

## Atrazine effects on meiobenthic assemblages of a modular estuarine mesocosm

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### Abstract

Atrazine is a widely used herbicide in the US found at levels ranging from <10 ng/L to 62.5 µg/L in estuaries throughout the southeast. Effects of atrazine on estuarine meiobenthic assemblages chronically exposed to environmentally relevant concentrations are unknown. The purpose of our research was to assess effects of atrazine on meiobenthos at concentrations near the proposed USEPA SWQC (26 µg/L) using modular estuarine salt marsh mesocosms as a field surrogate. Indigenous copepod and nematode densities were assessed after 28 days of exposure in transplanted colonization chambers. Cluster analysis showed a group characterized by low copepod densities, mostly atrazine exposed chambers, and a group containing all but one control chamber. The later group included chambers with high densities of the copepods *Paronychocamptus wilsoni* and *Enhydrosoma baruchi*. Compared to controls, copepod densities was ~70% lower in atrazine chambers, with three of the most common copepod species (*E. baruchi*, *Onychocamptus* sp. and *P. wilsoni*) showing an average of 50–70% reduction in population densities ( $p < 0.05$ ). Although nematode density did not differ between atrazine and control chambers, the nematode-to-copepod ratio was significantly higher in atrazine ( $9.95 \pm 7.61$ ;  $p = 0.011$ ) than in control chambers ( $0.61 \pm 0.35$ ). Our findings suggest that chronic exposures over multiple generations to atrazine at concentrations near the proposed USEPA SWQC could have significant effects on the abundance and composition of estuarine meiobenthic copepod assemblages.

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### 1. Introduction

Meiobenthos, defined as groups of benthic metazoans between 45 and 500 µm, and typically comprised of nematodes (60–90%) and copepods (10–40%) (Coull, 1999), are abundant in estuarine sediments (>10<sup>6</sup> meiobenthos/m<sup>2</sup>) (Coull, 1999). The meiobenthos play an important role in carbon and nutrient cycling (Coull,

1999), and are an essential component of estuarine food webs, serving as key prey for higher trophic levels (i.e., shrimp and juvenile fish) (Coull, 1990, 1999; Gee, 1989). This component of the estuarine fauna has been extensively used to assess the effects of contaminants on species-specific endpoints, and community structure and composition (Coull and Chandler, 1992; Kennedy and Jacoby, 1999). Advantages of using meiobenthos in contaminant assessment studies include their high abundance and species diversity, short generation time, fast turnover rates, ubiquitous distribution, and moderate to high sensitivity to contaminants (Kennedy and

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Jacoby, 1999). Recent studies with the meiobenthos have incorporated experimental designs with higher levels of environmental and biological complexity (i.e., mesocosms) (Farmer et al., 1995; Kreutzweiser et al., 2004; Medina et al., 2004). Aquatic mesocosms (i.e., experimental ponds and enclosures) are an attempt to better simulate and predict the environmental effects of stressors, particularly contaminants, on the structure and function of aquatic environments. They allow the simultaneous assessment of contaminant effects across a wide level of biological organization, from individual endpoints to whole ecosystems. Some mesocosm studies have been used to evaluate endpoints such as species composition, and population and community-level effects on meiobenthos chronically exposed to various pesticides (Kreutzweiser et al., 2004; Medina et al., 2004).

Our study used modular estuarine mesocosms to evaluate the effects of a widely used herbicide, atrazine, on indigenous meiobenthic communities in salt marsh sediments. Atrazine has been detected in estuarine systems and coastal environments at concentrations of 90 ng/L–63 µg/L, following agricultural runoff during peak application (Clark et al., 1999; Pennington et al., 2001; Pait et al., 1992). A previous study using the copepod *Amphiascus tenuiremis* chronically exposed for two generations to an atrazine concentration of 30 µg/L, found that this herbicide did not affect copepod development or survival, but did have an effect on overall reproductive success and offspring production (Bejarano and Chandler, 2003). Projected population growth using a stage-structured population growth model indicated that chronic exposure of *A. tenuiremis* to 30 µg/L atrazine concentration could result in a dramatic reduction of the population size relative to unexposed populations (Bejarano and Chandler, 2003). Consequently, the purpose of the current study was to examine further the effects of chronic exposures to atrazine on estuarine meiobenthos (i.e., copepod and nematode densities) in a field mesocosm system. We evaluated an atrazine concentration near the proposed USEPA seawater quality criterion (SWQC, 26 µg/L).

