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EFFECTS OF MUNICIPAL WASTEWATER ON FERTILIZATION, SURVIVAL, AND DEVELOPMENT OF THE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS*

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INTRODUCTION

Nearly one billion gallons (3.78×10^9 liters) of municipal wastewater are discharged daily into coastal waters of the Southern California Bight. Most of the wastewaters are unchlorinated and are injected into the ocean through deep ocean outfalls (60 m depth, several km offshore) fitted with multiport diffusers which initially dilute the wastewaters by factors of 80 to 200 (Hendricks, 1977). An important purpose of this dilution is to mitigate possible toxicity to marine life in the water column. However, marine organisms have rarely been used to assess the toxicities of these effluents. Instead, freshwater fish have been, and still are being used as 96-hour bioassay organisms (Kopperdahl, 1976) for the determination of wastewater toxicity. Because of this reliance on freshwater fish, it is uncertain whether or not water quality near the discharges is suitable for survival and growth of sensitive stages of marine organisms. Woelke (1972), Cardwell (1979), Cardwell *et al.* (1977, 1977b, and 1979), Stober *et al* (1977 and 1978) and Kobayashi (1971, 1972, and 1973) have demonstrated such toxicity can indeed occur in the sea near industrial and domestic discharges, and their results suggest that echinoderm and oyster eggs and larvae are quite sensitive bioassay test organisms.

Conversely, the freshwater fish used in the wastewater tests were not very sensitive and often demonstrated 100 percent survival in 50 to 100 percent effluent for 96 hours.

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Since fish had such low mortality in high concentrations of wastewater, the lowering of toxicity due to improvements in effluent quality, such as alterations in processing or source control, may go undetected.

To reduce this uncertainty, the Southern California Coastal Water Research Project began a program to develop a marine bioassay system for local receiving water monitoring. Bioassays were conducted to determine the concentrations of wastewater that were not harmful to sensitive life stages of the purple sea urchin, *Strongylocentrotus purpuratus*, to compare the sensitivites of *S. purpuratus* and freshwater fish used in effluent bioassays, and to test the toxicities of present and projected effluents.

To address the subject of wastewater toxicity in the ocean, we examined the effects of municipal wastewater and digested sludge on fertilization, survival, and development of purple sea urchin gametes, embryos, and larvae. These early life stages are both sensitive to marine pollutants and critical for normal growth of the urchins (Kobayashi, 1971).

MATERIALS AND METHODS

The experimental procedures were adapted from the bioassay techniques of Kobayashi (1971) on sea urchins, Stober *et al.* (1977) on sand dollars, and Woelke (1972) on oysters. Urchins were collected intertidally, held in a laboratory cold-bath and induced to spawn. The eggs and sperm were exposed, separately, to the test concentrations of effluent before being added together in the test solution. The eggs were subsampled after a short time and microscopically examined for the presence or absence of the fertilization membrane (fertilization), and later for the percentage of normally developed 48-hour larvae.

This study investigated the toxicities of six effluents:

City of Los Angeles' Hyperion Treatment Plant (Hyperion)

1. Present final effluent (5-mile)
2. Primary and secondary digested sludge (7-mile)

County of Los Angeles' Joint Water Pollution Control Plant (JWPCP)

3. Present final effluent
4. Projected final effluent: four parts fine-screened primary and two parts Eunox secondary effluent

County Sanitation Districts of Orange County, Plants 1 and 2 (ORCOSAN)

5. Present final effluent

6. Projected final effluent: one part present primary effluent and two parts secondary effluent

The average concentrations of general constituents, trace metals, and chlorinated hydrocarbons, as well as average flow rates for all the present effluents and Hyperion sludge are summarized by Schafer (1980). Young (1978) has identified the EPA "Priority Pollutants" for these wastewaters. All effluent samples collected by the treatment plant personnel were 24 to 36 hour composites. Glass bottles that had been acid-washed and kilned were used for collection to insure against metal or hydrocarbon contamination of the effluent samples. The Hyperion sludge samples were one-hour composites which had been collected in an acid-washed plastic bucket.

For the experiment, all effluents and sludge were diluted with filtered (3 μm) natural seawater. A list of experimental dilutions is given in Table 1. A 900ml portion of each dilution was poured into each of two one-liter beakers and a 50 ml portion was poured into each of two polypropylene cups. The beakers were used for egg exposure and development, and the cups for sperm exposure. The remaining portions of the effluent dilutions were sampled and measured for dissolved oxygen (Winkler titration), pH (Beckman pH meter) and salinity (refractometer). The beakers and cups were placed in a 12°C water bath and the gametes were added.

