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ENVIRONMENTAL IMPACT OF DRILLING FLUID DISCHARGES FROM AN OFFSHORE DRILLING OPERATION

by

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ABSTRACT

An offshore stratigraphic test well was drilled in Lower Cook Inlet, Alaska, between June 20, 1977, and September 15, 1977 (Fig. 1). This well was drilled from the OCEAN RANGER, a deep-water, self-propelled, semisubmersible drilling platform. On various occasions during the drilling operation drill fluids (used to lubricate the drill bit and seal off porous formations) and cuttings were discharged from the platform to the marine environment. The water-based drill fluid used for this well was composed mainly of the minerals barite and bentonite.

Two groups were involved in assessing any impact that these discharges might have on fish and other organisms in the area around the platform. The Fisheries Research Institute (FRI) performed static bioassays and live-box studies. Dames and Moore, a cooperating firm, studied the benthic community, physical characteristics of the discharge plume and monitored the sea bottom by television.

We conducted a total of 21 bioassays in a portable laboratory aboard the OCEAN RANGER. Shrimp, juvenile pink salmon, mysids, amphipods, and isopods collected from Lower Cook Inlet were used as test organisms for the bioassays.

Preliminary analysis of the results indicated that the 96-hr LC50 (concentration at which 50 percent of the test organisms died) for shrimp, amphipods, isopods, and mysids ranged from 1-10 percent by volume of drilling fluid to ambient seawater. The pink salmon were somewhat more sensitive, with a 96-hr LC50 range of 0.3-5 percent. Significantly lower LC50 values were noted for organisms tested in solutions that were well stirred, compared to organisms in solutions

where the heavier components of the mud were allowed to settle to the bottom of the test tank. The increase in suspended solids during stirring probably caused increased irritation and physical damage to the tissues (especially the gills) of the organisms.

Live-boxes containing pink salmon at depths of 40 ft and 105 ft (12 and 32 m) and containing shrimp, and hermit crabs on the bottom were placed at 300 and 600 ft (91.4 and 182.9 m) from the discharge pipe and at a control station 1 mi (1.6 km) from the discharge. No mortalities were observed over a 96-hr period in any of the live-boxes. Although the bioassays demonstrated that lethal effects could occur in the laboratory at high concentrations, the actual impact in the field was probably minimal because, as determined by Dames and Moore, the dilution and dispersion of the fluids at this specific site and the intermittent nature of the discharge probably did not cause sustained toxic concentrations of drill fluids.

INTRODUCTION

The Atlantic Richfield Company (ARCO) drilled an offshore stratigraphic test well in lower Cook Inlet between June 1977 and September 1977 (Fig. 1). This well was drilled from the OCEAN RANGER, a deepwater, self-propelled, semisubmersible drilling rig owned by the Ocean Drilling and Exploration Company (ODECO). On various occasions during the drilling operation, drill fluids and cuttings were discharged from the platform to the sea. Some concern was expressed about the possible impact that these intermittent discharges may have had on fish and other organisms in the area around the platform. Therefore, ARCO requested a study to determine the extent of any impact.

A two-part study was conducted to assess the possible impact: 1) a physical description of the discharge plume and the fate of its components, especially the cuttings; and 2) a biological study of the impact of the plume. Dames and Moore,[1] consulting engineers, performed the physical description studies. The biological studies were divided into: a) laboratory bioassays and in situ live-box tests; and b) benthic community analysis and television monitoring of the bottom around the drilling rig. The Fisheries Research Institute (FRI), University of Washington, conducted the bioassays and the live-box studies. Dames and Moore conducted the benthic studies and the television monitoring.

This report mainly concerns the studies by FRI.

Objectives

The specific objectives of the FRI studies were to:

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[1] 510 "L" Street, Anchorage, Alaska.

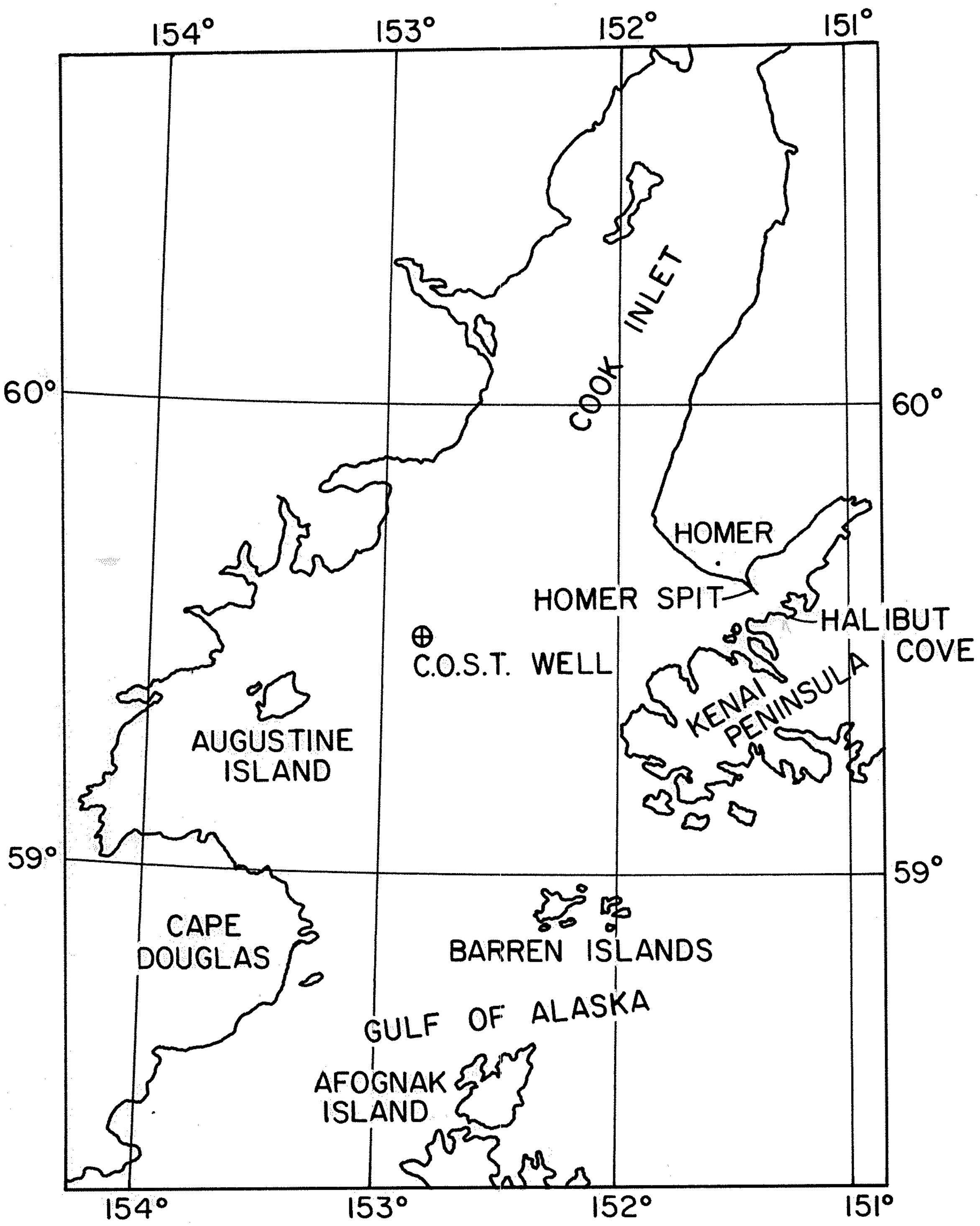

Fig. 1. Location of drilling site in lower Cook Inlet, Alaska.

1. Define the concentrations at which drill fluids discharged from the OCEAN RANGER might be toxic to a variety of organisms. This was accomplished using standard 48- and 96-hr static bioassays (American Public Health Association (APHA) 1977, U.S. Environmental Protection Agency (EPA) 1976, EPA 1975).

2. Determine if toxic conditions existed around the OCEAN RANGER due to drill fluid discharges. This was done by holding live organisms in live-boxes at various depths and distances from the discharge pipe.

3. Correlate the results from 1 and 2 above to determine if any impact occurred in the area around the OCEAN RANGER during the drilling operation.

The bioassays determined the responses of organisms exposed to high concentrations of drill fluid for sustained periods of time. The live-boxes indicated response under in situ conditions where dilution, dispersion, and intermittent discharge occurred.

Background: Drill Fluids

Drill fluids ("mud") are used extensively in both onshore and offshore drilling. The fluids are composed of a variety of different components, each of which has its own special function. The main components used in this particular well were barite, lignosulfonate, bentonite, carboxy methyl cellulose, caustic soda, and fresh water. The approximate composition of the mud used in the lower Cook Inlet well (percentage by weight) is provided in Table 1.

Monaghan et al. (1976) has categorized the main functions of these fluids as: 1) remove and transport cuttings; 2) cool and lubricate the bit and drill string; 3) control subsurface pressure; 4) coat the hole

Table 1. Whole mud composition on the OCEAN RANGER at various dates during drilling. (Data were provided by Dennis Lundquist, IMCO services.)

Bar = Barite
LDS = Low density solids, sand, and cuttings
Gel = Bentonite
CMC = Carboxymethylcellulose
VC-10 = Ferrochrome lignosulfonate
CS = Caustic Soda
WC = Water Content

Date	Bar (ppm)	LDS (ppm)	GEL (ppm)	CMC (ppm)	VC-10 (ppm)	CS (ppm)	WC %
7/3	256,803	142,668	85,601	2,140	3208	2853	88
7/10	199,735	128,402	85,601	171	5707	2853	85
7/13	228,269	285,337	85,601	0	5707	2853	84
7/18	228,269	285,337	85,601	0	5707	2853	84
7/24	228,269	285,337	85,601	0	5707	2853	84
7/30	228,269	285,337	85,601	0	5707	2853	85
8/7	228,269	285,337	85,601	0	5707	2853	86
8/22	228,269	285,337	85,601	0	5707	2853	86
8/29	228,269	199,735	85,601	0	5707	2140	87
9/3	228,269	199,736	92,734	0	5707	2853	87
9/6	228,269	199,736	92,734	0	5707	2853	87
9/8	228,269	199,736	92,734	0	5707	2853	87
9/9	228,269	199,736	92,734	0	5707	2853	87
$\bar{X}$	228,269	229,367	87,796	178	5515	2798	86

wall with impermeable filter cake; 5) suspend cuttings and weight material; 6) release undesirable solids at the surface; 7) support part of the drill pipe and casing weight; and 8) insure maximum information from the well and allow maximum drilling rates.

During drilling, the drill fluids were recirculated (Fig. 2). The major components of the mud were mixed on board. These components were fed into the mud pits or bins and are then pumped down the central shaft of the drill pipe to the drill bit. At this point, they passed through holes in the bit, picked up rock chips (cuttings) loosened by the bit and returned to the surface between the drill pipe and the bore hole. At the surface, the mud and cuttings passed through the shale shaker, where the cuttings and mud were separated. The cuttings were either saved for analysis or were washed overboard. The mud returned to the mud pits for recycling. As drilling proceeded, sand and silt not removed by the shale shaker were accumulated in the mud. The mud was separated from the sand and silt every 2 or 3 days as it was passed through desilters and desanders. The sand and silt were washed overboard and the mud was recycled. The desilters and desanders were operated for only 1 or 2 hr every 2 to 3 days. The actual concentration of drill fluids at the point of discharge during a typical desilter and desander discharge was estimated by Dames and Moore as approximately 0.25 percent volume of mud to volume of seawater.

At the end of the drilling operation, and occasionally during drilling, a large portion of the mud in the system was discharged (often referred to as a "mud dump").

