

Growth of Brittlestars Exposed to Sediments from a Municipal Wastewater Outfall Gradient off San Diego

The red brittlestar, *Amphiodia urtica*, is one of the most abundant organisms on the mainland shelf in the Southern California Bight (Jones 1969). However, its abundance is reduced in sediments near municipal wastewater outfalls (City of Los Angeles 1994, City of San Diego 1994, County Sanitation Districts of Orange County 1994). Abundances of *Amphiodia urtica* range as high as 960 individuals/m² in relatively unaffected sediments off Orange County and >2000 individuals/m² in relatively unaffected sediments in Santa Monica Bay. Near the municipal wastewater outfalls in these areas, brittlestar abundances decrease to zero (City of Los Angeles 1993, County Sanitation Districts of Orange County 1993) suggesting that the brittlestar is responding to altered sediment quality.

The reason for the decline in abundance is unknown, but growth of *A. urtica* is reduced when they are exposed to highly contaminated sediments from the Palos Verdes Shelf (SCCWRP 1994a). Growth and oocyte size of *A. urtica* are not affected when they are exposed to less contaminated sediments from outfall areas in Santa Monica Bay and off Orange County (SCCWRP 1994b).

The *Amphiodia urtica* population off Point Loma ranges from <100 individuals/m² 0.2 km from the municipal wastewater outfall to >1000 individuals/m² 10 km from the outfall (City of San Diego 1994). While the abundance gradient off Pt. Loma is similar to the gradient in Santa Monica Bay, the population size structures differ; larger animals occur with greater frequency at the reference site off Point Loma than at the reference site in Santa Monica Bay (SCCWRP 1994c).

The objective of this study was to measure growth and reproductive condition of adult *Amphiodia urtica* exposed in the laboratory to sedi-

ments from a gradient of brittlestar abundance near the wastewater outfall off Point Loma. Because previous investigations with outfall-impacted sediments from Santa Monica Bay and Orange County failed to measure a growth effect after eight weeks, the present experiment was conducted for a longer time to increase its sensitivity.

MATERIALS AND METHODS

Collection of Sediments and Animals

Sediments were collected from four stations along the 60 m isobath near the Point Loma outfall (Figure 1); the stations are part of the ocean monitoring program of the City of San Diego. Test organisms and reference sediments were collected from northern Santa Monica Bay (station C3). Five replicate sediment samples were collected by Van Veen grab in September 1993 from all stations. The top 2 cm of sediment from each grab was transferred to clean plastic jars. In the laboratory, sediments were passed through a 2 mm screen to remove resident organisms. Test organisms were collected from

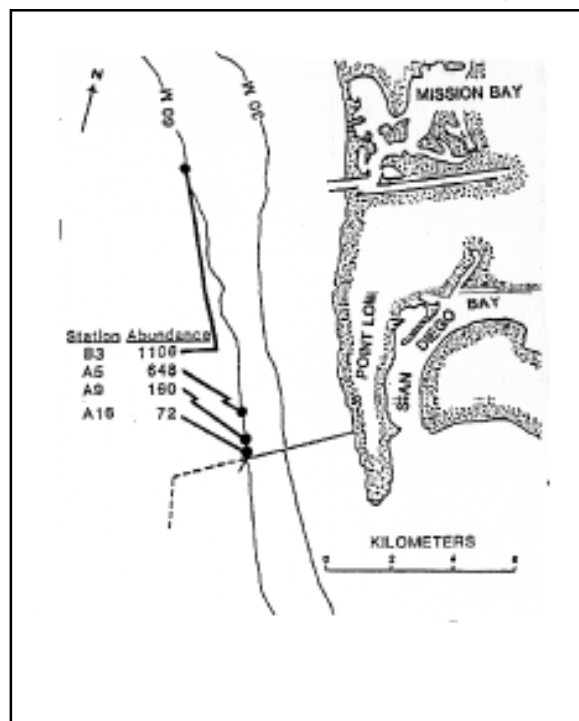
screened sediments from station C3. Animals were acclimated to flow-through conditions on screened C3 sediment prior to testing.

