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SAND DOLLAR (*Dendraster excentricus*) SPERM
AND EMBRYO BIOASSAY OF PUGET SOUND RECEIVING
WATER SAMPLES

by

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Final Report
for receiving water samples collected 11 July 1979

to

WASHINGTON STATE SHELLFISH LABORATORY
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for Robert L. Burgner, Director

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ABSTRACT

Comparative toxicity bioassays of Puget Sound receiving water samples were conducted using sperm cells and embryonic development of sand dollars (*Dendraster excentricus*) and oysters (*Crassostrea gigas*). This report details the results of the sand dollar bioassays conducted by Fisheries Research Institute personnel at our West Point Marine Laboratory. The oyster bioassays were conducted by researchers of the Washington State Shellfish Laboratory, Brinnon, Washington. The results of those tests will be reported under a separate cover.

Eleven receiving water samples (including three control samples, one carry-along control and West Point control water) proved non-toxic to sand dollar sperm and embryonic development. One water sample (from Everett, Washington) produced a significant reduction in egg fertilization success after 30- and 60-minute exposures of the sperm. This same water sample also produced a slight retardation in sand dollar embryonic development during a 72-hour exposure. The sperm bioassay seemed to provide a more precise definition of toxicity in this one toxic sample than did the embryonic development. One reason for this may be the relative insensitivity of early embryonic stages which would be subjected to the most toxic conditions. Later, more sensitive stages, are present only after the toxic compounds have partially degraded and/or been lost due to volatilization, absorption by test containers, etc.

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INTRODUCTION

Bioassay of receiving water quality requires sensitive, short-term tests capable of discriminating toxic effects after a waste effluent has undergone initial dilution by the receiving water.

Marine receiving waters of Washington State have been routinely bioassayed using a 48-h oyster (*Crassostrea gigas*) embryo bioassay originally developed by Woelke (1972). Similar marine bioassays have been conducted in Japan using embryonic development of several echinoderm species (Kobayashi 1972, 1974, 1977).

An ideal receiving water bioassay should be sensitive to a wide variety of pollutants at concentrations sub-lethal to fish and adult invertebrates; capable of testing discrete samples in short periods of time; and the results easily quantified in an objective manner. As such, embryo bioassays have two distinct limitations: 1) embryo development usually requires time frames of at least 24-48 hours and 2) results are usually based on a more-or-less subjective analysis of "abnormal development", which is often difficult to reconcile with "mortality" in the same test.

Presently, researchers of the Fisheries Research Institute, University of Washington, are investigating the use of a sperm bioassay for defining the toxicity of pollutants in routine laboratory testing. This sperm bioassay may prove especially useful as a receiving water bioassay, since typical exposure times are only 15-60 minutes and the results are defined in an objective manner as the presence or absence of subsequent egg fertilization. These results can then be assessed by most standard bioassay statistical methods.

The objective of this study was to comparatively bioassay receiving water samples collected from Puget Sound and Hood Canal, using sand dollar (*Dendraster*

eccentricus) sperm and embryo development tests and equivalent oyster (*Crassostrea gigas*) sperm and embryo development tests. The sand dollar tests were conducted by Fisheries Research Institute researchers at the West Point Bioassay Laboratory. The results of those tests are the object of this report. The equivalent oyster tests were conducted by researchers of the Washington State Department of Fisheries at the State Shellfish Laboratory, Brinnon, Washington. The results of the oyster tests are reported under a separate cover.

MATERIALS AND METHODS

Receiving water samples were collected by float plane on 11 July 1979. Two one-gallon samples were collected in polyethylene containers from each sample site (Table 1). One sample of each pair was delivered to the Fisheries Research Institute's Bioassay Laboratory, presently located at METRO's West Point Sewage Treatment Plant, for the sand dollar sperm and embryo bioassays. The second sample of each pair was delivered to the State of Washington Shellfish Laboratory, Brinnon, Washington, for bioassay with oyster sperm and embryos. An additional sample was collected from the West Point seawater supply system for use as a laboratory control for the sand dollar tests only.

Sperm Bioassays

The sand dollar sperm bioassays were conducted by exposing sperm from four separate males to 25-ml subsamples of each agitated receiving water sample for 30 and 60 minutes. Eggs were added to the samples after each respective

TABLE 1. Sample locations and temperature, salinity, pH, dissolved oxygen and Secchi disc readings of receiving water samples for sand dollar/oyster comparative toxicity testing. Collection date: 11 July 1979.