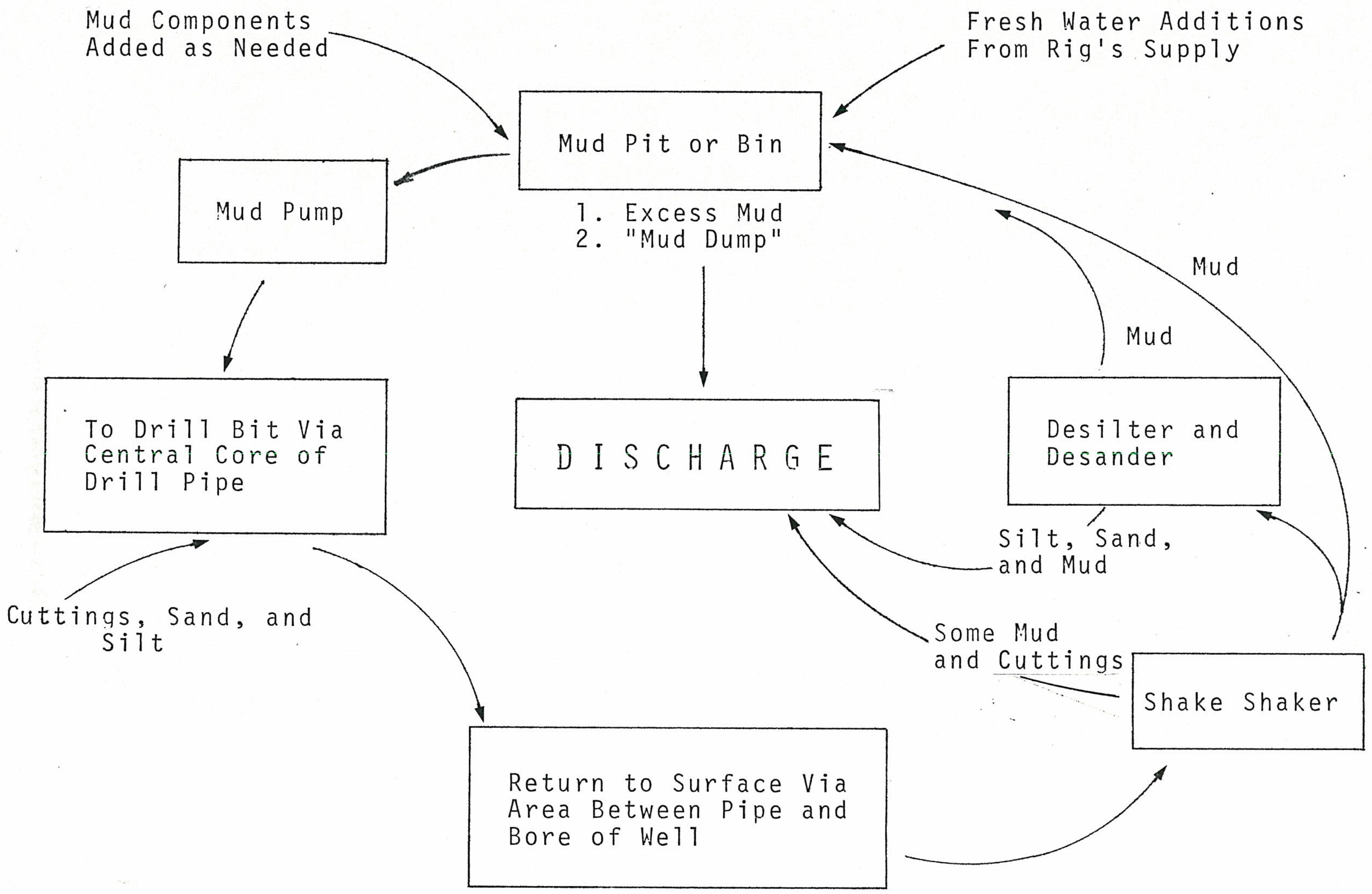

Fig. 2. Schematic drawing of mud circulating and discharge system on the OCEAN RANGER.

To summarize (Fig. 2), the main types of drilling discharges were: 1) cuttings with associated mud from the shale shaker; 2) silt and sand with associated mud; 3) excess mud that occurred during volume adjustments to the mud system; and 4) mud dumps. On the OCEAN RANGER, all of the discharges were made through a single downpipe that ran from the surface collector system to approximately 50 ft below the surface.

Site Characteristics

The drill site (Fig. 1) was located in lower Cook Inlet, Alaska, about 36 mi (57.6 km) southwest of Homer. The water depth at this location was approximately 200 ft (61 m). The water column was well mixed from the surface to the bottom, mainly because of the large tidal exchange in Cook Inlet. The oceanographic conditions at the site are described in detail in the Dames and Moore report.

LITERATURE REVIEW

Drilling a typical 10,000-ft well entails discharging approximately 250 tons of mud and 920 tons of cuttings (George 1975). The possibility of ecological damage due to this discharge has long been of major concern. As a result, toxicity testing has been done on both the whole mud and its components. Unfortunately, the information that has resulted from these tests is often contradictory and often noncomparable due to differences in testing procedures, in mud types used, and in different test organisms. Nevertheless, a brief summary of some of the literature may give a general idea of the toxic ranges encountered.

Components of Drilling Muds

Although there are over 600 compounds available for use in drilling fluids, the number actually used is relatively small. Of these, the most commonly used in water-based muds are barite, caustic soda, gels, dispersants, and lignosulfonates. The caustic soda and lignosulfonates are generally considered as the most toxic. Another concern is the suspended solids in the drilling mud that result mainly from the barite and bentonite.

Barite is used mainly as a weighting material. Daugherty (1950), testing various marine organisms, showed no toxicity with barite up to 7,500 ppm. Grantham and Sloan (1975), using sailfin mollies (*Mollienisias latipinna*) in fresh- and saltwater, found a 96-hr LC50 with barite of over 100,000 ppm. A 10 percent solution of mud (mud mixed with ambient seawater) from the OCEAN RANGER gave a barite concentration of approximately 22,800 ppm.

Caustic soda is used primarily to reduce bacterial growth in the mud by maintaining a high pH. Daugherty (1950) found caustic soda to be lethal to several marine fish and shrimp in concentrations of 70 to 200 ppm (pH up to 12). Logan et al. (1973), testing rainbow trout (*Salmo gairdneri*) in freshwater, showed a 96-hr LC50 of 105 ppm. A 10 percent mud solution from the OCEAN RANGER contained approximately 280 ppm caustic soda and could cause acute toxicity by creating high pH levels in test solutions. However, the high buffering capacity of seawater would tend to negate this effect.

Gel (usually bentonite) is used as a viscosity builder. Daugherty (1950) showed bentonite (Aquagel) to be nontoxic up to 7,500 ppm. Wallen (1951) showed no mortalities at 100,000 ppm. The gel used on the OCEAN RANGER was bentonite at concentrations of approximately 88,000 ppm.

Chrome lignosulfonate is used mainly as an emulsifier. Chesser and McKenzie (1975) found a 96-hr LC50 for chrome lignosulfonate of 465 ppm using white shrimp (*Paneus setiferus*). Hollingsworth and Lockhart (1975) showed 96-hr LC50's of 12,200 ppm in saltwater and 7,800 in freshwater. Logan et al. (1973) found a 96-hr LC50 of 1,530 ppm for ferrochrome lignosulfonate for rainbow trout in freshwater. A 10 percent mud solution from the OCEAN RANGER contained approximately 551 ppm chrome lignosulfonate.

Due to the insolubility of barite and gel, drilling muds have a high suspended solids content. Possible effects of suspended solids on organisms include suffocation, a decrease of disease resistance, behavioral changes, introduction of toxic substances and increased oxygen demand (Mortensen et al. 1976). Wallen (1951) tested montmorillonite clay, the type used in drilling mud, on estuarine fish and found it to be lethal within 2 hr in the range of 38,000 to 175,000 ppm.

Whole Muds

Logan et al. in Falk and Lawrence (1973) determined 96-hr LC50's of whole mud for rainbow trout. They found LC50 values ranging from 0.83 percent to 5.3 percent by volume. Lawrence and Scherer (1974) reported 96-hr LC50's for whole mud on rainbow trout of 7.5 percent by volume and on whitefish (*Coregonus clupeaformis*) of 2.5 percent by volume. Weir and Moore (1975) reported 96-hr LC50's for rainbow trout from 9 percent to 70 percent by volume. Monaghan et al. (1977) presented data on tests with whole mud on various marine invertebrates. They found 96-hr LC50's for polychaetes (*Nereis* sp.) ran from 2.3 percent to 56 percent by volume, for clams (*Mya* sp.), 1 percent to 56 percent by volume, for crabs (*Hemigrapsus nudis*), 5.3 percent to 56 percent by volume, and for shrimp (*Orchestea* sp.), 1.4 percent to 56 percent by volume. Monaghan et al. also showed that the 96-hr LC50 for rainbow trout ranged from 1.6 to 2.4 percent by volume and 1.5 to 19 percent for coho (*Oncorhynchus kisutch*).

These studies point out the variation in toxicity of different muds, even to the same organism, as well as the variations in tolerance between different organisms. In summary, most tests indicated that high concentrations (on the order of parts per hundred) were necessary to cause acute toxicity and that the majority of the mud components were relatively nontoxic. To determine the lethal concentrations of drilling fluid used in this well and if these concentrations were present in the receiving waters, the bioassays and live-box studies were conducted at the lower Cook Inlet drill site.

METHODS AND MATERIALS

Bioassays

Laboratory

The bioassays were conducted on board the OCEAN RANGER in a trailer laboratory constructed by ARCO (Fig. 3). The trailer was placed on the main deck which was 80 ft (approximately 24.4 m) above the waterline. Water was pumped to the trailer using a 5-hp stainless steel submersible pump. This pump setup was not entirely satisfactory and resulted in loss of a considerable amount of time and effort during the study due to setting, retrieving, and repairing the pump (Appendix 1). Despite these problems all test conditions (mainly temperature control) during any particular test were satisfactorily maintained.

All plumbing in the trailer was of polyvinyl chloride (PVC). The lab had approximately 300 ft^2 (approximately 31 m^2) of floor space. It contained six plywood troughs with flowing seawater and drainage, freshwater, two sinks, counter and cupboard space, a refrigerator, and an office area (Fig. 3). The troughs were constructed of fiberglass reinforced plywood measuring 3 ft x 8 ft x 1 ft (0.9 m x 2.4 m x 0.3 m) in height. Four of these troughs were used as water baths for temperature control during bioassays and two for holding organisms. Lighting for the lab was provided by fluorescent light fixtures that were turned on and off automatically. The light and dark periods were set to coincide with ambient daylight.

Test Organisms

Several different types of marine organisms were used for testing. These were: fish--juvenile pink salmon (*Oncorhynchus gorbuscha*) and

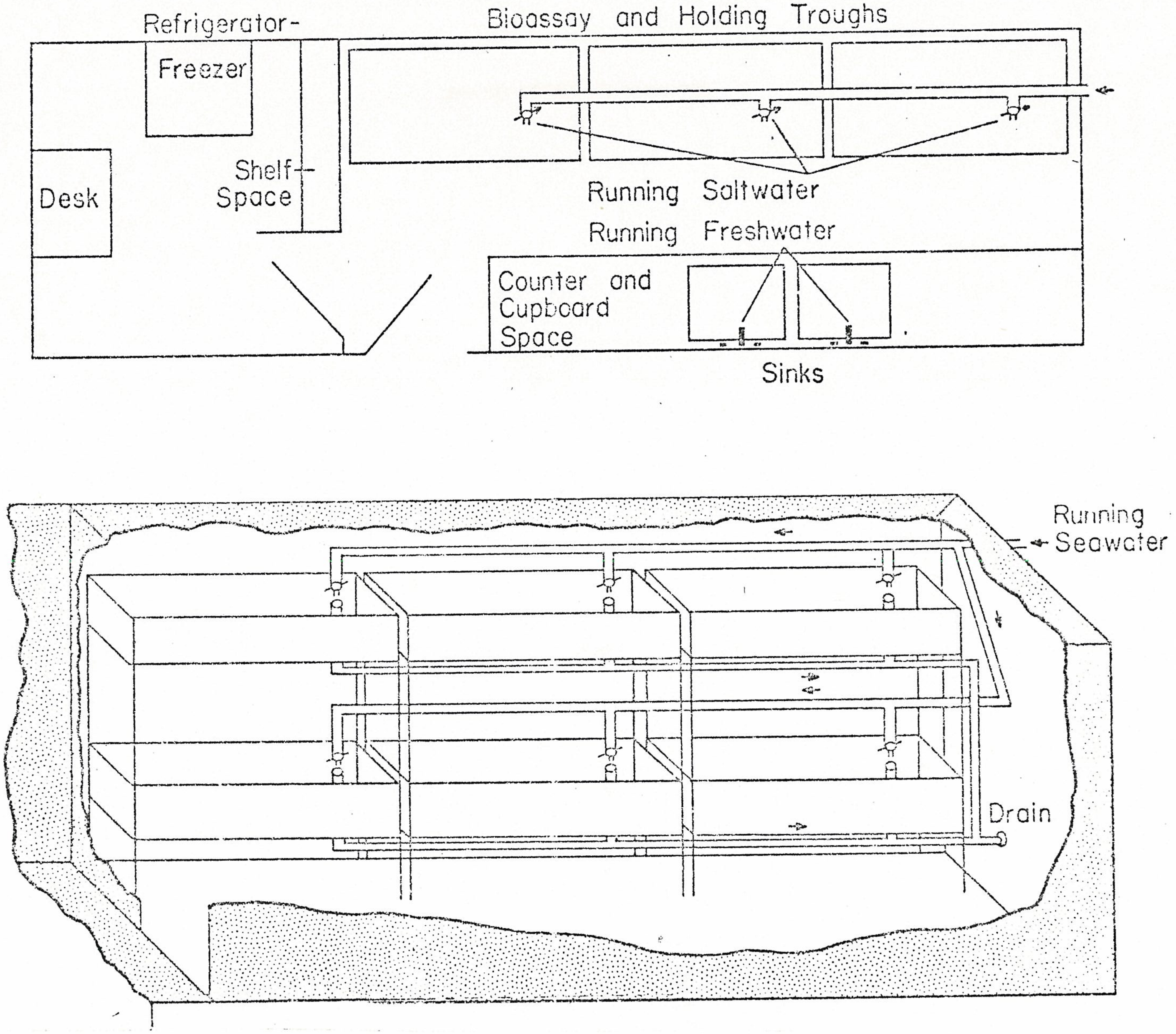

Fig. 3. Schematic drawing of the trailer laboratory used for bioassays on the OCEAN RANGER. The trailer was approximately 30 ft long and 10 ft wide.

sculpins (*Leptocottus armatus*); crustaceans--mature coonstripe shrimp (*Pandalus hypsinotus*), mysids (*Neomysis integer*), isopods (*Gnorimosphaeroma oregonensis*), amphipods (*Anisogammarus confervicolus*); and a bivalve--deepwater mussels (*Modiolus modiolus*). These organisms were selected either because they were economically important, they occurred in the drilling vicinity and/or they were readily available for testing. All of these organisms were acquired locally. An additional species, brine shrimp (*Artemia salina*) was selected for use as an "index species" for comparing to other pollutants bioassayed with brine shrimp.

