Response of the Brittlestar Amphiodia urtica to an Outfall Gradient

Amphiodia urtica is one of the most abundant invertebrates in sediments on the mainland shelf off Southern California (Jones 1969). However, it is rare or absent in sediments near municipal wastewater outfalls (City of Los Angeles 1993, County Sanitation Districts of Orange County 1993). The reasons for the decline in *A. urtica* abundance are unknown.

We set out to refine laboratory test methods that can be used to examine the response of *A. urtica* to contaminated sediments. Previous studies demonstrated that the adult brittlestars can be maintained in long-term cultures in the laboratory, and that growth was negatively affected by exposure to contaminated sediments from the Palos Verdes Shelf and Los Angeles Harbor (see *Sediment Toxicity Methods for the brittlestar, Amphiodia urtica* in this volume).

The objective of this study was to measure the response of adult *Amphiodia urtica* to sediments collected along two gradients of outfall effects in Santa Monica Bay and off Orange County. Because *A. urtica* is used as an indicator of outfall impacts, its abundance in sediments over the gradients tested ranged from low near the outfalls to levels typical of reference areas in the Southern California Bight.

Materials and Methods

Collection of Animals and Sediments

Santa Monica Bay. Four stations were chosen along the 60 m isobath near the Hyperion Wastewater Treatment Plant 5-mile outfall (Figure 1). The stations are part of the benthic monitoring program conducted by the Environmental Monitoring Division (EMD) of the City of Los Angeles. The abundance of *A. urtica* ranged from 0 to >2,000/m2 (City of Los Angeles 1993). Sediment samples were collected in November 1990. Sediment was removed from the top 2 cm of four replicate van Veen grab samples and stored in separate polyethylene jars at 5°C. Resident brittlestars were removed from the sediment by press-sieving it through a 2 mm screen.

Brittlestars for the sediment tests were collected in northern Santa Monica Bay (EMD station C3) and off Point Loma (San Diego benthic monitoring station B3). *Amphiodia urtica* was screened from the sediments in the laboratory and trans ferred to acclimation tanks containing C3 sediments.

Orange County. Six stations were chosen along the 60 m isobath near the County Sanitation Districts of Orange County (CSDOC) outfall (Figure 2). The stations are part of the CSDOC benthic monitoring program. The abundance of *A. urtica* ranged from 0 to 960/m2 (County Sanitation Districts of Orange County 1993). Sediment samples were collected in January 1993. Sediment handling procedures were described above. Brittlestars for the sediment tests were collected from northern Santa Monica Bay (station C3).

Sediment Exposures

Santa Monica Bay. Tests were conducted in 1 L polypro-pylene beakers modified for flow through for 28 days. There were four replicates for stations C4 and C5 and six replicates for stations C3 and C6. Each beaker received 2 cm of sediment, 2-4 mL/min flowing seawater, and aeration via a capillary tube. The beakers were randomly arranged in a water bath at 12°C and allowed to equilibrate overnight before animals were added.

Brittlestars were anesthetized with isotonic MgCl2 and oral width was measured with an ocular micrometer in a dissecting microscope. The animals were allowed to recover and five individuals with oral widths between 0.69-1.95 mm were added to each exposure container. Twice weekly, 0.03 g of 0.1

mm sieved Tabimin™ was added to each beaker for food. The oral width of each animal was measured after 28 days; individual change in oral width (growth) was calculated.

Orange County. Exposure conditions were the same as in the Santa Monica Bay experiment with some modifications. The duration of the test was increased to 56 days. There were five replicates per station; three additional replicates were included for C3 and sampled at 28 days. Beakers were allowed to equilibrate for three days before the brittlestars were added.

Five animals with oral widths between 0.71-1.79 mm were added to each test chamber. To determine initial gonad condition, a sample of female *A. urtica* were collected at the beginning of the exposure and prepared for histological examination. Twice weekly, 0.03 g of Argent Hatchfry Encapsulon I was added to the containers as food. After 56 days, the oral width of each surviving animal was measured and all female gonads were prepared for histological examination.

Reproductive Condition

This endpoint was only measured during the Orange County experiment. Female gonads were removed, fixed for 48 h in borax-buffered formalin, transferred to 70% ethanol, and decalcified with EDTA and mild HCl. Cross sections (6 μm) were prepared and stained with hematoxylin and eosin. The diameters of fifteen randomly selected oocytes from each of two sections per animal were measured with an ocular micrometer in a compound microscope. To avoid measurement duplication, non-contiguous sections were selected and only oocytes sectioned through the nucleolus were measured. The largest diameter was measured for oocytes with distorted shapes.

Data Analyses

Endpoint means for each exposure container were used to calculate the mean for each treatment group. Treatment groups were compared by analysis of variance (ANOVA; Wilkinson 1990).

