~~C-205~~
C-210

EVALUATION OF BIOASSAY METHODS FOR RED ABALONE, MYSID SHRIMP AND GIANT KELP

by

Steven M. Bay
and
Darrin J. Greenstein

Southern California Coastal Water Research Project
646 W. Pacific Coast Highway
Long Beach, CA 90806

for

State of California
Department of Fish and Game
1416 Ninth Street
Sacramento, CA 95814

Contract No. 7369

August, 1987

INTRODUCTION

This report summarizes the results of an evaluation of the draft bioassay protocols developed by the Department of Fish and Game. Current versions of the test methods for the red abalone larvae, mysid, and kelp spore tests were used at the Southern California Coastal Water Research Project (SCCWRP) to measure the no effect level (NOEL) of zinc on each organism. The objective of these tests was to evaluate the technical feasibility of these methods for use by personnel who were competent in bioassay testing, but had had limited experience in working with these specific organisms and methods. This project was expected to identify deficiencies or potential problems in the methods and suggest modifications to alleviate these problems, thereby making the methods more feasible for implementation in other laboratories.

Tests were conducted at SCCWRP with animals supplied by the Fish and Game Marine Pollution Laboratory. An effort was made to follow the draft protocols as closely as possible, with deviations occurring only when necessary to adapt the methods to a static culture system or to compensate for minor differences in equipment. These tests were conducted from June 23 to July 1, 1987. The data resulting from this work has been compared to previous data generated by the Marine Pollution Lab as presented in the Final Report on the Toxicity of Zinc and a Complex Effluent to Red Abalone, Mysid Shrimp and Giant Kelp (May 1987). All test results have been expressed in terms of the nominal concentrations of zinc used in each test.

48 HOUR ABALONE LARVAE TEST

Methods

The experimental methods used were as specified in the draft protocols except for the modifications described below. Gravid red abalone were supplied by the Department of Fish and Game Marine Pollution Lab. Two females were placed in the spawning chamber and supplied with flowing UV irradiated seawater at a rate of 50 ml/min (10 inch UV tube). Spawning of two males was initiated after the females began to spawn (4 hours later) so that the availability of eggs could be assured. One of the females and both of the male abalone spawned.

The zinc solutions used in this and the other tests were prepared in SCCWRP laboratory seawater using directions and materials supplied by the Marine Pollution Lab. An inoculation volume of 5.7 ml (1,000 eggs) was added to each bioassay chamber using an automatic pipet. Water bath temperatures during this assay ranged from 14.5 to 15.0 C. At the end of the assay, embryos were transferred to 10 ml culture tubes and preserved with 5 % borax buffered formalin. Larvae were examined under a compound microscope at a magnificaton of 100 X.

The concentrations of zinc used in the exposures were verified by flame atomic absorption spectroscopy. Some of the zinc concentrations used in this test were below the detection

limit for the analytical method. Substantial differences from the nominal values were found for the 32 and 56 ppb levels (Table 1). The bioassay results have been presented using the nominal values until these analyses can be verifid by another laboratory.

Results

The raw data resulting from the examination of larvae and water quality measurements are presented in the Appendix (Tables A1 & A2). In general, the percentage of normal larvae found in the various zinc exposures (Table 2, Figure 1) were very similar to those reported by the Marine Pollution Lab. Seawater controls had a high percentage of normal embryos and nearly 100 % of the eggs were fertilized. The water quality measurements stayed within typical values throughout the experiment. Variability in the percentage normal data was generally low except for the 32 ppb exposure, which had a coefficient of variation of 15 %. A summary of the statistical analysis of these data is shown in Table 3. Dunnett's Test indicated significant reductions in the percentage of normal larvae at nominal zinc levels of 32 ppb and greater. The NOEL for this test was estimated to be 18 ppb, lower than the value of 32 ppb reported by the Marine Pollution Lab.

Discussion

The NOEL for zinc obtained with this test was lower than that previously reported by the Marine Pollution Lab. Examination of the data (Figure 1) clearly indicates that this value is an accurate description of the abalone's response. In comparison to previous test data with zinc, the larvae had a greater response to concentrations of 32 ppb or greater. There are two likely explanations for this difference. It is possible that embryos of poor health or greater sensitivity were used in this test; this is possible since eggs from only a single female were used in the test. The high percentage of successful fertilization and the good performance of the control samples suggest that the organisms used were in good condition.

A second explanation for these data would be differences in recognition of the shell abnormality endpoint. Abnormalities in larval shell morphology were distinct and easily seen on most embryos, but there may have been slight differences in categorizing slightly affected animals as either normal or abnormal. Samples of larvae from this test have been saved. It would be advisable for the Marine Pollution Lab to examine them to see if variations in endpoint recognition have occurred.

The description of an abnormal shell as having 10 % or more of the visible shell area deformed is hard to visualize and may have been interpreted differently at SCCWRP. Photographs provided by the Marine Pollution Lab were helpful in distinguishing normal from slightly abnormal larvae and should be included in final versions of the protocol.

Table 1.

MEASURED ZINC CONCENTRATIONS
FOR ALL EXPOSURES

TEST	NOMINAL CONC. (ppb)	INITIAL MEASURED (ppb)	FINAL MEASURED (ppb)	INITIAL %NOMINAL
KELP	0	< 18	< 17	
	560	520	499	93
	1000	900	935	90
	1800	1707	1717	95
	3200	3136	2985	98
	5600	5410	5075	97
MYSID	0	< 18	78	
	56	40	36	71
	100	52	79	52
	180	137	151	76
	320	261	283	81
	560	477	494	85
ABALONE	0	< 17	< 18	
	10	< 18	< 17	
	18	< 18	< 18	
	32	18	21	50
	56	38	46	68
	100	96	81	96

Table 2. Effect of 48 hour zinc exposure on shell development of red abalone larvae (data are mean ± SE, N = 5).

