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T. Neuparth[a], R. Capela[a], S. P. P. Pereira[a], S. M. Moreira[a], M. M. Santos[ab] & M. A. Reis-Henriques[a]

[a] CIMAR/CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

[b] FCUP-Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

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TOXICITY EFFECTS OF HAZARDOUS AND NOXIOUS SUBSTANCES (HNS) TO MARINE ORGANISMS: ACUTE AND CHRONIC TOXICITY OF *p*-XYLENE TO THE AMPHIPOD *Gammarus locusta*

T. Neuparth[1], R. Capela[1], S. P. P. Pereira[1], S. M. Moreira[1], M. M. Santos[1,2], M. A. Reis-Henriques[1]

[1]CIMAR/CIIMAR–Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal
[2]FCUP–Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

Despite the recent focus on hazardous and noxious substances (HNS) spills preparedness and responses, much remains to be done regarding the threat posed by HNS spills on marine biota. Among the identified priority HNS, *p*-xylene was selected to conduct ecotoxicological assays. The aim of this study was to assess the performance of the amphipod *Gammarus locusta* under acute and chronic exposure to *p*-xylene simulating conditions of a spill incident. In the acute exposure (96 h) the *p*-xylene LC_{50} was estimated. In the chronic bioassay (36 d), an integration of organism-level endpoints (survival, growth rate, and sex ratio) with biochemical markers indicative of oxidative stress including catalase (CAT), glutathione *S*-transferase (GST), and superoxide dismutase (SOD) activities and lipid peroxidation (LPO) levels was determined. The aim was to increase the xylene ecotoxicological database and better predict its impact in aquatic environments. *p*-Xylene induced several chronic toxicity effects in *G. locusta*. Significant alterations in antioxidant enzymes and lipid peroxidation levels as well as growth rate and biased sex-ratio were observed. p-Xylene significantly affected the activities of CAT, SOD, and GST in *G. locusta* and produced oxidative damage by increasing levels of LPO in males. Further, impacts in key ecological endpoints, that is, growth and sex ratio, were noted that might be indicative of potential effects at the population level in a spill scenario. The present data may be useful to assist relevant bodies in preparedness and response to HNS spills.

Although the shipping of liquids in bulk is still largely dominated by oil, the percentage of chemicals transported by sea has been rising in the last decades (Le Floch et al., 2010). The postulation is that chemical maritime traffic induces an increase in the risk of accidents, and consequently, an enhanced risk of chemical spills. The wide variety of chemicals transported by sea, their varying physical and chemical properties, their range of behaviors in the environment (i.e., gas, dissolves, evaporates, floats, sinks), and the toxicity to marine organisms indicate that the operational response strategy to be defined in the case of a spill is not straightforward (International Tanker Owners Pollution Federation Limited [ITOPF], 2010). Therefore, to better identify the risks that chemicals transported by sea may present, the notion of Hazardous and Noxious Substances (HNS) emerged in Europe in 2000 (International Maritime Organisation [IMO], 2000). In this context, the Protocol on Preparedness, Response and Co-operation to Pollution Incidents by Hazardous and Noxious Substances (OPRC–HNS Protocol) was adopted by the IMO (2000) and enacted in

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Address correspondence to T. Neuparth, CIMAR/CIIMAR–Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 177, 4050-123 Porto, Portugal. E-mail: tneuparth@ciimar.up.pt

2007. Despite this protocol, the understanding, threat, and consequences of HNS spills in marine environments are not yet sufficiently developed and much remains to be done regarding preparedness and response to HNS spills.

In the context of the ARCOPOL project, Neuparth et al. (2011) developed a weight-of-evidence approach aimed at prioritizing HNS that pose major environmental risks to Atlantic European waters. The approach took into consideration the probability of HNS spills in European Atlantic waters and the HNS physicochemical and toxicity properties. The study also included a collection of marine toxicological data available for the 23 HNS identified as priority. Neuparth et al. (2011) stated that marine chronic toxicity data were missing for most of the priority HNS, and in some cases only freshwater acute toxicity data were available, supporting the need to gather additional toxicological data for priority HNS.