## 2. Material and methods

### 2.1. Modular estuarine mesocosms

The modular estuarine mesocosms used in this experiment were based on the original design by Lauth et al. (1996), modified as described by Pennington et al. (2004). Briefly, modular estuarine mesocosms, held in a greenhouse, were designed to independently simulate the tidal dynamics of estuarine tidal creeks. Each mesocosm contained an individual seawater reservoir for tidal water storage, holding 300 L of seawater. Seawater

was collected from Cherry Point Boat Landing on Wadmalaw Island, SC, USA, salinity adjusted to 20 ppt with deionized water and water placed into each mesocosm system. Each system held raised sediment trays representing the low, mid and high marsh. Whole sediment plots (27.3 cm wide × 29.8 cm long × 15.2 cm deep), containing rooted vegetation as well as epibenthic, benthic and meiobenthic fauna and flora, were randomly collected from a pristine reference site located along Leadenwah Creek on Wadmalaw Island, SC, USA. Sediment plots were placed into sediment trays, and later randomly allocated across replicate mesocosms ( $n = 6$ ). A semidiurnal tidal cycle was simulated daily using submerged, magnetic impeller pumps set to a central timer. Water flow and occurrence of the tidal cycle was consistent across mesocosms.

### 2.2. Atrazine dosing

Modular estuarine mesocosms were maintained for 3 months prior to atrazine addition. Six modular estuarine mesocosms were used in this experiment, with three mesocosms randomly assigned to either an acetone carrier control treatment (0.01%; CC) or to the atrazine treatment (26 µg/L target concentration). The replicates of each treatment were randomized within the greenhouse to account for spatial variability. The system was dosed with atrazine as a single pulse, and atrazine concentration monitored regularly over the following 28 days. Atrazine dose level was chosen based on the current USEPA seawater quality criterion for this herbicide (SQWC, 26 µg/L). We will like to notice that as of 2003 the revised draft atrazine SQWC is 17 µg/L. Water aliquots were collected at time intervals (0, 1, 4, 11, 14 and 25 days post-dosing) from all atrazine and control mesocosms and stored at  $-70^{\circ}\text{C}$  until further analysis. Water samples ( $n = 18/\text{treatment}$ ) were analyzed for atrazine using an atrazine magnetic-particle enzyme immunoassay (Atrazine RaPID Assay<sup>®</sup>, Strategic Diagnostics, Newark, DE, USA), with immunoassay precision (coefficient of variation, CV%) ranging from 4.4% to 7.6%. Samples were diluted on a 5-fold basis to accommodate for sample concentrations within the test detection range (0.04–5 µg/L).

### 2.3. Meiobenthos colonization experiment

Transplanted colonization chambers (150 mL) consisted of sealed PVDF (Teflon) bottles each with three  $3 \times 2$  cm windows covered with 53 µm-mesh. Mesh windows allowed meiobenthos colonization of sediments by larval-size individuals in a selective fashion. Chambers were loaded with 20 mL of meiobenthos-free and contaminant-free sediments ( $\leq 0.63$  µm particle size), and covered with 5 mL of overlying seawater. A week prior to herbicide dosing, chambers were totally submerged

and deployed in each of the lower trays of the mesocosms assigned as either control or atrazine. Prior to dosing, sediment samples were collected from the mesocosms, and chambers randomly retrieved from three of the six mesocosms to verify similar species composition and densities across mesocosms. Two chambers per mesocosm (i.e., a total of six chambers per treatment) were left in the lower tray for a period of 28 days to allow chamber colonization, and mesh windows gently brushed daily to maintain water flow. On day 28 of exposure, chambers were retrieved, and sediments enumerated for copepod and nematode densities. Copepods were identified to the lowest taxonomic level, analyzed for sex specific densities and clutch sizes per species, and counted for total copepodites and nauplius per chamber. Due to the inherent difficulty in nematode taxonomic identification, the abundance of this group is presented as total nematodes. Studies with meiobenthos traditionally present densities as individuals/10 cm<sup>2</sup>; however, due to the use of artificial experimental units (colonization chambers) our data are presented as individuals per chamber.

#### 2.4. Statistical analysis

Multivariate statistical analysis (i.e., hierarchical cluster analysis; PROC CLUSTER; SAS Institute, Cary, NC, USA) was used to detect natural groupings between control and atrazine chambers using total adult density per species as measured characteristics. The Ward's minimum variance method was chosen a priori to determine resultant distances (linkages) between chambers. All variables were standardized prior to cluster analysis. The number of clusters was determined by plotting the proportion of variance resulting from joining two clusters (semi-partial square root, SP-R<sup>2</sup>), versus the cumulative number of clusters.

