of the impact of various contaminants that can biologically affect endemic species will result in a better understanding and more effective means of monitoring and maintaining the integrity of the local ecosystem [53].

One of the major concerns for the conservation of marine organisms is the introduction of further contaminants into their ecosystem. Some of these contaminants may prompt genetic changes (e.g., [7,8]). With anthropogenic activities continuously increasing and affecting the marine environment, analyses of the genotoxic potential of both individual contaminants and group of contaminants is gradually becoming more relevant [65]. Genotoxins may have high ecotoxicological relevance when chronic exposure to multiple contaminants takes place even at low doses [66], providing an early warning to long-term effects of contaminants [67].

Biomonitoring is a common tool to assess the contamination level of multiple pollutants in the natural environment. Yet, the mortality and genotoxic effects of such a mixture of pollutants remain less often investigated (but see higher toxicity recorded in juvenile white shrimp *Penaeus setiferus* treated simultaneously with MeHg and Cd [68]), or are not conclusive, including synergistic as well as antagonistic effects of pollutants [69]. The results obtained in this study after exposing the endemic mangrove shrimp *P. khori* to various levels of MeHg, TM (Cr, Cd, Mn, Pb and V) and OT compounds (MBT, DBT and TBT) showed that, even at the concentrations reported in the environment, a combination of multiple types of contaminants can significantly reduce survival (see also [70]). In our study, a single type of contaminant reached the same effects only when dosed at ten times those concentrations. This is an important and critical finding, which stresses the known but often disregarded notion that mixtures of contaminants, even at moderate concentrations, might affect individuals more than we might have anticipated and calls for monitoring of multiple contaminants at the same time to be more effective in the detection of the condition of the ecosystem [71,72] and management interventions.

In marine crustaceans, the gills are regarded as the most sensitive of the epithelia and are in direct contact with the water and thus susceptible to harm through passive diffusion of pollutants conducted via the membranes. Sentinel species exposed to trace metals in the water matrix show that pollutants tend to accumulate in gill tissues [73] and this causes both cytological and histochemical injury to the gill epithelium. This results in gill malfunctions and inhibition of oxygen consumption [71,74], explaining the mortalities observed. The data also indicate that the survival probability did not change significantly when the shrimp were dosed with a single type of contaminant at ambient concentrations. However, single types of contaminants adversely affected survival when applied at ten times the ambient concentrations. This is line with previous studies conducted on the pink shrimp *Pandalus montagui*, where researchers a reported a 10% increase in the mortality levels with higher pollutant dosage [75]. Note, however, the reduced survival of shrimp when mercury was one of the pollutants involved in the combination of contaminants. This agrees with previous reports that point to the synergism of the mixture of Hg and other pollutants, as observed by [76] investigating the mussel *Perna viridis*. Mercury has a high affinity for protein binding sites, accounting for the increased toxicity at relatively elevated

concentrations in the environment [77]. The data also mimics that which was observed by [78,79], in which the crustacean *Neomysis integer* was exposed to combinations of metals in concentrations deemed to be individually safe.

Aneuploidy was reported in all treatments, including the control. Aneuploidy is known to be common in marine invertebrate populations, with animals recurrently presenting a low level of aneuploidy in their cells (e.g., [80–82]). In 2007, a scale for aneuploidy levels in the oyster *Magallana gigas* [83] was established, with aneuploidy levels less than 5% being classified as very weak, between 5 and 10% weak, between 10 and 14% normal weak, between 14 and 18% normal high, between 18 and 30% high, and more than 30% very high. In our study, the levels observed in the control situation both at T_4 and T_8 fall into the very weak stage of the above classification, whereas the levels found in the different treatments, at T_4 and T_8, ranged in most cases from normal high to high aneuploidy levels. All treatments presented significantly higher aneuploidy levels when compared to the control, highlighting the genotoxic nature of TM, MeHg and OT on *P. khori*. The genotoxic effect of TM observed in this study had been previously highlighted in several other species of marine invertebrates (e.g., Bolognesi & Hayashi, 2011, Cross & Rebordinos, 2003, Piló et al., 2017, Leitão et al., 2017), including in the Qatari local pearl oysters. However, previous studies on the genotoxicty of methylmercury and organotins in marine invertebrate were scarce. The higher levels of genotoxicity observed in this study were levied by the combined effect of more than one pollutant, highlighting the need of monitoring the mixture of contaminant types over single ones. No significant difference in aneuploidy levels was observed between exposure times, suggesting the reach of a threshold, however these results concern only treatments were comparisons were possible, since at eight weeks, 1/3 of the treatments could not be assessed, as not enough animals were still alive. All the used contaminants influenced the aneuploidy levels and their effect was detected already after four weeks of exposure. Further identification of which chromosomal pairs are being affected by chromosomal loss/gain in the different treatments. i.e., if aneuploidy is random or if it reveals a differential chromosomal susceptibility, would allow a better clarification of the relationship between the different genotoxic agents studied and the aneuploidy phenomenon in this species.

