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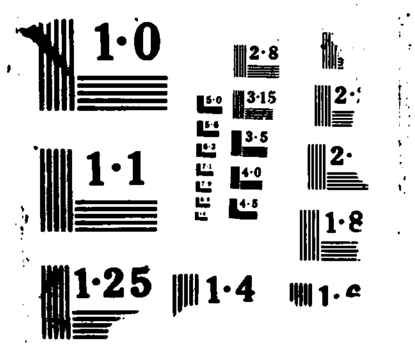
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SUBLETHAL EFFECTS OF TRIBUTYLTIN ON THE HARD SHELL CLAM, *Mercenaria mercenaria*

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Chronic bioassays, lasting at least seven days, show that veliger stages of clams, *Mercenaria mercenaria*, are the most sensitive to exposure to tributyltin compounds (TBT). Post-set clams survived exposures up to 7.5 µg/l for 25 days. No veligers, the planktonic larval stage, however, survived seven days in 750 ng/l. Valve length of veligers, an index of growth, was statistically less than controls in concentratons of 50 ng/L and above on day 14 of development. When clam embryos are exposed to TBT, effects on subsequent stages are the most severe, suggesting that TBT exerts its most damaging effects very early in larval development. Data from these studies suggest that acute toxicity to clams would not occur in most habitats due to exposure to TBT from antifouling coatings. Sublethal effects, however, could result from lower exposures typical of some sites. Field observations to assess ecological consequences would be most useful to confirm risk estimates.

INTRODUCTION

Experience in Europe with effects of tributyltin (TBT) antifouling coatings has shown molluscs to be among the most sensitive non-target groups (1-4). The work reported here on the hard shell clam, *Mercenaria mercenaria* was performed to determine if indigenous, commercially-significant western Atlantic bivalve species are at risk due to use of these compounds. We have attempted to identify the most sensitive life-history stage to chronic exposures to TBT and the range of active concentrations.

MATERIALS AND METHODS

All bioassays were performed using bis(tri-n-butyl)tin oxide (Alfa-Products). It was dissolved in acetone so that 10 µg stock per liter seawater yielded the desired nominal concentration for exposure. Exposure regimes were daily static renewal for larval bioassays. Tests with juvenile clams were flow-through from a reservoir of TBT in seawater mixed to the desired test concentration.

Clam gametes, larvae and juveniles were obtained from an experimental hatchery effort at Harbor Branch Oceanographic Institution. Adults for spawning were obtained from the Indian River Lagoon (Florida, USA). This population apparently spawns throughout the year so no laboratory conditioning is necessary. Spawning was induced by cyclical temperature exposure to 20°C first, followed by 30-32°C. During all bioassays, experimentals were fed microalgae, *Isochrysis galbana* Tahiti strain. TBT concentrations in seawater were measured by hydride derivatization, followed by purge and trap. They were quantified by atomic absorption spectrometry (5,6). Additional specific details of experiments summarized in this paper are available in (7) and (8).

RESULTS

Clam post larvae, the settlement stages which resemble very small clams, are the least sensitive portion of the life cycle we tested. In 21 days exposure to TBT, only groups exposed to 10 µg/l suffered complete mortality (Fig. 1). Survival of those in lower exposures were not markedly different from controls.

Clam veliger stages are much more sensitive to acute effects of TBT exposure. None survived eight days exposure to 1 µg/l or higher (Fig. 2). At the end of this period, controls had become pediveligers. Clam veligers exposed to 0.6 µg/l displayed some survival (less than controls) but there was very little growth and metamorphosis to pediveligers did not occur.

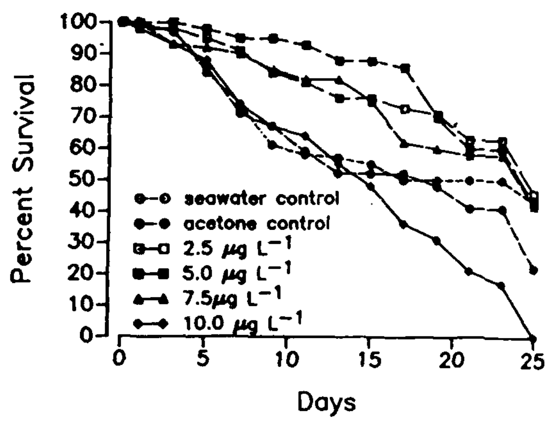

Fig. 1. Survival of hard shell clam, *Mercenaria mercenaria* post larvae exposed for 25 days to TBT. Only groups in 10 µg/l suffered complete mortality.