Density (i.e., by sex, female reproductive stage and total species density) of the most common copepod species between control and atrazine dosed colonization chambers were analyzed by one-way analysis of variance (ANOVA; PROC GLM). Variables failing normality were transformed via square root ( $\sqrt{x}$ ) or angular transformation ( $\arcsin(\sqrt{p})$ ) where appropriate.

### 3. Results

Water quality parameters were consistent across control and atrazine dosed mesocosms. Mean measured values ( $\pm 1$  SD) throughout the 28 days of exposure were: dissolved oxygen saturation (DO%) =  $7.90 \pm 1.21$  mg/L, salinity =  $22 \pm 0.5$  ppt, pH =  $7.9 \pm 1.2$ , turbidity =  $6.2 \pm 3$  NTU and water temperature =  $30 \pm 3$  °C. Atrazine concentration over the 28 days was on average  $24.8 \pm 6.9$  µg/L, with measured concentrations

26% above ( $35.17 \pm 0.12$  µg/L;  $n = 3$ ) and 76% below ( $14.75 \pm 0.74$  µg/L;  $n = 3$ ) nominal concentration (26 µg/L) on days 1 and 25, respectively.

A total of six exposure chambers per treatment were initially placed in the mesocosms. A single control chamber missing a window mesh was removed from the analysis at the end of the exposure. The number and density of meiobenthic assemblages (i.e., species composition) at the beginning of the exposure were similar across estuarine mesocosms, with 12 copepod species identified in the system. Copepod and nematodes were found in all colonization chambers, with seven copepod species common to all chambers. Three of those species, *Microarthridion littorale*, *Enhydrosoma propinquum* and *Halicyclops* sp. were found at low densities in control chambers (<12 adults/chamber) and were present at even lower densities (<5 adults/chamber) or absent in atrazine chambers. Thus, these species were not included in the analysis.

The Ward's minimum variance method identified two distinct clusters (SP-R<sup>2</sup> = 0.52; Fig. 1). The first cluster includes all atrazine chambers (A1–A6) plus one control chamber (C1), while the second cluster includes the remaining four control chambers (C2–C5). The first cluster is characterized by low copepod densities across all species, while the second group is dominated by chambers having high densities of *Paronychocamptus wilsoni* and *E. baruchi*.

Total adult copepod densities (including all adult copepod species) in atrazine chambers were reduced on average by 72%, relative to controls ( $p = 0.001$ ; Fig. 2); while early copepod life stages (i.e., nauplius and copepodites) and total nematode densities were similar between exposures ( $p > 0.05$ ). The reduction in adult copepod densities had a significant effect on the nematode-to-copepod ratio ( $p = 0.011$ ), which was  $0.61 \pm 0.35$  in control and  $9.95 \pm 7.61$  in atrazine chambers.

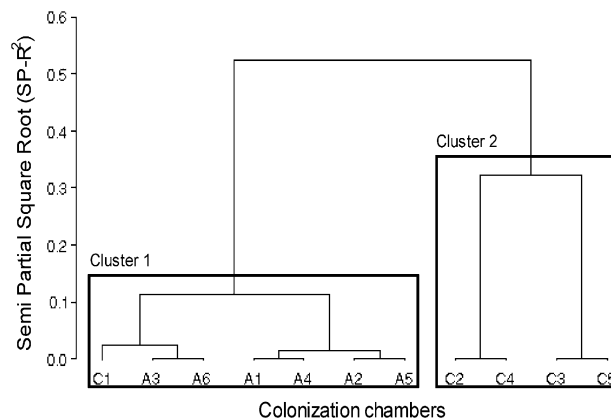


Fig. 1. Cluster analysis (Ward's minimum variance method) based on total adults per copepod species found in control and atrazine colonization chambers. C = control; A = atrazine.