Among the 23 HNS identified by Neuparth et al. (2011) as priority, *p*-xylene was selected in this study based on (1) limited ecotoxicological data available for marine organisms, (2) indication of being highly transported in the sea, and (3) involvement in previous accidental spills. This HNS is in position 8 in the traffic ranking of the 100 most transported HNS in Atlantic European waters (HASREP, 2005). Several xylene spills were reported globally, including the *Canon*, which sank in the Galicia, Spain, in 1987 with an unknown amount of xylene on board, and the *Grape one*, which carried 3041 tonnes of xylene and sank in the United Kingdom (Mamaca et al., 2009). However, knowledge regarding the environmental impacts of xylene spills in aquatic ecosystems is limited since no monitoring programs were implemented following the reported incidents (Purnell, 2009).

Monocyclic aromatic hydrocarbons, such as xylenes, present in gasoline, paints, rubber products, plastics, detergents, dyes and pesticides are classified as environmental priority pollutants (Plaza et al., 2007). Because of the large amounts of xylene transported by sea and their relatively high water solubility and low K_{ow} values, xylenes are highly mobile in the environment (Dou et al., 2008). Despite the ecological concern due to its toxicity and ability to bioaccumulate through food chains (Plaza et al., 2007), the toxic effects of xylene on marine organisms have been little studied. Neuparth et al. (2011) found no xylene chronic toxicity data were available for marine organisms and acute toxicity studies have seldom been addressed. In addition, to the best of our knowledge, no studies are available that assessed biochemical alterations induced by xylene in crustaceans. However, oxidative stress biomarkers were applied to assess the toxicity of monocyclic aromatic hydrocarbons, such as toluene, ethylbenzene, and xylene, in insects, earthworms, and macrophytes (Liu et al., 2010; Yan and Zhou, 2011; Singh et al., 2009). Studies demonstrated alterations on activity of superoxide dismutase (SOD) and catalase (CAT) activities as well as lipid peroxidation (LPO) levels when organisms were exposed to monocyclic aromatic hydrocarbons, including xylene.

Oxidative stress biomarkers may be useful as a complementary approach to the traditional endpoints typically analyzed in ecotoxicological testing such as survival and growth responses to investigate the effects of xylene toxicity on crustaceans. Amphipods are ubiquitous in the aquatic ecosystem and are considered reliable bioindicators of contamination. Thus, these organisms have been successfully used in ecotoxicology for decades (Ré et al., 2009). In this study, the amphipod *Gammarus locusta* was experimentally exposed to *p*-xylene to simulate the conditions of a spill incident and several biomarkers of oxidative stress were analyzed in conjunction with responses at individual level. *Gammarus locusta* was selected as a test organism due to its ecological relevance in the European Atlantic coast. This epibenthic species has a wide geographical distribution along the northeast Atlantic, from Norway and Iceland to the Strait of Gibraltar, including the British Isles and Baltic Sea (Neuparth et al., 2005); it is among the main prey of many fishes, birds, and invertebrate species (Costa and Costa, 2000). Moreover, *G. locusta*

is recognized as a relevant test species since it possesses a number of advantages for application in ecotoxicological studies (Costa et al., 2005), such as sensitivity to a wide variety of contaminants, ecological importance, and easy culturing, but also because of short generation time (Neuparth et al., 2002). The aim of this study was to assess the performance of *G. locusta* under acute and chronic exposure to *p*-xylene. The parameters examined organism-level endpoints including survival, growth rate, and sex ratio, as well as biochemical markers indicative of oxidative stress: CAT, glutathione S-transferase (GST), and SOD activities and LPO levels.

MATERIAL AND METHODS

Amphipod Collection and Maintenance

The amphipods were obtained from a permanent lab culturing system (Neuparth et al., 2002). The culture is partially renewed once a year by introducing specimens collected from a clean site located on the lower part of the south margin of the Sado estuary (38° 27′ N, 08° 43′ W), Portugal (Neuparth et al., 2002). Animals were fed with *Ulva* sp. that, together with the seawater sediments and small stones, were collected from coastal areas near Porto, Portugal, in a site devoid of direct contamination sources. The seawater passed through a circuit with sand and carbon filters before being used.