Results

Santa Monica Bay

There was no mortality in any treatment group during the exposure and there was no evidence of sediment avoidance or altered behavior. The mean change in oral width (growth rate) for animals in the control (C3) was 0.02 mm/week. Growth rates for brittlestars exposed to sediments from stations near the Hyperion outfall were not significantly different from the control (Figure 3).

Orange County

Mortality occurred in all treatments and ranged from 4% for OC-10 to 40% for C3 (Figure 4). Mortality occurred between days 35 and 56, and was highly variable within treatment groups. There was no significant difference in mortality of *A. urtica* among the six Orange County stations (ANOVA, $p=0.33$). Two of the five collection site controls (C3) had 100% mortality at 56 days while the remaining three beakers had no mortality. No other test containers had 100% mortality. Animals that eventually died first moved to the sediment surface, and subsequently their arms fragmented. Beakers with mortality often had discolored sediment (e.g., dark or orange patches).

Growth rates of brittlestars in Orange County sediments were equal or greater than growth rates of animals in the collection site control sediment (Figure 5). There were no significant differences in growth rates among the six stations (ANOVA, $p=0.22$). The lowest mean growth rate (0.020 mm/week) occurred among animals in sediment from the station closest to the outfall (OC-0), while the highest mean growth rate (0.033 mm/week) occurred among animals in sediment from the next closest station (OC-1). Animals exposed to collection site (C3) sediments for 28 days had a mean growth rate of 0.044

mm/week; those surviving to 56 days had a mean growth rate of 0.02 mm/week.

There was no apparent change in gonad condition among female *A. urtica* during the experiment. There was no significant difference among initial mean oocyte diameters and final oocyte diameters in all treatment groups (ANOVA, p=0.53; Figure 6). Mean maximum oocyte diameters in the treatments were also not significantly different from the initial condition (data not shown). Oocyte size-frequency distributions in the treatments were similar to the initial size-frequency distribution (data not shown).

Discussion

There was no relation between the field abundance of *A. urtica* and the growth of adults in sediments from the same site in the laboratory. Adult *A. urtica* mortality and growth were not deleteriously affected by exposure to sediments where their field abundances were several orders of magnitude lower than abundances in reference areas. Furthermore, *A. urtica* arm regeneration rates were not affected by exposure to sediments from the same stations in Santa Monica Bay (unpublished data).

Mortality of *A. urtica* in Orange County sediments was probably a laboratory artifact resulting from degradation of sediment quality in some test chambers. Previous experiments used the same exposure design, but were limited to 28 days. This phenomena began to affect the animals after five to six weeks. We are investigating sediment renewal as a possible remedy.

Increasing the exposure duration from 28 to 56 days did not increase the sensitivity of the test. However, growth effects were observed in *A. urtica* during 28-day exposures to highly contaminated sediments from the Palos Verdes Shelf and inner Los Angeles Harbor (see *Sediment Toxicity Methods for the brittlestar Amphiodia urtica* in this volume).

Growth in oral width was not constant over the course of the experiment. In the second half of the Orange County experiment, little or no growth occurred among brittlestars exposed to C3 sediments. This may have been related to the sediment quality problems discussed above. However, the 56-day growth rate (0.02 mm/week) was equal to the 28-day growth rate in the Santa Monica Bay experiment.

No significant change in gonad condition was detected based on measurements of oocyte diameters. The mean initial oocyte diameter (50 µm) was within the range of means (30-54 µm) in the *Amphiodia urtica* field study at C3 (see *Population biology of the brittlestar, Amphiodia urtica* in this volume), but higher than the means in January (40 µm) and February (33 µm) 1990 samples from C3. Further refinements in techniques for assessing reproductive endpoints will be necessary to investigate reproductive impacts. Gonad developmental stages and gonad histopathology may provide more useful information than measuring oocyte diameters.

The impact of municipal wastewater discharges on *Amphioida urtica* populations in the field may be due to effects on the larval or juvenile stages, which may be more sensitive to sediment quality than the adults.

Conclusions

Adult *Amphiodia urtica* were not affected by laboratory exposures to sediments collected near municipal wastewater outfalls, although they are rare or absent in these same sediments in the field. The experiments indicate that test sensitivity was not enhanced by lengthening the exposure to 56 days or by examining oocyte sizes. Further research with post-settlement juveniles is probably necessary to identify the factors that affect the abundance of *A. urtica* in the field. Future work, which will build on the results

of laboratory exposures and field surveys, will include a long-term exposure of adult and juvenile *A. urtica* to sediments collected near municipal wastewater outfalls.

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FIGURES

Figure 1.
Locations of sediment collection stations in Santa Monica Bay. *Amphiodia urtica* abundances for July 1990 (Abundance data from City of Los Angeles 1992).