The coonstripe shrimp (*Pandalus hypsinotus*) were collected in commercial shrimp pots at depths of 30 to 50 ft (approximately 9-15 m) approximately 1/4 mi (0.4 km) southeast of the Homer Spit. The shrimp were placed in 30-gal (approximately 114-liter) plastic garbage containers equipped with portable aerators and transported to the Homer airport. From the airport, they were transported via helicopter to the lab on the OCEAN RANGER. A few mortalities (< 3 percent) occurred in almost all shipments of shrimp, probably due to temperature shock and handling stress. The shrimp were maintained on a diet of commercial fish food (Oregon Moist Pellets) and dead shrimp (mortalities), which were often consumed by other shrimp before they could be removed. Pellets were fed once or twice daily. Holding tanks were cleaned of decaying food and waste at least twice a week and dead shrimp were removed when noticed.

All organisms were held for at least 48 hr before they were used for bioassay. This allowed an observation period to check for any residual mortalities due to handling and transport. Test organisms were not fed for 24 hr prior to bioassay.

Pink salmon fry were obtained from an Alaska Department of Fish and Game pen-rearing site in Halibut Cove (on Kachemak Bay), Alaska (Fig. 1). They were transported to the lab in 30-gal (approximately 114-liter) plastic garbage containers in a manner similar to that used for the shrimp. The fry were placed in a holding trough on the OCEAN RANGER. Feeding and tank-cleaning schedules were the same as for the shrimp.

Mysids were collected at low tide in the vicinity of the Homer Spit. They were transported to the lab via helicopter in 1-gal (3.785-liter) jars and placed in small plastic mesh containers (*see* section on test containers) in the holding tanks. Mortalities due to transport were minimal.

The amphipods and isopods were collected at low tide in intertidal areas in the vicinity of the Homer Spit and transported to the lab in 1-gal (3.785-liter) jars.

The sculpins were collected by beach seine from the Homer Spit. Aeration problems during shipment to the lab caused approximately 20 percent mortality. The sculpins were held 48 hr before testing. No additional mortalities occurred during this period.

Mussels were collected by divers in the vicinity of Homer and shipped in aerated containers to the lab. Survival was 100 percent.

Table 2 shows the average weights and lengths of the test organisms (where applicable) for the various bioassays.

Test Containers

The test solutions of drilling fluids were highly turbid, which made direct observation of the test organisms difficult. Therefore, several different types of test containers were used.

Table 2. The various drilling fluid bioassays and the conditions under which they were run.

#	Date begun	Duration of test (hr)	Aeration	Stirring	Range tested (% by vol.)	*Length (mm)	Weight (g)	Total no. used
Shrimp								
1	7/8	96	1 stone	minimal	0.025-10	28.1±2.9	14.8	140
4	7/13	96	1 stone	minimal	.5-20	28.1±2.9	14.4	120
8	7/24	96	2 stones	minimal	.5-10	30.9±2.9	20.2	60
9	7/30	96	2 stones	minimal	2.5-20	30.2±2.4	19.8	120
13a	8/29	96	1 stone	paddles	.1-10	28.4±2.8	16.7	120
13b	8/29	96	1 stone	minimal	5-15	28.4±2.8	16.7	120
17	9/3	48	1 stone	paddles	5 and 10	29.2±2.6	19.0	150
19	9/6	48	1 stone	paddles	5 and 10	28.4±3.1	21.3	60
Isopods								
6	7/18	96	2 stones	Dynaflo	.5-7		0.02	120
Brine Shrimp								
15	8/29	48	none	minimal	1-10			120
21	9/9	48	none	minimal	1-10			120

Table 2. The various drilling fluid bioassays and the conditions under which they were run. (Cont'd.)

#	Date begun	Duration of test (hr)	Aeration	Stirring	Range tested (% by vol.)	*Length (mm)	Weight (g)	Total no. used
<u>Pink Salmon</u>								
5	7/18	96	2 stones	Dynaflo	0.5-7	60.5±.9	2.3	120
11a	8/7	96	1 stone	paddles	0.1-3	66.0±1.3	2.3	120
11b	8/7	96	1 stone	minimal	0.1-3	66.0±1.3	2.3	120
<u>Amphipods</u>								
2	7/10	48	glass tube	minimal	0.5-20		0.07	120
3	7/11	48	none	minimal	0.5-20		0.07	120
7	7/18	96	2 stones	Dynaflo	0.5-7		0.07	120
10	7/30	96	2 stones	minimal	2.5-20		0.07	120
<u>Mysids</u>								
12	8/22	48	none	minimal	1-20			70
14a	8/29	96	1 stone	paddles	1-10			120
14b	8/29	96	1 stone	minimal	5-15			120
16	9/3	48	glass tube	minimal	1-15			120
20	9/8	48	glass tube	minimal	1-10			120
<u>Modiolus</u>								
23	8/11	326	1 stone	minimal	1-3			48
<u>Sculpins</u>								
22	9/16	48	1 stone	paddles	5-20	14.3±1.5		48

*In shrimp this is carapace length, in fish total length.

Larger organisms were tested in standard 20-gal (75.7-liter) all-glass aquaria. For smaller organisms, 0.16-gal (600-ml) plastic containers with plastic netting inserts[2] (Fig. 4) were either suspended in the 20-gal (75.7-liter) aquaria and run simultaneously with another bioassay, or placed in 0.26-gal (1-liter) beakers.

Artemia bioassays were conducted in standard petri dishes.

Pens constructed of plastic netting were placed in all of the glass aquaria and organisms to be tested were placed inside the pens (Fig. 4). The pens were raised or lowered for observation of the organisms.

Mixing of the Test Solutions

In all of the bioassays, test solutions were made by mixing mud with ambient seawater. Initially, the test solutions were thoroughly mixed but if they were allowed to stand, some of the components would settle out, especially the barite (specific gravity 4.2). Thus, it was desirable to completely mix the solution to suspend all of the components. Two different types of mixing devices were used. One was a Dynaflo II water circulator[3] which drew water from the bottom of the aquarium and reinjected it into the aquarium near the surface. The other device was a plexiglass paddle driven by a windshield wiper motor. This paddle was placed at the bottom of selected 20-gal (75.7-liter) aquaria. The back-and-forth motion of the paddle forced the barite off the bottom of the tank and into suspension.

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[2] The plastic netting was 1/8-inch Vexar plastic netting (a product of DuPont).

[3] Metaframe.

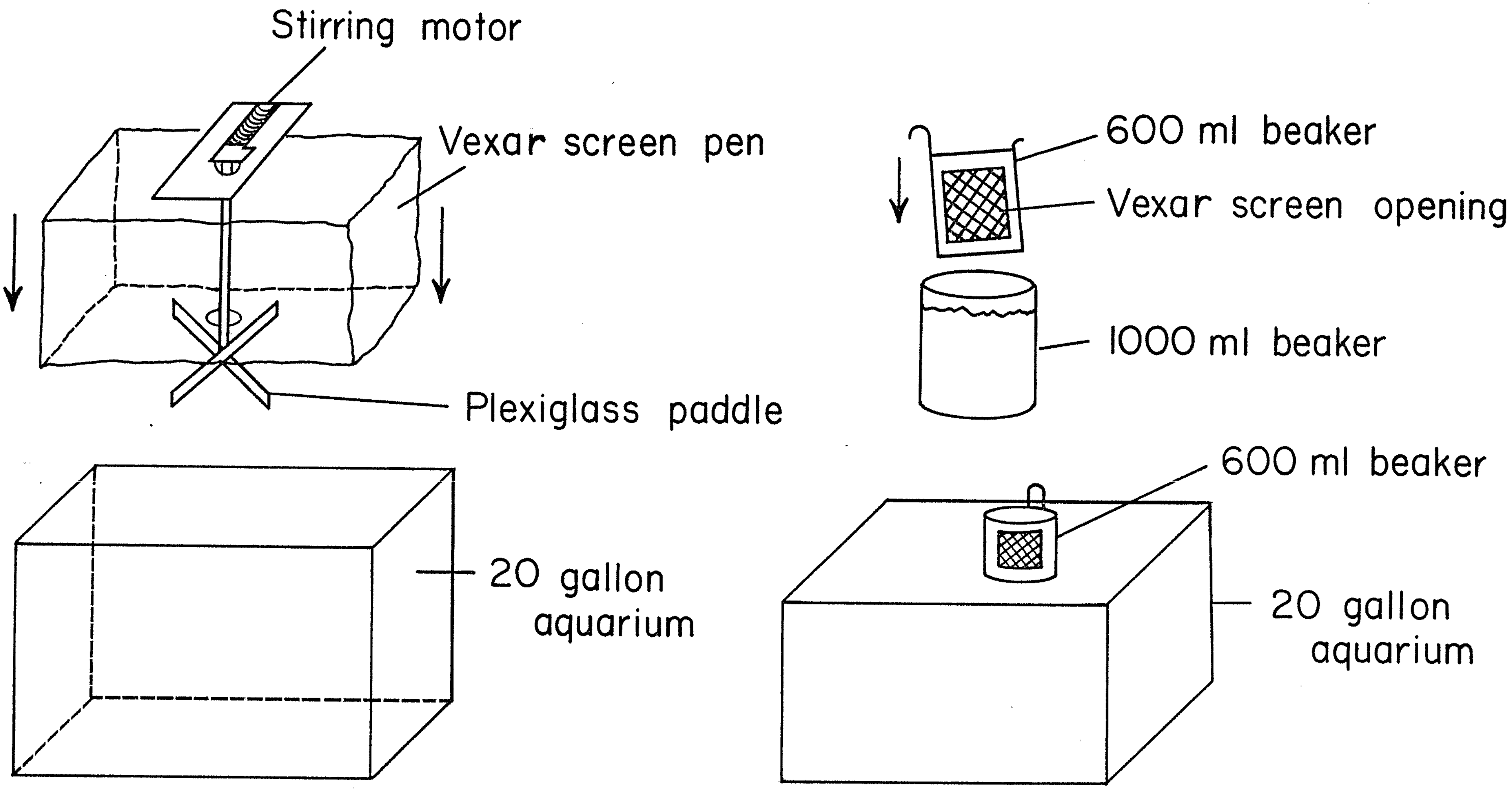


Fig. 4. Test containers and equipment used in the various bioassays.

The tests were performed with three methods of mixing, as follows:

1. Minimal - Initial mixing with minimal stirring, but no further stirring except that provided by aeration. The aeration tended to keep all of the soluble components in circulation, but the heavier materials settled to the bottom.

2. Dynaflo II circulator - This tended to circulate some of the barite, but was not successful in suspending all of the materials.

3. Paddle - These were quite successful in keeping most of the components in suspension (Fig. 4).

Both biological and physical tests were made to determine the relative effectiveness of mixing methods 1 and 3. The physical tests were based on total nonfilterable residue (TNFR) tests on water taken from the upper layers (near surface) of selected test aquaria. Samples were filtered through a standard glass fiber filter paper in accordance with methods recommended in "Standard Methods for the Examination of Water and Wastewater" (APHA 1976). The biological tests were bioassays that directly compared toxicity differences between well-stirred solutions and minimally stirred solutions at the same concentrations.