Acute Bioassay

The acute bioassay was conducted at 20–21°C and 33–35‰ salinity and under a 12-h photoperiod in a semistatic system for 96 h. The bioassay was conducted with juveniles (2–4 mm length, 3 wk old) produced in the lab. At least 24 h before the beginning of the test, a substock of animals was isolated from the lab culture and acclimated to the assay water temperature with unlimited food conditions (*Ulva* sp.). Fifteen amphipods were randomly allocated to glass test vessels,with 2 L capacity and were exposed to 5 different treatments (control: natural seawater at 33–35‰ salinity, and 4 *p*-xylene concentrations: 0.1, 0.5, 1, or 1.5 mg/L) with three replicates per treatment. These concentrations were selected based on our preliminary studies. During the exposure, seawater was renewed daily and organisms were not fed. When the experiment was finished the contents of the test chambers were sieved through a 250-μm screen and number of amphipods was recorded as alive or dead. Toxicity data generated in this study were statistically analyzed by Probit analysis using SatPlus Portable (AnalystSoft Inc.) software and the 96-h LC_{50} was determined.

Chronic Bioassay

A partial life-cycle bioassay was carried out with neonates (1–1.5 mm length; <8 d), obtained in our lab culture, in a semistatic system during 36 d (i.e., until the age of the first maturity). The assay was conducted at 20–21°C under a 12-h photoperiod in 5 L-glass aquaria, covered with appropriate lids sealed with parafilm to minimize the *p*-xylene volatilization. A 1-cm-deep layer of natural sediment was placed in each aquarium the day before the start of the assay. Aeration was provided with plastic tips placed at least 1 cm above the sediment surface and small stones were furnished to provide shelter. The assay started the following day with the allocation of exactly 60 amphipods in each test chamber. The organisms were fed with macroalgae *Ulva* sp. on an ad libitum basis, assuring that food was never in shortage. The water of each aquarium was renewed daily. Test chambers were inspected daily for aeration and feeding requirements and to remove dead animals. Four treatments were established, with three replicates each (control: natural seawater at 33–35‰ salinity, and 3 *p*-xylene nominal concentrations: 19, 75, or 300 μg/L). These concentrations were selected based on the *p*-xylene LC_{50} obtained in this study.

p-Xylene Analytic Quantification by SPME-GC-MS

The actual concentrations of *p*-xylene were determined once during the assay, at 0 and

24 h before one of the daily water changes. Three samples of seawater per treatment were directly analyzed by solid-phase microextraction gas chromatography–mass spectrometry (SPME-GC-MS). This method enabled the *p*-xylene detection with a detection limit of 0.5 ng/L and a quantification limit of 10 ng/L. Isolation of *p*-xylene was performed by solid-phase microextraction (SPME) with a 100-μm PDMS fiber. The SPME fiber samples were desorbed thermally in a split/splitless injection port to allow desorption. At the same time, the GC-MS run was started. GC/MS was performed using a Varian 3900 gas chromatograph coupled to a Varian Saturn 2000 ion trap mass spectrometer.

Analysis of Biochemical Markers and Ecological Endpoints

Ecological and biochemical parameters were determined just after amphipods' sexual maturation. At d 36, 3 aquaria from each treatment were sampled, and their contents were sieved through 250-μm screens to collect the amphipods. The ecological endpoints analyzed comprised survival, sex ratio, and individual growth. All these endpoints were determined separately in each replicate. The number of surviving males and females was converted to percentages and expressed as percent of control. Sex ratio in each replicate was determined as the number of surviving males divided by the number of surviving females. The individual lengths of all males and females were measured to the nearest 0.1 mm on a stereomicroscope (Nikon SMZ 1000). The metasomatic length was used, which is defined as the distance between anterior end of the rostrum and posterior end of the last metasomatic segment (DeWitt et al., 1992).

