Philip S. Oshida, Steven M. Bay, Ann Haeckl, T. Kim Goochey, and Darrin Greenstein

SEAWATER AND WASTEWATER TOXICITY STUDIES

In order to set meaningful discharge criteria for municipal wastewater disposal in the sea, it is useful to know the relative toxicities of wastewater effluents, ocean waters near wastewater outfalls, harbor waters, and open coastal waters. The new bioassay based on echinochrome synthesis by larval sea urchins (Bay *et al.*, this report, and Bay *et al.*, in press) has been used to address this problem and to assess the toxicities of these waters. The four water sample locations named above provided comparative information about toxicity and helped us evaluate the new bioassay's sensitivity and applicability. Wastewaters diluted by a factor of about 100 were found to be toxic in the laboratory, but water collected near a wastewater outfall had no detectable toxicity. Samples from both harbor and offshore stations were occasionally toxic when tested monthly over a period of 14 months.

Major goals of this project were to evaluate the bioassay's ability to detect toxicity in complex seawater and wastewater samples and to estimate the range of toxicity present in harbor and offshore areas. While the wastewater and outfall site bioassays were specifically designed for toxicity analysis by echinochrome measurements, the bioassays done monthly for the offshore and harbor samples were often conducted using three methods as indicators of toxicity: echinochrome production, fertilization percentage, and developmental morphology.

MATERIALS AND METHODS

The sea urchin echinochrome bioassay was conducted with seawater from harbor and offshore stations, seawater collected from near an outfall, and wastewater effluents from five treatment plants. Gametes of the purple sea urchin, *Strongylocentrotus purpuratus* were used in all experiments. The gametes were collected by injecting 0.5 M KCl to induce spawning. The isolated gametes and fertilized eggs were exposed to the experimental solutions for 15 minutes and 48 hours, respectively, according to the methods of Oshida *et al.* (1981). After 48h of incubation, the echinochrome pigment was extracted from the sea urchin larvae and quantified by the method described in Bay *et al.* (this report) and Bay *et al.* (in press). When compared to the controls, decreased pigment production by the sea urchin larvae in the experimental solutions was an indicator of toxicity in the solutions. Eggs, embryos, and larvae exposed to offshore and harbor samples were also examined microscopically for percentage fertilized and percentage gastrulated (Oshida *et al.* 1981).

The five wastewater effluents used in this study were taken from five treatment plants which discharge effluents into the ocean: Oxnard Wastewater Treatment Plant (Oxnard), City of Los

Angeles Hyperion Treatment Plant (Hyperion), County of Los Angeles Joint Water Pollution Control Plant (JWPCP), County Sanitation Districts of Orange County (ORCOSAN), and City of San Diego Point Loma Treatment Plant (Pt. Loma).

The average concentrations of general constituents, trace metals, and chlorinated hydrocarbons, as well as average flow rates for all the effluents and sludge have been summarized by Schafer (this report). All effluent samples collected by the treatment plant personnel were 24-hour composites. Acid-washed, kilned glass bottles were used for collection to insure against metal or hydrocarbon contamination of the effluent samples. The Hyperion sludge effluent samples were one-hour composites which had been collected in an acid-washed plastic bucket. All effluents were held at 11°C until used in the experiments. In these experiments, effluents were diluted with filtered (3 μm) natural seawater. The experimental effluent dilutions are listed in Table 1. All wastewater dilutions were triplicated for the bioassay. Blanks containing wastewater only were also processed to correct for interference in the pigment extracts from effluent particulates.