<u>WDF Sample</u>	<u>Location (Depth)</u>	<u>Temperature</u> (°C)	<u>Salinity</u> (o/oo)	<u>pH</u>	<u>Dissolved Oxygen</u> (ppm)	<u>Secchi Disc</u> (Feet)
231	Point Whitney (0')	16.0	28.2	8.2	8.2	17
232	Point Whitney (30')	14.6	28.8	8.2	8.2	17
233	Poulsbo (10')	16.3	29.2	8.1	7.4	7
234	Sinclair Inlet (10')	13.4	29.5	8.0	7.4	13
235	Twanoh (30')	10.9	29.8	7.8	6.3	7
236	Budd Inlet (10')	14.8	29.0	7.8	8.7	8
237	Ketron Island (10')	12.5	30.2	8.0	7.4	30
238	ASARCO (10')	12.9	29.7	8.0	7.6	27
239	Hylebos Waterway (10')	14.0	26.2	7.9	6.8	4
240	Everett (10')	12.6	29.2	7.4	6.3	5
141	Carry Along Control (45')	---	---	7.8	6.1	---
---	West Point (25')	11.7	29.4	7.9	8.4	---

exposure period. The sperm and eggs were left in contact for 15 minutes before fixing the samples in 10% formalin in sea water. The eggs were later tabulated for percent fertilization by scoring the presence or absence of the fertilization membrane in a subsample of 100 eggs.

The gametes were obtained by injecting 0.5 ml of 0.5 M potassium chloride through the oral aperture (Tyler 1949) and collecting in approximately 100 ml of filtered (5 μ m) seawater.

The sperm samples were adjusted to a concentration of approximately 50×10^6 sperm/ml and 0.1 ml (5×10^6 sperm) was added to each 25-ml water sample. The eggs were washed twice with fresh seawater and adjusted to a concentration of 5×10^3 eggs/ml. One ml of eggs was added to each 25 ml sample after the sperm exposure periods to yield a sperm/egg ratio of 1000/1.

Embryo Bioassays

The embryo bioassays were conducted by adding approximately 5×10^3 freshly fertilized eggs to triplicate 100-ml subsamples of each receiving water sample. Six replicate samples of West Point seawater were included as laboratory controls. Development was allowed to proceed in an ambient seawater temperature bath for 72-hours followed by fixation in 10% formalin in seawater. The samples were later tabulated for stage of development and abnormalities.

RESULTS

Sperm Bioassays

The results of the 30- and 60-minute sperm exposure bioassays are tabulated in Tables 2 and 3, respectively. The maximum average percent fertilization in most samples was only about 90% due to the presence of approximately 10% immature eggs which were not capable of fertilization.

TABLE 2. Sand dollar fertilization success after exposure of sperm to WDF receiving water samples for 30 minutes. Sperm from four separate males were used. Sample size was 100 eggs.

WDF Sample No.	% Fertilization				
	Male #1	Male #2	Male #3	Male #4	Average
231	94	91	92	91	92.0
232	83	91	92	92	89.5
233	89	80	82	94	86.2
234	85	94	95	89	90.8
235	89	88	92	96	91.2
236	85	90	84	91	87.5
237	92	81	91	88	88.0
238	75	91	87	90	85.8
239	86	92	93	93	91.0
240	37	59	50	45	47.8
141	91	91	84	87	88.2
West Point	88	87	86	88	87.2

TABLE 3. Sand dollar fertilization success after exposure of sperm to WDF receiving water samples for 60 minutes. Sperm from four separate males were used. Sample size was 100 eggs.

WDF Sample No.	% Fertilization				
	Male #1	Male #2	Male #3	Male #4	Average
231	92	89	86	90	89.2
232	93	80	91	82	86.5
233	95	87	84	89	88.8
234	94	90	87	86	89.2
235	75	92	96	89	88.0
236	89	97	83	92	90.2
237	87	89	87	89	88.0
238	89	85	91	89	88.5
239	88	81	85	85	84.8
240	48	48	27	40	40.8
141	93	88	86	88	88.8
West Point	86	90	84	83	85.8

The average percent fertilization in all samples (except Sample #240) for both 30- and 60-minute sperm exposures times fell within the range of 84.8-92.0%. The average percent fertilization in Sample #240 was substantially less for both 30-minute (47.8%) and 60-minute (40.8%) tests. The 95% confidence intervals for each of the samples are listed in Table 4. All samples except #240 exhibit overlapping confidence intervals which range from 71.2-100.0% fertilization. Sample #240 has 95% confidence intervals of 30.9-64.7% for the 30-minute test and 22.6-59.0% for the 60-minute test. Based on non-overlapping 95% confidence intervals, the fertilization success in Sample #240 was significantly less than all other samples. Thus, water sample #240 had an apparent toxic effect on the sand dollar sperm resulting in reduced fertilization success. Fertilization in all other samples was essentially the same as in the various control samples (Samples #231, 232, 237 and West Point). Temperature, salinity, pH, dissolved oxygen and Secchi disc readings for each sample site are presented in Table 1.

Embryo Bioassay

The stages of development of sand dollar embryos exposed to the receiving water samples for 72 hours are enumerated in Table 5. Two samples (231C and West Point C) exhibit a marked retardation in development compared to all other samples. Some presently unknown isolated factor was probably responsible for this retardation, since both samples were control samples and this pattern is not reflected in their equivalent replicates. Thus, these two samples should be considered abnormal and rejected from further consideration.