Test Procedures

A total of 23 bioassays were conducted between July 8 and September 10. These were identified chronologically from nos. 1 to 23.

In preparation for a test, solutions were mixed on a volume-to-volume basis; mud to seawater. Treatments were randomly assigned to test containers held in water-bath troughs. In general, six concentrations, including controls, were tested per bioassay. Each concentration had two replicates (Table 2, test conditions).

Drilling fluids were collected by grab sampling from the mud bins. The bins were "downstream" from the shale shaker (Fig. 2) and, therefore, there were no cuttings in the samples. Cuttings were not included in any of the bioassays because of their proprietary nature and because it was assumed that any impact of the cuttings would be a physical smothering of benthic organisms and in general cuttings would not contain toxic substances.

The mud was usually much warmer (> 45° C or 113° F) than the ambient seawater (10°-14° C). Thus, several hours of cooling were required before test tank temperatures reached those of the holding tanks. When the solutions had cooled, test organisms were randomly added, two at a time to the test tanks, until there were 10 per tank.

During the first few tests, the selection of concentrations was based on procedures suggested by EPA (1976). EPA recommends that the dilution of samples for initial test concentrations should be 10 percent, 0.1 percent, 0.001 percent, and 0.0001 percent dilutions, by volume, of the whole mud sample. These concentrations were selected for use; however, results from the initial tests indicated that the 96-hr LC50 value would possibly be larger than 10 percent. Therefore, when possible in the majority of the remaining tests, concentrations greater than 10 percent were also included.

In general, two steps were made to determine the LC50 value for a particular substance. The first step was a wide-range test which gave some indication of the LC50 value. The concentrations were spaced widely as recommended by EPA. Once an approximate LC50 value was determined, a second series of tests was made with a narrower range of concentrations to determine more precisely the LC50. For the tests on the OCEAN RANGER, it was nearly impossible to conduct any narrow-range tests because of the changing composition of the mud with time, pump failures (*see* Appendix 1), and lack of sufficient numbers of organisms at any one time to run both wide-range tests and narrow-range tests consecutively. Therefore, the range of concentrations for the narrow-range tests were somewhat larger than would normally be tested so that an approximate LC50 value could be determined. When applicable, LC50 values were estimated both by "visual inspection" of graphs, and by computation of a "least squares" curve using the BMD03S computer program developed at the University of California at Los Angeles (Dixon 1970). The BMD03S program could only be used in certain instances where three or more concentrations for a bioassay produced mortalities between 0 and 100 percent. The program gave a more precise estimation of the LC50 value than visual inspection.

Aeration for each aquarium was supplied by Hush III air pumps. Airstones were used in the large tanks and disposable pipettes were used in the smaller containers. Aeration of the test solutions was done for all bioassays except nos. 3, 12, and the *Artemia* bioassays (Table 2).

Tests were conducted for either 48 or 96 hr. Observations for mortalities were made frequently during the first 24 hr and thereafter, every 24 hr. Water quality measurements of pH, temperature, dissolved oxygen (D.O.), and salinity were made prior to the introduction of the test animals and at 24-hr intervals during the test. Temperatures were measured to 0.1° C by mercury thermometers. A Yellow Springs Instrument Co. (YSI) dissolved oxygen meter (Model 54A) was used for D.O. readings.

Salinity measurements were made with a YSI salinometer (Model 33). The pH readings were made with either an Analytical Measurements, Inc. meter (Model 107--used for bioassays nos. 1-4), or a Photovolt Model 85A meter (bioassays nos. 5-22). In addition, water samples from the low, median, and high concentrations of later bioassays (nos. 11-22) were frozen for metals analysis or suspended solids determinations. Also, organisms from selected bioassays were frozen for metals analysis.

Specific Bioassays

In general, at least one of the tests for a particular species was done to establish the 48- or 96-hr LC50 value for that species and for comparison of sensitivity to the other organisms tested. Other tests were made to directly test the differences between well-mixed solutions and minimally mixed solutions. A third type of test (preliminary) was designed to determine if certain components of the mud were more toxic than others. Before drilling began at this site, it was anticipated that muds from depths greater than 4,800 ft (1,462 m) might be more toxic than muds taken at shallower depths because of a slightly different mud composition. Therefore, some preliminary tests were made to determine if, in fact, the deeper mud was more toxic. Table 3 describes the specific purposes for each individual bioassay.

In some instances, it was advantageous to test several species in one aquarium. This was most frequently done with shrimp and smaller organisms such as isopods, amphipods, or mysids. Each species was isolated in separate cages and was treated as a separate bioassay.

A brief description of each type of bioassay is made below:

Table 3. Major purposes for each individual bioassay.

Bioassay #	Species	Purpose
1	Shrimp	To define the 96-hr LC_{50} value for mud above the 4800 ft level.
4,8,9	Shrimp	To define the 96-hr LC_{50} value for mud below 4800 ft.
13a + 13b	Shrimp	To compare differences in toxicity between a solution well mixed by paddles (#13a) and a solution mixed only by initial agitation and aeration (#13b).
17,19	Shrimp	To obtain preliminary indications about the relative toxicity of various components of the mud.
6	Isopods	To define the 96-hr LC_{50} value for this species for mud from below 4800 feet.
5,11a,+11b	Pink Salmon Fry	To define the 96-hr LC_{50} value for this species for mud from below 4800 feet. Also, the mixing of the test solution was different in each case and this provided information on the difference in toxicity between a solution well mixed by paddles (#11a) and one that was mixed only by initial agitation and aeration.
2	Amphipods	To determine the 48-hr LC_{50} value for this species using minimal mixing (by aeration only and initial agitation).
3	Amphipods	To determine the 48-hr LC_{50} value using no mixing (initial agitation and no aeration) beyond initial agitation.
7	Amphipods	To determine the 96-hr LC_{50} value for this species using Dynaflo II mixing.
10	Amphipods	To determine the 96-hr LC_{50} value for this species using minimal mixing (initial agitation and aeration only).
12	Mysids	To determine the 96-hr LC_{50} value for this species using minimal mixing (initial agitation and aeration only).

Table 3. Major purposes for each individual bioassay - (cont'd).

Bioassay #	Species	Purpose
14a, 14b	Mysids	To determine the comparative differences between solutions well mixed by paddles (#14a) and minimally mixed solutions (#14b).
16, 20	Mysids	To determine the 96-hr LC_{50} value for this species under minimal mixing conditions (initial agitation and aeration only)
22	Sculpins	To determine the 48-hr LC_{50} value for this species.
23	*Modiolus*	To determine the relative sensitivity of this species to drill fluids and to determine if this species might accumulate heavy metals that may have been present in the fluids.
15,21	Brine Shrimp	To determine the suceptibility of this species to drill fluids. This species was chosen for comparison to other species.

Shrimp. A total of eight shrimp bioassays was made. The large 20-gal (75.7-liter) aquaria were used for these bioassays. Total volume of the test solution was 15.85 gal (60 liters). Large plastic cages were used to contain the organisms and to assist in making observations.

Mysids. Mysids were contained in the 0.16-gal (600-ml) plastic containers during tests. These were then suspended in either large aquaria (bioassay no. 14) or a 0.26-gal (1-liter) plastic beaker (bioassays nos. 12, 16, and 20).

Pink Salmon. The 20-gal (75.7-liter) aquaria with plastic net pens were used as test chambers for all salmon bioassays. In the stirred tests (nos. 5 and 11a), either the Dynaflo II circulators or plexiglass paddles were used. Mixing was minimal in unstirred tests.

Artemia. Open petri dishes were used as test containers for the *Artemia* bioassays. Total volume per petri dish was 0.03 gal (100 ml). Test solutions were mixed in larger containers and poured into the dishes. The *Artemia* were exposed to the solutions for 48 hr and only one observation was made at the end of the test. *Artemia* used for bioassay were 1-day-old larvae. A preliminary test was made to determine the approximate LC50 value. Due to the turbidity in the test solutions, it was difficult to determine if the *Artemia* were dead or alive. Therefore, a second test was made using a staining technique proposed by Crippen and Perrier (1974). A stock solution of 1:1,000 (weight to volume ratio) was made using neutral red stain granules. At 48 hr, 1 ml of this was added to each container (1:100,000). After 6 hr, live *Artemia* were brightly stained and easily visible to the naked eye.

Amphipods. In bioassay no. 2, test solutions were mixed in 0.26-gal (1-liter) beakers. Aeration with glass pipettes kept the mud evenly suspended in these small containers. Bioassay no. 3 was run under identical conditions, except aeration was not used. Bioassay no. 10 was run simultaneously and in the same glass aquaria as bioassay no. 9, a shrimp test.

Isopods. Bioassay no. 6 was run simultaneously with no. 5 (a salmon bioassay) and isopods were exposed to the same solutions and conditions as the salmon fry.

Mussels. The tests with mussels (*Modiolus*) were primarily designed to determine if this organism would accumulate any heavy metals if exposed to drilling fluids. However, because the organisms were held in test solutions for an extended period of time, the results were useful for determining a relative index of toxicity.

The mussel bioassays were run in the large glass aquaria with a total volume of 15.85 gal (60 liters). Observations of test organisms were made at 7 and 14 days of exposure.

Sculpins. The sculpins were tested in the 20-gal (75.7-liter) aquaria in a manner similar to shrimp and salmon. Duration of the test was 48 hr. Only a limited number of sculpins was available, therefore, only six fish were tested per container and only three concentrations and controls were bioassayed.

Histopathological Analysis

Some preserved specimens of both salmon and shrimp were submitted to Dr. Marsha Landolt (Assistant Professor, College of Fisheries, University of Washington) for histopathological analysis to determine the possible cause of death. Of particular importance were the gills of both species. Both control and test groups exposed to various concentrations were submitted.

Live-Box Studies

Two live-box studies were conducted around the OCEAN RANGER from July 18 to July 23 and from August 27 to August 31, 1977.

Positioning of Live-Boxes

Live-box arrays were set at three distances from the rig: 300 ft (91.4 m) NNE and 600 ft (182.9 m) NNE for the experimental arrays and 1 mi (1.6 km) WNW for the controls (Fig. 5). The experimental arrays at 300 and 600 ft were positioned along the axis of maximum drill fluid concentration (NNE to SSW) as determined by plume-modeling studies by Dames and Moore. The control array 1 mi from the rig was assumed to be out of the area affected by the mud. Live-boxes were set at three depths (surface, midwater, and bottom) at the three locations.

Due to boat traffic (supply vessels) near the rig, surface buoys to mark the live-boxes could not be used at the 300- and 600-ft locations. Therefore, an acoustic release mechanism with subsurface floats served as markers at these locations during both live-box studies. No acoustic release was used on the control array.

Test Organisms

The test organisms were either mature hermit crabs (*Elassochirus gilli*), pink salmon fry, or mature coonstripe shrimp. This particular species of hermit crabs was found throughout the subtidal areas of Cook Inlet. They were collected by divers in the vicinity of Homer and

