Sediment Toxicity Test Methods for the Brittlestar *Amphiodia urtica*

The red brittlestar *Amphiodia urtica* is one of the most common benthic invertebrates on the mainland shelf off Southern California (Jones 1969); abundances range from 78/m^2 to 1342/m^2 (Thompson *et al*. 1994a). The brittlestar occurs less frequently, or not at all, in contaminated sediments near sewage outfalls (Word and Mearns 1979). The causes of its reduced abundance are unknown. Consequently, there has been a lot of interest in developing sediment toxicity tests for *A. urtica* for experimental manipulations in the laboratory.

This article reports on two studies aimed at developing *A. urtica* as a sediment toxicity test organism. The objectives of the first study were to: 1) determine how long brittlestars could be held in the laboratory, and 2) determine the optimum bioassay exposure period. Survival, growth, and reproductive status were measured to judge culturing success. The objectives of the second study were to: 1) determine the effects of contaminated sediments on growth and survival, and 2) evaluate growth, arm regeneration, and calcium uptake as indicators of sublethal stresses.

Sorting a sediment sample.

Materials and Methods

Animals for both experiments were collected in 60 m of water in northern Santa Monica Bay (Environmental Monitoring Division (EMD) of the City of Los Angeles benthic monitoring station C3) with a van Veen grab. Brittlestars were collected by gently rinsing the sediment through a 0.5 mm screen.

Long-Term Culture

Fifteen animals with oral widths ranging from 0.75 to 1.69 mm were placed in each of two chambers. The chambers were 21 cm x 11 cm polypropylene food storage boxes and contained 2 cm of clean sediment from northern Santa Monica Bay (EMD benthic monitoring station C3). The chambers were placed in a 12°C water bath and supplied with aerated flowing seawater. Temperature varied between 12° and 15°C during the experiment. Each chamber received 0.045 g of ground Tetramin™ tablets twice a week.

Brittlestars were screened from the test chambers after 7, 12, 16, 26, 31, 39, 47, 52, 60, and 69 weeks. The animals were anesthetized with $MgCl_2$ and their oral width and longest arm were measured. Oral width — the distance across the mouth between the outer edge of the oral and arm plates (Lie 1968) — was measured with an ocular micrometer in a dissecting microscope. Oral width was a surrogate for growth. To assess maturity, the color and relative size of the gonad was recorded. Fresh sediment was placed in the chambers and the animals were returned.

Sediment Exposure

Sediments were collected by van Veen grab from northern Santa Monica Bay (EMD station C3), the Palos Verdes Shelf (County Sanitation Districts of Los Angeles County benthic monitoring station 7-3), and Los Angles Harbor (East Turning Basin). Sediments from northern Santa Monica Bay station were 69% sand, 2% clay, and 0.5% total organic carbon (TOC) (City of Los Angeles 1991). Sediments from the Palos Verdes Shelf (28% sand, 11% clay and 4.2% TOC) and Los Angeles Harbor (42% sand, 19% clay and 1.1% TOC) contain elevated contaminant concentrations and are toxic to other invertebrates (Anderson *et al.* 1988). No contaminant or sediment characterization was done for this study.

The top 2 cm of sediment from several grabs were removed and stored in plastic jars at 5°C. Exposure chambers were plastic 1 L tri-pour beakers. Each chamber contained 2 cm of sediment and about 700 ml of aerated seawater replaced at 2-4 ml/min. There were eight replicates for each sediment type. Four replicates were used for oral width change and calcium uptake; they contained five animals that had been measured for oral width. The four remaining replicates were used for arm regeneration and contained five animals, each of which had one arm severed at the 10th segment. Temperature was maintained at 12°C during the 28-day exposure. Twice a week, 0.03 g of ground Tetramin™ tablets were added to each beaker.

At the end of the regeneration experiment, oral width and length of the regenerated arm were measured on each animal. Three animals of different sizes were selected for the calcium uptake experiment and placed in beakers containing ^{45}Ca for 24 h. Oral width was measured on the remaining animals. After exposure to ^{45}Ca, the soft parts of the animals were solubilized. The hard parts were then dissolved and the radioactivity of the ossicles was measured with a liquid scintillation counter.

Differences in oral width and calcium uptake between reference and contaminated stations were tested by analysis of variance and Dunnett's multiple comparison test (Zar 1984). Differences in arm regeneration between reference and contaminated stations were tested by analysis of covariance, with oral width as the covariate, followed by pairwise contrasts (Wilkinson 1990).