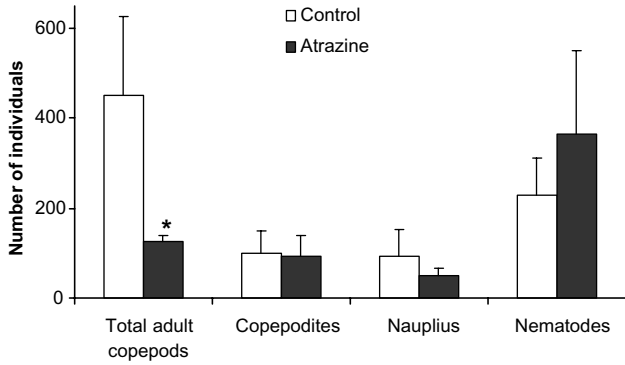


Fig. 2. Total density (mean number of individuals  $\pm$  1SD) of adult copepod, copepodites, nauplius and nematodes in control ( $n = 5$ ) and atrazine ( $n = 6$ ) colonization chambers. Total density includes all adult copepod species found in colonization chambers. (\*) Represents significant difference ( $\alpha = 0.05$ ) versus controls.

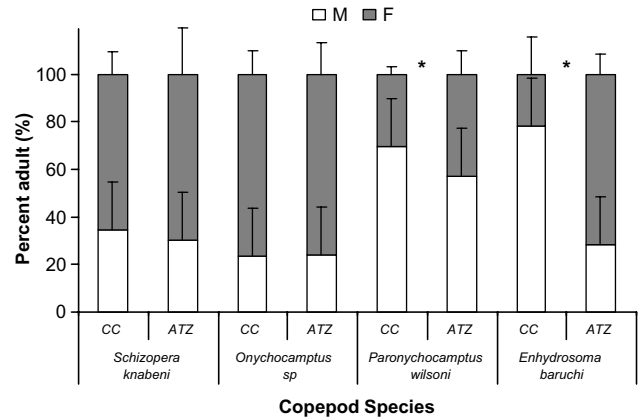


Fig. 3. Percent surviving female and male copepods (mean %  $\pm$  1SD) of the most common copepod species in control (CC,  $n = 5$ ) and atrazine (ATZ,  $n = 6$ ) colonization chambers. (\*) Represents significant difference ( $\alpha = 0.05$ ) versus controls.

Analysis of the most common species (*P. wilsoni*, *E. baruchi* and *Onychocamptus* sp.) in both treatments showed a significant reduction (50–70%;  $p < 0.05$ ) in male and female densities (all three species), and a reduced number of gravid females (*P. wilsoni*) in atrazine exposures, relative to controls (Table 1). However, brood size by copepod species was not statistically significant between exposures ( $p > 0.05$ ). The copepod *Schizopera knabeni*, on the other hand, did not show any differences in density across treatment and control.

In *S. knabeni* and *Onychocamptus* sp., male-to-female ratios were similar across exposures (i.e.,  $\sim 1:2$  and  $1:3$ , respectively;  $p > 0.05$ ; Fig. 3), but in *P. wilsoni* and *E. baruchi* these ratios were altered following chronic exposure to atrazine. For example, in *P. wilsoni* the female proportion doubled in atrazine exposures (ratio  $\sim 1.25:1$ ) relative to control chambers (ratio  $\sim 2.25:1$ ;  $p = 0.032$ ); while the male-to-female ratio in *E. baruchi* exposed to atrazine was reversed (ratio  $\sim 1:2.5$ ) compare to that of controls (ratio  $\sim 2.25:1$ ;  $p = 0.032$ ).

Table 1

Adult female and male mean densities ( $\pm$ 1SD) of the most common copepod species in control ( $n = 5$ ) and atrazine ( $n = 6$ ) colonization chambers