Sediment Exposures

New 1 L polypropylene Tripour beakers were acid and seawater rinsed, and supplied with 2 cm of sediment. Beakers were allowed to equilibrate overnight before the animals were added. Three sub-replicates were created from each of the five replicate samples from the five stations for a total of 75 beakers.

Brittlestars were anesthetized with isotonic MgCl₂ and the oral width was measured with an ocular micrometer

FIGURE 1. Locations of sediment collection stations off Point Loma. Not shown is the animal collection site in northern Santa Monica Bay.



under a dissecting microscope. The animals were allowed to recover in seawater and five individuals with oral widths between 0.84-1.84 mm were added to each exposure beaker. Concurrently, a random sample of female *A. urtica* was prepared for histological examination to determine initial gonad condition.

Beakers were maintained at 12°C under flow through conditions with 0.45 µm filtered seawater regulated at 2-4 mL/min and air supplied via capillary tubing. Watch glasses covered each beaker. Containers were examined daily for mortality and air flow. Dead animals were removed and their oral width was measured. Twice weekly, each container received 0.03 g Argent Hatchfry™ 50-150 µm micro-encapsulated invertebrate food.

After seven weeks, the sediment in two of the three sub-replicates was replaced with screened fresh sediment from C3. At this time, the third beaker in each sub-replicate group was terminated. Animal survival was determined, oral widths were measured, and a sample of females from each station was preserved for histology.

After 16 weeks, the oral width of the surviving animals was measured and female gonads from the two stations at opposite ends of the field abundance gradient were preserved for histology. *Amphiodia urtica* growth was calculated as the difference between initial and final oral width measurements.

Reproductive Condition

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 15 randomly selected oocytes from each of two sections per animal were measured using a compound microscope with an ocular micrometer. 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 Analysis

Endpoint means for each sub-replicate group were used to calculate the mean for each treatment group. Equality of variance was tested with the F_{\max} test (Sokal and Rohlf 1969). A one way analysis of variance (ANOVA) or

Kruskal-Wallis test (Zar 1984) was used to evaluate variations in survival and growth among the Point Loma stations. A t-test was used to compare mean oocyte diameters. Oocyte size frequency histograms were prepared using the interval width determination method of Sturges (Sturges 1926) and compared using the Kolmogorov-Smirnov test for discrete data (Zar 1984).

RESULTS

There was no significant difference in mortality among the four Point Loma stations after 16 weeks (ANOVA, $F=1.46$, $p=0.23$). Mortalities occurred in each treatment and ranged from 2% for A9 to 34% for A16 (Figure 2). Stations C3 (reference), A5, and A16 each had one sub-replicate with 100% mortality. Most of the mortalities occurred after week 7, but the survival rate during the last nine weeks of the experiment was not significantly different from the survival rate during the first seven weeks (Mann-Whitney U test, $p=0.34$).

Brittlestar oral width growth rates were not constant during the experiment (Figure 3); most of the growth occurred by seven weeks. Brittlestars exposed to C3 sediments grew an average of 0.035 mm/week during the first seven weeks and 0.008 mm/week from seven to 16 weeks. Over 95% of the total growth for brittlestars exposed to sediments from stations A16 and B3 occurred in the first seven weeks; at the remaining stations, 56-77% of the growth occurred in the first seven weeks.

Brittlestar oral width growth rates were not significantly different among the four Point Loma stations at

FIGURE 2. Survival of *Amphiodia urtica* after exposure to sediments collected off Point Loma (see Figure 1 for locations). Means (\pm SE) were based on $n = 15$ for week 7 and $n = 10$ for week 16. Seven week survival in reference sediment is indicated by the dashed line; 16 week survival is indicated by the dotted line. Week 16 data are cumulative.