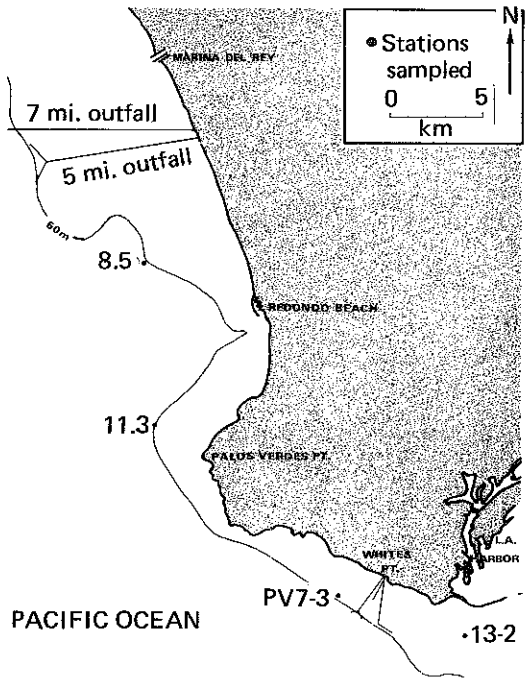

Figure 1. Offshore stations sampled for seawater

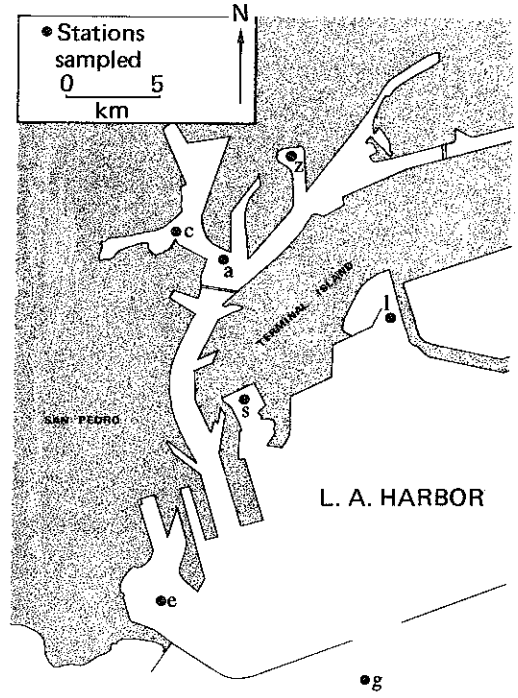

Figure 2. Los Angeles Harbor stations sampled for seawater toxicity.

Seawater samples were from three general locations: offshore stations (Figure 1), in Los Angeles Harbor (Figure 2), and near the Hyperion sludge outfall (Figure 3). Samples were taken near the Hyperion outfall on only one occasion, 19 May 1982,while the other offshore and harbor stations were sampled monthly for about one year, on the dates listed in Table 2. The samples taken near the Hyperion sludge outfall were from four stations (Figure 3) at two depths per station: 25 m and three meters from the bottom. The other offshore samples were taken from four stations at three depths per station: one meter, three meters below the thermocline (or at mid-depth if the thermocline was not distinct), and three meters from the bottom. All seawater samples were collected with a PVC Van Dorn sampler. The samples were drained into acid-washed and kilned glass bottles, brought to the laboratory, and kept at 11°C until used (undiluted) in the bioassays. All seawater samples were tested in duplicate.

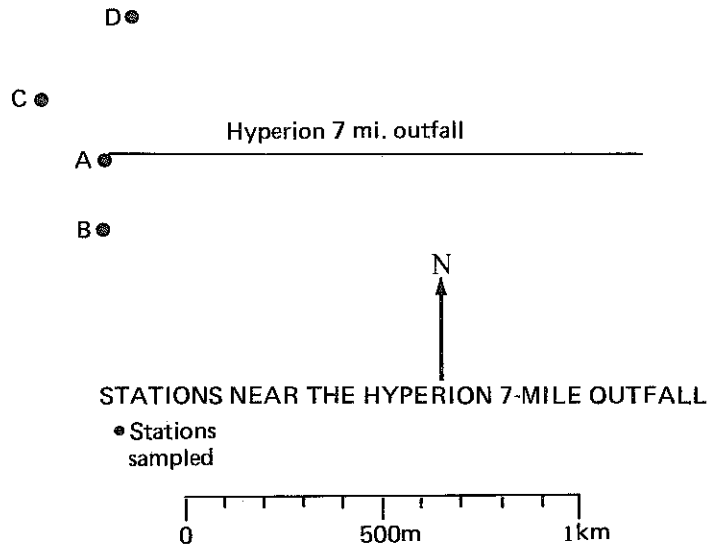

Figure 3. Stations near the Hyperion 7-mile sildge outfall sampled for seawater toxicity.

Table 1. Results from the echinochrome bioassays that tested the toxicities of final wastewater effluents. Note levels at which effluents are non-toxic.

Concentration (percentage effluent)	Dilution seawater: effluent	Treatment Plant				
		Hyperion	JWPCP	Orange County	Ventura	San Diego
Control		○	○	○	○	○
.07	1500:1	○	○	○	○	○
.2	500:1	○	○	○	○	○
.5	200:1	●	●	●	○	○
1	100:1	●	●	●	○	○
4	25:1	●	●	●	○	●
7	14.3:1	●	●	●	○	●
15	6.7:1	●	●	●	●	●

○ Non-toxic

● Toxic*

*Significantly reduced echinochrome production ($p \leq 0.05$).