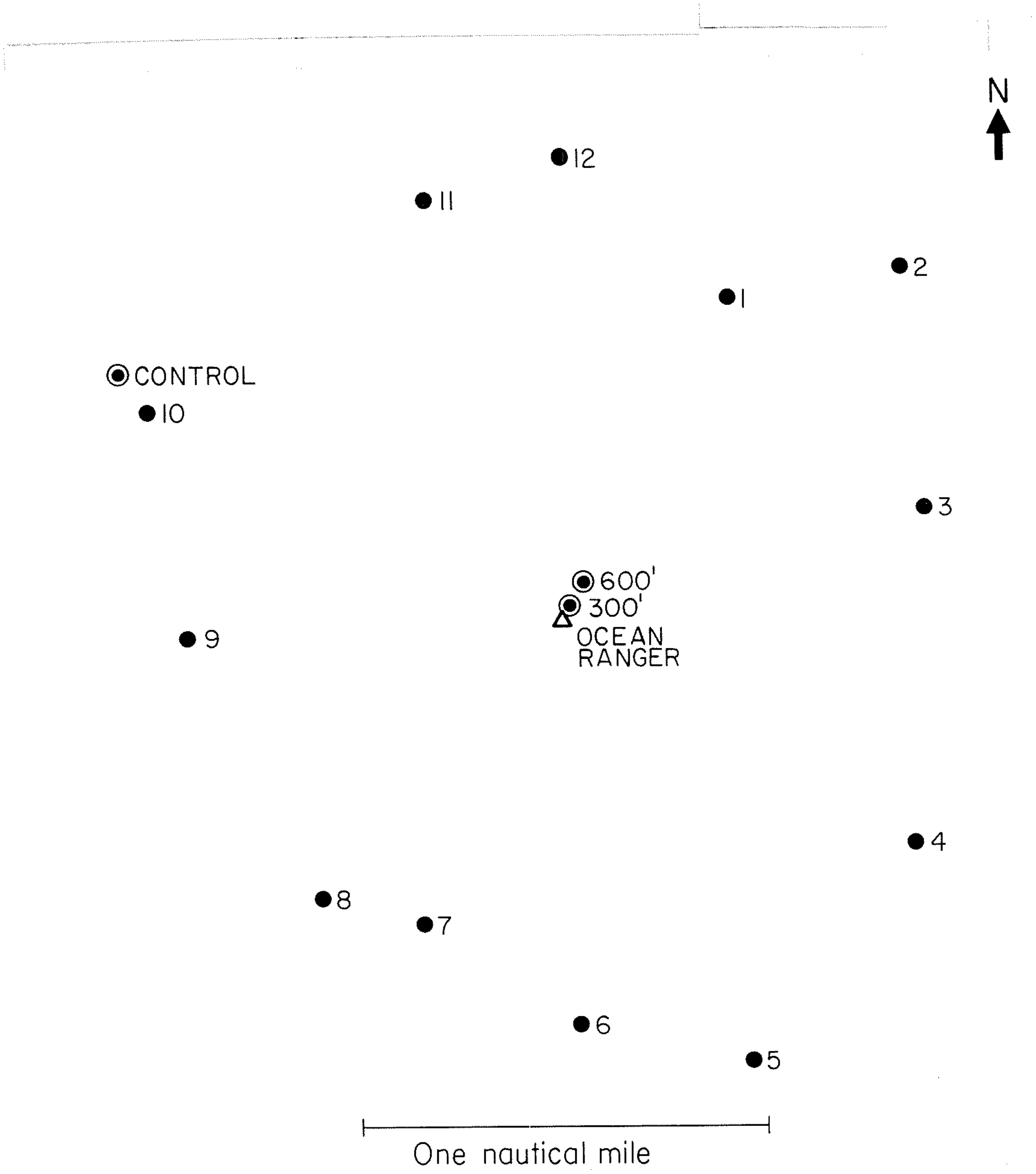


Fig. 5. Live-box locations for first and second tests.

shipped in aerated containers to the lab. The methods of obtaining shrimp and salmon were the same as previously described.

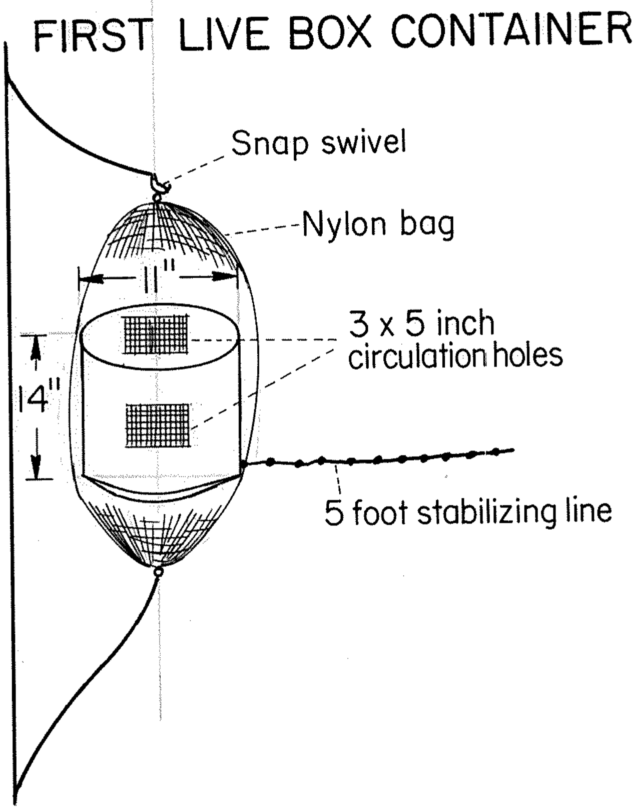
Test Containers

First Test Series (July 18-July 23). The surface and midwater containers held pink salmon fry. The containers were constructed from 3-gal (12-liter) plastic buckets with 3- x 5-inch plastic screened holes cut into the sides and bottom (Fig. 6). Each bucket was placed in a nylon mesh bag and attached to the cable supporting the apparatus. A 5-ft knotted 1/4-inch rope was attached to the bucket to orient it so that the circulation holes were not pointed directly into the current. The bottom containers were standard commercial shrimp pots.

Second Test Series (August 27-August 31). The buckets proved unsatisfactory in the first test, therefore new containers were made from 38-inch long sections of 8-inch diameter PVC pipe (Fig. 6). The ends were covered with PVC caps, one of which could be detached for entry into the container. This end also had a 3-inch diameter circular hole covered with plastic netting for water circulation. Circulation was further increased by cutting slits in the sides of the container and then covering the slits with plastic netting. Commercial shrimp pots were used for the bottom cages.

Test Procedures

First Test Series. The control array at 1 mi from the rig was set on July 18. The surface and midwater containers were placed at depths of 40 ft (12.2 m) and 105 ft (32 m), respectively, below mean lower low


FIRST LIVE BOX CONTAINER

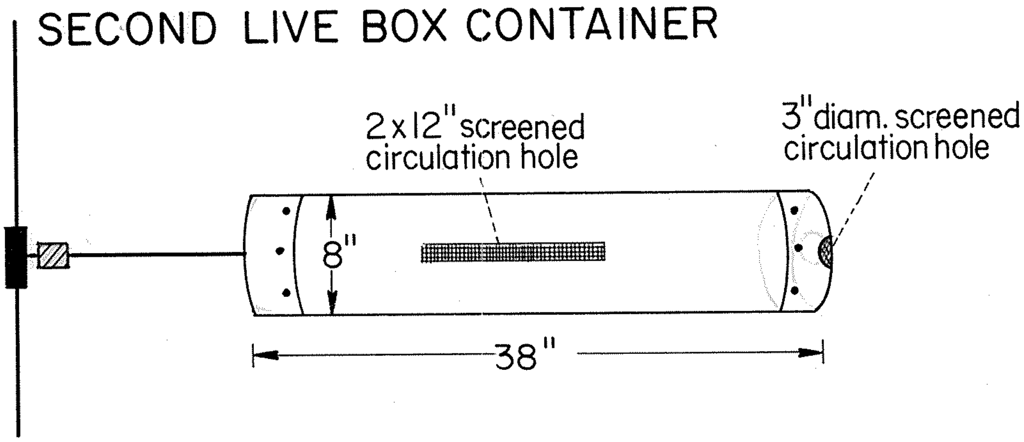

SECOND LIVE BOX CONTAINER

Fig. 6. Live-box containers for first and second live-box tests.

water (m.l.l.w.). The shrimp pot was set on the bottom, 200 ft (61.0 m) below m.l.l.w. (Fig. 7).

The arrays at 300 ft (91.4 m) and 600 ft (182.9 m) from the rig were set on July 19. The surface and midwater containers were put at 50 ft (15.2 m) and 105 ft (32 m), respectively, below m.l.l.w.

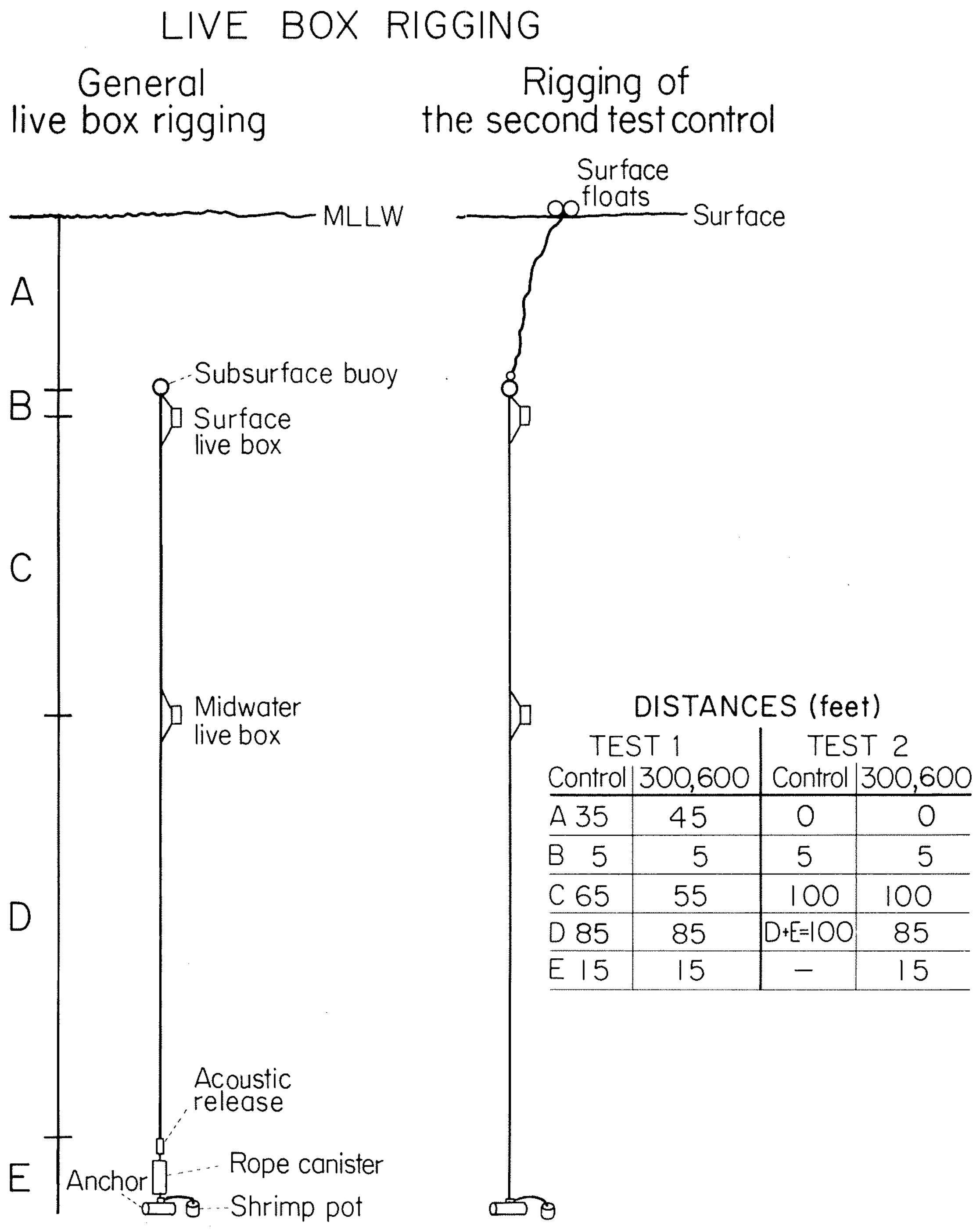
The arrays were fished for 4 days.

Ten organisms were placed in each live-box.

Second Test Series. The live-box arrays at 300 ft (91.4 m) and 600 ft (182.9 m) from the rig were set on August 27. Ten hermit crabs were put in the shrimp pot and 10 pink salmon fry were placed in each live-box at 5 ft and 105 ft below m.l.l.w. (Fig. 7). The apparatus was rigged similar to the first live-box series.

The control live-box array was also set on August 27. Test organism distribution among the live-boxes was identical to the other arrays.

These arrays were also fished for 4 days.



DISTANCES (feet)

	TEST 1		TEST 2	
	Control	300,600	Control	300,600
A	35	45	0	0
B	5	5	5	5
C	65	55	100	100
D	85	85	D+E=100	85
E	15	15	–	15

Fig. 7. General live-box rigging for first and second live-box studies.

RESULTS

Bioassays[4]

Table 4 shows the 48- and 96-hr LC50 values calculated (both by eye fit and where applicable, via the BMDO3S program) for the shrimp bioassays. In some tests (e.g., nos. 1, 8, and 19), LC50 values could not be estimated because the range of concentrations was not wide enough to "bracket" the LC50 (selection of test concentrations was previously discussed in the section on test procedures). No confidence limits were calculated for any of the LC50 values because of the small sample sizes.

Shrimp

Test No. 1. No LC50 value could be estimated because at the highest concentration of 10 percent no mortalities occurred. Only one mortality was observed and that was at the 0.5 percent concentration. This was probably a latent mortality due to an injury sustained during transport to the rig because the other shrimp in the tank appeared healthy and even consumed the dead shrimp, leaving only the empty shell. The agitation in this test was minimal and therefore, the barite component of the mud settled to the bottom of the tank. Even at 10 percent concentration, the shrimp were able to support themselves on top of the barite layer and actively walked on it.