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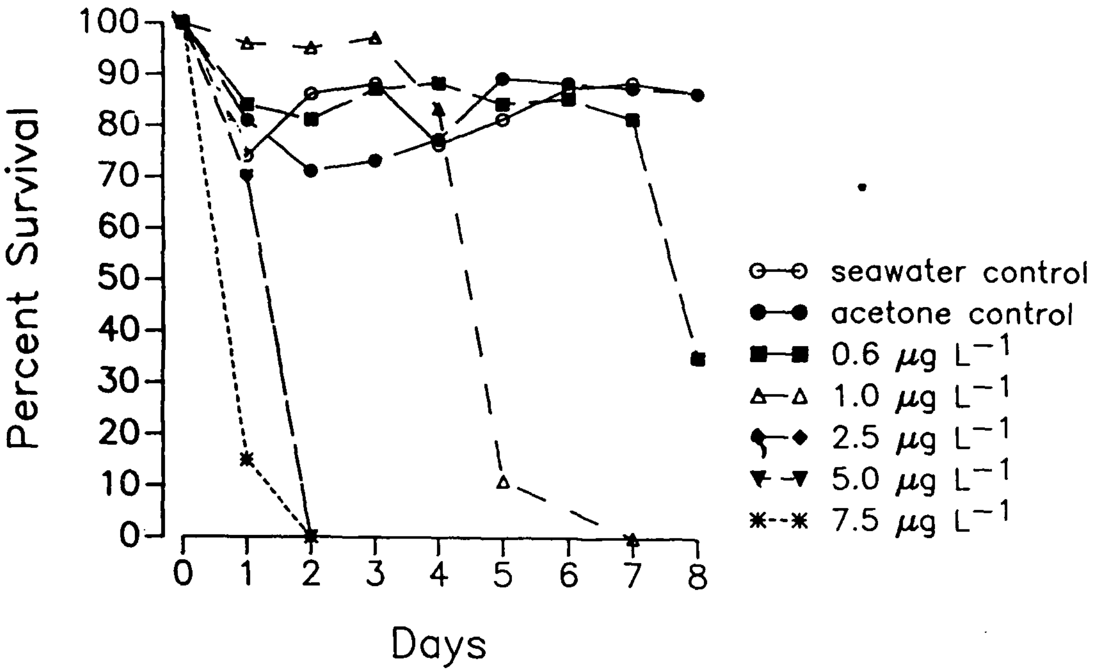

Fig. 2. Survival of hard shell clam veligers, Mercenaria mercenaria, exposed to TBT. No veligers survived exposure of 1 µg/L and above.

In another set of experiments, exposure began with embryos and continued for 14 days. TBT concentrations were between 10 and 500 ng/l. In addition to continuous exposure, a second group was exposed for the first five days to TBT, then kept in clean water for the next 8 to determine if effects of TBT were reversible. Survival was not exposure-dependent, or affected by a recovery period (Fig. 3).

Growth, however, was consistently reduced by exposure to low TBT exposures (Fig. 4). Although an eight day recovery did produce a slight increase in mean value length of experimentals, in no case was it statistically significantly larger than continuously exposed animals (Fig. 5).

Chemical measurements showed that initial concentrations were ~80% of nominal. At the end of 24 hours, they declined to ~20-30% of nominal. It should be noted that recovery of added TBT to water containing microalgae is only about half that of "clean" seawater. Thus, the 24 hour values likely reflect only dissolved TBT and does not completely quantify TBT bound to food material and potentially available through ingestion.

DISCUSSION

Embryos and veligers of clams are the most sensitive ones tested. Juvenile clams are much more tolerant of prolonged TBT exposure. Mortality of embryos and veligers was not different from controls in concentrations even as high as 500 ng/l, but growth was very sharply reduced with increasing concentration. It is likely that TBT exposures above 50 ng/l are too high to allow successful recruitment from larval into adult populations because growth is insufficient to allow metamorphosis before the larvae lose competence to complete development.

This work was spurred, in part, by an unpublished LC50 (96 hour) value of ~15 ng/l for clams (9). Our results do not confirm such a low acute value, but we have observed effects on growth at these concentrations. The source of these differences is unclear, but suggest possible significant variation in the response of clam populations to this, as well as other, xenobiotics. It would be speculative to attempt to extrapolate effects of these differences in laboratory bioassays to ecological effects in the field. It is, however, quite possible that release of TBT from antifouling coatings on boats in waters over clamming grounds would influence larval growth and recruitment.