Males and females from each treatment were frozen in liquid nitrogen and stored at –80°C until further analysis of the biomarkers CAT, SOD, and GST activities and levels of LPO, which were determined according to the methods described in the following. Pools of 2 to 3 males and 5 to 6 females were homogenized in 1 ml of ice-cold sodium phosphate buffer. Mitochondrial fractions were obtained after centrifugation at 15,000 × g for 20 min, at 4°C, and supernatant was used for biochemical determinations of CAT, GST, and SOD activities and LPO levels. CAT activity, expressed as micromoles per minute per milligram protein, was determined by measuring the consumption of H_2O_2 at 240 nm as described previously by Ferreira et al. (2007). GST activity was determined according to the method of Habig et al. (1974) adapted to a microplate and expressed in nanomoles per minute per milligram protein measured every 20 s, at 340 nm, during the first 5 min. SOD activity was measured by an indirect method involving the inhibition of cytochrome *c* reduction at 550 nm as described in Ferreira et al. (2010). The activity was given in SOD units (1 SOD unit = 50% inhibition of the xanthine oxidase reaction) per milligram of protein. The peroxidative damage to lipids that occurs with free radical generation and results in the production of malondialdehyde (MDA) was assessed by determination of thiobarbituric acid-reactive substances (TBARS), as described in Ferreira et al. (2008). LPO levels were expressed as nanomoles of malondialdehyde (MDA) equivalents per milligram of protein. Due to technical constraints, the female material collected for LPO determination was damaged and LPO levels could only be quantified in males.

Statistical Analyses

Data from each studied variable (ecological endpoints and biomarker markers) were first checked for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene´s test) and subsequently analyzed by one-way analysis of variance (ANOVA) to determine whether differences in responses between exposed and control organisms could be attributed to exposure to *p*-xylene. Significant differences were set at $p < .05$. The Fisher´s least significant difference test (LSD) was used for multiple comparisons between pairs of means.

RESULTS

Acute Toxicity Assay

The mean 96-h LC_{50} test resulted in an estimated LC_{50} of 1.1 mg/L with a 95% confidence interval of 0.8–1.4 mg/L. There were 6.6% mortality in the control and 71% mortality in the highest tested *p*-xylene concentration (1.5 mg/L).

Chronic Toxicity Assay

***p*-Xylene Analytic Quantification** Table 1 summarizes the concentration of *p*-xylene measured once in the chronic assay. No *p*-xylene was detected in controls. Results show that *p*-xylene concentrations at the initial time were slightly higher than nominal. After 24 h, immediately before the water change, the amount of *p*-xylene in water at all *p*-xylene treatments lower than nominal. The reason for this loss might be related to high volatilization of *p*-xylene. However, to ensure the exposure of amphipods to a *p*-xylene concentration close to the nominal concentration throughout the assay, water was changed daily.

Biochemical Markers CAT, SOD, and GST enzymatic activities in *G. locusta* males and females and the LPO levels in *G. locusta* males exposed to p-xylene over 36 days are displayed in Figure 1. Significant differences in all oxidative stress biomarkers analyzed were noted after 36 d of p-xylene exposure. CAT activity in males was significantly higher from control at 19 μg/L p-xylene concentration; in females no significant induction was reported, but higher activity values were observed at the higher concentration of 300 μg/L. SOD activity in males increased significantly at concentrations of *p*-xylene of 75 and 300 μg/L. In females, SOD activities showed the opposite profile with a significant activity reduction observed for all concentrations of *p*-xylene. An induction of GST in males was also observed at all *p*-xylene concentrations; in females only 300 μg/l *p*-xylene concentration led to a significant induction. The LPO levels in males rose significantly at concentrations of *p*-xylene 75 and 300 μg/L.