Nominal Conc. (ppb)	Percent Normal
0	95.1 ± 2.5
10	94.6 ± 1.5
18	93.9 ± 1.5
32	78.2 ± 11.4
56	6.1 ± 3.8
100	0.2 ± 0.3

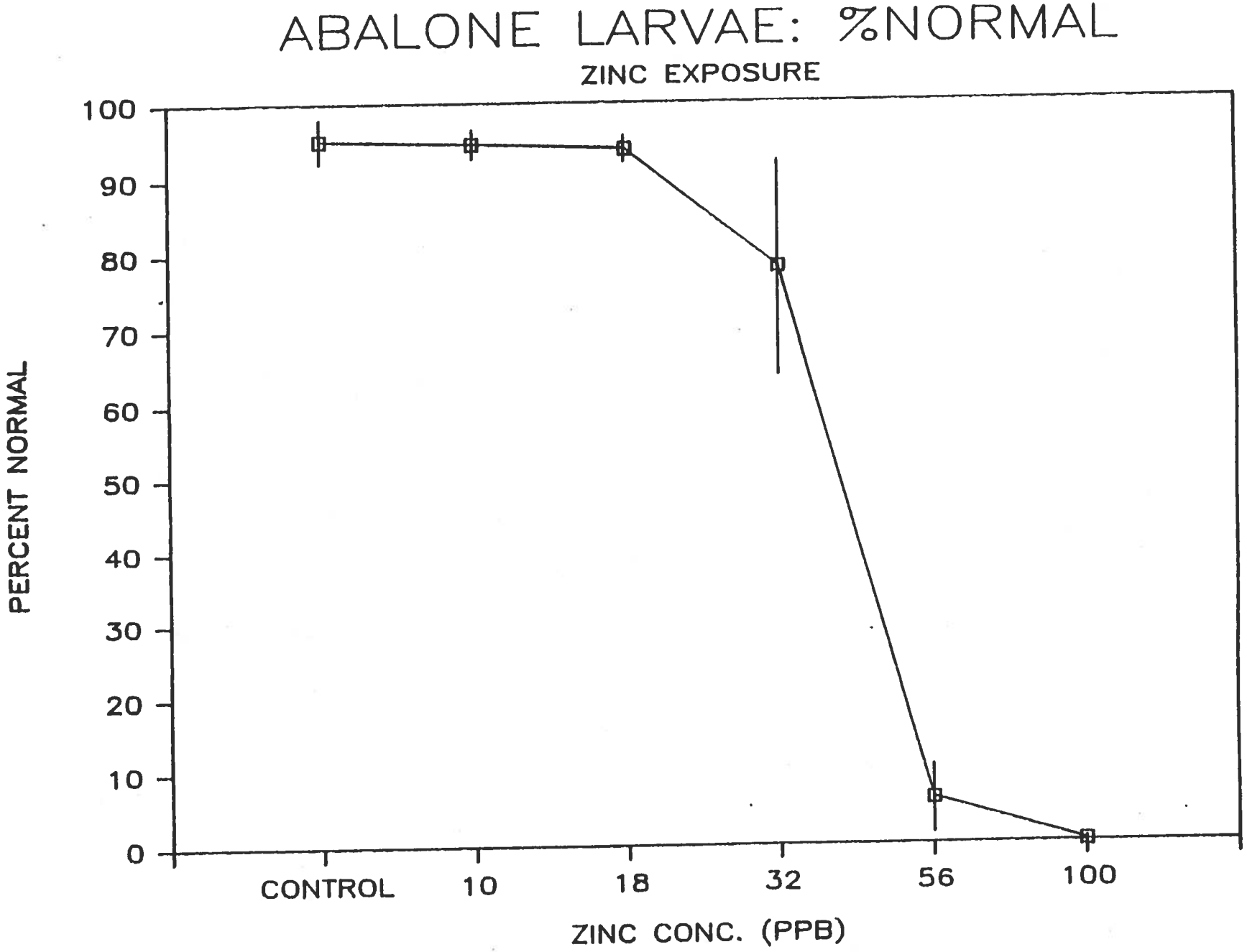

Figure 1. Response of red abalone larvae to zinc exposure (data are mean ± SD).

Table 3.

SUMMARY OF THE ANALYSIS OF VARIANCE
48 HR ABALONE TEST WITH ZINC (% NORMAL ARCSIN TRANSFORMATION)

SOURCE OF VARIATION	SS	DF	MS
TOTAL	30172.59	29	
GROUPS	29700.94	5	5940.189
ERROR	471.644	24	19.65183

F= 302.2715
F 0.05(1),5,24= 2.62

DUNNETT'S MULTIPLE RANGE TEST
48 HR ABALONE TEST WITH ZINC (% NORMAL ARCSIN TRANSFORMATION)

SAMPLE	N	MEANS	DIFFERENCE FROM CONTROL	SE	q'	p
CONTROL	5	77.5	0	2.8037	0.0000	1
10 PPB	5	76.7	0.8	2.8037	0.2853	2
18 PPB	5	75.8	1.74	2.8037	0.6206	3
32 PPB	5	62.8	14.72	2.8037	5.2502	4
56 PPB	5	13.6	63.94	2.8037	22.8056	5
100 PPB	5	1.8	75.74	2.8037	27.0143	6

q 0.05(1),24,6= 2.36
q 0.05(1),24,4= 2.17
q 0.05(1),24,3= 2.01

Fragmentation of the larvae in the preserved samples was probably an important factor in the apparent increase in sensitivity of the test. The velum and other portions of the soft tissue of the larvae often separated from the rest of the organism. When these fragments were encountered under the microscope, a subjective decision had to be made as to whether these pieces were entire abnormal larvae or pieces of normal larvae. In the controls, it was fairly obvious which pieces of tissue were fragments, but in those samples affected by zinc, this determination was more difficult because highly deformed shells often separated from the embryo, leaving a mass of soft tissue in varying degrees of fragmentation. Including significant numbers of fragments in the counts of the affected samples would tend to reduce the calculated percentage of normal larvae in these samples. The best remedy for this problem would be to minimize fragmentation through modifications of techniques for larval preservation or counting.