Results

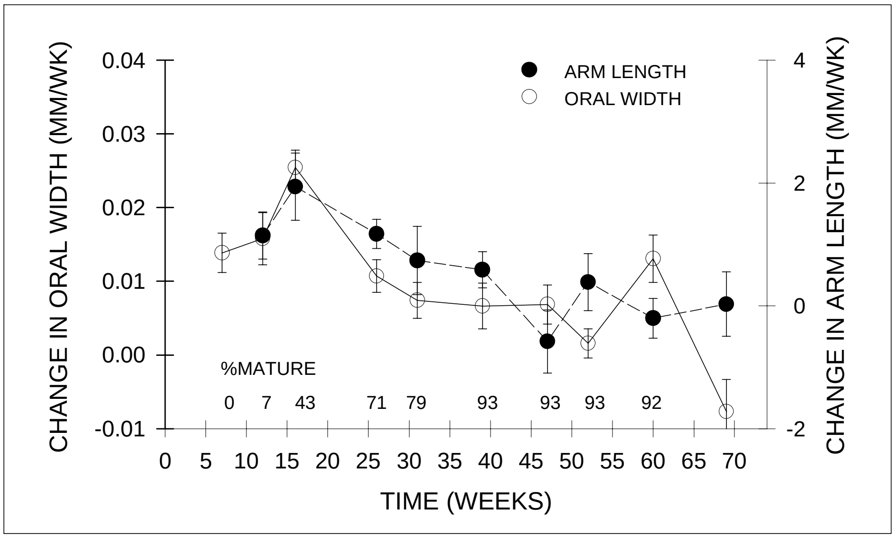
Long-Term culture

Survival of *Amphiodia urtica* was 80% and 40% in the two tubs. Lower survival in the second tub was caused by technical problems affecting the seawater and air delivery systems to that tub only. Results presented here are for the chamber with higher survival.

The brittlestars grew and matured during culture (Figure 1). Changes in oral width and arm length were most rapid during the first 26 weeks; change in oral width ranged from 0.011 to 0.025 mm/week and growth of arms ranged from 1.1 to 1.9 mm/

Figure 1.

Results of the long-term culture test with *Amphiodia urtica*. Data are mean ±1 standard error for animals in one chamber (n=15 at the starting and n=12 at the end). Arm length was the length of the longest arm. Percent maturity was the proportion of animals with mature gonads based on a visual examination of gonad size and color.



week. After 26 weeks, change in oral width ranged from -0.008 to 0.013 mm/week and growth of arms ranged from -0.6 to 0.6 mm/week. The mean increase in oral width for the entire experiment (69 weeks) was 0.57 mm; oral width in several animals exceeded 2.0 mm. Over 90% of the survivors had visible gonads by 39 weeks. While the gonads appeared to be mature, there was no evidence of spawning.

Sediment Exposure

Brittlestar survival was not affected in any of the sediment exposures. Only two animals for the entire experiment were lost (one in a control beaker and one in a beaker containing Palos Verdes sediment). Oral width, arm regeneration, and calcium uptake were significantly reduced among animals exposed to Palos Verdes sediments (Table 1). Oral width and arm regeneration were significantly reduced among animals exposed to Los Angeles Harbor sediments. Change in oral width showed the largest difference from the control, followed by calcium uptake, and arm regeneration. Arm regeneration was the most precise [mean coefficient of variation (CV)=9%], followed by oral width (CV=55%), and calcium uptake (CV=374%).

Discussion

Long-Term Culture

Survival of *Amphiodia urtica* was high for 69 weeks of laboratory culture. The reduction in growth rate after 26 weeks was probably due to natural causes. The largest brittlestars in the long-term culture were larger than any *A. urtica* collected thus far from the field (Thompson 1994b). As the animals approach maximum size, the growth rate is expected to decrease. As the animals grow, increased expenditures of energy for reproductive development divert energy away from growth. In the experiment, gonad tissues were increasing in size while growth rates were declining. The reductions in growth of arms near the end of the study may have been due to handling. As the length of the arms increased, it became more difficult to handle them without breaking off the fragile tips.

In this and subsequent long-term experiments with *A. urtica*, survival and growth were occasionally poor and mortality increased noticeably after 6-8 weeks. Renewing the sediment every 4-6 weeks reduced the mortality and improved the health of the cultured brittlestars. Higher mortalities as holding time increased may have been related to degradation of sediment quality in the test chambers over time.

