Using a Novel Sediment Exposure to Determine the Effects of Triclosan on Estuarine Benthic Communities

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Introduction

Triclosan (TCS) (5-chloro-2-(2,4-dichlorophenoxy)-phenol; Figure 1)

- An antimicrobial compound in many personal care and consumer products including:
  - liquid hand soaps, deodorants, cosmetics, toothpastes
  - toys, kitchen utensils, socks

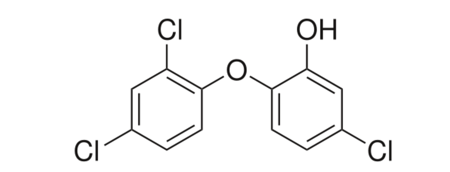


Figure 1. Chemical structure of triclosan.

Environmental Concern

- Enters estuarine environments primarily via wastewater treatment plant effluent
- Sorbs to organic matter and accumulates in sediments (Cantwell et al. 2010)
- Has been found in sediment cores dating back to the mid-1960s at levels up to 800 ng/g (ppb) (Miller et al. 2008, Cantwell et al. 2010)
- TCS is toxic to marine organisms at the ppb level **(Perron in prep**, DeLorenzo et al. 2008).

Objectives:

1.) Determine the effect of triclosan on intact marine benthic communities
2.) Test a novel sediment exposure method for efficacy in evaluating contaminants of emerging concern

Methods

Collection

- Sediment cores (height: ~17 cm, diameter: 15 cm) were collected from Narrow River, Narragansett, RI in June 2010

Maintenance

- 24 cores were maintained in the laboratory in a flow-through, filtered seawater system with aeration (Figure 2)
- Avg. 6.4 h turnover rate; 30 ‰, 20ºC, 16:18 light:dark cycle

Treatments (6 cores/treatment)

- **Field Control** (no sediment added)
- **Lab Control** (non-toxic Long Island Sound (LIS) sediment)
- **Low Triclosan** (nominal: 30 mg triclosan/kg dry LIS; measured: 13.9 mg/kg)
- **High Triclosan** (nominal: 300 mg triclosan/kg dry LIS; measured: 181 mg/kg)

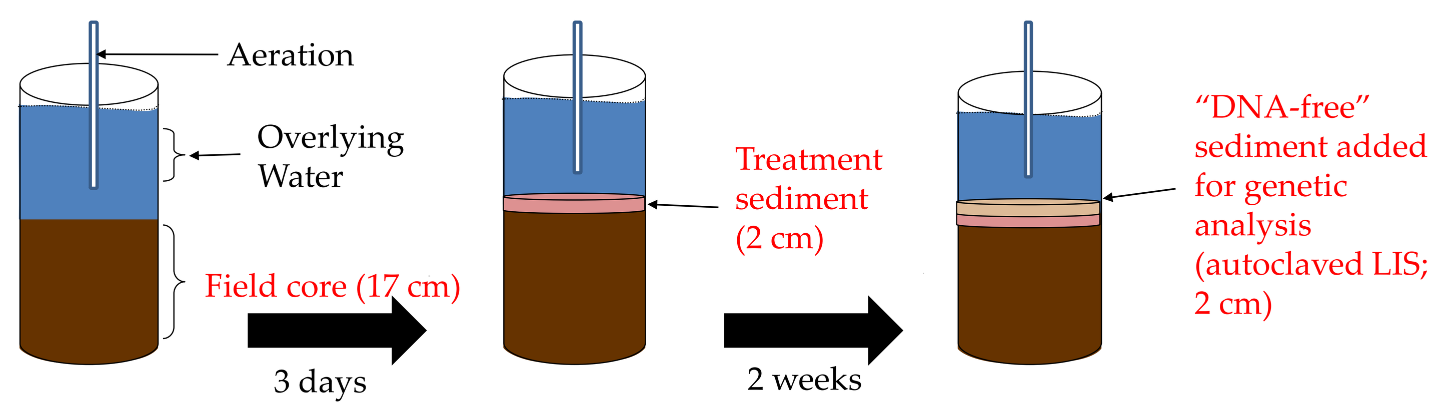


Figure 2. Schematic of core set-up in laboratory.