In general, the protocol for this test was easy to follow and worked well. Most labs should not have a problem in conducting this test. There are two additional aspects of the method which might benefit from modification. First, covering of the bioassay containers is not recommended in the protocol. This should be recommended in order to achieve better quality control and safety. Fallout from the ceiling or air may contaminate some containers, resulting in inconsistent results. Second, it was inconvenient to work with the recommended volume (200 ml) of sample in the plastic assay containers supplied. The greatest problem was in adjusting the water bath levels so that the best temperature control was achieved. With the relatively small water depth in each container, some of the containers began to float after small samples (40 ml) were removed for water quality analysis. An increase in the recommended sample volume or reduction in surface area of the container would minimize this difficulty. This recommendation also applies to the kelp and mysid tests.

GIANT KELP ZOOSPORE TEST

Methods

The methods used for the giant kelp tests were as specified in the draft protocol except for the modifications described below. Macrocystis sporophylls from Monterey were provided by the Marine Pollution Lab. Approximately 36 hours elapsed between sporophyll collection and use. An accurate light meter was not available to measure actual light levels. Lighting in the growth chamber was adjusted to produce an irradiance level close to the value specified in the protocol. Lighting was uniform among the test containers. Water bath temperatures ranged from 14.8 to 15.7 C during the test.

Results

The raw data for the spore examinations are included in the Appendix (Table A3). A very low germination percentage was obtained in this test, as shown in Table 4. Only 20 % of the control zoospores germinated successfully, compared to an expected control germination of at least 80 %. Only a very slight trend of reduced germination with increasing zinc concentration was found (Figure 2). These changes in germination success were not statistically significant, so the estimated NOEL value for this endpoint would be at least 5,600 ppb.

Reductions in germ tube length with increasing zinc concentration were found with this test (Table 4 and Figure 3). The control tube length, response to zinc, and variability of the data were similar to results reported previously by the Marine Pollution Lab. Statistical analysis of the tube length data indicated significant differences from the control at all concentrations tested (Table 5). The NOEL for this endpoint was therefore $\leq$ 560 ppb, the same as that found by the Marine Pollution Lab.

The water quality data for this test are listed in the Appendix (Table A4). The measured values of DO, pH, salinity, and total ammonia stayed within normal ranges during the test.

Discussion

Both the percentage germination and germ tube length measurements were unambiguous and relatively easy to measure. The examination of the slides was time-consuming, however. It would certainly be preferable if a method could be developed to preserve the samples so that the slides could be examined at times other than immediately after the 48 hr exposure period.

Unsatisfactory results were obtained for the spore germination endpoint, apparently because of a low number of viable zoospores. The density of spores in the release beaker (100,000/ml) was within the range indicated in the protocol. One cause of this problem may have been the extended storage of the sporophylls before use. It is also possible that the

Table 4. Effect of zinc on *Macrocystis* zoospore germination and germ tube growth (data are mean ± SE, N = 5).

Nominal Zinc Conc. (ppb)	% Germination	Germ Tube Length (microns)
0	21.2 ± 6.7	20.5 ± 1.6
560	16.2 ± 5.1	16.2 ± 1.3
1000	16.0 ± 2.3	14.7 ± 0.6
1800	17.2 ± 4.1	15.1 ± 0.8
3200	14.8 ± 3.7	13.0 ± 0.8
5600	13.6 ± 5.8	12.5 ± 1.2