Ecological Parameters

Survival and Sex Ratio The mean total survival, expressed as percent of control, obtained for each *p*-xylene treatment for 36 d of exposure is presented in Figure 2. Survival did not differ significantly between control and *p*-xylene treatments. The mean total survival for control was 53.3%, which was within the expected range for long-term bioassays with *G. locusta* (full life cycle 35–40 d) and other gammarids (Nair and Anger, 1979; Neuparth et al., 2002, Costa et al., 2005, Prato et al., 2013). The life history of *G. locusta* in experimental conditions was previously detailed by Neuparth et al. (2002) using life table analysis. From 14 to 46 d old, control mortality ranged from 40 to 50%. In addition, Nair and Anger (1979) noted that in the lab, a bimodal mortality pattern was typical for the marine amphipod *Jassa falcate* with high juvenile mortality rates (up to 50%) especially from the time of the first and third moult, low mortality during the reproductive phase, and again, high mortality rates toward the end of the life cycle (especially during the last two moults).

Surviving males significantly exceeded females in some of the *p*-xylene treatments, as evidenced by the sex ratios above 1 (Figure 2). Compared with control, an abnormal marked low proportion of surviving females

TABLE 1. Nominal and Measured Concentrations of *p*-Xylene Concentrations (mg/L) in Seawater Samples of Each Treatment Collected at 0 h, and 24 h Before One of the Daily Water Changes

Time (h)	Control	19 μg/L	75 μg/L	300 μg/L
0	N.D.	32 ± 0.006	118 ± 0.08	440 ± 0.08
24	N.D.	0.08 ± 0.0001	0.48 ± 0.0004	0.0013 ± 0.000001

Note. Data expressed as mean ± standard deviation ($n = 3$).

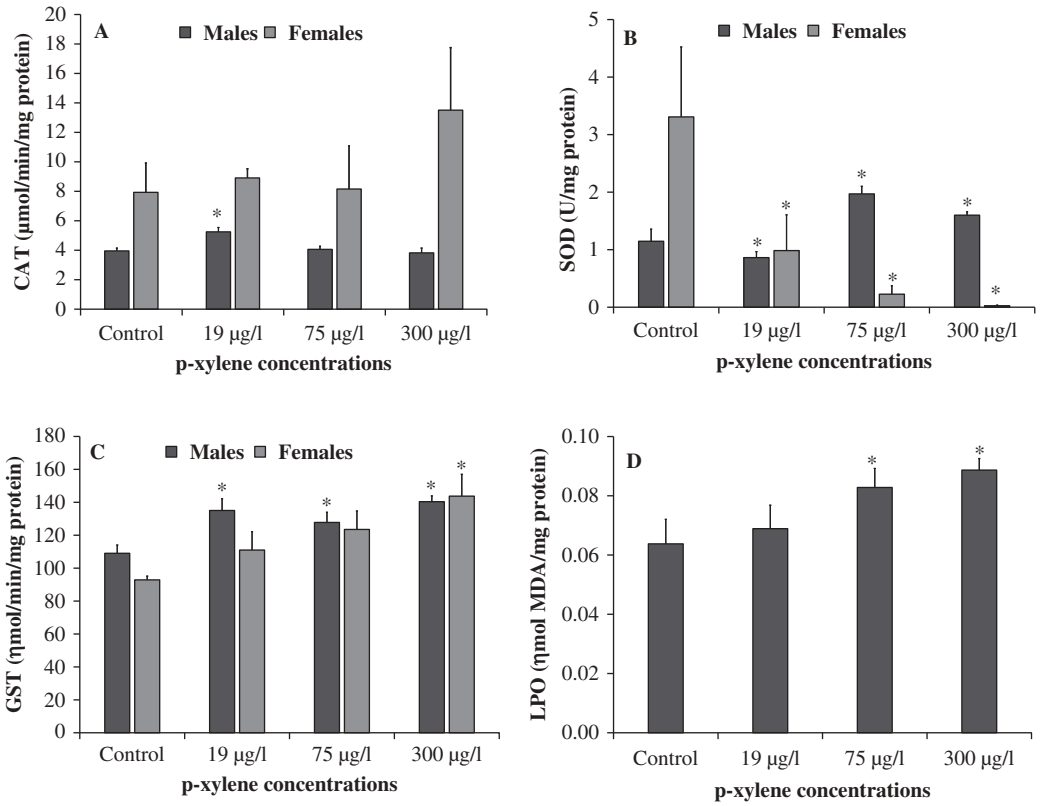

FIGURE 1. Chronic effects of *p*-xylene on (A) catalase (CAT), (B) superoxide dismutase (SOD), (C) gluthatione S-transferase (GST), and (D) lipid peroxidation (LPO) of *Gammarus locusta* after an exposure of 36 d. Error bars indicate the standard errors; asterisks indicate significant differences from control ($p < .05$).