- Following addition of each sediment layer (to all except Field Control cores), organisms migrate to the top, better oxidized layer (Chandler et al. 1997)

Samples (from top 2-cm layer one week after application of DNA-free sediment)

- Meiofauna (retained on a 50 µm sieve)
- Macrofauna (retained on a 500 µm sieve)
- Genetic samples (described in **Chariton in review**)

Analysis

- Chemical analysis of background and spiked sediments were performed with an automated solvent extraction (ASE) unit and GC/MS analysis
- Statistical:
  - community indices and the abundances of selected meio- and macrofauna taxa were analyzed with a balanced, single-factor ANOVA, followed by a post hoc Student Newman-Keuls (SNK) test
  - non-metric multidimensional scaling (nMDS)
  - permutational multivariate analysis of variance (PERMANOVA)

Figure 3. Average number of meiofaunal (a) and macrofaunal (b) individuals of selected taxa (grouped by Class or higher taxonomic level) in each treatment community.

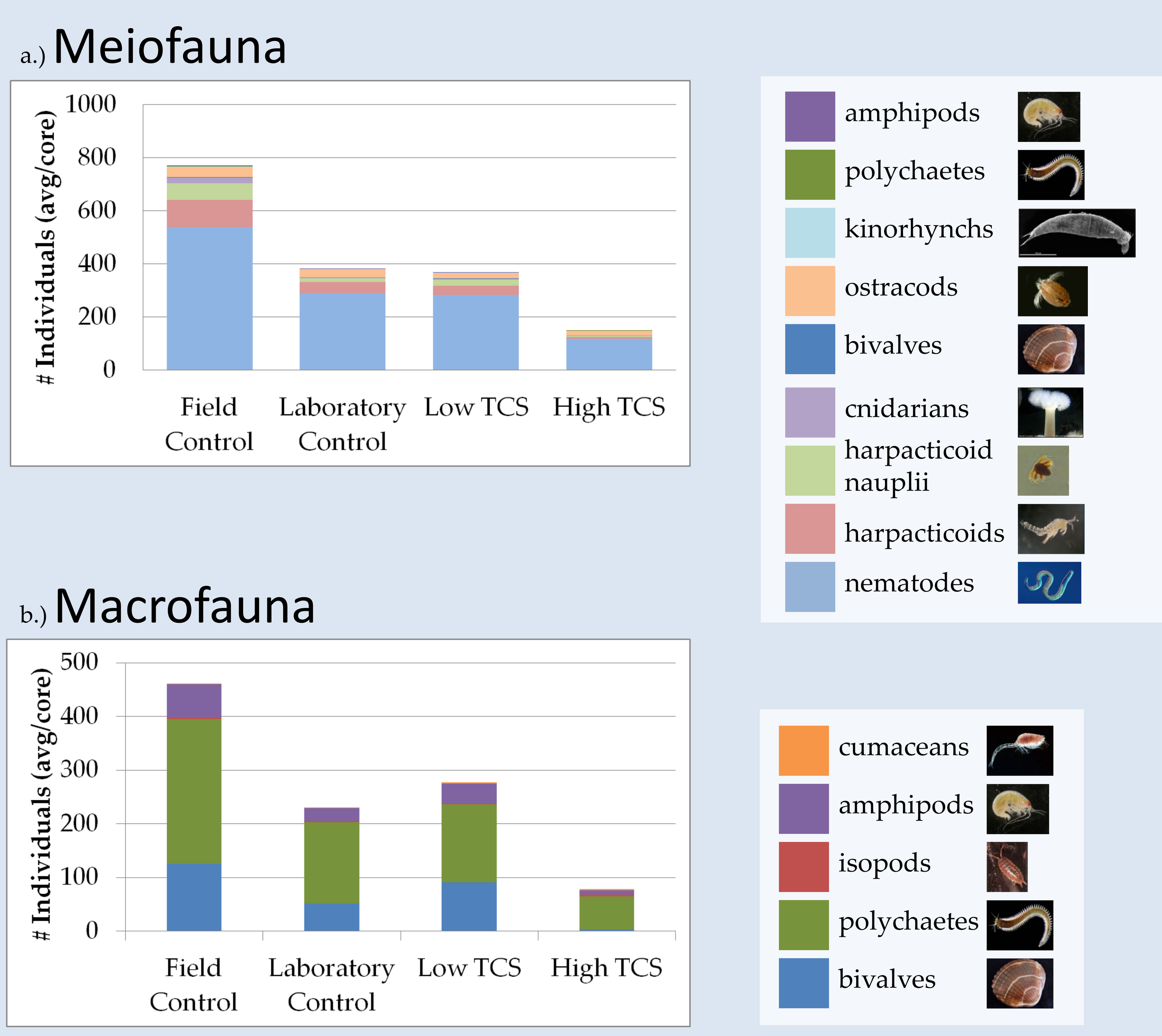


Figure 4. Summary Venn Diagram indicating sensitivity of meiofaunal (a) and macrofaunal (b) selected taxa and univariate community metrics to sedimentation, triclosan, or both. Diagram displays significant results from a Student Newman-Keuls post-hoc test, interpreted as follows: Field>(Lab=Low=High): Sensitive to sedimentation **(Sediment)**; (Field=Lab=Low)>High: Sensitive to triclosan **(Triclosan)**; Field>(Lab=Low) >High: Sensitive to sedimentation and triclosan **(Both)**. Taxa and metrics that were not significantly affected by treatment are not shown.