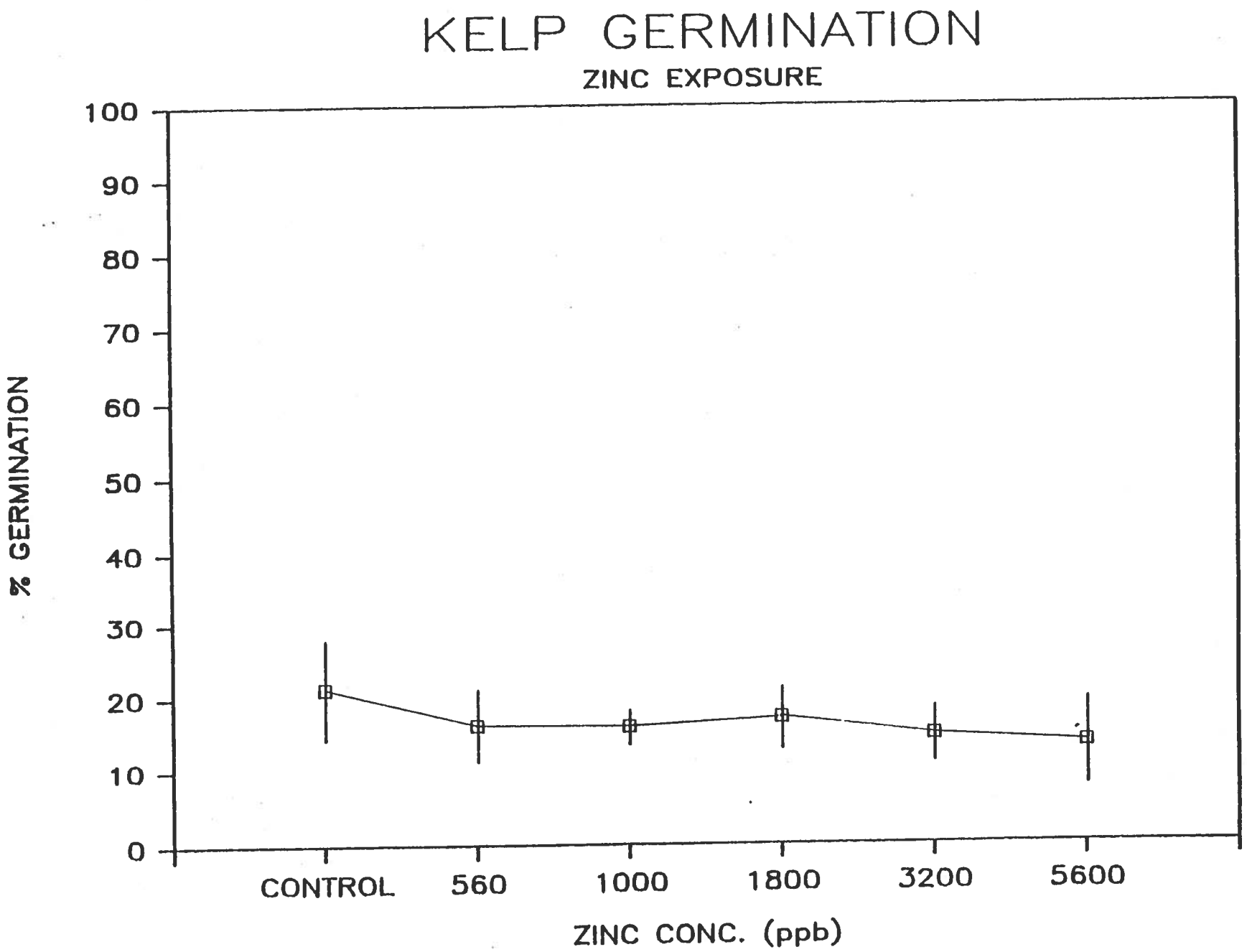

Figure 2. Changes in giant kelp zoospore germination following zinc exposure (data are mean ± SD).

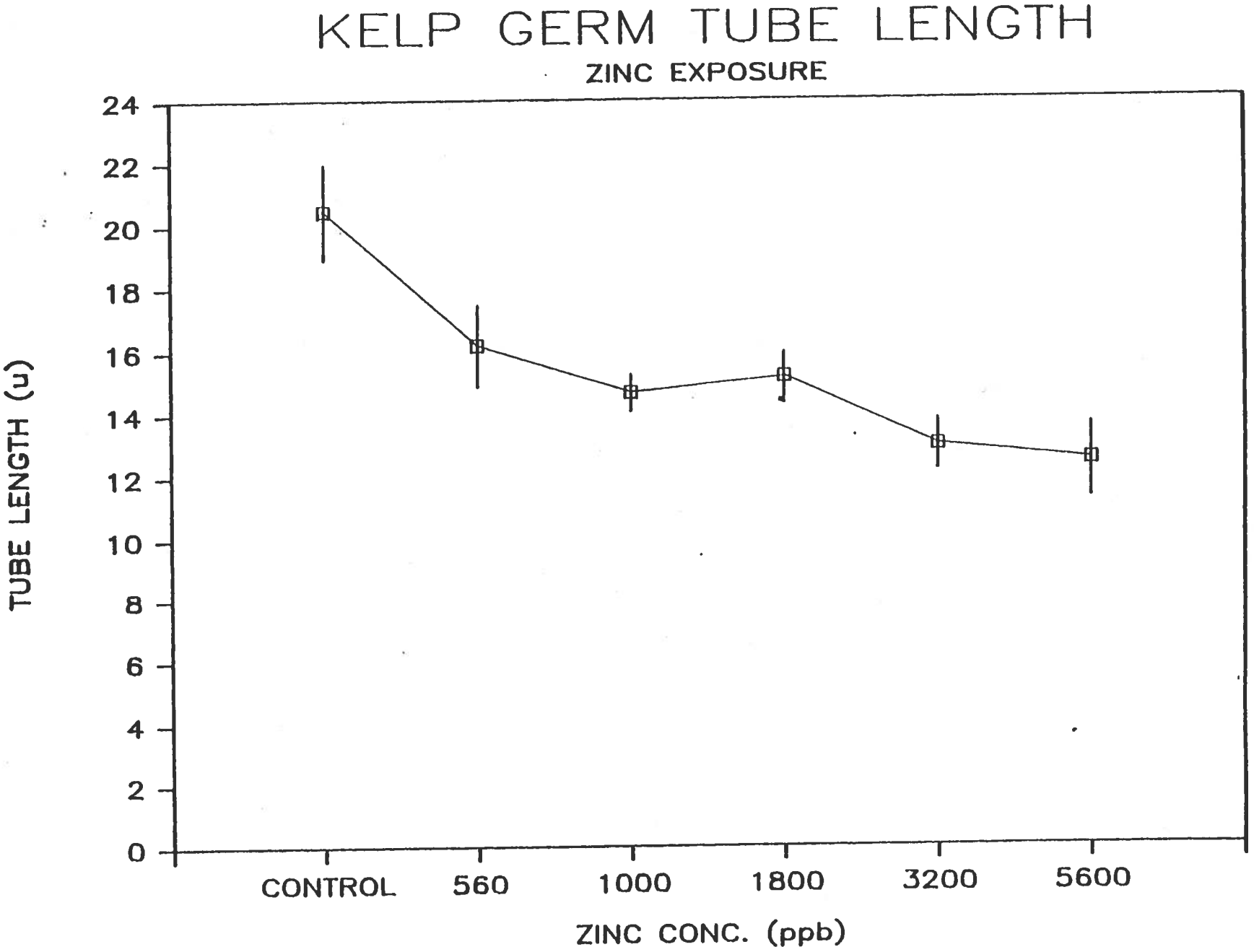

Figure 3. Changes in giant kelp germ tube growth following zinc exposure (data are mean ± SD).

Table 5.