5. Conclusions

This study experimentally evaluated the effects of methylmercury, trace metals and organotins administrated as a single type or in different combinations at environmental or very high dosages on the shrimp *P. khori* during long-term exposure (eight weeks). Our results showed that survival decreased in shrimp exposed to a combination of multiple types of chemicals, even at the concentration reported in the environment. A genotoxic response (significantly higher aneuploidy levels than the control) was recorded in all treatments. This study highlights the importance of regular environmental biomonitoring of multiple genotoxic contaminants within the marine ecosystem, as a mix of pollutants (even at moderate concentrations) can significantly affect survival. Genotoxic effects (increase in the aneuploidy levels) were observed under laboratory conditions after only four weeks of exposure. A combined approach with chemical and biological assessments [50] in local invertebrates [83] used as key sentinel species could be a valuable tool to detect early warnings of pollution in the marine environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse10070843/s1, Figure S1: Forest plots of hazard ratios: A individual treatments for experiment 1 (for actual concentration please refer to Table 1); C treatments for experiment 1 grouped in categories (control; EnvSingle: single contaminant at environmental concentration; 10*EnvSingle: single contaminant at 10 times the environmental concentration; EnvCombined: combined chemicals at environmental concentration; 10*EnvCombined: combined chemicals at 10 times the environmental concentration). Number of events, global *p*-values and AIC and Concordance Index can be found below each plot; Figure S2: Survival curves of *Palaemon khori* for eight weeks of the study, under different treatments (experiment 1). Curves are grouped by

categories: A single contaminant at reported environmental concentrations; B single contaminants at 10 times the reported environmental concentration; C combination of contaminants at reported environmental concentrations; D combination of contaminants at 10 times the reported environmental concentrations. Refer to the legend for colours. *p* values (long-rank model) are reported in each panel. Values of concentrations can be found in Table 1; Figure S3: Forest plots of hazard ratios: A individual treatments for experiment 2 (for actual concentration please refer to Table 1); C treatments for experiment 2 grouped in categories (control; EnvSingle: single contaminant at environmental concentration; 10*EnvSingle: single contaminant at 10 times the environmental concentration; EnvCombined: combined chemicals at environmental concentration; 10*EnvCombined: combined chemicals at 10 times the environmental concentration). Number of events, global *p*-values and AIC and Concordance Index can be found below each plot.; Figure S4: Survival curves of *Palaemon khori* for eight weeks of the study, under different treatments (experiment 2). Curves are grouped by categories: A single contaminant at reported environmental concentrations; B single contaminants at 10 times the reported environmental concentration; C combination of contaminants at reported environmental concentrations; D combination of contaminants at 10 times the reported environmental concentrations. Refer to the legend for colours. *p* values (long-rank model) are reported in each panel. Values of concentrations can be found in Table 1. Table S1: Pairwise comparisons among treatments on survival, using log-rank test for experiment 1. Significative differences are expressed in bold. Table S2: Pairwise comparisons among treatments on survival, using log-rank test for experiment 2. Significative differences are expressed in bold. Table S3: Pairwise comparisons among treatments on survival, using log-rank test for experiment 1 and 2 combined. Significative differences are expressed in bold. Table S4: Number of observed metaphases (in ten animals) with corresponding number of aneuploid metaphases for two replicated tanks (except the control) at four and eight weeks after dosage (T4 and T8). Aneuploidy levels were calculated as the ratio between aneuploid and total metaphases. Average aneuploid numbers were calculated for replicated tanks and used for statistical analyses.

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