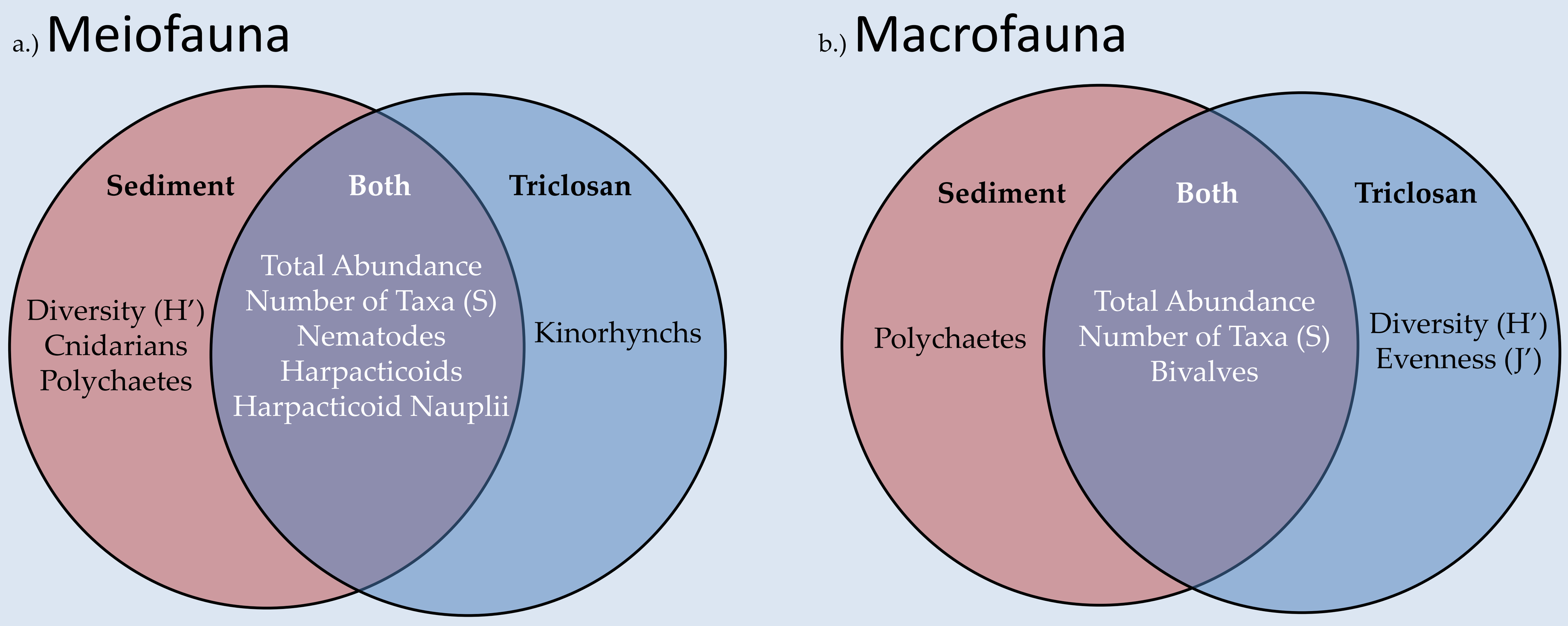
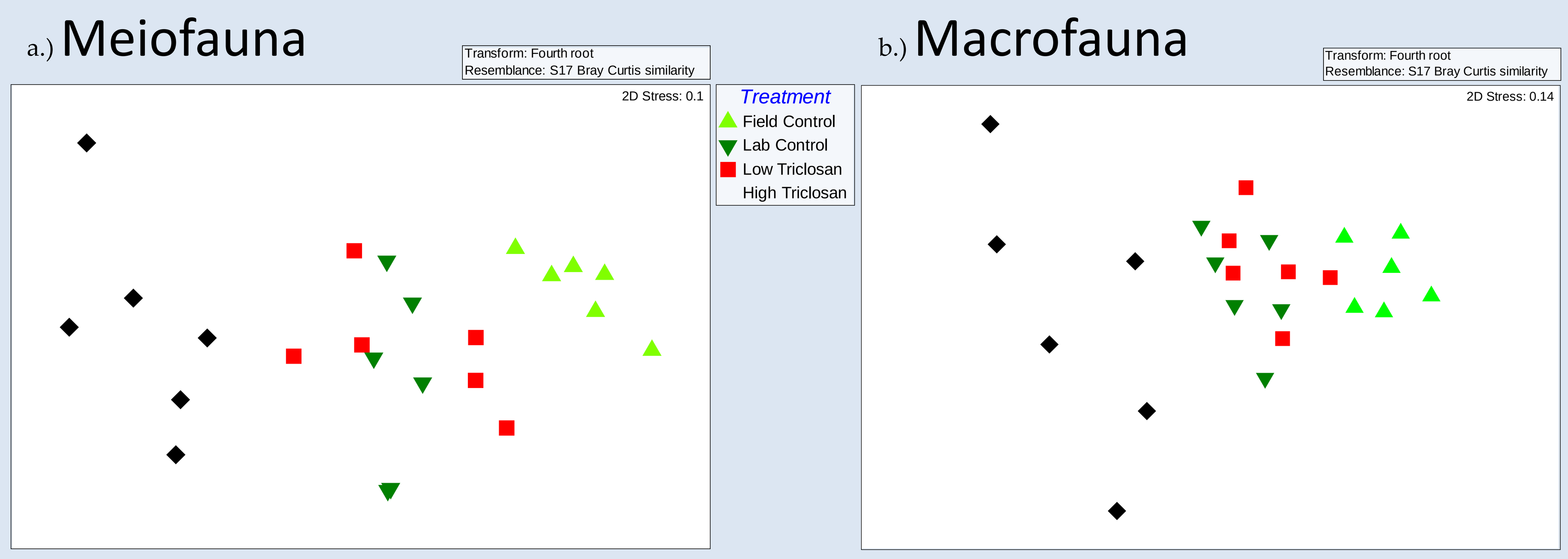


Figure 5. nMDS ordination plot for the meiofaunal (a) and macrofaunal (b) communities sampled from the four treatments.