Copepod species	Control	Atrazine	<i>p</i> -value
<i>Schizopera knabeni</i>			
Total adult male	6.67 $\pm$ 2.49	4.14 $\pm$ 1.4	>0.05
Total adult female	13.67 $\pm$ 6.18	18 $\pm$ 16.88	>0.05
Total gravid female	6 $\pm$ 4.08	9 $\pm$ 7.26	>0.05
Female brood size	17.89 $\pm$ 6.16	17.04 $\pm$ 6.05	>0.05
Total adult	20.33 $\pm$ 8.06	22 $\pm$ 16.27	>0.05
<i>Paronychocamptus wilsoni</i>			
Total adult male	162.33 $\pm$ 75.84	37.33 $\pm$ 16.82	0.001
Total adult female	71.33 $\pm$ 36.43	25 $\pm$ 7.26	0.005
Total gravid female	11 $\pm$ 2.16	3.67 $\pm$ 3.77	0.006
Female brood size	13.55 $\pm$ 4.16	13 $\pm$ 3.79	>0.05
Total adult	233.68 $\pm$ 111.67	62.33 $\pm$ 23.1	0.001
<i>Enhydrosoma baruchi</i>			
Total adult male	8 $\pm$ 3.74	1.33 $\pm$ 0.47	0.0002
Total adult female	2 $\pm$ 1.23	3.33 $\pm$ 0.47	0.03
Total gravid female	0.67 $\pm$ 0.94	0.67 $\pm$ 0.47	>0.05
Female brood size	10 $\pm$ 4	13 $\pm$ 0	>0.05
Total adult	10.33 $\pm$ 2.63	4.67 $\pm$ 0.47	0.002
<i>Onychocamptus</i> sp.			
Total adult male	28.67 $\pm$ 16.68	8 $\pm$ 2.83	0.005
Total adult female	100.33 $\pm$ 54.78	28.67 $\pm$ 9.84	0.004
Total gravid female	8.67 $\pm$ 6.34	5.67 $\pm$ 2.06	>0.05
Female brood size	12.69 $\pm$ 2.81	15.18 $\pm$ 3.24	>0.05
Total adult	129 $\pm$ 65.79	36.67 $\pm$ 7.36	0.002

#### 4. Discussion

Modular estuarine mesocosms provide multi-phylum, structural and functional complexity for a more comprehensive assessment of toxicant fate and effects in salt marshes (Pennington et al., 2004). In the current study, data from these mesocosms satisfactorily identified the potential effects of atrazine on meiobenthos chronically exposed to a concentration near the proposed USEPA seawater quality criterion (SWQC, 26 µg/L). The copepod community found in our mesocosms included the indigenous copepod species commonly found in estuarine salt marshes and salt marsh sediments (Coull, 1978; Bell, 1979). Four copepod species (*S. knabeni*, *P. wilsoni*, *E. baruchi* and *Onychocamptus* sp.) were common to all the experimental chambers, with predominance of *P. wilsoni* and *E. baruchi*.

Chronic exposure to atrazine resulted in an overall reduction of adult copepod densities, while early copepod life stages (i.e., nauplius and copepodites) and nematodes densities appeared unaffected. In contrast to a previous study examining water-borne atrazine in laboratory lifecycle assays (Bejarano and Chandler, 2003), we did not find any differences in mean brood sizes resulting from the chronic 28-day atrazine exposure. Additionally, we found species-sensitivity differences with adult *P. wilsoni*, *E. baruchi* and *Onychocamptus* sp. being more sensitive to atrazine exposure than *S. knabeni*. The latter species has previously shown less sensitivity to acute organic contaminant toxicity (i.e., phenanthrene) than copepods such as *Nitocra lacustris* (Lotufo and Fleeger, 1997). Other aquatic mesocosm studies have also found differences in sensitivities among copepod species (Kreutzweiser et al., 2004; Medina et al., 2004). In exposures to the plant-derived insecticide Neemix 4.5, adult calanoid copepods were more sensitive to Neemix than cyclopoids as shown by reductions in the abundance of the calanoid copepod *Skistodiatomus oregonensis* (Kreutzweiser et al., 2004). In that study, adult copepods were also more sensitive than early life stages. Also, in marine mesocosms dosed with cypermethrin, Medina et al. (2004) found decreased copepod diversity compared to controls. At the end of the 14-day exposure period, cypermethrin dosed mesocosms were dominated by copepods in the families Temoridae (67%) and Acartiidae (33%), while control mesocosms were dominated by copepods in the families Acartiidae, Temoridae, and Calanidae (Pseudocalanidae and Paracalanidae) accounting for 55%, 25% and 20%, respectively. In the present study, we also found that male densities of *P. wilsoni* and *E. baruchi* were significantly depressed by atrazine exposure; however, densities of *E. baruchi* were low in both, controls and treatment chambers. In contrast, female to male *Onychocamptus* sp. ratio in atrazine exposure chambers was similar to control. The ecological

significance of skewed sex ratios, in particular reduced male density, could potentially result in lower female fertilization and, consequently, reduced population growth (Bejarano et al., 2005).