SUMMARY OF THE ANALYSIS OF VARIANCE
KELP BIOASSAY
ZINC EXPOSURE
GERM TUBE LENGTH

SOURCE OF VARIATION	SS	DF	MS
TOTAL	237.9586	29	
GROUPS	207.8186	5	41.56373
ERROR	30.14	24	1.255833

F= 33.09653

F 0.05,(1),5,24=2.60

DUNNETT'S MULTIPLE RANGE TEST
GERM TUBE LENGTH

SAMPLE	MEANS	DIFFERENCE FROM CONTROL	SE	q'	p
CONTROL	20.5	0.0	0.70875477	0	1
560 PPB	16.2	4.3	0.70875477	6.066978	2
1800 PPB	15.2	5.3	0.70875477	7.477903	3
1000 PPB	14.7	5.8	0.70875477	8.183366	4
3200 PPB	13.0	7.5	0.70875477	10.58193	5
5600 PPB	12.5	8.0	0.70875477	11.28740	6

q 0.05,(1),24,2=1.71

inoculation samples removed from the spore release beaker were not taken near enough to the surface and thus included a large proportion of nonviable spores. The spore release and inoculation methods in the protocol should be carefully examined and modified if necessary to minimize this problem in the future. Guidance should also be supplied as to the acceptable range of control germination success. It is apparent from these data that the sensitivity of the test may vary significantly when there is low control germination.

The results obtained from the kelp germ tube length data were remarkably similar to that reported previously by the Marine Pollution Lab. This result is surprising considering the poor control germination success and the reduced sensitivity of germination endpoint in the test encountered at SCCWRP. One point of concern about this test is the shape of the response curve for zinc. Although there is an initial sharp decrease in tube length with zinc, the curve changes shape and appears to reach a limit at a value representing less than a 50 % decrease. If this curve is typical for other toxicant samples, this test may not be useful for generating EC50 values.

Several minor difficulties with the kelp test protocol were also encountered which might benefit from modification. These are described below.

1. When the zinc stock solution (100 ppm) was prepared entirely in seawater as suggested, a precipitate formed immediately. Initially dissolving the zinc sulfate in a small volume of distilled water eliminated the immediate precipitation, although precipitates still formed after 24 hr of storage at room temperature. The stock concentration should be reduced or distilled water used to produce a more stable stock solution.

2. We were fooled by the description of the color of the zoospore solution. The actual color in our release beaker was so light that we did not think release had occurred until a sample was examined under the microscope. This resulted in a delay of about an hour in the test setup.

3. No recommendation is given as to the cleanliness of the glass slides used in the test. Even though new slides are used each time, there may be some residue from the manufacturing process or dust contamination from a partially opened box. Slides should be at least acid washed before use.

4. As was recommended for the abalone test, a greater water depth in the containers and covers should be used.

5. The protocol states that the zoospore radius is 1.5 u, but our measurements indicate it is closer to 3 u.

MYSID TEST

Methods

Test procedures used were those of the draft protocol except for the following modifications. Mysids (Holmesimysis costata) were collected from Monterey and transported to SCCWRP, where they were held under static conditions at 15 C. Both juveniles and brooding females were supplied. The test was conducted with 3 day old juveniles released within 24 hrs of collection in Monterey. Only 244 juveniles were available for the test instead of the recommended 300. Variable numbers of animals per bioassay container (7 - 9) were therefore used instead of the recommended number of 10. Brine shrimp nauplii were fed to the mysids during the exposure at the rate of 40 nauplii/mysid/day. A single daily feeding was used instead of the recommended ration of 20 nauplii/mysid every 12 hours. After 48 hr of exposure, the food ration was reduced to 20/mysid/day in order to reduce the accumulation of uneaten nauplii in the bioassay containers. The temperature range during the experiment was 14.5 to 15.7 C.

Results

The daily counts of living and dead mysids are listed in the Appendix (Table A5). A summary of this data is shown in Table 6. Control survival after 96 hr was 82 %, lower than obtained previously by the Marine Pollution Lab. The response of these mysids to zinc was generally similar to that reported by the Marine Pollution Lab, except that higher variability about the mean was found and survival at the highest concentrations was greater. Statistical analysis of these data indicated significant reduced survival at 320 and 560 ppb (Table 7). The estimated NOEL for these data was 180 ppb, higher than that reported by the Marine Pollution Lab (100 ppb). Examination of the response data (Figure 4) indicates that these animals were affected by zinc levels $\geq$ 100 ppb, but that high variability reduced the statistical significance of the data.

The water quality analyses (Appendix Table A6) yielded acceptable values for DO, pH, and salinity during the experiment. An elevation in total NH_3 was observed during the course of the test. Initial ammonia values of 0.02 mg/l increased to approximately 0.47 mg/l by the end of the 96 hr test. These ammonia values appeared to be little influenced by the number of mysids present in each container.

Discussion

This test produced unacceptable control mortality and highly variable data. An obvious explanation for the high control mortality is not evident. Examination of the raw data indicates that cannibalism was not a problem during this test, even though the food ration was reduced. It is possible that the juveniles used were stressed by their transport from

Table 6. Effect of zinc on juvenile mysid survival during a 96 hr exposure (data are mean ± SE, N = 5).

Nominal Zinc Conc. (ppb)	PERCENTAGE MYSID SURVIVAL			
	24 hr	48 hr	72 hr	96 hr
0	91 ± 12	87 ± 12	87 ± 12	82 ± 12
56	95 ± 6	93 ± 11	93 ± 11	88 ± 12
100	87 ± 10	82 ± 14	79 ± 14	68 ± 18
180	91 ± 9	89 ± 11	82 ± 10	61 ± 13
320	80 ± 14	80 ± 20	77 ± 24	34 ± 26
560	80 ± 21	80 ± 21	65 ± 22	15 ± 16