Table 2. Results from the 48-hour development test that tested the toxicities of water samples collected monthly from the offshore and Los Angeles Harbor stations. Note that there is increased toxicity in June 1981, January 1982, and February 1982, but relatively little toxicity otherwise.

Year		1981									1982			
Month		Mar	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Station	Z	●		●	○	○		○	○	○	○	●	●	○	○	○
	C	○		○	●	○	○	○	○	○	●	●	○		○
	A	○		●	●	○	○	○	○	○	○	○	○	○	○
	S	○		○	○	○	○	○	○	○	●	●	●	●	○
	L	○		●	○	○	○	○	○	○	●	●	○	●	●
	E	○		●	○	○	○	○	○	○	●	●	○	○	○
	G	○		○	○	○	○	○	○	○	●	●	○	●	○
13.2	surf.	○	○	○	○	○	○	○	○	○	●	○	○	○	○
	bot.	○	○	○	○	○	○	○	○	○	●	○	○	○	○
7.3	surf.	○	○	○	○	○	○	○	○	●	●	○	○	○	○
	bot.	○	○	●	○	○	○	○	○	○	●	○	○	○	○
11.3	surf.	○	○	○	○	○	○	○	○	○	●	●	○	●	○
	bot.	○	○	○	○	○	○	○	○	●	●	○	○	○	○
8.5-3	surf.	○	○	○	○	○	○	○	○	○	●	●	○	○	○
	bot.	○	○	○	○	○	○	○	○	○	●	●	○	○	○

○ Non-toxic

● Toxic*

*Significant reduction in the percentage of normal gastrulas after 48-hr ($p \leq 0.01$)

For the 15-minute fertilization, 48-hour development, and echinochrome tests, the samples were examined for percentage fertilization, percentage normal and echinochrome absorbance, respectively. All percentage data underwent arcsine transformation for statistical analysis. The samples in each experiment were compared respective to their controls by one-way analysis of variance. Those experiments that had significant F values ($p \leq 0.01$) for harbor and offshore experiments, $p \leq 0.05$ for wastewater and outfall area experiments were further analyzed by Student-Newmann-Keuls multiple range test (Nie *et al.*, 1975). Those samples that were significantly less than the controls were noted and considered "toxic."

RESULTS

Eight seawater samples were collected within a few hundred meters of the Hyperion 7-mile sludge outfall at depths of 25 m and three meters above the bottom. Elevated ammonia levels

Table 3. Results from the echinochrome bioassays that tested the toxicities of seawater samples collected from stations near the Hyperion 7-mile outfall (final effluent). Note the lack of toxicity in the regions where a high ammonia level indicates wastewater.

Station	Depth	Ammonia (mg/l)	Toxic ● */Non-toxic ○
Control		0.010	○
A	25	0.037	○
A	98	0.136	○
B	25	0.029	○
B	88	0.064	○
C	25	0.101	○
C	127	0.007	○
D	24	0.082	○
D	129	0.028	○

*Significantly reduced echinochrome production ($p \leq 0.05$).

in the water samples indicated that some of them were within the influence of the sludge effluent plume (Table 3). No evidence of toxicity was found in these water samples (Table 3), although past experiments with dilutions of Hyperion sludge indicated it was five to ten times more toxic than other wastewater effluents (Oshida *et al.* 1981). These new data suggest that the sludge effluent did not create a large area of toxic water at the depths sampled.

Examination of water samples from harbor and offshore stations with the three different sea urchin bioassay methods (fertilization, development, and echinochrome) showed only sporadic toxicity over a 14 month period. We did not determine the specific compounds causing the toxicity and we recognize that the urchins may have been responding to natural toxins (e.g. dinoflagellates) as well as anthropogenic contaminants.

It appeared that the echinochrome (Table 4) and development tests (Table 2) were not only more sensitive to harbor and offshore seawater toxicity than the 15-minute fertilization test (Table 5), but also elicited the same results 75 percent of the time.

DISCUSSION

After conducting these three types of sea urchin bioassays on different types of water samples we have concluded that the echinochrome test is faster, simpler, more reliable, and therefore the best bioassay for general use. The echinochrome measurement procedure requires only about 25 percent of the time needed for microscopic examination necessary in the development test, and requires less technical and biological expertise.