Test No. 4. This test was conducted during a period when the mud was being changed from a shallow type (< 4,800 ft) (1,462 m) to a deeper type (> 4,800 ft) mud. Certain additives were mixed into the mud at

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[4] Only the major results are presented in this section. The details of the entire collection of data on mortalities and water quality during each bioassay are presented in Appendices 2 and 3.

Table 4. 48- and 96-hr LC$_{50}$ values for the various bioassays as determined by BMD03S program and visual inspection of log probit graphs.

Organisms	Bioassay #	LC$_{50}$ values (% by volume)	Remarks
Shrimp	1	>10	96-hr test, minimal stirring, mud from above 4800 ft.
	4	3.2*	96-hr test, minimal stirring.
	8	>10	96-hr test, minimal stirring.
	9	8.6*	96-hr test, minimal stirring.
	13a	4.4*	96-hr test, stirring by paddles.
	13b	15.0*	96-hr test, minimal stirring.
	17	5< x <10+	48-hr test, stirring by paddles.
		5< x <10ǂ	48-hr test, stirring by paddles.
		>10‡	48-hr test, stirring by paddles.
	18	Pump failure:	No results
	19	>10⧧	48-hr test, stirring by paddles.
Salmon	5	1.9*	96-hr test, Dynaflo stirring.
	11a	0.3*	96-hr test, stirring with paddles.
	11b	2.9*	96-hr test, minimal stirring.
Amphipod	2	1< x <5	48-hr test, minimal stirring. Aerated.
	3	1< x <5	48-hr test, minimal stirring. No aeration.
	7	>7	96-hr test, Dynaflo stirring. Run with bioassay #5.
	10	15< x <20	96-hr test, minimal stirring.
Isopod	6	>7	96-hr test, Dynaflo stirring. Run with bioassay #5.

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*Value obtained from BMD03S program.
+Whole mud.
ǂWhole mud but pH adjusted to ambient level with hydrochloric acid.
‡Barite only - the concentration is equivalent to the amount found in a whole mud sample.
⧧Gel only.

Table 4. 48- and 96-hr LC_{50} values for the various bioassays as determined by BMD03S program and visual inspection of log probit graphs. (Cont'd.)

Organisms	Bioassay #	LC_{50} values (% by volume)	Remarks
Mysid	12	$10 < x < 15$	48-hr range finding test.
	14a	$1 < x < 5$	96-hr test, run with bioassay #13a, stirred with paddles.
	14b	$10 < x < 15$	96-hr test, run with bioassay #13b, minimal stirring.
	16	>10	48-hr test, stirring by aeration.
	20	7.4*	48-hr test, stirring by aeration.
Artemia	15	>10	48-hr test, minimal stirring.
	21	>10	48-hr test, minimal stirring.
Sculpins	22	$10 < x < 20$	48-hr test, stirred with paddles.
Modiolus	23	>3	Sublethal test where 3% was highest concentration.

*Value obtained from BMD03S program.

this time. The main additive was Drillaid 405.[5] The 96-hr LC50 value fell to 3.2 percent (Table 4) which was significantly lower than the results found in test no. 1.

Test No. 8. Conditions during this test were essentially identical to those of test no. 1 except fewer shrimp were used per aquarium due to a lack of availability at this particular time. Results from test no. 4 indicated that the 96-hr LC50 value for muds from depths greater than 4,800 ft was between 1 and 5 percent. The solutions for test no. 8 were made accordingly but the toxicity of this particular mud sample decreased, and therefore the 96-hr LC50 value was not found. The 96-hr LC50 was greater than the highest concentration tested (10 percent).

Test No. 9. Conditions for test no. 9 were similar to conditions for test no. 8 except more shrimp were available and a larger range of test concentrations was used. The 96-hr LC50 value in this test was 8.6 percent, which was similar to previous tests. Mortalities at the 20 percent concentration occurred rapidly and all of the shrimp were dead within 3 hr. At 15 percent, all of the shrimp were dead at 24 hr. At the 20 percent concentration, some of the shrimp were obviously irritated because they would jump completely out of the tank when placed into the test solutions. This behavior was not noted at lower concentrations. This jumping behavior was also noted at high concentrations ($\geq$ 15 percent) in other tests with shrimp.

Tests No. 13a and b. These bioassays compared 96-hr LC50 values between well-mixed solutions and minimally mixed solutions. The 96-hr

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[5] Amoco Chemicals Co.

LC50 value for the well-mixed solution was 4.4 percent and for the minimally mixed solution was 15 percent (Fig. 8). Although the small sample size precluded statistical analysis, it was readily apparent that the stirred solutions were more toxic.

Tests No. 17 and 19. These bioassays tested the effects of various major mud components on shrimp. Because of limited space, these tests were run with a limited number of concentrations. The results from test no. 17 showed that pH adjustment did not significantly reduce toxicity and that only the barite (added in proportions comparable to a similarly mixed mud solution) was less toxic than the whole mud. Test no. 19 showed that only bentonite was less toxic than whole mud.

Salmon Fry

The results from test no. 5 are presented in Table 4 and results from tests no. 11a and 11b are presented in Fig. 9. The tests with a Dynaflo circulator resulted in a 96-hr LC50 of 1.9 percent (test no. 5). This was only slightly lower than the 96-hr LC50 value for the minimally stirred mixtures in no. 11b and the results were probably not significantly different. However, a comparison was tenuous because two different subsamples of mud were used.

Muds from the same subsample were used in nos. 11a and 11b. As with tests no. 13a and 13b with shrimp, the well-stirred mixtures with salmon fry produced a 96-hr LC50 value considerably lower (0.3 percent) than with the nonstirred mixture (2.9 percent).

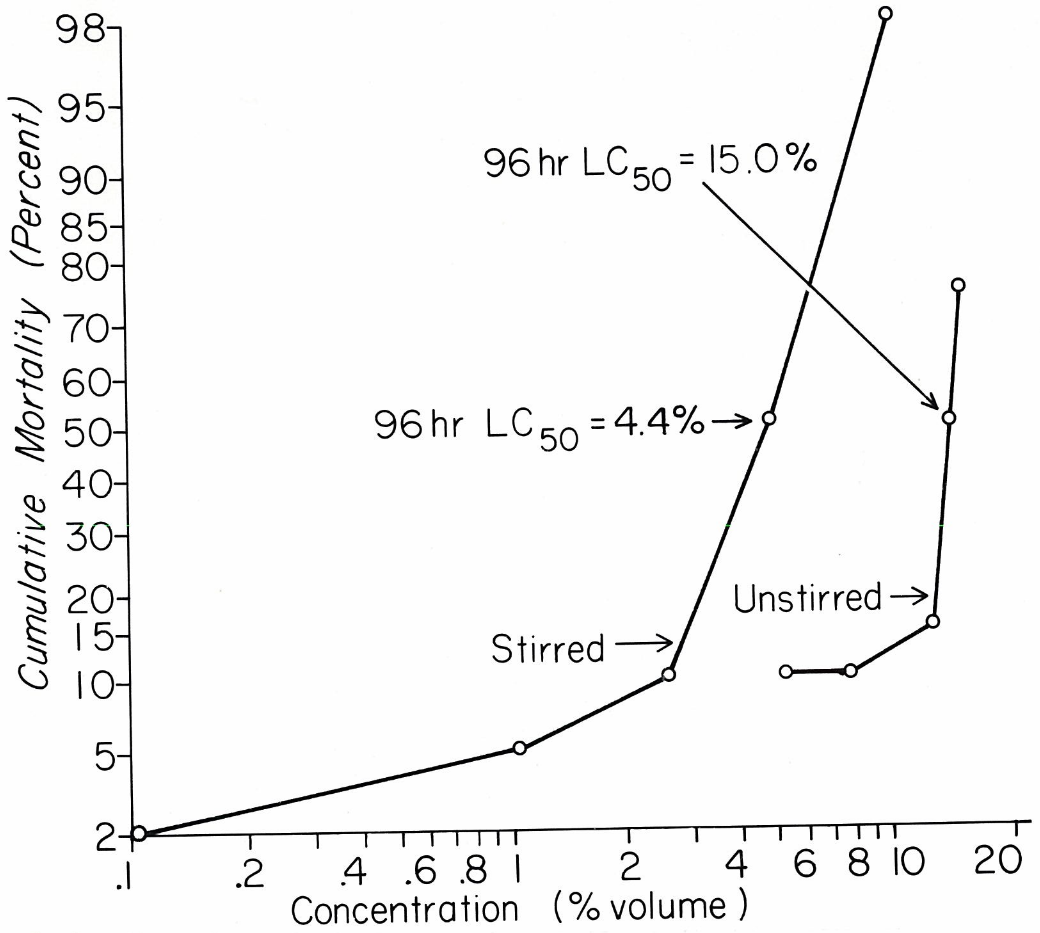

Fig. 8. Cumulative mortality and 96-hr LC_{50} values for both stirred and unstirred shrimp tests (bioassay no. 13).

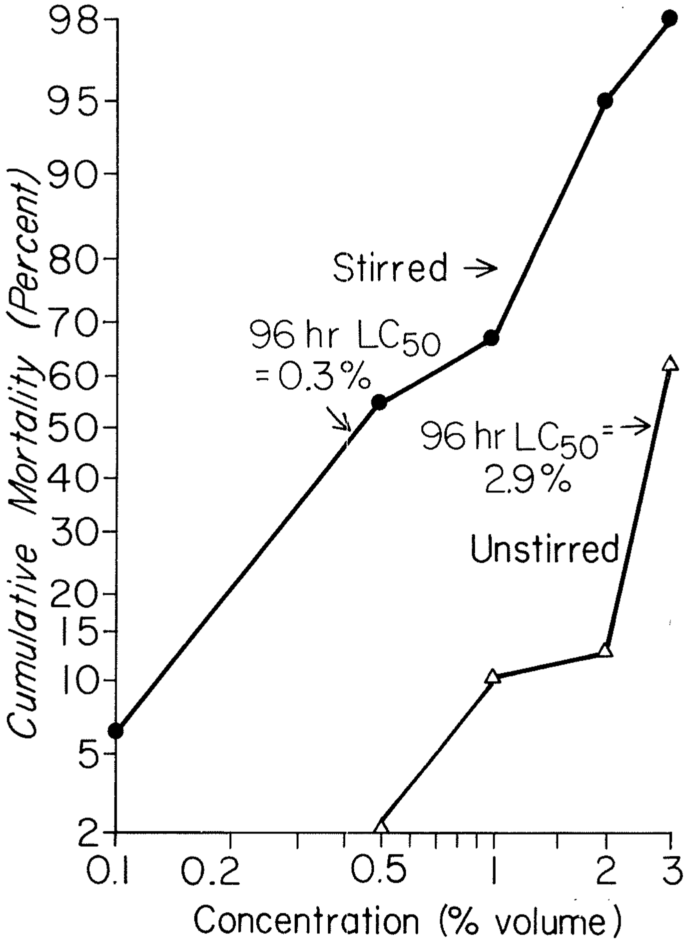

Fig. 9. Cumulative mortality and 96-hr LC_{50} values for both stirred and unstirred salmon fry tests (bioassay no. 11).

Amphipods

A variety of techniques was used with the amphipod bioassays. The results of these tests are discussed below:

Test No. 2. A layer of barite was present on the bottom of the small test containers used in this test. Many mortalities occurred because the amphipods came in contact with the barite. Unlike the shrimp, the amphipods were unable to support themselves on top of the mud. They would swim above it but eventually would fall into the mud, become partially buried, and die. The 48-hr LC50 value for this particular test was estimated to be between 1 and 5 percent. The subsample for the test was taken before the 4,800-ft level was reached.

Test No. 3. Test conditions were similar between this test and test no. 2 except no aeration was used. However, D.O. values (> 5 ppm) were adequate. The 96-hr LC50 value was similar between the two tests. A barite layer was present in this test and the amphipods eventually fell into the mud and died, as they did in test no. 2.

Test No. 7. No barite layer was present in this test due to a modification of the test containers. A screen was placed in the bottom of the test container and the barite was allowed to pass out of the container (Fig. 4). Circulation was provided by a Dynaflo filter. A 7 percent concentration was the highest tested and no significant mortalities occurred. Therefore, the 48-hr LC50 value was greater than 7 percent.

Test No. 10. Higher concentrations were used in this test than in tests no. 3 or 7. The range of concentrations encompassed the 48-hr LC50, which occurred between 15 and 20 percent solution. Minimal mixing was used in this bioassay and, therefore, the observed effects were probably due to the soluble components or suspended solids in the mud because no barite layer was present.

Mysids

Test No. 12. When no aeration and minimal stirring were used with the mysids, the 96-hr LC50 value was between 10 and 15 percent.