Differences in species sensitivity to the highly soluble herbicide atrazine, may have resulted from different copepod lifestyles (i.g., epibenthic or endobenthic) and/or copepod feeding strategies and differences in prey items. Copepods spending longer periods of time in the water column (epibenthic) would likely be exposed to contaminants with elevated water solubility, i.e., atrazine. Copepods that live in the sediment–water interface (epibenthic) or burrowed in sediments (endobenthic) would be exposed to contaminants present in pore water and sediments. Although most copepod species in the present study were epibenthic, they may have differed in the amount of time they were exposed to atrazine while in the water column. Likewise, effect differences between adult copepods and juveniles stages may have resulted from differences in life stage ecologies; adults could have been exposed to atrazine most frequently in the water column than the less mobile juveniles, which may have spent more time burrowed in sediments.

An alternative or additional explanation for the observed differences in species sensitivity could be the indirect effects of atrazine on the phytoplankton communities present in the mesocosms, which in turn may have caused negative effects on copepod density. In fact, an earlier study with tidal creek mesocosms dosed with 40 µg/L atrazine (DeLorenzo et al., 1999), reported a two- and five-fold reduction in chlorophyll-*a* in artificial substrates and the water column, respectively. The same study also showed that chronic exposures to atrazine had a negative effect on the algal assemblages, primarily by eliminating eight of the 28 taxa present in control mesocosms. Hence, copepods feeding on phytoplankton differentially affected by atrazine could exhibit negative indirect effects resulting from chronic herbicide exposure. However, little is known regarding the specific feeding preferences of the copepod species presented here.

Studies have suggested that copepods tend to be more sensitive to pollution than nematodes (Van Damme et al., 1984; Gee et al., 1985; Warwick et al., 1988). Consequently, Raffaelli and Mason (1981) proposed the nematode-to-copepod ratio as a pollution index; an index that is generally more sensitive when considering only interstitial nematodes and copepods in sandy sediments (Coull and Chandler, 1992; Shiells and Anderson, 1985). In our study, sediments were sandy muds, and the ratio was on average 94% higher in atrazine exposed colonization chambers than in controls, resulting from decreased copepod density and slight increases in nematode densities. Although the utility of this index has been debated (Coull et al.,

1981), another study suggested that this index can respond strongly to contaminant exposure (Peterson et al., 1996).

One of the greatest difficulties encountered in this study was the high variability among treatment replicates, partially resulting from the inherent difficulties of working with meiobenthic assemblages (i.e., spatial and temporal variability). Such variability could explain the clustering of one control chamber with all atrazine chambers in the hierarchical cluster analysis. Additionally, although the level of complexity in these estuarine mesocosms is lower than that of field salt marshes, these mesocosms likely had differences in variables such as phytoplankton composition and starting meiobenthic densities which may have contributed to high variance. Despite these difficulties, these estuarine mesocosms were still able to detect chronic atrazine effects on the indigenous meiobenthic community.

Finally, chronic exposures to an atrazine concentration near the proposed USEPA SWQC (26 µg/L) were deleterious to the meiobenthic assemblages present in colonization chambers. Farmer et al. (1995) stated that differences less than 50%, relative to controls, may be irrelevant in natural ecosystems because of large seasonal variation, rapid generation time, and recovery of meiobenthos. This suggests that the depression of copepod colonization found in this study (~70% lower in atrazine treatment than controls) is likely of environmental relevance. Under field conditions, such reduction in copepod abundance could result in meiobenthic community structure changes and reduced biomass. In addition to our results, laboratory studies with copepods chronically exposed to atrazine at a concentration near the proposed USEPA SWQC, have also shown atrazine induced developmental delays (Forget-Leray et al., 2005) and negative reproductive effects (Bejarano and Chandler, 2003). In 10-day chronic exposures to atrazine (25 µg/L) with the calanoid copepod *Eurytemora affinis*, Forget-Leray et al. (2005) found a 4-day delay in nauplius development into copepodites relative to controls (i.e., 8-day maturation from nauplius to copepodite). Also, a multigenerational lifecycle study of atrazine (30.3 ± 0.7 µg/L) with the harpacticoid copepod *Amphiascus tenuiremis* showed a 21% increase in reproductive failure and a 27% decrease in fecundity in copepods chronically exposed for one generation. These studies and our mesocosm study indicate that copepod populations and communities are at risk (directly or indirectly) when chronically exposed to an atrazine concentration near or below 26 µg/L.

Further studies should examine the effects of this herbicide on various meiobenthic copepod species with different life cycle ecologies, under both laboratory and mesocosm conditions. Additionally, similar experiments evaluating environmentally realistic atrazine concentrations will be useful in determining the lowest observable

effect concentration (LOEC) for chronically exposed meiobenthic assemblages.

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