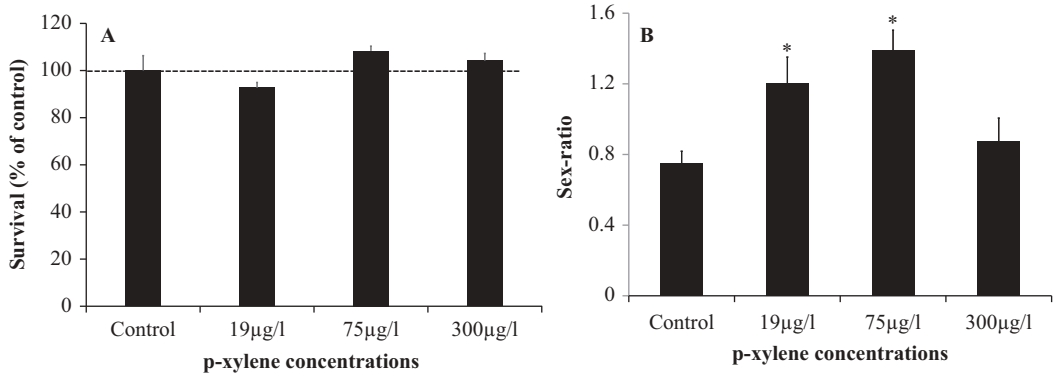

FIGURE 2. Chronic effects of *p*-xylene on (A) survival and (B) sex ratio of *Gammarus locusta* after an exposure of 36 d. Survival results are expressed as percent of control group. Error bars indicate the standard errors; asterisks indicate significant differences from control ($p < .05$).

was observed for 19 and 75 µg/l *p*-xylene treatments.

Individual Growth The individual growth results are outlined in Figure 3. The average length of males and females in the control was significantly higher compared to all *p*-xylene treatments. The males from the control, with an average length of 9.9 mm, exceeded between 7.5% and 9.8% the male size from *p*-xylene treatments. Similarly, the average length of control females (8.1 mm) exceeds between 5.8 and 9.2% the female length from *p*-xylene treatments.

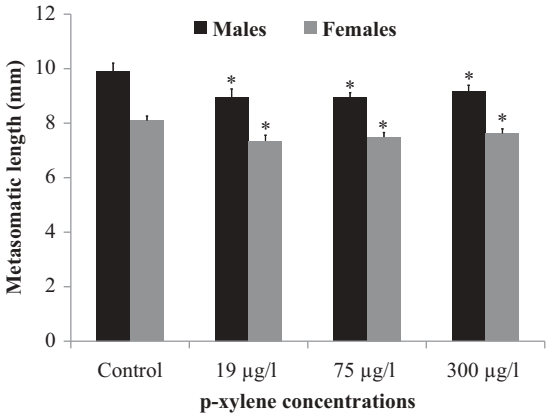

FIGURE 3. Chronic effects of *p*-xylene on *Gammarus locusta* growth after an exposure of 36 d. Error bars indicate the standard errors; asterisks indicate significant differences from control ($p < .05$).

DISCUSSION

Xylene is present in the aquatic environments primarily from discharge of industrial and municipal effluents. Nevertheless, because of the widespread use of this HNS and large amounts of xylene transported by sea, there is a correspondingly serious potential for contamination due to accidental spills with important ecological consequences to marine biota. Despite the ecological concern of xylene in aquatic environments (Li et al., 2013), the sublethal effects of xylene to marine organisms have seldom been addressed. There is a current paucity of knowledge regarding ecological hazards and consequences associated with a xylene accidental spillage.