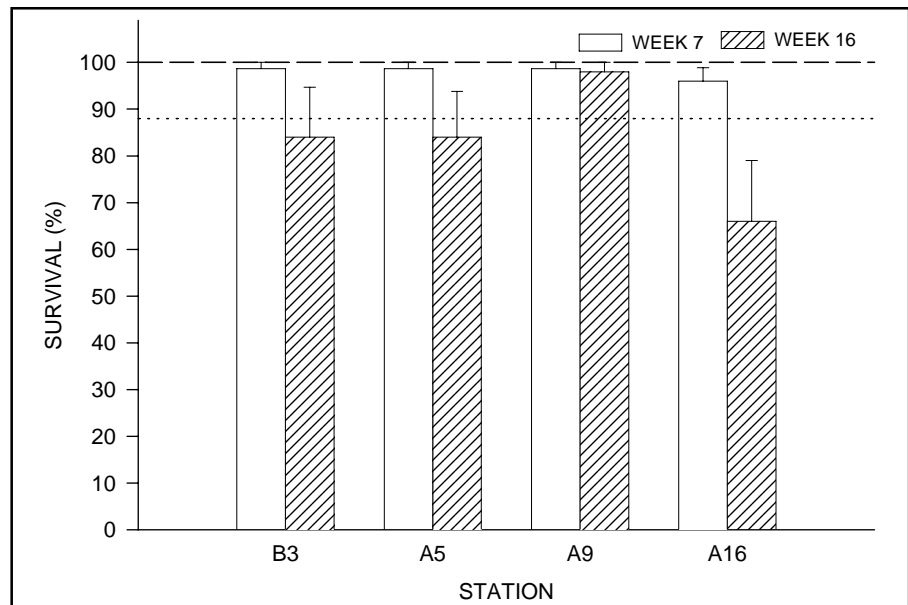
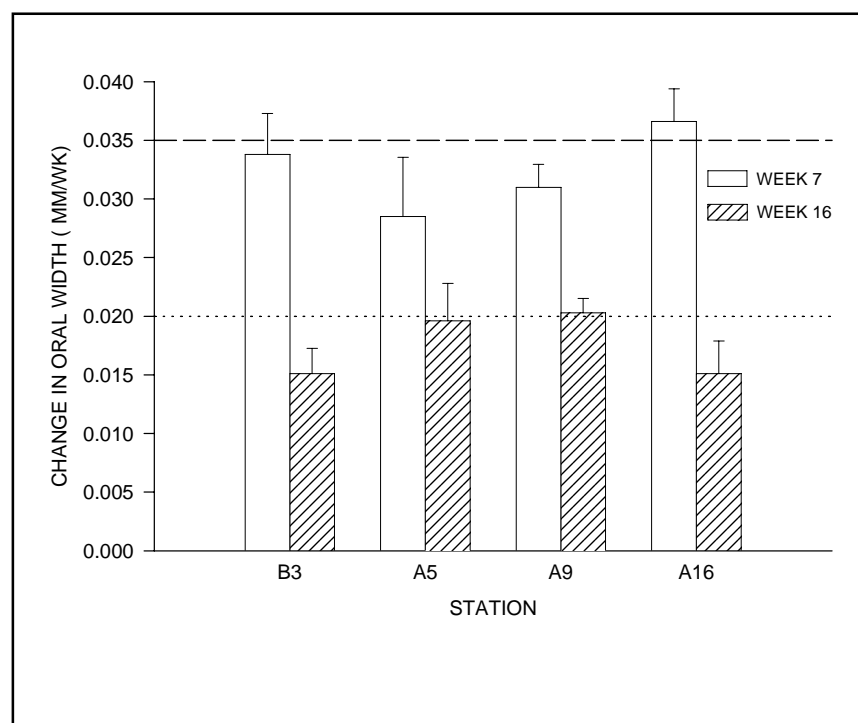


FIGURE 3. Change in oral width of *Amphiodia urtica* after exposure to sediments collected off Point Loma (see Figure 1 for locations). Means (\pm SE) were based on $n = 5$ for week 7; $n = 10$ for week 16 for B3 and A9; and $n = 9$ for week 16 for A5 and A16. Seven week growth in reference sediment is indicated by the dashed line; 16 week growth is indicated by the dotted line. Week 16 data are cumulative.



seven weeks (ANOVA, $F=0.99$, $p=0.42$) or at 16 weeks (ANOVA, $F=1.41$, $p=0.26$). At seven weeks, animals exposed to sediments from the station closest to the Point Loma outfall (A16) had the highest mean growth rate (0.037 mm/week), while animals exposed to sediments from a transition station (A5) had the lowest mean growth rate (0.028 mm/week). At 16 weeks, animals exposed to sediments from the station closest to the outfall (A16) and the station farthest from the outfall (B3) had the lowest mean growth rates (0.015 mm/week).