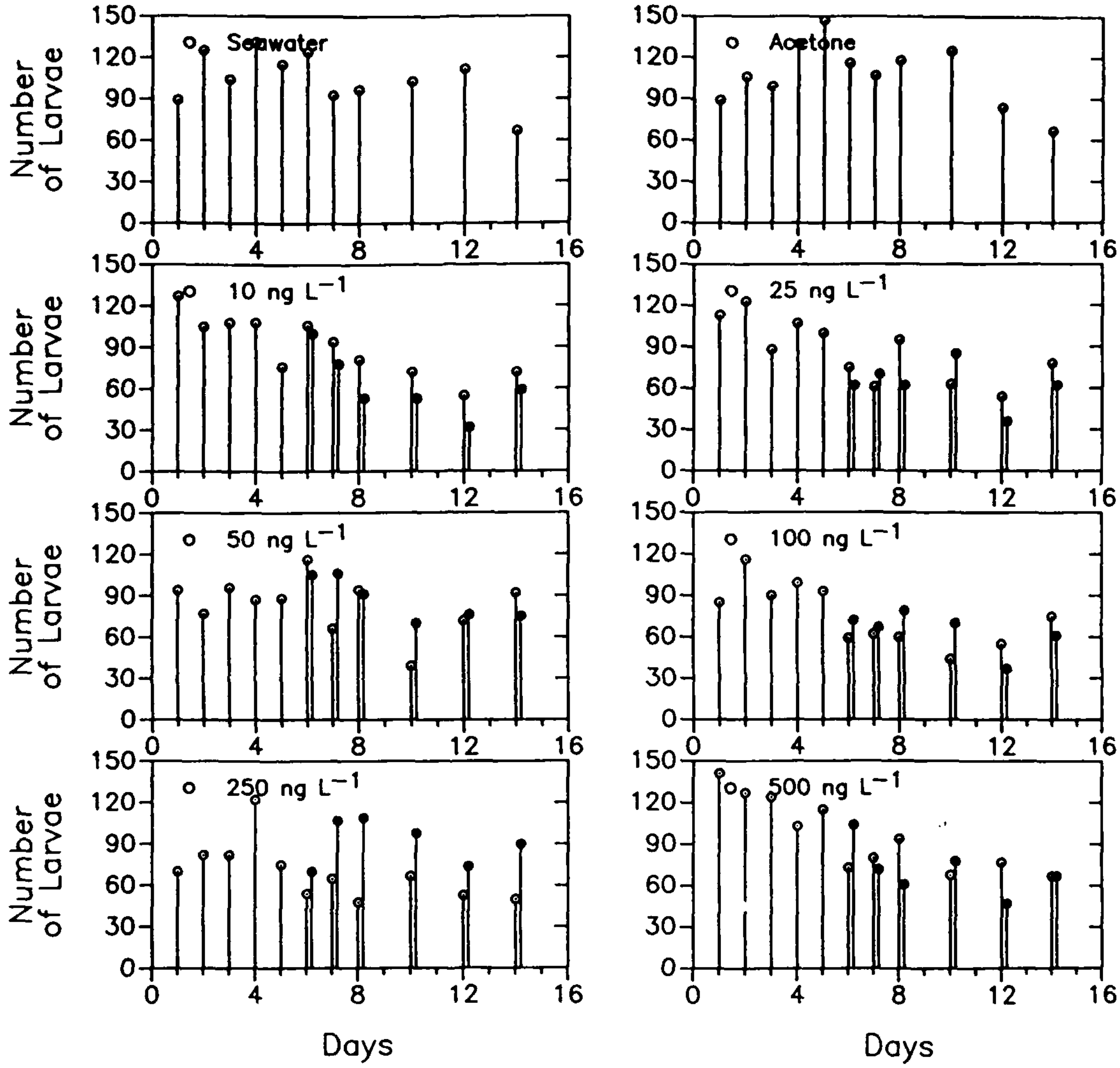

Fig. 3. Survival of hard shell clams, Mercenaria mercenaria, exposed from fertilization through metamorphosis to TBT. There were no exposure dependent reductions in survival. Open symbols: continuous exposure; Closed circles: recovery groups. Recovery group symbols are offset for clarity.