In the acute toxicity assay, a 96-h LC_{50} of 1.1 mg/L was obtained. According to the marine toxicological data collected by Neuparth et al. (2011), only three other LC_{50} evaluations were reported for *p*-xylene considering saltwater organisms. The 96-h LC_{50} value obtained for the Crustacea *Crangon franciscorurm* and for the fish *Moreno saxatilis* was 2 mg/L (Benville and Korn, 1977), and there were 24-h LC_{50} values of 27.8 mg/L and 17.7 mg/L for *Artemia* sp. (MacLean and Doe, 1989) and *Artemia franciscana* (Neuparth, personal communication) respectively. The LC_{50} obtained here for *G. locusta* was the lowest available for seawater organisms, suggesting that *G. locusta* is more sensitive to *p*-xylene than other crustaceans surveyed thus far. Determination of the median lethal concentration (LC_{50}) is the test most frequently used to estimate the toxicity of chemicals in aquatic organisms. However, chronic toxicity assays are a more realistic representation of the type of exposure expected in environmental situations, and biological effects other than survival are of greater ecological relevance for understanding the impact of contaminants on organisms and ecosystems (Emery et al., 1997; Ingersoll et al., 1998; U.S. Environmental Protection Agency–U.S. Army Corps of Engineers [USEPA-USACE], 2001). Therefore, a chronic toxicity assay with *p*-xylene was also performed, integrating individual-level endpoints (survival, growth, and sex ratio) with biochemical markers (antioxidant enzymes and lipid peroxidation levels). A number of biochemical markers have been developed and applied successfully to various invertebrate species (Livingstone, 2001; Hyne et al., 2003; Galloway et al., 2004), but they have been rarely integrated in conventional chronic toxicity assays and/or linked to individual and population-level effects. However, the application of multiple biomarkers in chronic tests may be advantageous for study of contaminant mechanism of action and to provide evidence of the cause–effect relationship between exposure to contaminants and ultimately organism and population responses.

In the present study, *p*-xylene induced several chronic adverse effects in *G. locusta*. Significant alterations at subindividual level (antioxidant enzymes and lipid peroxidation) and at individual level (e.g., reduced growth rate and biased sex ratio) were observed. Crustaceans, like fish and mammals, possess well-developed antioxidant defense systems designed to reduce or neutralize the toxicant effects of reactive oxygen species (ROS) (Zhu et al., 2008). A number of studies suggested that toxicity of xylene might be mediated by ROS (Costa et al., 2006; Sawicka and Dlugosy, 2008), thereby altering the activity of antioxidant enzymes. The overproduction of ROS such as H_2O_2 and

superoxide radical ($O_2^{\bullet -}$) generates oxidative stress (Livingstone, 2001). The adverse effects of ROS may be counteracted by cellular antioxidant enzymes such as SOD, CAT, and GST. Therefore, changes of these enzymatic activities indirectly indicate the adverse effects of contaminants on organisms (Sun et al., 2007; Liu et al., 2010). In the present study a differential antioxidant response was observed for males and females, mainly in SOD activity. In males, the same tendency was observed for SOD and GST activity. *p*-Xylene significantly induced the activity of SOD and GST. These results may be attributed to activation of depuration/detoxification mechanisms to overcome oxidative stress produced by *p*-xylene. Conversely, CAT activity was maximally inducted at the lowest *p*-xylene concentration, with a tendency toward recovery to control levels in the higher *p*-xylene concentrations. This might be attributed to high production of $O_2^{\bullet -}$, which was found to inhibit CAT activity (Kono and Fridovich, 1982; Zhu et al., 2008). In contrast, in females, despite GST and CAT presenting similar pattern to males, a marked inhibition of SOD was recorded. In agreement with our results, although not specifying sex differences, other studies found a depression in SOD activity in humans and rats after exposure to xylene (Costa et al., 2006; Kum et al., 2007), but elevated levels of this enzyme were reported in other studies with humans and insects (Georgiena et al., 2002; Singh et al., 2009). No extensive study has been undertaken to better understand the sex-specific responses of SOD following xenobiotic exposure, and therefore future studies are necessary to establish the cause of sex-linked differences in SOD activity. Nevertheless, female SOD inhibition after xylene exposure may reflect damage of this enzyme due to ROS production and this suggests that females might have lower defense capacities against oxidative stress produced by xylene than males. In fact, considering that xylene accumulates in tissues with high lipid content due to its lipophilicity (Reyna, 2009), it is likely that female amphipods that display higher lipid content than males (Gismondi et al., 2012; Sroda and Cossu-Leguille., 2011), may accumulate more xylene.