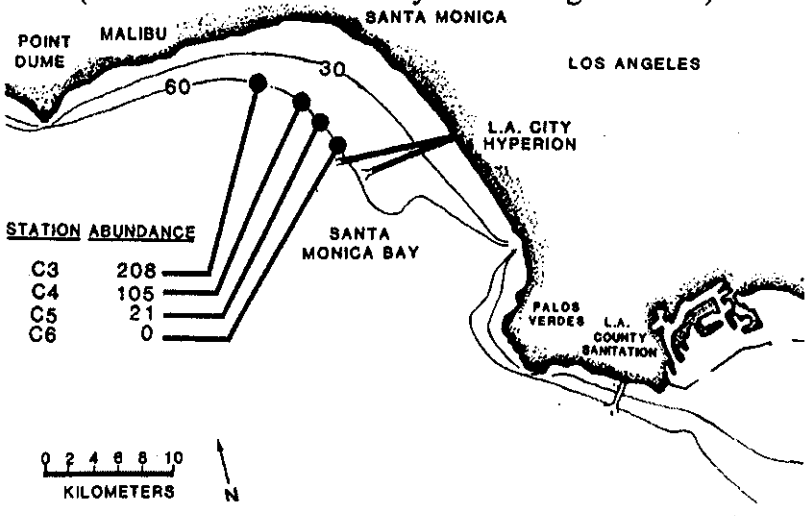


Figure 2.
Location of sediment collection sites off Orange County. *Amphiodia urtica* abundances are averages for

1985-1991 (Abundance data from County Sanitation Districts of Orange County 1993).

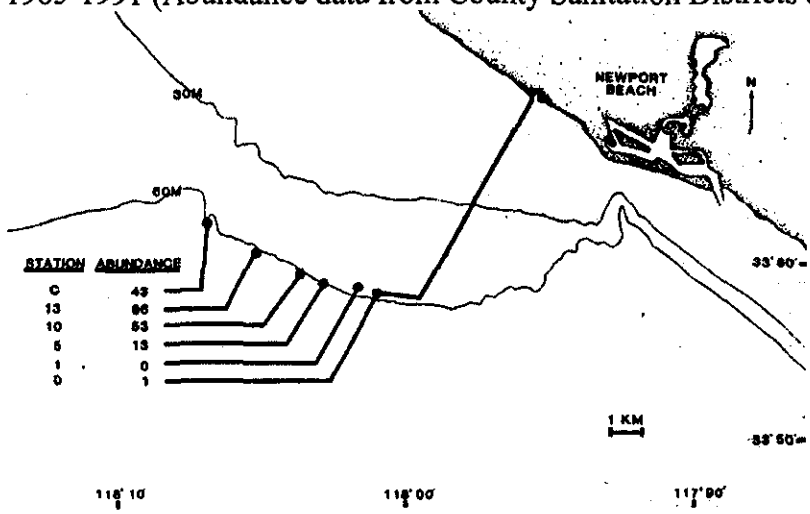


Figure 3.
Change in oral width of *Amphiodia urtica* during 28-day exposure to sediments from Santa Monica Bay. Treatment means are based on n=4 for C4 and C5, and n=6 for C3 and C6 (see Figure 1 for station locations). Error bars are one standard error.

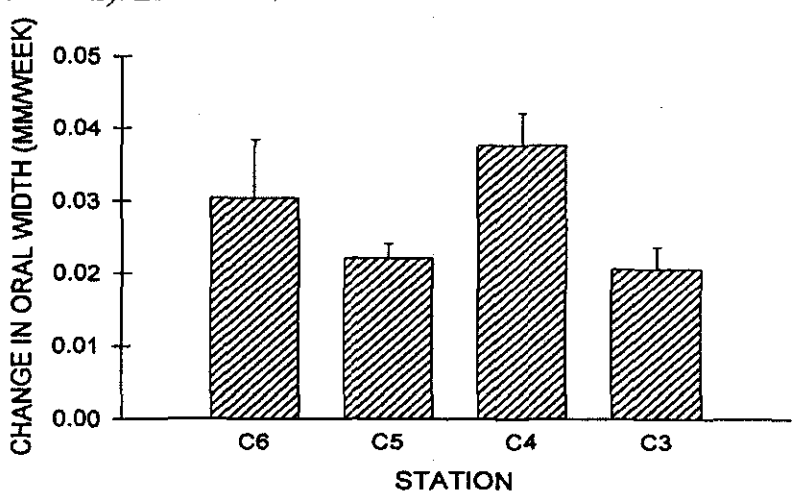


Figure 4.
Mortality of *Amphiodia urtica* after 56-day exposure to sediments off Orange County (see Figure 2 for station locations). Treatment means are based on n=5. Error bars are one standard error. Station C3 is the brittlestar collection site in Santa Monica Bay.