Salinity was monitored to determine variability in gamete and egg-development due to salinity. The salinity dilutions, which ranged from 65 percent seawater (21 ppt, parts per thousand) to 90 percent (29 ppt), were made by adding deionized water to natural seawater (33 ppt). These dilutions were set up and measured in the same manner as the effluent dilutions.

Four replicate beakers and four replicate cups containing filtered natural seawater were set up as controls in a manner similar to the effluent and salinity dilutions and measured for dissolved oxygen, pH, and salinity.

The urchins used in this study were collected intertidally by hand from rocks at Malaga Cove, Palos Verdes Peninsula, California. They were packed with brown algae (*Egregia* sp.) to prevent desiccation and transported to the laboratory where they were held in recirculating seawater aquariums at 12°C and fed brown algae, *Egregia* sp. Urchins were induced to spawn by injecting 0.5 ml of 0.5 M potassium chloride into the coelom (Hinegardner, 1967). Spawning female urchins were inverted, aboral side downwards, onto 100ml Pyrex beakers filled with 12°C seawater. The diameter of the beaker was smaller than the diameter of the urchin test and allowed the urchin to remain "perched" on the beakers with the gonadopores immersed in seawater. Eggs were collected only

TABLE 1. Effluent and sludge dilutions used in each experiment.

Test Sample	Test Date	Concentrations (sw:effluent)
Hyperion - present final effluent	12/19/78	500:1, 108:1, 23:1, 5:1
	1/24/79	108:1, 39:1, 14:1, 5:1
	3/29/79	103:1, 64:1, 45:1, 35:1, 24:1, 19:1, 15:1, 14:1
JWPCP - present final effluent	11/28/78	500:1, 108:1, 23:1, 5:1
	1/ 9/79	108:1, 39:1, 14:1, 5:1
	2/20/79	108:1, 39:1, 14:1, 5:1
JWPCP - projected final effluent	11/28/78	500:1, 108:1, 23:1, 5:1
	1/ 9/79	108:1, 39:1, 14:1, 5:1
	2/20/79	108:1, 39:1, 14:1, 5:1
ORCOSAN - present final effluent	12/ 5/78	500:1, 108:1, 23:1, 5:1
	2/ 6/79	108:1, 39:1, 14:1, 5:1
	3/13/79	103:1, 64:1, 45:1, 35:1, 24:1, 19:1, 15:1, 14:1
	4/ 9/79	300:1, 75:1, 60:1, 43:1, 35:1, 26:1, 19:1, 16:1
ORCOSAN - projected final effluent	12/ 5/78	500:1, 108:1, 23:1, 5:1
	2/ 6/79	108:1, 39:1, 14:1, 5:1
Hyperion - sludge	2/13/79	500:1, 300:1, 160:1, 130:1, 108:1, 80:1, 65:1, 23:1
	3/ 6/79	1000:1, 700:1, 600:1, 500:1, 300:1, 160:1, 130:1, 108:1

during the first 15 minutes of spawning to minimize the use of immature eggs that might be shed with prolonged spawning. Eggs collected from an average of six females were combined, passed through gauze to remove spines and debris, and rinsed twice with seawater (Hinegardner, 1967). They were then mixed with seawater to achieve a uniform density and a volumetric sample was taken to count the numbers of eggs released. Using a technique similar to Woelke's (1972), approximately 31,500 eggs were then added to each beaker of test sample with an automatic pipet and maintained for exactly 30 minutes prior to the addition of the sperm to the egg-exposure beakers.

Spawning male sea urchins were kept out of water with their oral side on wet paper towels to collect sperm under "dry" conditions (Hinegardner, 1967), so as not to activate the sperm. Sperm of several males were removed with a pasteur pipet to a test tube and kept below 5°C. Prior to the addition of sperm to the test sample cups, nine drops of sperm were added to 45 ml of seawater in a 100ml beaker and thoroughly stirred. This addition of the sperm to seawater "activated" the sperm. Fifteen minutes after we had added the eggs to the egg-exposure beakers, 1.2 ml of sperm solution were added to each of the sperm-exposure cups. At this time, the eggs and sperm were being simultaneously and separately exposed to the test samples.

Fifteen minutes after the sperm had been added to the sperm-exposure cups, which was also 30 minutes after the eggs had been added to the egg-exposure beakers, the sperm solutions were added to their respective egg-exposure beakers to allow for fertilization. Fifteen minutes later the eggs in each beaker were mixed to achieve a uniformly dense solution, and a 10 ml sample was taken from each beaker with an automatic pipet. Each sample was placed in a vial, preserved with ten percent borax-buffered formalin, and saved for future microscopic examination.