Tests No. 14a and 14b. These tests directly comparing a well-mixed solution with a poorly mixed solution showed that the mysids were less resistant to the well-mixed solutions (a 96-hr LC50 value between 1 and 5 percent) compared to the unmixed solutions (an LC50 value of between 10 and 15 percent).

Tests No. 16 and 20. Whole mud was used in test no. 16 and only the supernatants from similarly mixed solutions were used in test no. 20. The supernatant appeared to be less toxic with a 48-hr LC50 value greater than 10 percent. The whole mud solutions produced a 48-hr LC50 value of 7.4 percent.

Isopods

In test no. 6 it was found that the 96-hr LC50 value under these test conditions was greater than 7 percent.

Brine Shrimp

Test no. 15 was a preliminary test. Determining whether shrimp were dead or alive was very difficult due to the high turbidity of the test solutions. No dead *Artemia* were found, even at the 10 percent concentration. Therefore, the 48-hr LC50 was greater than 10 percent, but concentrations higher than that level were not tested. In test no. 21, a dye was used to make live-dead determinations and again, no dead shrimp were found even at 10 percent.

Sculpins

This test was preliminary, as it was conducted with a limited number of test organisms and test concentrations. The resulting 48-hr LC50 value was between 10 and 20 percent.

Modiolus

A limited number of concentrations was used in these tests and no mortalities occurred in 14 days at the highest concentration of 3 percent.

Histopathological Analysis

A full description of each sample analyzed can be found in Appendix 4. However, the overall results from Dr. Landolt are summarized below:

> "It appears both in the shrimp and salmon that the presence of the test material in high concentration has profound effects on the respiratory surfaces. This influence is further heightened when the test material has not been stirred. In both species, the test material is capable of inducing necrosis of respiratory epithelium, of hyperplastic changes in target cell types, and of accumulation of debris within the interlamellar areas. The control tissue for the shrimp and the low level exposure in the salmon appear to be innocuous and to have resulted in minimal damage, if any."

Suspended Solids

Table 5 presents the data on suspended solids levels in selected test aquaria. Although the data cannot be extrapolated to all test conditions, it was readily apparent that the stirring significantly increased the suspended solids levels. Background levels remained fairly constant throughout the sampling period with a range of 4.5 to

Table 5. TNFR values (in mg/ℓ) for selected samples from bioassays 9, 11, and 13.

Bioassay	Concentrations							
	Control	1%	2.5%	3.0%	5%	10%	15%	20%
9	8.7	--	212	--	1,590	3,367	12,205	17,105
11 stirred	5.2	3,513	--	11,823	--	--	--	--
11 unstirred	5.7	53	--	338	--	--	--	--
13 stirred	4.5	--	--	--	15,948	32,385	--	--
13 unstirred	--	--	--	--	17	277	--	--

8.7 mg/liter. The LC50 values were correlated inversely with suspended solids; however, the correlations were not linear. Also, the suspended solids concentrations were not linearly correlated to mud concentrations, probably because of variability in mixing and settling rates of solutions between containers.

pH

Figure 10 shows pH values from various bioassays. In general, the values increased with increasing mud concentration. Appendix 3 shows the entire data for the individual tests. The values varied from approximately 7.3 for ambient seawater to 8.6 for a 20 percent concentration.

Salinity

Figure 11 shows salinity values for ambient seawater during the study. Some measurements are missing because of pump failures. In general, the salinity was between 29 and 30 ppt, but fell to between 26 and 27 ppt near the end of the study. Additional values for each bioassay are listed in Appendix 3.

Temperature

Figure 12 shows temperatures of ambient seawater during the study. As with the salinity measurements, some of the data are missing due to pump failures. The temperature values tended to fluctuate, with higher temperatures noted near the beginning and near the end of the study, and lower temperatures near the middle. Overall, test temperatures varied between 10° and 14° C. Appendix 3 shows the complete temperature records during the bioassays.

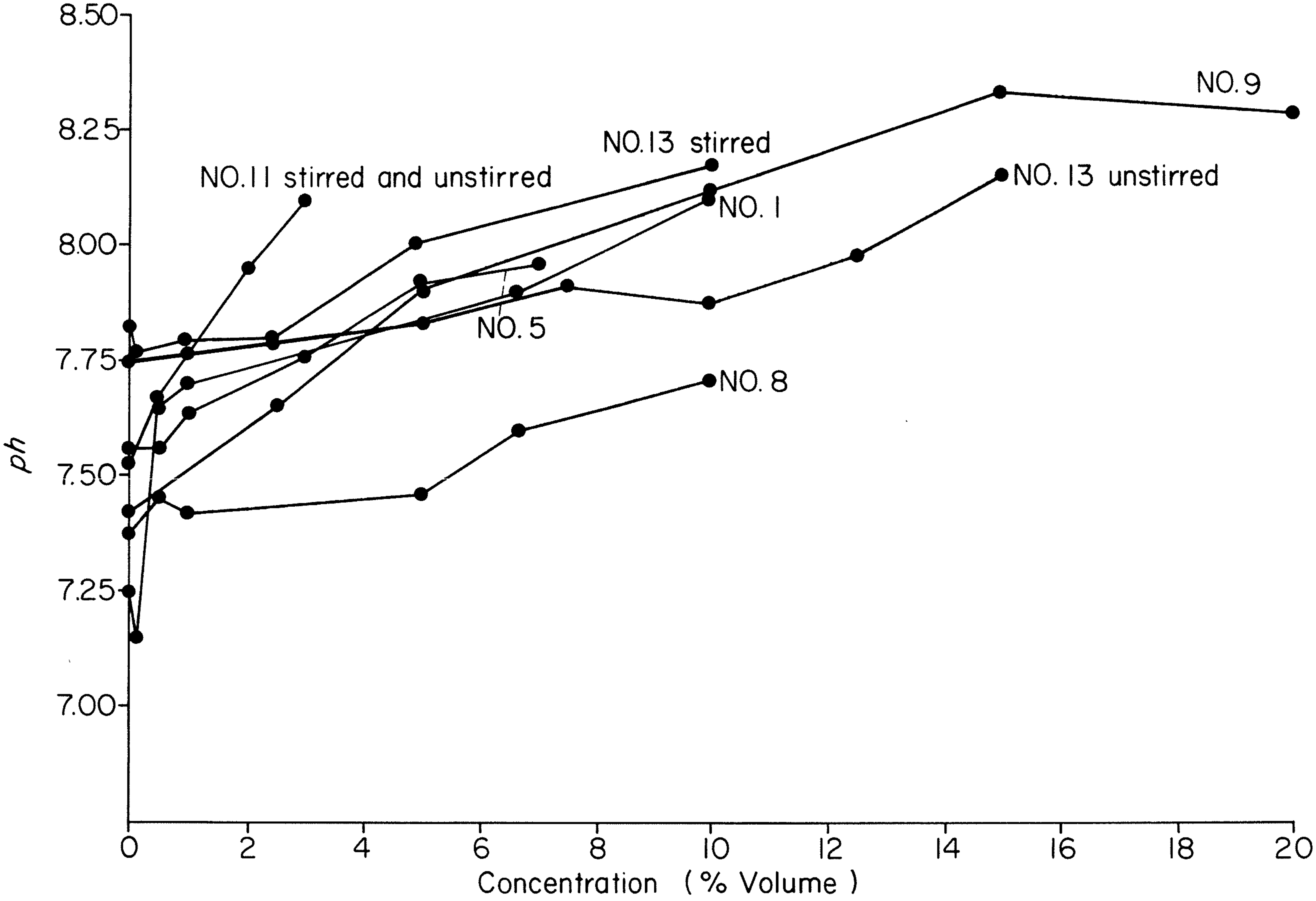

Fig. 10. pH values of test water from selected bioassays.

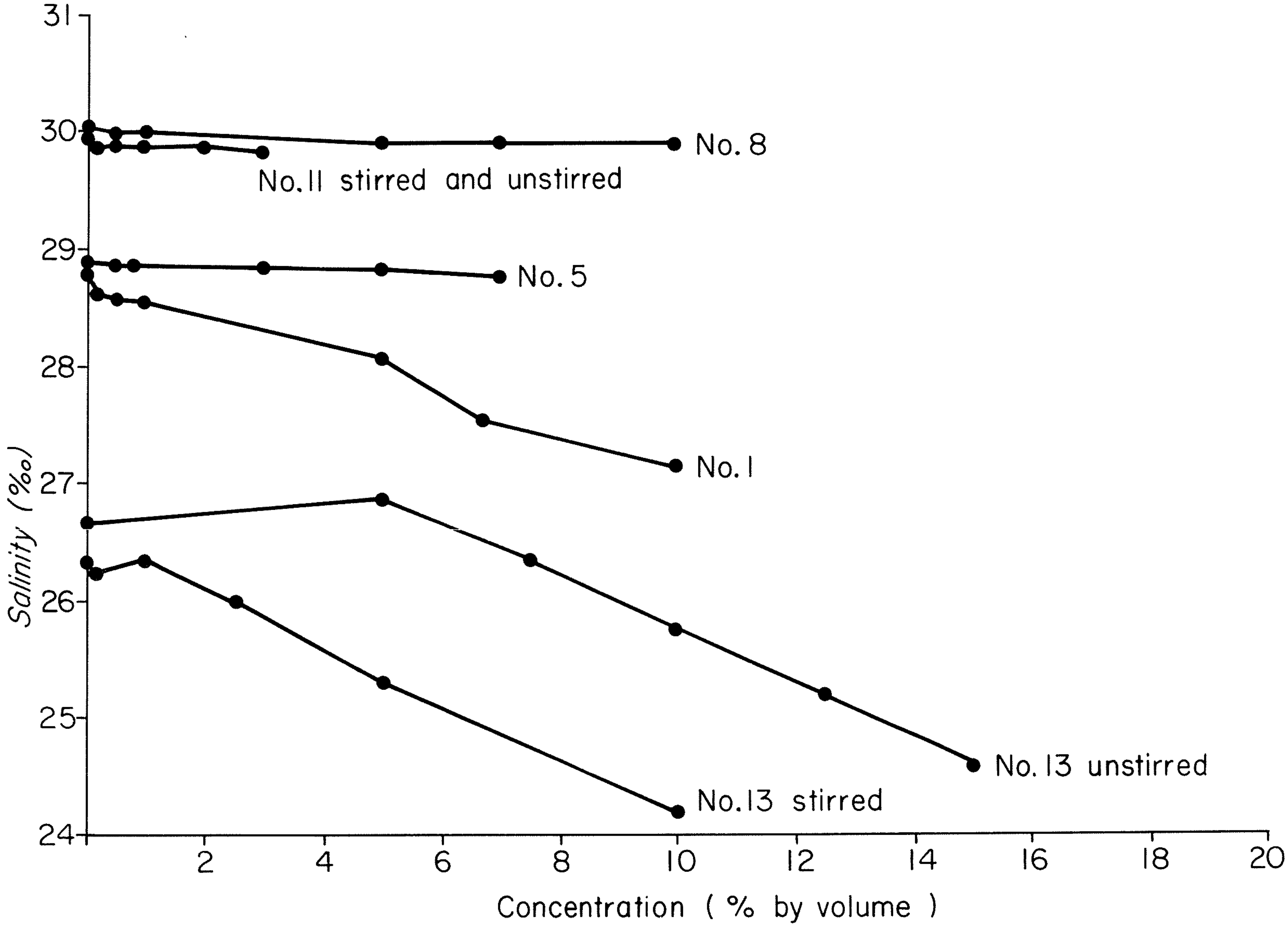

Fig. 11. Salinity values of test water from selected bioassays.