The percent-abnormal column includes only those embryos exhibiting gross abnormalities and/or immature eggs which failed to develop. The percent-abnormal in all stages ranged from 2-15% with no marked increase evident for any given set of replicates.

TABLE 4. 95% confidence intervals of the average % fertilization for the 30- and 60-minute sperm bioassays of the WDF receiving water samples.

WDF Sample	30-Minute Sperm Exposure	60-Minute Sperm Exposure
231	89.4 - 94.6	84.6 - 93.8
232	82.5 - 96.5	74.6 - 98.4
233	74.4 - 98.0	80.3 - 97.3
234	82.3 - 99.3	82.6 - 95.8
235	84.6 - 97.8	71.2 - 100.0
236	81.1 - 93.9	79.4 - 100.0
237	78.9 - 97.1	85.9 - 90.1
238	72.3 - 99.3	83.9 - 93.1
239	84.8 - 97.2	79.5 - 90.1
240	30.9 - 64.7	22.6 - 59.0
141	81.9 - 94.5	83.3 - 94.3
West Point	85.4 - 89.0	80.1 - 91.5

TABLE 5. Results of 72-hour sand dollar embryo exposure to WDF receiving water samples. All samples were tested in triplicate. Six replicates of West Point control water were tested. One hundred embryos were counted from each sample.

WDF Sample No.		Stage of Development (%)			
		Abnormal	Gastrula	Early Pluteus	Pluteus
231	A	7			93
	B	4			96
	C	7	10	83	
232	A	4			93
	B	3			97
	C	8			92
233	A	5		2	93
	B	2		2	96
	C	4		1	95
234	A	9			91
	B	5		3	92
	C	4			96
235	A	5	1		94
	B	6		1	93
	C	7	1		92
236	A	6		1	93
	B	7		1	92
	C	4	1	3	92
237	A	5			95
	B	7		1	92
	C	8			92
238	A	3			97
	B	9			91
	C	5			95
239	A	7		1	92
	B	7		2	91
	C	11		1	98
240	A	11	7	6	76
	B	4	5	5	86
	C	8	2	4	86

(Continued on next page)

Table 5 - continued

WDF Sample No.	Stage of Development (%)			
	Abnormal	Gastrula	Early Pluteus	Pluteus
141 A	2			98
B	5			95
C	7	1		92
West A	5			95
Point B	3			96
C	11	81	8	
D	15	3		82
E	7			93
F	6			94

Sample #240 is the only sample which showed an apparent retardation in development. All three replicates of Sample #240 showed small but consistent increases in the percent-gastrula and early pluteus stages and concomitant decreases in the number reaching full pluteus. All samples other than #240 showed average pluteus counts \geq 92% while the average pluteus count for the replicates of Sample #240 was 82.7%. Thus, the receiving water of Sample #240 exhibited a slight toxic effect on the normal development of sand dollar embryos while all other samples were apparently free of substantial toxic components.

DISCUSSION

The results of both the sperm and embryo bioassays are in close agreement that Sample #240 (from Everett) contained substances toxic to both sand dollar sperm and embryonic development. All other receiving water samples appeared to be non-toxic under the conditions of the present testing regimen.

The sperm bioassay appeared to give a more clear-cut response to the toxic components of Sample #240 than did the embryo assay. One reason for this might be the rapidity of the sperm test. This test may be a more effective bioassay of chemical substances which rapidly degrade or volatilize upon addition to the receiving water. Evidently, the various stages of embryonic development differ in their sensitivity to pollutants. Kobayashi (1977) found that toxic effects are more pronounced during pluteus formation than in any other prior stages in the sea urchin, *Anthocidaris crassispina*, and the sand dollar, *Peronella japonica*. Recent sea urchin (*Strongylocentrotus droebachiensis*) embryo tests of silver conducted at our West Point Laboratory suggest that pre-

hatch embryos (i.e., pre-gastrula stages still protected by the fertilization membrane) are less sensitive to silver than the post-hatch stages (Stober et al. 1979). Thus, embryo tests may be least sensitive to toxicants during the initial hours of exposure and then become gradually more sensitive after the toxic components have partially degraded or been lost by volatilization, absorption to the test container, etc. Thus, the use of later stages of development might produce the most sensitive embryo test, but this method would require additional time, effort and coordination of sample collection and distribution.

The relative sensitivity between sand dollar and oyster sperm and embryos is of primary interest in this set of experiments. We hypothesize that the sperm cells of a wide variety of marine invertebrates exhibiting external fertilization should be of similar sensitivity to toxic substances. Should this prove to be true, then a wide variety of animals could be useful for providing a "standardized" test "organism" (i.e., sperm cells) throughout the world. A uniform test "organism" of this nature would be valuable for providing comparative toxicity data for an array of chemicals from many different research laboratories.

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