The eggs remaining in the beakers were stirred, with electric stirrers, at about 60 rmp, aerated, and kept at 12°C. The result was a nearly homogeneous solution with very little sedimentation, even with the digested sludge. Sampling at 48 hours was accomplished in the same manner as the sampling at 15 minutes. Egg survival was carefully monitored in each experiment.

The eggs and larvae which had been sampled and preserved were examined microscopically (100X) on a Sedgewick-Rafter counting chamber. For the 15-minute samples, "fertilization" of the eggs was defined by the presence of an obvious fertilization membrane around each egg; for the 48 hour samples, "normal" development was defined by the presence of

the archenteron at the gastrula stage. The toxicity of a test sample was defined as the inhibitory effects of the test sample on fertilization, and the further development of sea urchin eggs (Kobayashi, 1972).

In the statistical analysis of the data, comparisons were restricted to those between the control and each of the treatments (individual effluent, sludge, or salinity dilutions). The control and treatment data were compared by calculating the T-statistic with respect to the difference between the two independent sample means. The treatments that differed significantly at $p \leq 0.05$ were identified.

RESULTS

Municipal wastewater significantly reduced fertilization of sea urchin eggs at effluent concentrations generally higher than one to seven percent effluent (Table 2). There was a trend of decreased percentage of fertilization with increased concentrations of effluent that paralleled reductions in the percentages of normal development of the 48-hour larvae (Table 3). The concentrations of effluent that did not elicit toxic responses were found to be similar in repeated experiments; usually only changed due to different experimental concentration levels. Hyperion digested-sludge (7-mile) concentrations at, and above 0.2 percent, significantly reduced fertilization of the eggs.

Urchin gametes and fertilized eggs exposed to the control solutions of uncontaminated natural seawater showed very little variability, with a mean percentage of fertilization of 94.7 percent (n = 40, standard error, S.E. was 0.84 percent) and a mean percentage of normal development of 92.1 percent (n = 48, S.E.: 0.80 percent).

The salinity tests that were conducted concurrently with effluent and sludge tests generally showed that fertilization and normal 48-hour development were reduced at salinity levels lower than 28 ppt. The results of salinity tests showed that the effects of lowered salinity were negligible at "present" effluent concentrations that caused harmful effects to the urchins. The salinities in the lowest "present" effluent concentrations that caused reduction in fertilization and development were at or higher than 30 ppt in all but one instance (Hyperion test, 12/19/78), while in the salinity tests, the salinities tested that caused significantly harmful effects were at or below 28 ppt.

The "projected" effluent from ORCOSAN caused significant reductions in normal development at 20 percent effluent, which had salinity of about 26 ppt. The toxicity in these 5:1

TABLE 2. A summary of the highest effluent concentrations that had no effect on fertilization of sea urchin, *Strongylocentrotus purpuratus, eggs,* and the lowest concentrations that significantly ($p \leq 0.05$) reduced fertilization.

		Highest concentration of effluent tested that caused no effect on fertilization		Lowest concentration of effluent tested that caused significantly reduced fertilization	
Test Sample	Test Date	Dilution	% Effluent	Dilution	% Effluent
Hyperion - present final effluent	12/19/78	23:1	4.3	5:1	20
	1/24/79	39:1	2.6	14:1	7:2
	3/20/78**	14:1	7.2		
JWPCP - present final effluent	1/ 9/79*	–	–	108:1	0.9
	2/20/79*	–	–	108:1	0.9
JWPCP - projected final effluent	1/ 9/79*	–	–	108:1	0.9
	2/20/79	39:1	2.6	14:1	7.2
ORCOSAN - present final effluent	2/ 6/79	108:1	0.9	39:1	2.6
	3/13/79*	-	-	103:1	1.0
	4/ 9/79	43:1	2.3	35:1	2.9
ORCOSAN - projected final effluent	2/ 6/79	39:1	2.6	14:1	7.2
Hyperion - sludge	2/13/79*	-	-	500:1	0.2
	3/ 6/79	700:1	0.14	600:1	0.17

*All concentrations of effluent tested caused significantly reduced fertilization.

**No experimental concentrations of effluent caused significantly reduced fertilization.

dilutions of effluent may not have been attributable to projected effluent, but, in fact, may have reflected lowered salinity effects.