Results and Discussion

The treatments had a significant effect on the benthic community

- PERMANOVA for differences between treatments (p=0.001 for both meio- and macrofauna)
- All treatments significantly different from each other except lab control and low TCS
- No significant differences between treatments in:
  - meiofaunal evenness
  - abundances of ostracods, meiofaunal amphipods, isopods, and cumaceans

Lab Controls suffered adverse affects from sedimentation

- Field controls more abundant and taxonomically rich (S) than lab controls (Figure 3a,b)
- Meiofauna field controls more diverse (H') than lab controls (Figure 4a)
- "Smothering" effect (Thrush et al. 2004, Sandulli and De Nicola-Giudici 1990)
- Silty autoclaved sediment layer = little interstitial space for oxygenated water to penetrate into the sediment (Figure 4a,b)

Low triclosan levels (~14 ppm) did not impact the benthic communities more than Lab Controls

- Ordination plots did not differ between lab control and low TCS treatments (Figure 5)

High triclosan levels (~180 ppm) decreased abundance and negatively impacted community structure

- Abundance (Figure 3a,b)
  - 1/5 of the field controls
  - 1/2 (meiofauna) or 1/3 (macrofauna) of the lab control and low triclosan treatments
- Univariate community metrics (Figure 4a,b)
  - Less taxonomically rich than all other treatments
  - Macrofauna community also less even and diverse than all other treatments

Conclusions

- The benthic community was adversely affected by high levels of triclosan (~180 ppm).
- However, many organisms also suffered from the addition of sediment to the tops of the cores, thereby confounding the effects of triclosan on the communities.
- Efforts will focus on minimizing this effect in future experiments.
- Although the triclosan concentrations used in this experiment were higher than what is currently found in the environment, the use of triclosan is widespread and its popularity is increasing. Therefore, concentrations could reach levels that significantly affect benthic communities.

References

Cantwell, M. G., Wilson, B. A., Zhu, J., Wallace, G. T., King, J. W., Olsen, C. R., Burgess, R. M. and Smith, J. P. 2010. Temporal trends of triclosan contamination in dated sediment cores from four urbanized estuaries: Evidence of preservation and accumulation. *Chemosphere* 78: 347-352.

Chandler, G. T., Coull, B. C., Schizas, N. V. and Donelan, T. L. 1997. A culture-based assessment of the effects of chlorpyrifos on mulitple meiobenthic copepods using microcosms of intact sediments. *Environ Toxicol Chem* 16: 2339-2346.

DeLorenzo, M. E., Keller, J. M., Arthur, C. D., Finnegan, M. C., Harper, H. E., Winder, V. L. and Zdankiewicz, D. L. 2008. Toxicity of the antimicrobial compound triclosan and formation of the metabolite methyl-triclosan in estuarine systems. *Environmental Toxicology* 23: 224-232.

Miller, T. R., Heidler, J., Chillrud, S. N., DeLaquil, A., Ritchie, J. C., Mihalic, J. N., Bopp, R. and Halden, R. U. 2008. Fate of Triclosan and Evidence for Reductive Dechlorination of Triclocarban in Estuarine Sediments. *Environ Sci Technol* 42: 4570-4576.

Sandulli, R. and De Nicola-Giudici, M. 1990. Pollution effects on the structure of meiofaunal communities in the Bay of Naples. *Mar Pollut Bull* 21: 144-153.

Thrush, S., Hewitt, J., Cummings, V., Ellis, J., Hatton, C., Lohrer, A. and Norkko, A. 2004. Muddy waters: elevating sediment input to coastal and estuarine habitats. *Front Ecol Environ* 6: 299–306.