The echinochrome bioassay is a convenient way of detecting wastewater toxicity. The method seems to be more sensitive to wastewater toxicity than the 48-hour development test. This is indicated by the fact that concentrations of effluent identified as toxic by the echinochrome test were slightly lower than previously reported for the 48-hour development test (Oshida *et al.* 1981). It is also sensitive to wastewater concentrations of about one percent (Table 5)

Table 4. Results from the echinochrome bioassays that tested the toxicities of water samples collected monthly from the offshore and Los Angeles Harbor stations. Note the increased toxicity in October 1981 and January 1982 at the offshore stations, but relatively little toxicity otherwise.

Year		1981				1982			
	Months	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Station	Z	●	○	●	●	○	○	○	○
	C	○	○	○	○	○	○		○
	A	○	○	○	○	○	○	○	○
	S	○	○	○		○	○	○	○
	L	○	○	○	○	●	○	●	○
	E	○	○	○	○	○	○	○	○
	G	○	○	○	○	○	○	●	○
13.2	surf.	●	○	○	●	○	○	○	○
	bot.	○	○	○	●	○	○	○	○
7.3	surf.	●	○	○	●	○	○	●	○
	bot.	○	○	○	○	○	○	○	○
11.3	surf.	●	○	○	●	○	○	●	○
	bot.	○	○	○	○	●	○	○	○
8.5-3	surf.	●		○	○	○	○	○	○
	bot.	○	○	○	●	○	○	○	○

○ Non-toxic
● Toxic*

*Significant reduction in echinochrome production ($p < 0.01$)

which is about the level present at the edge of each outfall's zone of initial dilution (Gibson 1978). The sensitivity of the echinochrome bioassay makes the method a useful indicator of possible wastewater toxicity outside the zone of initial dilution.

REFERENCES

Bay, S. M., K. D. Jenkins, and P. S. Oshida. A simple new bioassay based on echinochrome synthesis by larval sea urchins. Mar. Env. Res. In Press .

Gibson, C. 1978. Evaluation of initial dilution for ocean discharge. Report to State Water Resources Control Board.

Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Brent. 1975. SPSS: Statistical Package for the Social Sciences, New York: McGraw-Hill.

Table 5. Results from the 15-minute fertilization bioassays that tested the toxicities of water samples collected monthly from the offshore and Los Angeles Harbor stations. Note that there is very little toxicity evident.

Year	1981									1982				
Month	Mar	May	Jun **	Jul	Aug	Sep	Oct	Nov	Dec	Feb	Jan	Mar	Apr	May
Station														
Z	○		●	○	○	○	○	○	○	○	○	○	○	○
C	○		●	○	○	○	○	○	○	○	○	○		○
A	○		●	○	○	○	○	○	○	○	○	○	○	○
S	○		○	○	○	○	○	○	○	○	○	○	○	●
L	○		●	○	○	○	○	○	○	○	○	○	○	●
E	○		●	○	○	○	○	○	○	○	○	○	○	○
G	○		●	○	○	○	○	○	○	○	○	○	○	○
13.2 surf.	○	○	○	○	●	○	○	○	○	○	○	○	○	○
bot.	○	○	○	○	○	○	○	○	○	○	○	○	○	○
7.3 surf.	○	○	○	○	●	○	○	○	○	○	○	○	○	○
bot.	○	○	○	○	○	○	○	○	○	○	○	○	○	○
11.3 surf.	○	○	○	○	●	○	○	○	○	○	○	○	○	○
bot.	○	○	○	○	○	○	○	○	○	○	○	○	○	○
8.5-3 surf.	○	○	○	○	○	○	○	○	○	○	○	○	○	○
bot.	○	○	○	○	○	○	○	○	○	○	○	○	○	○

○ Non-toxic

● Toxic*

*Significant reduction in percentage of eggs that were fertilized ($p \leq 0.01$)

** The specific contaminant/s causing toxicity in June were not determined.

Oshida, P. S., T. K. Goochey, and A. J. Mearns. 1981. Effects of municipal wastewater on fertilization, survival and development of the sea urchin, *Strongylocentrotus purpuratus. In* Biological Monitoring of Marine Pollutants. Eds. F. J. Vernberg; A. Calabrese; F. P. Thurberg and W. B. Vernberg. New York: Academic Press.