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ACUTE TOXICITY OF TRIBUTYLTIN ON THE MARINE COPEPOD *Tisbe biminiensis*

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Antifouling systems (AFS) are physical or chemical treatments that control or prevent biological fouling on submerged surfaces (IMO, 2001). Tributyltin-based compounds have been used as AFS since the 1960s. These chemicals were soon recognized as ideal biocides and became the main AFS applied to ship hulls for several decades. Champ (2001) estimated that tributyltin (TBT) was applied to approximately 60,000 vessels of the world's merchant fleet in 2001. The widespread use of TBT as an AFS was identified to be a marine pollution issue in the 1970s when oyster shell anomalies and population declines were observed in Arcachon Bay, France (ALZIEU et al., 1986). Other TBT toxic effects have been identified in marine invertebrates, including imposex in mollusks, reduced reproductive success in amphipods and biased female-to-male ratio in copepod populations (HUANG et al., 2006; AONO; TAKEUCHI, 2008; BIGATTI et al., 2009).

In 2001, the International Maritime Organization (IMO) proposed an international convention for controlling the use of harmful AFS on ships (IMO, 2001). The initial goal of banning TBT by 2008 was challenging and did not succeed. Officially, the convention came into force only on September 17, 2008 and it currently has 63 Parties whose combined fleet represents 81% of the world's merchant ships (IMO, 2012). After proposal of the AFS convention in 2001, TBT concentrations in seawater began to decline although concentrations are still considered high in some parts of the world (ANTIZAR-LADISLAO, 2008). Liu et al. (2011) reported seawater concentrations ranging from 17 to 274 ng L^{-1} at mariculture sites in Taiwan. Water samples collected in the vicinity of the Port of Gdnyia, Poland exhibited TBT concentrations ranging from 13 to 191 ng L^{-1} (RADKE et al., 2012). Martí et al. (2011) reported TBT levels of 26 ng L^{-1} in waters off the Mediterranean coast of Spain. These recent studies demonstrate that TBT concentrations in seawater still exceed the limits of 0.2 to 10 ng L^{-1} set by various regulatory agencies (CCME, 1999; CONAMA, 2005; CEC, 2006).

Copepods are particularly suitable as a role model for bioassays since they are cosmopolitan, have a short life cycle, and are easy to handle and maintain in the laboratory. However, the TBT toxicity for copepods has been estimated for just a few species such as brackish (*Nitocra spinipes*) and marine (*Tigriopus japonicus*, *Tisbe battagliai*) benthic species, and brackish (*Eurytemora affinis*) and marine (*Acartia tonsa*, *Pseudodiaptomus marinus*, *Schmackeria poplesia*) planktonic species (LINDEN et al., 1979; BUSHONG et al., 1988; MACKEN et al., 2008; HUANG et al., 2010).

Tisbe biminiensis is an epibenthic copepod that has been recently proposed as a model for sediment samples of tropical areas (ARAÚJO-CASTRO et al., 2009). This marine species is distributed along the Atlantic coastal zone and lives associated with benthic macroalgae and sandy substrate (VOLKMANN-ROCCO, 1973). *T. biminiensis* cultivation in the laboratory yields high rates of population growth, and provides healthy organisms for bioassays throughout the year (RIBEIRO; SOUZA-SANTOS, 2011). Its epibenthic habit makes this species suitable for evaluation of toxicity in both water and sediment samples (ARAÚJO-CASTRO et al., 2009). The aim of this study was to assess the acute toxicity of TBT in seawater for the marine copepod *T. biminiensis*.

Neat tributyltin oxide (TBTO) was purchased from Sigma-Aldrich and used to prepare a stock solution in acetone (ACS reagent grade) at a concentration of 5.7 g TBT L^{-1}. Intermediate and working solutions were diluted in seawater prior to each bioassay. Concentrations of the working solutions ranged from 20 to 137 µg TBT L^{-1}. All reported values are based on nominal concentrations as suggested by other authors (BUSHONG et al., 1988; OHJI et al., 2002). According to Bushong et al. (1988), nominal and measured concentrations of TBT in seawater bioassays typically agree within the range of 10-15%. No light exposure and no feeding also contribute for maintenance of nominal concentrations during toxicity tests.