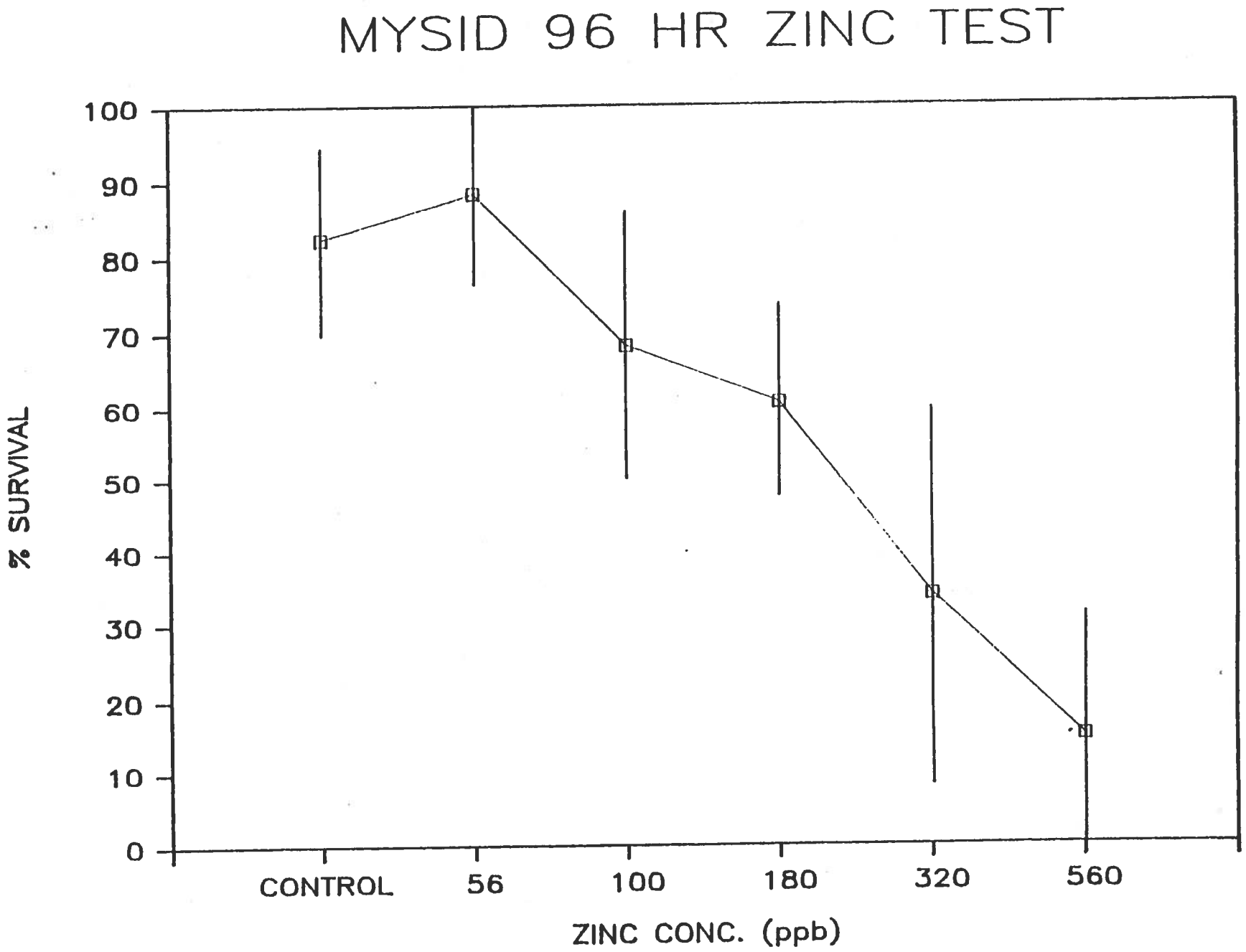

Figure 4. Survival of juvenile mysids following zinc exposure (data are mean ± SD).

Table 7.

SUMMARY OF THE ANALYSIS OF VARIANCE
MYSID EXPOSURE TO ZINC

%ALIVE(ARCSIN TRANS)

SOURCE OF VARIATION	SS	DF	MS
TOTAL	17024.96	29	
GROUPS	11693.36	5	2338.673
ERROR	5331.6	24	222.15

F= 10.52745

F 0.05(1),5,24=3.15

DUNNETT'S MULTIPLE RANGE TEST

SAMPLE	MEANS	DIFFERENCE FROM CONTROL	SE	q'	p
56 PPB	74.6	-6.6	9.426558	-0.70014	1
CONTROL	68.0	0.0	9.426558	0	2
100 PPB	56.4	11.6	9.426558	1.230565	3
180 PPB	51.0	17.0	9.426558	1.803415	4
320 PPB	32.2	35.8	9.426558	3.797780	5
560 PPB	17.6	50.4	9.426558	5.346596	6

q 0.05,(1),24,6=2.36
q 0.05,(1),24,5=2.28
q 0.05,(1),24,4=2.17

Monterey or that the laboratory seawater was slightly toxic. The control mortality of the mysids might have been reduced if juveniles from animals acclimated to SCCWRP lab conditions had been used. This would not have been possible for this experiment since only about 150 juveniles were obtained at an appropriate time from the brooding mysids supplied by the Marine Pollution Lab.

The excessive variability in the data is probably a result of a lack of experience with the mortality endpoint used in this test. The definition of mortality (no appendage movement) was taken in the strict sense for the 96 hr counts. An animal was classified as alive even if there was only very slight movement in any of its appendages when examined under a dissecting microscope. This technique resulted in several obviously moribund animals being counted as survivors, and may have contributed to the variability of the test.

The large increase in total ammonia observed during the test was apparently due to the metabolism of the brine shrimp nauplii. The nauplii were concentrated and washed before addition to the exposure containers so that the addition of ammonia from the nauplii culture water should have been negligible. Additional ammonia measurements should be taken in subsequent experiments to determine if this is a consistent problem in the mysid test. Increases in ammonia may create unwanted stresses during the test.

The methods outlined in the draft protocol were generally clear and easy to follow. Those areas which presented difficulties or could benefit from modification are listed below.

1. The recommendation that only 3 day old juveniles be used in a test requires that large numbers of brooding mysids be available for each test. For this experiment, a sufficiently large hatch (300 individuals) could not be obtained during the desired time period. Logistics for this test would be greatly simplified if the acceptable age range of test animals could be expanded (eg. 3-6 day old mysids).

2. Concern arose during the test because of a buildup of uneaten nauplii in the test containers. A decision was made to reduce the ration, although no guidance was given in the protocol. It would be helpful to address this situation and also provide a suggested method for making accurate counts of the living nauplii for feeding.