The pH, dissolved oxygen, and salinity levels in the controls, effluent dilutions, and sludge dilutions showed only minimal changes during the 48-hour experimental periods. The pH mean values were 7.90 (n=11, S.E.: 0.011), 7.78 (n=17, S.E.: 0.041), and 7.89 (n=72, S.E.: 0.008) for control, sludge and effluent solutions, at the start of the experiment with mean changes in pH after 48 hours of 0.15 (S.E.: 0.013), 0.11 (S.E.: 0.020), and 0.13 (S.E.: 0.007) units respectively. The same samples were also analyzed for dissolved oxygen and salinity, and the mean dissolved oxygen values at the start of the experiments were 7.56 (S.E.: 0.049), 6.69 (S.E.: 0.364), and 7.27 (S.E.: 0.070) mg/l, with mean changes in dissolved oxygen over 48 hours of 0.51 (S.E.: 0.095), 0.52 (S.E.: 0.062), and 0.97 (S.E.: 0.075) mg/l, for control, sludge and effluent solutions, respectively. The mean salinities at the start of the experiments were 32.5 (S.E.: 0.144), 32.6 (S.E.: 0.445), and 30.9 (S.E.: 0.267) ppt with mean changes after 48 hours of 0.33 (S.E.: 0.128), 0.19 (S.E: 0.101) and 0.27 (S.E.: 0.050) ppt for control sludge and effluent solutions, respectively. The lower mean salinity for the effluent solutions was due to the lower seawater dilutions used with these freshwater effluents.

DISCUSSION

This sea urchin fertilization and larval bioassay appears to be an accurate, reliable, and feasible test for measuring the toxicities of seawater dilutions of municipal wastewaters and sludges. These results are consistent with information found by other researchers. Cardwell *et al.* (1979) conducted 48-hour toxicity tests on fertilized eggs of the Pacific oyster, *Crassostrea gigas*, with chlorinated and unchlorinated sewage-treatment plant effluents from Olympia, Washington; both effluents were found to be toxic to developing oysters at ten percent effluent, but innocuous at one percent effluent. Stober *et al.* (1977) conducted a success of fertilization test with only the sperm of the sand dollar, *Dendraster excentricus*, exposed to both chlorinated and unchlorinated West Point, Seattle, Washington effluent prior to fertilization and showed that fertilization was reduced by 50 percent in 4.4 percent chlorinated effluents. In the Stober *et al.* (1977) test, the eggs were not exposed to effluent prior to fertilization, thereby measuring only the effects of

TABLE 3. A summary of the highest effluent concentration tested that had no effect on 48-hour sea urchin, *Strongylocentrotus purpuratus*, devolopment and the lowest concentrations that significantly ($p \leq 0.05$) reduced normal development.

		Highest concentration of effluent that caused no effect on development		Lowest concentration of effluent that caused significantly reduced normal development	
Test Sample	Test Date	Dilution	% Effluent	Dilution	% Effluent
Hyperion - present final effluent	12/19/78	108:1	0.9	23:1	4.3
	1/24/79	108:1	0.9	39:1	2.6
	3/20/79**	14:1	7.2	-	-
JWPCP - present final effluent	11/28/78	23:1	4.3	5:1	20
	1/ 9/79*	-	-	108:1	0.9
	2/20/79*	-	-	108:1	0.9
JWPCP - projected final effluent	11/28/78	23:1	4.3	5:1	20
	1/ 9/79	108:1	0.9	39:1	2.6
	2/20/79	108:1	0.9	39:1	2.6
ORCOSAN - present final effluent	12/ 5/78	23:1	4.3	5:1	20
	1/ 9/79	108:1	0.9	39:1	2.6
	3/13/79*	-	-	103:1	1.0
	4/ 9/79	300:1	0.3	75:1	1.3
ORCOSAN - projected final effluent	12/ 5/78	23:1	4.3	5:1	20
	2/ 6/79	14:1	7.2	5:1	20
Hyperion - sludge	2/13/79*	-	-	500:1	0.2
	3/ 6/79*	-	-	1000:1	0.1

*All concentrations of effluent caused significantly reduced normal development.

**No concentrations of effluent caused significantly reduced normal development.

the effluent on the ability of the sperm to fertilize the eggs. The sea urchin experiment described here showed very similar results; the significantly harmful effluent concentrations being above one to seven percent effluent.