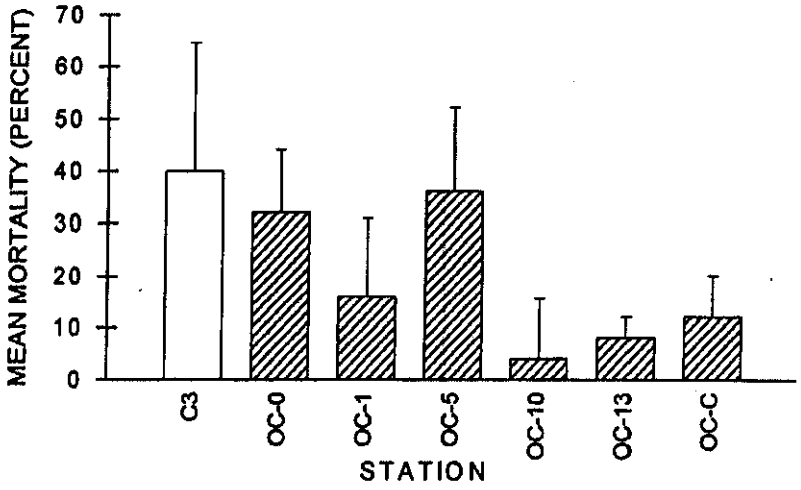


Figure 5.
Change in oral width of *Amphiodia urtica* after 56-day exposure to sediments off Orange County (see Figure 2 for station locations). Treatment means are based on n=5, except for C3 where n=3 due to 100% mortality in two replicates. Error bars are one standard error. Station C3 is the brittlestar collection site in Santa Monica Bay.

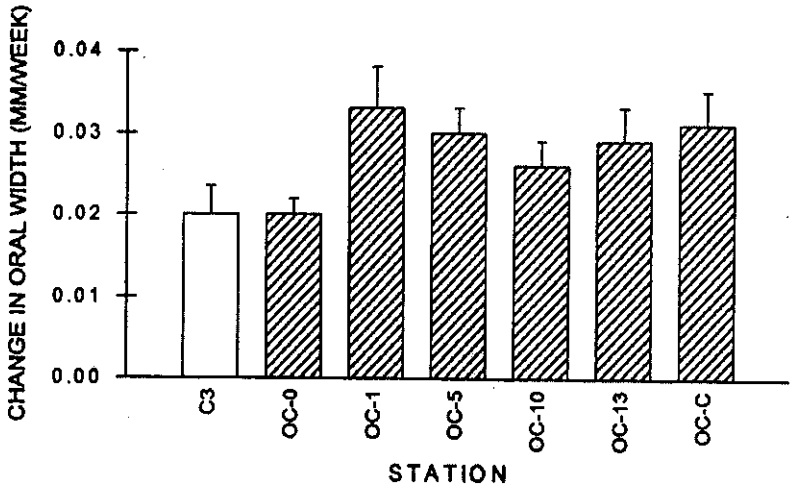
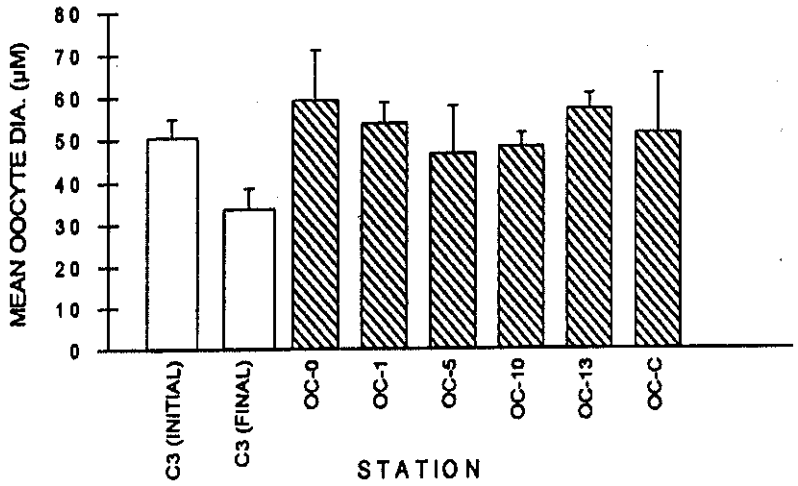


Figure 6.
Oocyte diameters of *Amphiodia urtica* after 56-day exposure to sediments off Orange County. Treatment means are n=12 for initial sampling and n=3-5 for the rest. Error bars are one standard error. Station C3 is the brittlestar collection site in Santa Monica Bay.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OC-5
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OC-C
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