T. biminiensis has been cultivated at our laboratory for several generations (RIBEIRO; SOUZA-SANTOS, 2011). Seawater used in the cultivation and bioassays was collected at Barra de Sirinhaém beach (08° 35'S, 35° 06'W), Pernambuco, Brazil. Copepods were cultivated in seawater filtered through 25 and 3 µm cartridges with salinity 34 ± 2, temperature of 28 ± 2°C and photoperiod of 12/12 h (dark/light) under constant aeration. Copepods were fed twice a week on diatoms (*Thalassiossira*

weissflogii or *Chaetoceros calcitrans*) and Alcon® fish food. Diatoms were cultivated in f/2 medium (ARAÚJO-CASTRO; SOUZA-SANTOS, 2005). Eight to eleven days prior to the bioassay, ovigerous females (>250 µm in length) were isolated from the maintenance cultures in order to produce nauplii. After 24 h, the newly hatched nauplii were isolated for beginning a new culture with controlled age to be used as test animals in the bioassay.

In the bioassays, standard water quality parameters were as follows: salinity 34, dissolved oxygen 6 ± 1 mg L^{-1} (saturation above 70%), pH 8.1 ± 0.1 and temperature 29 ± 1°C. Toxicity tests were carried out according to the protocol described by Araújo-Castro et al. (2009), with modifications. Briefly, five bioassays were performed with adult (7-10 day old), ovigerous females of *T. biminiensis*. Exactly 10 mL of filtered seawater (0.45 µm) was added to each glass container (5 cm height x 3.5 cm diameter). Ten ovigerous females were carefully taken from the age-controlled cultures and placed in each of the containers. TBT working solutions were then added to the glass containers to yieldconcentrations ranging from 10 to 68 µg TBT L^{-1} in 20 mL of seawater. Both controls (seawater only and seawater plus acetone) and treatments had four replicates each. Concentration of acetone in the test tubes did not exceed 24 µL L^{-1}. The bioassays lasted for 48 h with no feeding and no light exposure. Dead test organisms were counted and removed at 24 and 48 h. Mortality was typically defined as a position on which the animals urosome is perpendicular to its prosome (KWOK; LEUNG, 2005). In addition, no moving organisms were observed at the bottom of the recipients under a stereoscopic microscope after careful inspection. Copepods with no movement of both appendices and digestive system after an observation period of 30 seconds were also assumed to be dead.

The 24 h and 48 h LC_{50} were calculated according to the trimmed Spearman-Karber method (HAMILTON et al., 1977). The paired Students *t*-test was used to compare the mean mortality between controls. The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were estimated using an Analysis of Variance (ANOVA) followed by the post hoc one-tailed Dunnetts test. The mortality data were transformed using arcsin function to meet the assumptions of the ANOVA (ZAR, 1999). Normality and homogeneity of variances were evaluated using the Lilliefors and Bartlett tests, respectively. The critical level of significance for all statistical analyses was set at 0.05.
Mortality in the controls averaged 0.12% and 0.25% for seawater only and seawater plus acetone, respectively. There was no significant difference in mortality between controls (paired *t*-test, t = -0.53, df= 4, p = 0.62). This indicates that acetone used as a carrier solvent for TBT is non-toxic for *T. biminiensis* at a concentration of 24 µL L^{-1}. Acetone seems to be slowly taken up by marine copepods (ARA et al., 2010). The lack of acetone toxicity is in agreement with the limit of 100 µL L^{-1} for bioassays (OECD, 2000). The median NOEC and LOEC after 48 h of exposure were 29 and 34 µg TBT L^{-1}, respectively. The NOEC for *T. biminiensis* was similar to that estimated for *T. japonicus* (20 µg L^{-1}), indicating that these species have similar TBT sensitivities (LEE et al., 2007). The mean (1 standard deviation) 24 and 48 h LC_{50} were 37 ± 9 and 36 ± 9 µg TBT L^{-1}, respectively (Fig. 1).

T. biminiensis was as sensitive to TBT as other benthic copepods, including *T. battagliai*, *N. spinipes* and *T. japonicus* (KARLSSON et al., 2006; LEE et al., 2007; MACKEN et al., 2008; BAO et al., 2011). In general, these four benthic copepod species seem to be less sensitive to TBT than planktonic species such as *P. marinus*, *A. tonsa*, *E. affinis* and *S. poplesia* (Table 1). The lower sensitivity of benthic species may be related to more efficient detoxification mechanisms since they are usually exposed to higher concentrations of hydrophobic pollutants such as TBT. Alternatively, sensitivity differences between benthic harpacticoids and planktonic copepods (e.g., calanoids) may be a consequence of phylogenetic distances (DI TORO et al., 1991).