It is now possible to demonstrate how the toxic effects of wastewater might be distributed in the ocean by relating the effluent concentrations that caused toxic reactions in sea urchins to the measured and theoretical concentrations of wastewater around sewage outfalls. Hendricks (1977) made *in situ* measurements of the initial dilution zones of Hyperion outfall, JWPCP outfall, ORCOSAN outfall, and San Diego City's Point Loma outfall in October 1976. His goals were to compare various methods of predicting initial dilution zones with the actual measured dilutions produced by the existing outfall systems. Hendricks' results indicated that the "minimum initial dilutions" associated with the four outfalls ranged from a low of 100 to 1 seawater to effluent, to a high of 290 to 1, for the well-stratified conditions existing at the time of measurement. Using the 100 to 1 level as a hypothetical "minimum initial dilution", the Hyperion and ORCOSAN effluents would not be toxic to sea urchin fertilization and 48-hour development, after initial dilution. The JWPCP present effluent would still be toxic to sea urchins in this test at the 100 to 1 dilution, but may not be toxic at the 135 to 1 dilution that Hendricks (1977) had measured for this outfall in 1976.

The Hyperion digested-sludge discharge is still very toxic as the dilutions at which there were no effects on sea urchin fertilization or development were in the 600-1000 to 1 range. The "minimum average dilution" for the sludge outfall is about 100-150 to 1 (Hendricks, personal communication). Therefore, the sludge discharge and effluent discharge from the Hyperion Treatment Plant should be toxic and non-toxic, respectively, after initial dilution.

Since California has no standard marine bioassay for the measurement of the toxicity of wastewaters discharged into the ocean, the toxicities of such effluents have been, and still are, measured using freshwater fish exposed to freshwater dilutions of effluents (Kopperdahl, 1976). The results of the dischargers' freshwater fish bioassays and this study's sea urchin bioassays appear on Table 4. It appeared that the 48-hour sea urchin fertilization test was five to ten times more sensitive to wastewater effluent toxicity than the freshwater fish test.

The sea urchin test has several advantages when studying wastewater toxicity in the marine environment. It utilizes a marine organism in seawater solutions to measure toxicity and is sensitive to alterations in effluent toxicity that

TABLE 4. Summary of 96-hour LC50 results for freshwater fish, *Pimephales promelas*, (fathead minnows), bioassays with wastewaters (1/78-5/79) and effluent concentrations that caused 50 precent reduction in fertilization of sea urchin, *Strongylocentrotus purpuratus, eggs*.

Present Effluents Tested	Freshwater Bioassay with Fathead Minnows 96-hr LC50 (% effluent)			Marine Bioassay with Sea Urchin Gametes 50% reduction in fertilization occurred between		
	n	mean	standard error	n	——— and ——— (% effluent)	
Hyperion Final	16	98.6[A]	1.01	2	2.6	20
ORCOSAN Final	16	77.1[B]	3.29	3	0.97	7.1
JWPCP Final	9	26.6[C]	2.64	2	0.92	2.6

[A] Hyperion Treatment Plant and Santa Monica Bay 1978 Annual Summary Report and personal communication with Phil Chang.

[B] County Sanitation Districts of Orange County, California: Annual Report 1978, Operations and Marine Monitoring, and personal communication with Monica Farris.

[C] County Sanitation Districts of Los Angeles County, Joint Water Pollution Control Plant Annual Summary, 1979.
County Sanitation Districts of Los Angeles County, Monthly Monitoring Reports (January 1978 to December 1978).

occur when changes in plant operations or source control are implemented. Investigators can measure the toxicities of hypothetical effluents by adding and reducing specific contaminants to wastewaters or by mixing different proportions of primary, secondary, or tertiary treated wastewater.

Further studies will be conducted with the sea urchin fertilization and development test. Seawater sampling to measure toxicity in and near the zones of initial dilution will be conducted using a tracer (e.g. ammonia) in the effluent to confirm dilution measurements. The toxicity of these samples will be measured and compared to similar samples diluted in the laboratory to determine how the laboratory dilutions relate to actual *in situ* dilutions of wastewater and seawater. Seawater samples will also be taken at various depths in coastal regions to measure the natural variability in seawater toxicity, as well as to check for the influence of storm run-off and upwelling. Future tests will compare the sea urchin to other marine species to measure and calibrate interspecific differences in sensitivity to complex effluents and isolated contaminants.

SUMMARY

1. Municipal wastewaters reduced fertilization of urchin eggs at concentrations generally higher than one to seven percent effluent.

2. Using a 100 to 1 seawater to effluent ratio as a hypothetical "minimum initial dilution" of effluents and sludge injected into the ocean, two of three effluents tested did not affect fertilization outside the zone of initial dilution.

3. Digested sludge, as discharged into Santa Monica Bay, California, was very toxic within and near the zone of initial dilution.

4. This urchin fertilization test was 5-10 times more sensitive than the 96-hour freshwater fish bioassay now being used by the dischargers.

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