APPENDIX

Experimental Data From Tests Conducted

Table A1.

ABALONE LARVAE EXPOSURE TO ZINC
MICROSCOPIC EXAMINATION DATA

BEAKER NUMBER	NOMINAL CONC. (PPB)	NO. NORMAL	NO. ABNORMAL	NO. FERTILIZED UNDEVELOPED	PERCENT ABNORMAL
11	0	217	9	2	4.8
13	0	313	15	0	4.6
18	0	241	6	0	2.4
23	0	241	8	1	3.6
29	0	229	23	0	9.1
1	10	147	9	-	5.8
6	10	195	7	4	5.3
10	10	197	5	1	3.0
17	10	235	7	1	3.3
30	10	206	11	1	5.5
3	18	234	10	-	4.1
21	18	219	11	1	5.2
24	18	181	15	0	7.6
25	18	213	12	2	6.2
27	18	209	17	0	7.5
5	32	128	48	2	28.1
12	32	175	45	2	21.2
15	32	188	22	3	11.7
20	32	189	18	4	10.4
26	32	129	75	3	37.7
2	56	14	181	-	92.8
4	56	15	170	-	91.9
7	56	2	232	2	99.2
14	56	19	162	0	89.5
19	56	9	209	1	95.9
8	100	0	211	2	100.0
9	100	0	208	3	100.0
16	100	0	154	2	100.0
22	100	1	163	1	99.4
28	100	1	168	0	99.4

Table A2

ABALONE LARVAE EXPOSURE TO ZINC
WATER QUALITY DATA

DATE	BEAKER NUMBER	NOMINAL CONC. (PPB)	D.O. (mg/L)	pH	SALINITY (ppt)	BATH TEMP. (C)	TOTAL AMMONIA (mg/L)
6/30/87	INTIAL	0	7.3	8.01	34	15.0	0.035
		10	7.4	7.99	34		0.036
		18	7.3	8.01	34		0.025
		32	7.2	7.96	34		0.026
		56	7.4	7.99	34		0.029
		100	7.4	8.00	34		0.031
7/1/87	13	0	7.8	7.75	34	14.6	0.037
	30	10	8.0	7.97	34		0.036
	21	18	8.0	8.01	34		0.032
	20	32	8.1	8.00	34		0.031
	14	56	8.2	8.00	34		0.031
	8	100	8.2	7.99	34		0.031
7/2/87	18	0	8.0	8.46	34	14.5	0.058
	6	10	8.0	8.00	34		0.032
	24	18	8.0	8.00	34		0.031
	15	32	8.0	8.00	34		0.033
	4	56	8.1	8.00	34		0.028
	9	100	8.1	8.00	34		0.027

TAble A3.

MACROCYSTIS ZOOSPORE EXPOSURE TO ZINC
PERCENT GERMINATION AND GERM TUBE LENGTH DATA
(REPLICATE MEASUREMENTS SEPERATED BY COMMAS)

BEAKER NO.	NOMINAL CONC. (ppb)	NO. GERM.	NO. UNGERM.	GERM TUBE LENGTH (MICRONS)
1	0	18,21	82,79	20,27.5,22.5,17.5,13.8,20,23.8,20,25,27.5
2	0	36	85	20.3,17.4,17.4,8.7,24.6,8.7,29,17.4,18.8,20.3
3	0	12,16	97.84	15,25.2,22.5,23.8,18.8,22.5,23.8,20,25.2,23.8
4	0	15,21	91,98	23.2,14.5,15.8,20.3,11.6,29,20.3,18.8,23.2,21.8
5	0	29,31	93,75	20,25.3,20,25,21.2,12.5,20,23.8,21.2,12.5
6	550	23,21	82.85	17.5,25,17.5,15,23.8,13.8,20,12.5,20,17.5
7	550	24	85	14.5,8.7,17.4,14.5,14.5,15,14.5,17.4,14.5
8	550	6,18	94,89	20.3,20.3,17.4,11.6,11.6,17.4,17.4,17.4,8.7,13
9	550	25	212	10.2,20.3,8.7,17.4,17.4,14.5,14.5,15,21.8,17.4
10	550	16,14	84,86	16.2,21.2,17.5,20,12.5,12.5,16.2,16.2,17.5,17.5
11	1000	17	104	14.5,14.5,15,24.6,17.4,8.7,8.7,11.5,14.5,11.6
12	1000	14,16	86,87	12.5,15,15,17.5,16.2,16.2,15,12.5,15,12.5
13	1000	23	124	16,17.4,20.3,16,8.7,11.6,17.4,13,11.6,16
14	1000	9,23	91,88	11.2,16.2,12.5,17.5,12.5,16.2,12.5,16.2,13.8,12.5
15	1000	21,19	80,84	16.2,15,15,20,13.8,16.2,13.8,15,17.5,13.8
16	1800	12,13	90,87	13.8,15,17.5,15,16.2,16.2,21.2,13.8,16.2,17.5
17	1800	15,16	85,86	15,16.2,20,15,17.5,13.8,16.2,15,12.5,13.8
18	1800	30,16	70,84	13,10.4,15.6,15.6,18.2,13,15.6,15.6,15.6,15.6
19	1800	27	115	17.4,13,17.4,16,8.7,17.4,14.5,14.5,11.6,17.4
20	1800	13,20	87,80	13.8,12.5,15,15,15,15,11.2,15,15,12.5
21	3200	16,15	84,85	10.4,14.8,13,15.6,15.6,14.3,10.4,13,15.6,15.6
22	3200	14,14	98,88	15,15,12.5,10,13.8,15,13.8,15,13.8,15
23	3200	14,24	91,92	14.5,16,14.5,10.2,16,14.5,8.7,10.2,10.2,13
24	3200	25	106	14.5,11.6,11.6,16,8.7,16,8.7,10.2,11.6,14.5
25	3200	10,12	94,98	13,13,10.2,5.8,11.6,14.5,11.6,14.5,16,14.5
26	5600	10.8	101,93	15,12.5,10,12.5,13.8,13.8,17.5,15,12.5,15
27	5600	11,10	98,97	15,12.5,16.2,16.2,13.8,15,10,12.5,13.8,11.2
28	5600	13	102	14.5,11.6,17.4,8.7,8.7,11.5,14.5,16,8.7,11.6
29	5600	13	85	11.6,13,14.5,8.7,14.5,11.6,8.7,11.6,14.5,8.7
30	5600	22	80	13,8.7,14.5,11.6,8.7,11.6,13,11.6,8.7,7.2

Table A4.