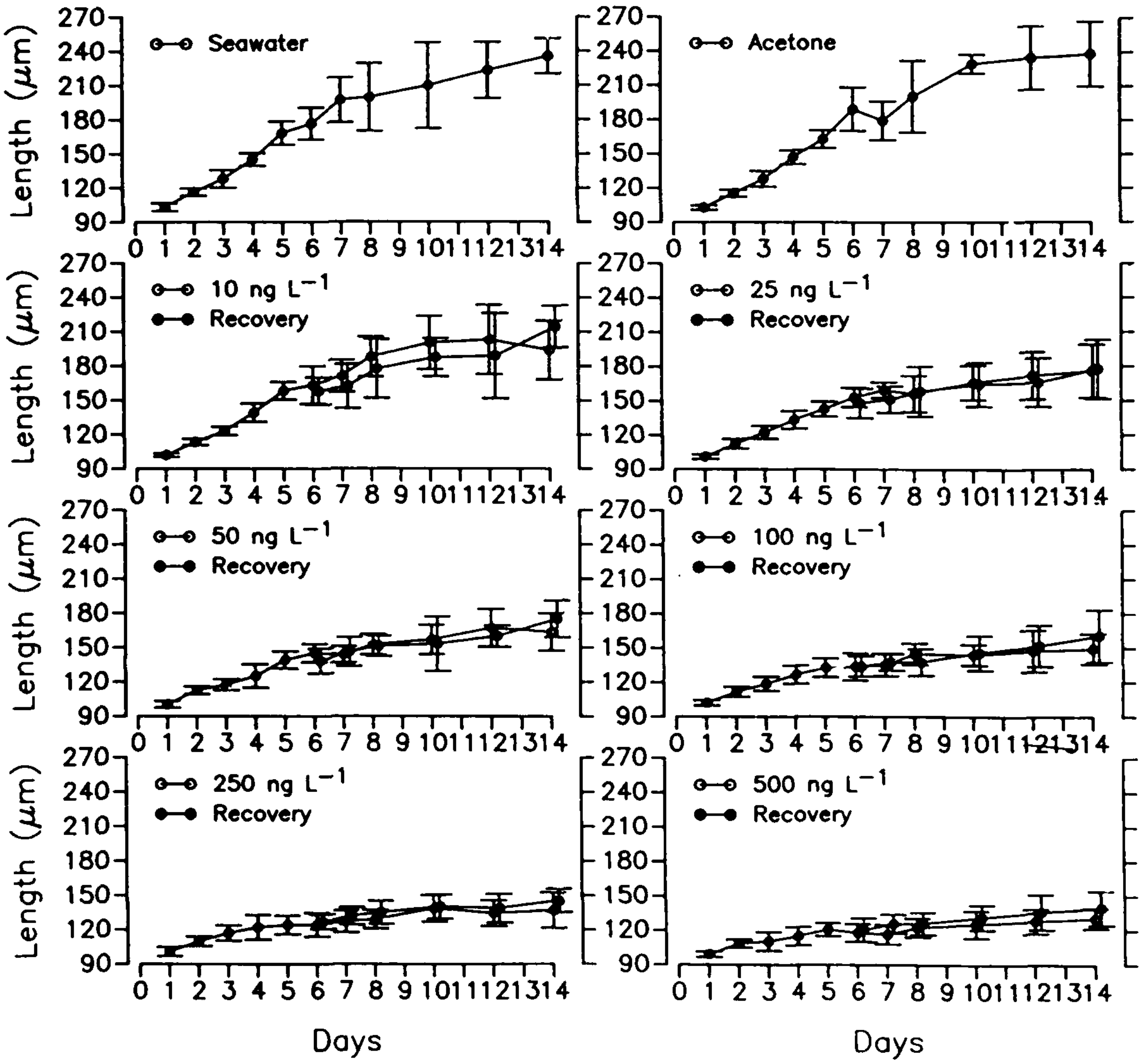

Fig. 4. Cumulative growth of hard shell clams, *Mercenaria mercenaria*, exposed from fertilization through metamorphosis to TBT. Reductions in growth were apparent with the first five days, and were consistantly exposure-dependent. Open symbols: continuous exposure; Closed symbols: recovery groups; Error bars: 1 standard deviation. Recovery group symbols are offset slightly for clarity.

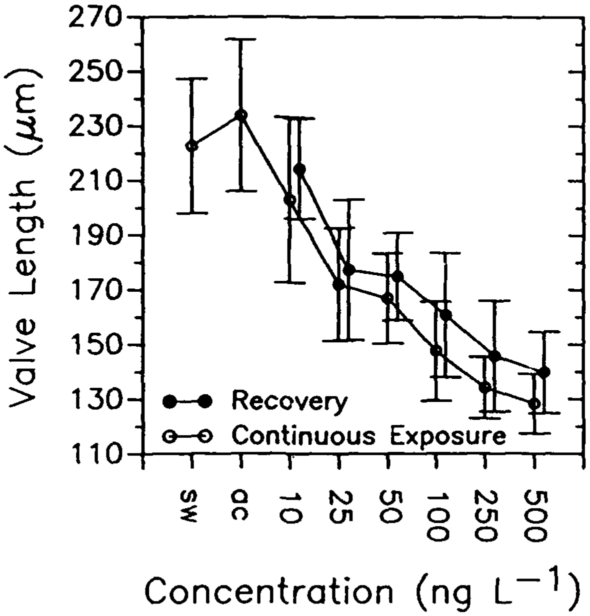

Fig. 5 Comparison of final size of hard shell clams, Mercenaria mercenaria exposed to TBT for 14 days. An eight day recovery period had little apparent effect on size compared to continuous exposure. Error bars: 1 standard deviation.

ACKNOWLEDGEMENTS

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