There was no significant difference in oocyte diameter at 16 weeks for the stations closest to, and farthest from, the outfall (A16 and B3; t -test, $t=1.77$, $p=0.10$). The mean oocyte diameter at the beginning of the experiment was similar to the diameters at seven and 16 weeks (Figure 4). The size frequency distributions were not significantly different between stations A16 and B3 (Kolmogorov-Smirnov test for grouped data, $D_{\max}=5.0$, $0.2 < p < 0.5$) nor were the mean maximum oocyte diameters significantly different (data not shown).

DISCUSSION

The survival, growth, and reproductive condition of adult *Amphiodia urtica* were not affected by laboratory

exposure for 16 weeks to sediments from the area influenced by the municipal wastewater outfall on the mainland shelf off Point Loma. This is consistent with the results of previous laboratory experiments with adult *Amphiodia urtica* exposed to sediments collected near municipal wastewater outfalls in Santa Monica Bay and off Orange County (SCCWRP 1994b). Increasing the exposure time from four or eight weeks (Santa Monica Bay and Orange County studies) to 16 weeks did not increase test sensitivity.

There was some mortality in all treatments during the experiment. The highest mortality occurred among brittlestars exposed to sediments from the station closest to the outfall (A16); however, mortality was lowest in sediments from the next closest station (A9). Within a treatment, mortality was generally concentrated in one beaker rather than spread equally among the beakers. The sediment in some of the beakers with high mortality was discolored (e.g., dark patches or algal growth) and mortality may have been due to the degradation of sediment quality as the experiment progressed. Renewing the sediments at seven weeks did not prevent this

phenomenon; more frequent renewal may be necessary for long experiments.

Oral width growth rates were not constant during this experiment, nor in the experiment with sediments collected off Orange County (SCCWRP 1994b). Most of the growth occurred by the midpoint of the experiments. *Amphiodia urtica* exposed to reference sediments (station C3) attained 77% of their final oral width by week seven in the present experiment and 100% of their final oral width by week four (of eight) in the Orange County experiment. The decrease in growth rate may be an artifact caused by deteriorating sediment quality as the experiment progressed.

The growth rate of brittlestars on reference sediments at seven weeks in the present study (0.035 mm/week) was higher than the growth rate on reference sediments at four weeks (0.021 mm/week, t -test, $t=3.04$, $p=0.01$) and eight weeks (0.020 mm/week, t -test, $t=2.81$, $p=0.03$) in the previous studies. The higher growth rates in the present study may have been due to: temporal variability in the brittlestar condition at the time of collection; spatial or temporal differences in the sediment characteristics at station C3; an artifact of conducting the experiments in different laboratories (the Point Loma study was conducted