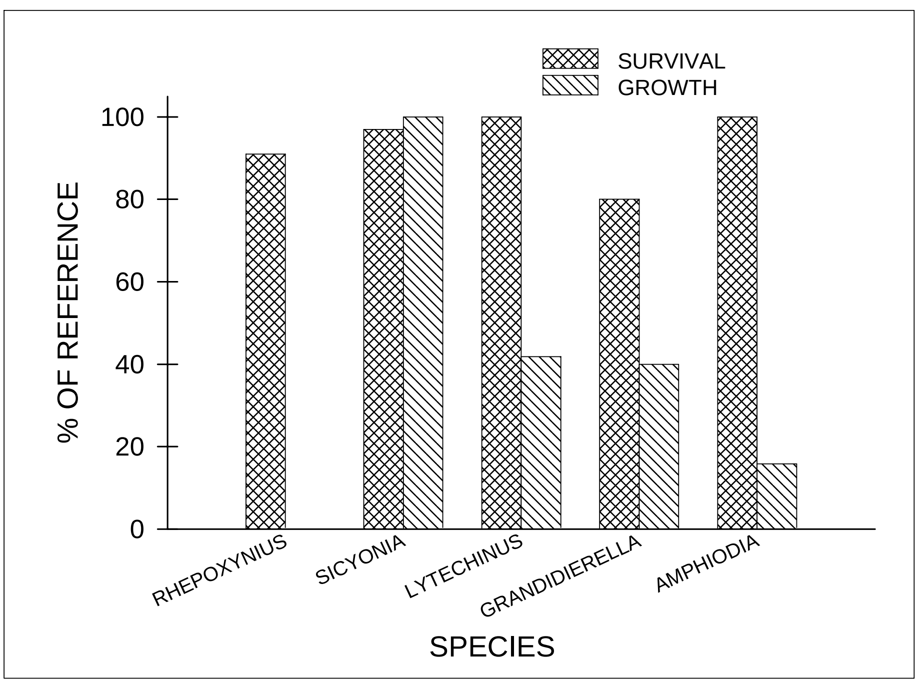
Table 1.

Responses of the brittlestar *Amphiodia urtica* to sediments from northern Santa Monica Bay (Hyperion station C3=reference), Palos Verdes Shelf (County Sanitation Districts of Los Angeles County station 7-3), and Los Angeles Harbor (East Turning Basin). Data are means of four replicates and one standard deviation. Means with an asterisk (*) are significantly different from the reference site (p0.05).

STATION	ORAL WIDTH CHANGE (mm/28 days)		ARM REGENERATION (mm/27 days)		CA UPTAKE (DPM/mg ossicles)	
Santa Monica Bay	0.19	(0.08)	7.2	(0.05)	117	(59)
Los Angeles Harbor	0.01*	(0.07)	4.6*	(0.6)	53	(33)
Palos Verdes Shelf	0.03*	(0.09)	5.2*	(0.3)	47*	(26)

Figure 2.

Responses of invertebrates to sediments from the Palos Verdes Shelf (County Sanitation Districts of Los Angeles County monitoring station 7-3). Data are expressed as percent of reference sediment exposure. Testing was done by several laboratories with samples collected over 5 years: *Rhepoxynius abronius* (Ferraro *et al*. 1991); *Sicyonia ingentis* (SCCWRP 1988); *Lytechinus pictus* and *Grandidierella japonica* (Anderson *et al.* 1988).



Sediment Exposure

Sediments from the Palos Verdes Shelf have been collected and tested with marine organisms in the laboratory for several years. Growth in amphipods (*Grandidierella japonica*) exposed to this sediment was reduced 68%; growth in sea urchins (*Lytechinus pictus*) was reduced 57% (Anderson *et al*. 1988); and growth was not affected in shrimp (*Sicyonia ingentis*; SCCWRP 1988) (Figure 2). The growth response of *Amphiodia urtica* — 84% reduction — was one of the most sensitive sublethal measures of sediment quality for sediments from PV 7-3.

Exposure of brittlestars to sediment from inner Los Angeles Harbor caused significant reductions in growth in *A. urtica*. In previous tests with these sediments, the growth of sea urchins and amphipods was not affected; however, amphipod survival was reduced (Anderson *et al.* 1988).