MACROCYSTIS ZOOSPORE EXPOSURE TO ZINC
WATER QUALITY DATA

DATE	BEAKER NUMBER	NOMINAL CONC. (PPB)	D.O. (mg/L)	pH	SALINITY (ppt)	BATH TEMP. (C)	TOTAL AMMONIA (mg/L)
6/24/87	1	0	7.6	7.94	34	15.2	0.063
	6	560	7.6	7.94	34		0.034
	11	1000	7.6	7.94	34		0.025
	16	1800	7.6	7.93	34		0.024
	21	3200	7.6	7.93	34		0.024
	26	5600	7.8	-	34		-
6/25/87	5	0	7.8	7.98	34	15.4	0.035
	8	560	7.9	7.95	34		0.034
	13	1000	8.0	7.95	34		0.021
	19	1800	8.0	7.93	34		0.021
	22	3200	8.0	7.91	34		0.019
	27	5600	7.9	7.81	34		0.029
6/26/87	3	0	7.6	8.02	34	15.6	0.032
	7	560	8.0	7.87	34		0.031
	14	1000	8.0	7.94	34		0.030
	17	1800	8.1	7.94	34		0.030
	23	3200	8.2	7.90	34		0.030
	28	5600	8.2	7.89	34		0.030

Table A5.

MYSID EXPOSURE TO ZINC
CUMMULATIVE SURVIVAL AT EACH TIME POINT

BEAKER NUMBER	NOMINAL CONC. (PPB)	24 HR		48 HR		72 HR		96 HR			
		LIVE	DEAD	LIVE	DEAD	LIVE	DEAD	LIVE	DEAD	MISSING	%SURVIVAL
13	0	7	0	7	0	7	0	7	0	0	100
18	0	7	2	7	2	7	2	7	2	0	78
29	0	9	0	7	2	7	2	6	2	1	67
23	0	9	0	9	0	9	0	8	1	0	89
11	0	7	2	7	2	7	2	7	2	0	78
30	56	8	1	8	1	8	1	7	2	0	78
6	56	7	1	6	2	6	2	5	2	0	75
1	56	7	0	7	0	7	0	7	0	0	100
10	56	9	0	9	0	9	0	8	1	0	89
17	56	7	0	7	0	7	0	7	0	0	100
21	100	8	0	7	1	7	1	7	1	0	88
24	100	5	2	4	3	4	3	3	3	1	43
3	100	8	1	8	1	8	1	7	1	1	78
27	100	7	1	7	1	7	1	6	2	0	75
25	100	6	1	6	1	5	2	4	3	0	57
20	180	8	0	8	0	7	1	4	4	0	50
15	180	7	2	7	2	6	3	5	3	0	57
5	180	9	1	7	2	7	2	4	5	0	44
26	180	8	0	8	0	7	1	6	2	0	75
12	180	9	1	8	1	8	1	5	3	0	67
14	320	7	1	7	1	7	1	4	4	0	50
4	320	5	3	4	4	3	5	0	8	0	0
7	320	8	0	8	0	8	0	5	3	0	62
2	320	7	1	8	1	8	1	4	4	1	44
19	320	5	2	5	2	5	2	1	6	0	14
8	560	6	2	6	2	5	3	2	6	0	25
9	560	4	3	4	4	4	4	3	5	0	38
16	560	7	0	7	0	6	1	0	7	0	0
22	560	8	0	8	0	7	1	1	7	0	12
28	560	6	2	6	2	3	5	0	8	0	0

Table A6.

MYSID EXPOSURE TO ZINC
WATER QUALITY DATA

DATE	BEAKER NUMBER	NOMINAL CONC. (PPB)	D.O. (mg/L)	pH	SALINITY (ppt)	BATH TEMP. (C)	TOTAL AMMONIA (mg/L)
6/26/87	INTIAL	0	7.4	7.98	34	15.7	0.02
		56	7.4	7.98	34		0.02
		100	7.2	7.99	34		0.02
		180	7.2	7.93	34		0.02
		320	7.0	7.96	34		0.02
		560	7.2	7.84	34		0.02
6/27/87	13	0	7.6	7.93	34	14.8	0.045
	30	56	7.6	7.94	34		0.054
	21	100	7.8	7.93	34		0.039
	20	180	7.8	7.94	34		0.044
	14	320	7.8	7.91	34		0.048
	8	560	7.8	7.92	34		0.046
6/28/87	18	0	7.4	8.00	34	14.6	0.15
	6	56	7.6	7.94	34		0.13
	24	100	7.7	7.96	34		0.12
	15	180	7.6	7.94	34		0.13
	4	320	7.7	7.96	34		0.12
	9	560	7.8	7.93	34		0.11
6/29/87	29	0	7.7	8.04	34	14.6	0.38
	1	56	7.8	7.94	34		0.25
	3	100	7.6	7.90	34		0.36
	5	180	7.8	7.91	34		0.32
	7	320	7.9	7.93	34		0.30
	16	560	8.1	7.93	34		0.23
6/30/87	2	0	7.8	7.92	34	14.8	0.35
	10	56	7.6	7.88	34		0.47
	22	100	7.7	7.89	34		0.48
	23	180	7.7	7.94	34		0.54
	26	320	7.8	7.92	34		0.49
	27	560	7.7	7.93	34		0.48