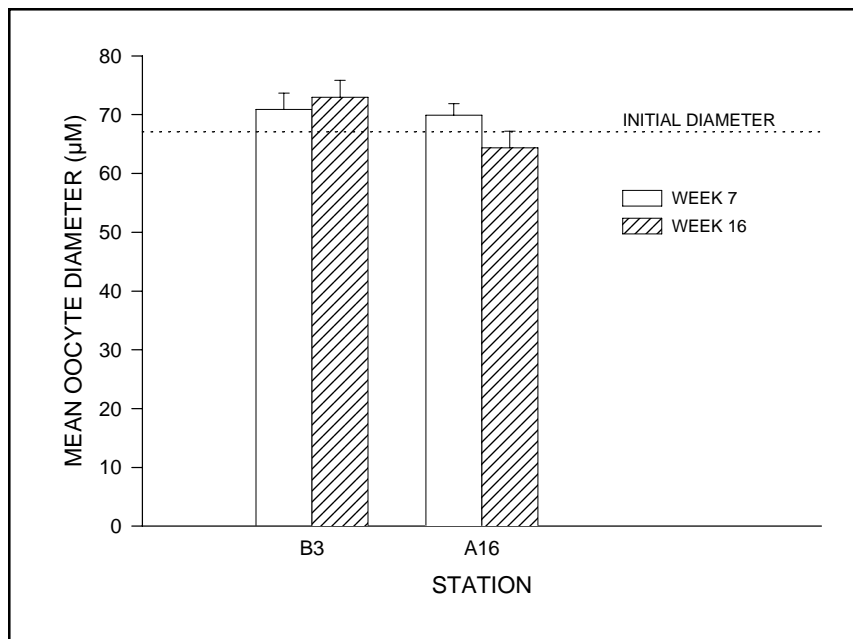


FIGURE 4. Mean oocyte diameter (\pm SE) for female *Amphiodia urtica* after exposure to sediments collected off Point Loma (see Figure 1 for locations). Means (\pm SE) were based on $n = 5$ beakers for week 7 at B3; $n = 4$ beakers for A16; $n = 10$ beakers for week 16 at B3; $n = 5$ beakers for A16. The dotted line indicates the initial mean oocyte diameter of a random subsample of 14 females from the original animal collection at the beginning of the exposure.

in Westminster while the other experiments took place in Long Beach); or inconsistent handling of the animals or sediments prior to the experiment.

The lack of a significant change in the oocyte size during the present experiment may have been related to the initial condition of the brittlestars. The initial mean oocyte diameter ($67\ \mu\text{m}$) was greater than the average oocyte diameter measured among *A. urtica* collected from station C3 in previous studies ($54\ \mu\text{m}$; SCCWRP 1994b). Oocyte size-frequency patterns were, however, similar to those reported for *A. urtica* collected at C3 in September 1990 (SCCWRP 1994c). It does not appear that *A. urtica* oocytes in laboratory cultures grow to atypical sizes even when held for several months (SCCWRP 1994b and unpublished data). If the oocytes of the brittlestars used in the present experiment were close to their maximum size, it would have been difficult to detect further growth.

The lack of effects on growth and reproduction of *Amphiodia urtica* exposed to sediments from three major municipal wastewater outfalls suggests that either laboratory exposures do not simulate field conditions, or that something other than toxicity to adults is responsible for the reduced brittlestar abundances around the outfalls. However, the growth of adult *A. urtica* was reduced significantly by exposure to highly contaminated sediments from Los Angeles Harbor and the Palos Verdes Shelf (SCCWRP 1994a).

Factors such as predation, sediment resuspension, or reduced juvenile survival may be responsible for reduced *A. urtica* abundances in moderately contaminated sediments near sewage outfalls. Brittlestars are preyed on by bottom dwelling fishes such as spotted ratfish, English

sole, Pacific sanddab, and Dover sole (Allen 1982).

Sediment resuspension may affect population patterns by altering the availability of organic material to benthic animals. The population size structure of *Amphiodia urtica* in reference areas is different from the population size structure in areas influenced by sewage discharge (SCCWRP 1994c). Juvenile mortality is higher in areas near sewage outfalls suggesting that early life stages of *A. urtica* are more sensitive than adults to municipal wastewater discharges. We are currently investigating the relative sensitivity of juvenile *Amphiodia urtica* in laboratory exposures.

CONCLUSIONS

The survival, growth, and reproductive condition of *Amphiodia urtica* in the laboratory was not affected by exposure to sediments collected near three municipal wastewater outfalls (City of San Diego, County Sanitation Districts of Orange County, and City of Los Angeles). However, adult *Amphiodia urtica* are rare or absent at these stations. Future investigations focusing on the early life stages of *Amphiodia urtica* explain the decreased abundances near municipal wastewater outfalls in Southern California.

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