Lipid peroxidation is an important consequence of oxidative stress due to the production of oxygen radicals that reduces the protective capacity of the organisms (Livingstone et al., 1993; Solé et al., 1995; Xu et al., 1999). In this study, a significant increase was found on LPO levels in male exposed to the higher *p*-xylene concentrations, which indicated that generation of ROS overwhelmed the antioxidant defenses of *G. locusta*. The significant augmentation in the activity of antioxidant enzymes observed in males was a response to reduce the adverse effect of ROS generated by *p*-xylene, but apparently the antioxidant defenses were not sufficient to overcome ROS toxicity produced by the highest *p*-xylene concentration, since significant augmentation in LPO levels was also observed. Data suggest that production of ROS associated with the higher *p*-xylene concentrations is no longer within the elimination capacity of amphipods and therefore oxidative damage of lipids occurs. These results are consistent with a number of previous studies that observed significant induction of antioxidant enzymes along with elevation in LPO levels in insects and humans exposed to monocyclic aromatic hydrocarbons like xylene, benzene, and/or toluene (Georgiena et al., 2002; Singh et al., 2009). The biomarker responses obtained in this study suggest that the use of CAT, SOD, GST, and LPO as biomarkers of exposure and effects in crustaceans has potential to be incorporated into the environmental impact assessment of xylenes after spill incidents. Taking into consideration that determination of these biomarkers is cost-effective, rapid, and easy to use, it will be useful to assist relevant bodies in impact assessment after HNS spills.

ROS are known to affect the physiology and growth of aquatic organisms (Baker et al., 1997; Pandey et al., 2003; Zhu et al., 2008). In the present study, despite no marked effects being apparent on survival, an impaired condition of the amphipods expressed by biased sex ratio and a significant reduction in growth rate (lower average length) of males and females

was detected at *p*-xylene concentration as low as 19 μg/L, suggesting that oxidative stress/oxidative damage observed may be sufficient to produce adverse health effects. These individual level effects observed are probably related to increased metabolism demands due to energy requirements to cope with oxidative stress induced by *p*-xylene, which may lead to mobilization of energy reserves allocated for normal growth and other physiological needs. Decrease in the growth of an organism may exert direct effects at the population level. In organisms such as crustaceans, the fecundity and reproduction are positively correlated to the size (Callow, 1979; Graney and Giesy, 1986). Thus, a reduction in growth decreases future breeding potential and decline in offspring recruitment rates of *G. locusta* exposed to xylene. Additionally, an alteration of normal sex ratio, with prevalence of male-biased sex ratios, affects the population structure. The reduction of females in the population reinforces the decrease of juvenile recruitment. Therefore, there are costs associated with reduction in growth and biased sex ratio that might be extrapolated from individual level to potential effects at the population level.

Given the lack of reliable information on ecological hazards and consequences of HNS spills, including acute and chronic toxicity of HNS in representative species of marine taxa, a better and relevant understanding of the effects inherent to HNS spills is a priority. Therefore, studies to gather HNS-related toxicological data on marine biota require more attention. Hence, the type of research presented here is considered essential to assist relevant bodies in preparedness and response to HNS incidents. In conclusion, the present study gathered essential information on acute and chronic toxicity of a priority HNS (*p*-xylene) to the amphipod *G. locusta*. Further, the first steps were taken to better understand adverse effects of *p*-xylene in crustaceans. The present findings indicate that oxidative stress induced by chronic exposure may exert a significant impact in key ecological endpoints, such as sex ratio and growth, which can have important implications at population level. Considering the present findings, further studies are warrant to address additional ecological relevant endpoints, such as reproduction and potential transgenerational effects.

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