TBT is several orders of magnitude more toxic for *T. biminiensis* than ammonia (24 h LC_{50} of 12,060 µg L^{-1}) and chromium (48 h LC_{50} of 7,510 µg L^{-1}) (ARAÚJO-CASTRO et al., 2009). The average 24h/48h LC_{50} ratio for TBT is 1.0. The calculated ratio for ammonia is 2.3, whereas the 48h/72h LC_{50} ratio for chromium is 1.6 (ARAÚJO-CASTRO et al., 2009). These ratios suggest that *T. biminiensis* takes up organic contaminants such as TBT faster than inorganic contaminants such as ammonia and chromium. As a consequence, TBT seems to reach a steady-state in *T. biminiensis* within 24 h of exposure while ammonia and chromium may not reach a steady-state even after 72 h of exposure. The TBT lipophilic nature and the high lipid content of *T. biminiensis* (~35% dry weight) may facilitate the pollutant uptake (LIMA et al., 2013).

In summary, *T. biminiensis* was as sensitive to TBT as other benthic copepod species including *T. battagliai*, *T. japonicus* and *N. spinipes*. Nonetheless, benthic copepods are generally less sensitive to TBT pollution than planktonic copepods. *T. biminiensis* is several orders of magnitude more sensitive to TBT thanother inorganic contaminants such as ammonia and chromium. *T. biminiensis* also seems to absorb organic contaminants (e.g., TBT) faster thaninorganic contaminants.

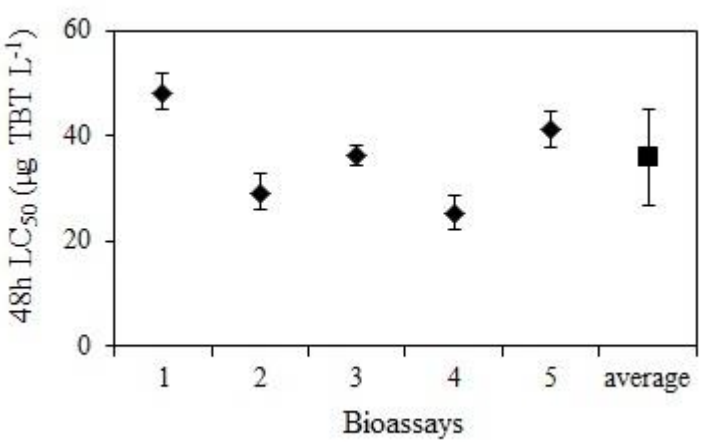

Fig. 1. Lethal concentrations for 50% of the test animals (i.e., ovigerous females of *Tisbe biminiensis*) after 48 hours of exposure to tributyltin (TBT). Error bars for square and diamonds represent the coefficient of variation and the 95% confidence intervals, respectively.

Table 1.Sensitivity of several copepod species to tributyltin (TBT) based on lethal concentrations (µg TBT L^{-1}) for 50% of the test animals (LC_{50}) after the exposure time.

Species	Life Stage	LC_{50} (µg TBT L^{-1})	Exposure (days)	Reference
Benthic copepods				
Tisbe biminiensis	Adult	36	2	This study
Tisbe battagliai	Copepodite	17	2	Macken et al. (2008)
Nitocra spinipes	Adult	1,96	4	Linden et al. (1979)
	Adult	12,7	4	Karlsson et al. (2006)
	Adult	1,88	4	Linden et al. (1979)
Tigriopus japonicus	Adult	50	4	Lee et al. (2007)
	Adult	18	4	Bao et al. (2011)
	Adult	0,85	2	Ara et al. (2010)
	Adult	0,51	2	Ara et al. (2010)
	Adult	0,15	4	Kwok and Leung (2005)
Planktonic copepods				
Pseudodiaptomus marinus	Adult	1,2	2	Huang et al. (2006)
Acartia tonsa	Adult	0,98	4	U'renet al. (1983)
	Adult	0,24	2	Kusk and Petersen (1997)
	Subadult	1,1	2	Bushong et al. (1988)
Eurytemora affinis	Subadult	2,2	2	Hall et al. (1988)
	Subadult	2,5	2	Bushong et al. (1988)
	Subadult	1,4	2	Bushong et al. (1988)
Schmackeria poplesia	Adult	0,41	4	Huang et al. (2010)

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