Amphiodia urtica is usually not found at Palos Verdes station 7-3 and is not expected to occur at the shallow station in Los Angeles Harbor. However, depth and grain size characteristics at PV7-3 were within the ranges tolerated by *A. urtica*. The PV7-3 sediment was not acutely toxic to adults, but caused strong sublethal effects that may prevent the sediments from supporting a healthy population of *A. urtica*. Additional studies with more stations and sublethal biological indi cators (e.g., reproduction, juvenile growth, and larval settlement) are needed before the relation between sediment contamination and *A. urtica* population abundance can be explained.

Calcium uptake was a sensitive sublethal endpoint that was comparable to the results of the growth and regeneration tests, at least for sediments from PV7-3. While sufficiently sensitive, it was a difficult and time consuming procedure. It may be a good choice when a shorter exposure period is necessary.

Variability of the oral-width measurements was quite high. Subsequent experiments have produced lower variability (CV=89%) and precision can be further increased by measuring under higher magnification or using a computerized image analysis system to make the measurements.

Conclusions

Amphiodia urtica was a good sediment toxicity test subject; it was relatively easy to collect in the field and hold for long periods in the laboratory. This study provided the first laboratory evidence that *A. urtica* was sensitive to contaminated sediments. While survival was not a sensitive endpoint, growth (oral width) and arm regeneration were easily measured, sublethal indicators as sensitive as indicators currently used in sediment tests with other marine invertebrates. *Amphiodia urtica* is a sensitive, ecologically important species from the coastal waters off Southern California. Additional studies are in progress that focus on the growth and reproduction of adults and survival and growth of juveniles.

References

- Anderson, J.W., S.M. Bay, and B.E. Thompson. 1988. Characteristics and effects of contaminated sediments from Southern California. Final Rep. to Calif. St. Wat. Resour. Contr. Bd., Sacramento. Contrib. #223. Southern California Coastal Water Research Project, Long Beach. 120 pp.
- City of Los Angeles. 1991. Hyperion Treatment Plant and Santa Monica Bay monthly, quarterly, semiannual and annual monitoring report. Environmental Monitoring Division, Bureau of Sanitation, City of Los Angeles. 289 pp.
- Ferraro, S.P., R.C. Swartz, F.A. Cole, and D.W. Schults. 1991. Temporal changes in the benthos along a pollution gradient: Discriminating the effects of natural phenomena from sewage-industrial wastewater effects. *Estuar. Coast. Shelf Sci.* 33:383-407.
- Jones, G. 1969. The benthic macrofauna of the mainland shelf of Southern California. Allan Hancock Monographs in Marine Biology, No. 4. Univ. Southern California, Los Angeles. 219 pp.
- Lie, U. 1969. A quantitative study of benthic infauna in Puget Sound, Washington, USA, in 1963-1964. *Fiskerdirektoratets Skrifter, Serie Havundersokelser*. 14:229-556.
- SCCWRP see *Southern California Coastal Water Research Project*
- Southern California Coastal Water Research Project. 1988. Effects of contaminated sediments on three benthic invertebrates. Pp. 58-64, *In*: Southern California Coastal Water Research Project 1987 Annual Report. Southern California Coastal Water Research Project, Long Beach.
- Thompson, B.E., D. Tsukada, and D. O'Donohue. 1993. 1990 Reference Survey. Contribution #355. Southern California Coastal Water Research Project, Westminster. 58 pp..
- Thompson, B.E., D. Tsukada, D. O'Donohue, A. Jirik, and G. Jones. 1994a. Biology of the Ophiuroid, *Amphiodia urtica* off Southern California, I: Distribution, abundances, fecundity and ecological relationships. *In review*.
- Thompson, B.E. D. Tsukada, A. Jirik, L. Cooper, and G. Hendler. 1994b. Biology of the Ophiuroid, *Amphiodia urtica* off Southern California, II: Population biology. *In review*.

•Wilkinson, L. 1990. SYSTAT: The system for statistics. SYSTAT, Inc. Evanston, IL.

•Word, J.Q. and A.J. Mearns. 1979. 60-meter control survey off Southern California. Contribution #133. Southern California Coastal Water Research Project. El Segundo. 58 pp.

•Zar, J.H. 1984. Biostatistical analysis, second edition. Prentice-Hall, Inc., Englewood Cliffs, NJ. 718 pp.

Acknowledgements

Authors Steve Bay and Darrin Greenstein thank J. Brown and D. Tsukada for their help; H. Stubbs and D. Diehl for collecting sediment and animals; the crews of the R/V *La Mer* (Bureau of Sanitation, City of Los Angeles) and R/V *Ocean Sentinel* (County Sanitation Districts of Los Angeles County) for shiptime and help at sea.