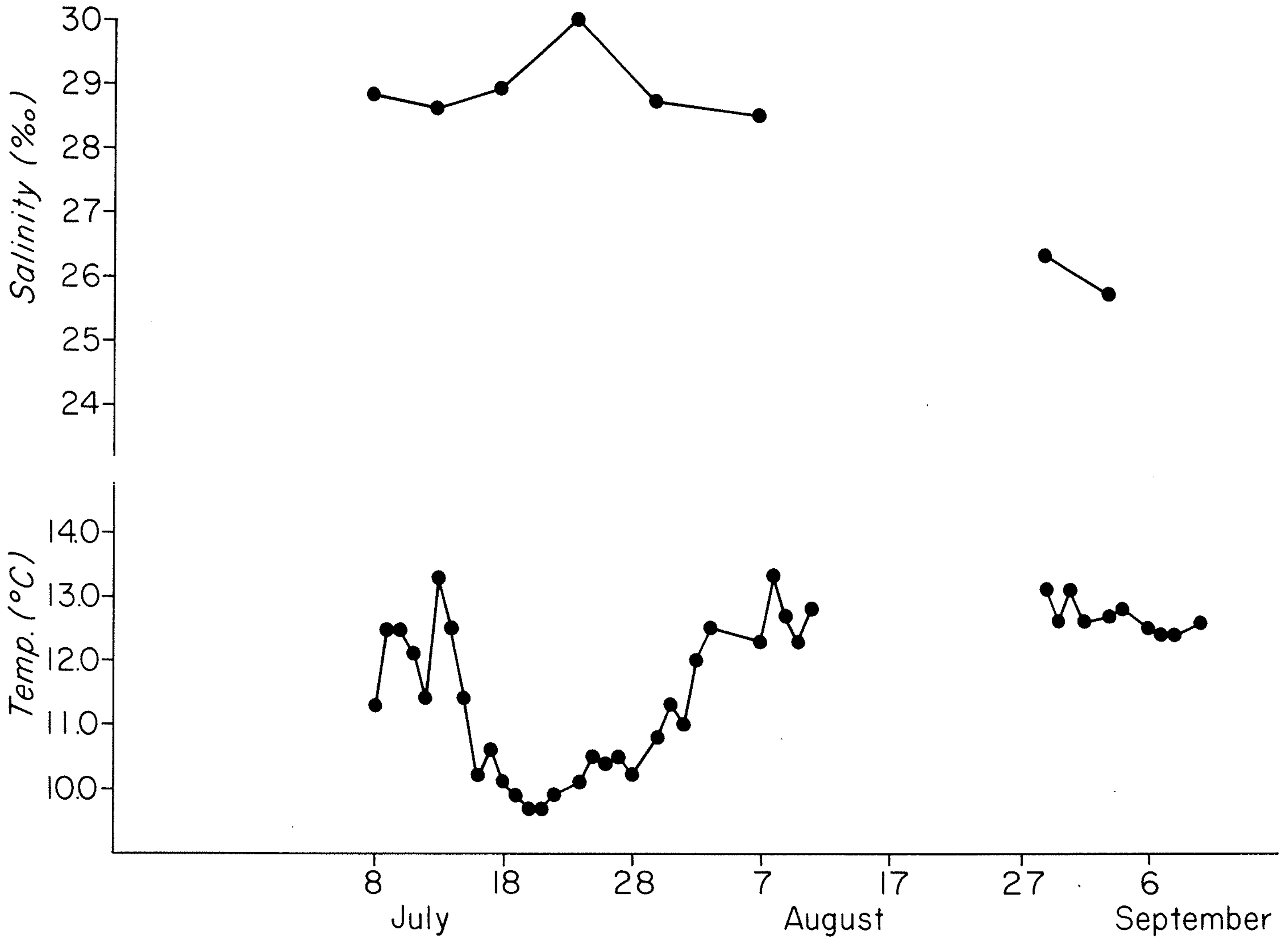

Fig. 12. Temperature and salinity determination from seawater sampled in the vicinity of OCEAN RANGER during onsite bioassays.

Live-Box Studies

First Study

The results from this study are shown in Table 6a. Neither the plastic buckets nor the nylon bags withstood the 2- to 3-knot water current. Consequently, most of the containers were either lost or damaged. In addition, the array located 300 ft from the rig was fouled in the anchor line of a supply boat, which caused the loss of most of that equipment. Only one live-box with fish was retrieved intact and all fish in that box were in good condition. Some fish were found in the other boxes that were retrieved. Many of these fish were in poor condition or dead because of direct physical damage (abrasion and laceration) from the poorly functioning live-boxes.

The shrimp missing from the shrimp pots were assumed to have escaped as no carapaces were found which might otherwise have indicated the shrimp had died. All other shrimp were alive and were in good condition.

Second Study

All of the fish and hermit crabs (Table 6b) in the control live-box apparatus were alive and appeared healthy. The pot containing the hermit crabs had been caved in on one side, apparently due to an anchor landing on it.

The fish and hermit crabs in the array 600 ft NNE of the rig were alive when recovered. However, the fish had a red coloration on the top of their heads. They appeared in good health otherwise. The red coloration disappeared about 2 hr after retrieval of the live-box.

The acoustic release mechanism of the apparatus 300 ft from the rig did not function properly. The apparatus was recovered by trawling.

Table 6a. Summary of live-box test results July 18-23, 1977.

	Depth (ft) below MLLW	Species	Exposure (hrs)	Number Recoveries Well	Number Recoveries Injured	Number Recoveries Dead	Number Missing	Comments
Control	50	Salmon	94	0	2	7	1	Live box damaged by current
	105	Salmon	95	0	0	0	10	Live box lost
	Bottom	Shrimp	96	10	0	0	0	All okay
600 ft	50	Salmon	122	1	0	2	7	Live box cover loose, bag ripped
	105	Salmon	122	10	0	0	0	All okay
	Bottom	Shrimp	123	9	0	0	1	Nine okay, no trace of missing shrimp
300 ft	50	Salmon	124	0	0	0	10	Live box destroyed by work boat anchor line
	105	Salmon	125	0	0	0	10	Live box destroyed by work boat anchor line
	Bottom	Shrimp	125	9	0	0	1	Cage dented - no trace of missing shrimp

Table 6b. Summary of live-box test results August 27-31, 1977.

	Depth (ft) below MLLW	Species	Exposure (hrs)	Number Recoveries			Number missing	Comments
				Well	Injured	Dead		
Control	5	Salmon	88	10	0	0	0	All okay
	105	Salmon	88.5	10	0	0	0	All okay
	Bottom	Hermit crabs	89	10	0	0	0	All okay
600 ft	5	Salmon	95	10	0	0	0	Reddish coloration
	105	Salmon	95.5	10	0	0	0	Reddish coloration
	Bottom	Hermit crabs	96	10	0	0	0	All okay
300 ft	5	Salmon	101	10	0	0	0	Reddish coloration
	105	Salmon	101.5	10	0	0	0	Reddish coloration
	Bottom	Hermit crabs	-	0	0	0	10	Cage lost

The anchor and the shrimp pot with the hermit crabs were not recovered due to a broken rope. The fish recovered were all alive and appeared healthy except for reddish coloration on the head which eventually disappeared.

DISCUSSION

Bioassays

The bioassay tests of drilling fluids demonstrated that high concentrations and prolonged periods of exposure to drilling mud were required to cause mortalities of selected organisms under conditions of static bioassay in a laboratory. The 96-hr LC50 for the most sensitive species tested never occurred in less than a 0.3 percent solution. These results compared closely with results reported in the literature in that the magnitude of most LC50 values was on the order of parts per hundred or volume percent. This conclusion pertains only to similar mud types. It should also be emphasized, however, that most water-based lignosulfonate muds are basically the same and although these additional components are available, they are used infrequently.

In general, the crustaceans (shrimp, mysids, isopods, and amphipods) were more tolerant than the salmon (96-hr LC50 values of approximately 3 percent or greater for shrimp and 0.3 to 3 percent for salmon). The reason for this difference was not determined but may have been due to some structural adaptation (especially of the gills) that allowed the crustaceans to clear the mud more readily or they may have had a higher tolerance to gill irritation and damage. It was also possible that the salmon may have been less tolerant to some soluble component in the mud such as the lignosulfonate or the caustic soda. Further tests would be necessary to determine the exact reason for the difference in tolerance between species.

The cause of death in all animals was difficult to determine. However, it was fairly obvious that gill irritation and damage were largely responsible in both shrimp and salmon. This irritation was especially noticeable in the salmon because of the large amounts of mucous that were visible on the gills at the 1 and 3 percent mud concentrations. The factors in the mud that caused this irritation could have been the suspended solids, the high pH due to the caustic soda, and/or some other additive in the mud. The fact that some of the shrimp responded by jumping out of the water at higher concentrations (15 percent and 20 percent) indicated some type of irritation. Some of the mortalities may have been due to latent injuries incurred during handling and holding, although the minimum 48-hr holding period prior to bioassay probably minimized this effect.

In the tests where solutions were either stirred or not, it was apparent that the higher suspended solids levels were more toxic. It was somewhat surprising that the histopathological analysis showed a lesser degree of gill damage in stirred than in unstirred tests at the same concentration. The unstirred solutions may have caused more gill damage because the organisms were exposed to these test solutions long before they died, thus increasing the likelihood of physical damage.

Preliminary results indicated that a direct linear correlation between LC50 values and suspended solids was not clearly established (Table 5). This suggests that suspended solids were not the only toxic agent and that the interactions between the other components (lignosulfonate, caustic soda, gel, and any other additives) were partly responsible for the toxic response. The component tests on bentonite and barite showed that these components, taken individually, were not responsible for the lethal effects. Also, tests in which pH was adjusted suggested that the high pH due to the caustic soda may not have been the lethal component (by itself) because no significant difference in mortalities was noted when the pH was adjusted to ambient. Furthermore, the supernatant tests (nos. 16 and 20) with mysids suggested that the supernatant was less toxic than the whole mud. Obviously, more testing of the components in various combinations is necessary to define the effects.

Although it was suggested that muds from above the 4,800-ft level would be less toxic, the data presented here do not clearly indicate such a difference. The one shrimp test that was made with a mud sample taken above the 4,800-ft depth indicated that the 96-hr LC50 value was greater than 10 percent. However, in most other shrimp tests except nos. 4 and 13a, the 96-hr LC50 value was greater than or equal to 10 percent. The only other tests with mud taken from less than 4,800 ft were tests no. 2 and 3 with amphipods. A comparison between LC50 values for the amphipods, however, is inappropriate because different methods were used among the tests and the presence of a barite layer in tests no. 2 and 3 caused mortalities that resulted more from physical entrapment than from chemical toxicity of the mud per se.

Live-Box Studies

First Period

The results of all tests indicated that the drilling fluid was not lethal. According to the Dames and Moore plume description studies, the maximum concentration of drilling mud occurred in the area of the 300- and 600-ft live-boxes. Therefore, the tests approximated a "worst possible case" in which the fish were held in maximum concentration for an extended period of time. This exposure period was approximately 3-4 hr during flood tide. The lack of mortality in the live-boxes was expected in view of the high concentration of drilling fluid needed in laboratory experiments to cause mortality and the relatively low concentration which was present in the plume.

Second Period

The results of the second live-box test were much more conclusive than those of the first test. In the second test, all test organisms survived. Clearly, the drilling fluids in the plume were not concentrated enough for a sufficient period of time to cause mortalities.

The cause of the reddening of the heads of the fish remains unknown. The rapid ascent to the surface when the acoustic apparatus was released may have caused localized hemorrhaging. However, another possible cause could be a stress reaction to the drilling fluid. The control fish did not show the coloration, but they were subjected to neither the rapid ascent nor (presumably) the drilling fluid. A few additional studies will be needed to determine the cause of this reddening.

Neither of the live-box studies showed lethal effects. The second live-box study was of particular importance because the desilters and desanders were operated for 3 hr at 16 to 17 barrels/hr, which was maximum discharge except for a complete mud dump. This discharge did not cause mortalities.

Although a live-box array should have been set closer to the rig, this was technically impossible because of anchors, anchor lines, and rigging. Dames and Moore studies indicated that concentrations necessary to cause mortality probably could not have been sustained for periods long enough to elicit that response because of the strong currents and rapid dilution.

SUMMARY AND CONCLUSIONS

Under the conditions of static bioassay where the test organisms were held in the test solutions for long periods of time, very high concentrations relative to ambient were needed to cause mortality. Although stirring lowered the LC50 values, the lowest values for any test organisms were never less than about 0.25 percent (no. 11b). The calculated value for the concentration of drilling fluid at the point of discharge was estimated by Dames and Moore studies to be 0.25 percent during a normal desilter and desander discharge lasting 2 hr or less. Concentrations during a mud dump were considerably greater, but the release of all the mud was made infrequently. Thus, considering the high dilution and dispersion that occurred at the lower Cook Inlet drill site and the intermittent nature of the discharge schedule, it was extremely unlikely that sustained lethal concentrations of drill fluids occurred.

The live-box studies verified that the discharge of drilling fluids in the field had no lethal effects on the test organisms.

The overall results of the FRI studies and the studies by Dames and Moore demonstrated that the impact at this particular drill site was probably minimal. The following conclusions can be made about the studies:

1. Concentrations of 0.25 to 20 parts per hundred were needed to cause mortality among selected test organisms. These concentrations probably were not sustained long enough in the drilling plume to cause mortality.

2. Of the various species tested, the crustaceans tolerated the drill fluids more readily than salmon. The reason or reasons for species difference is unknown.

3. Increased agitation of the test solutions and consequent increase in suspended solids levels resulted in increased toxicity of the mud. The correlation, however, was not linear and thus, preliminary indications would suggest that the suspended solids were not entirely responsible for toxicity.

4. The live-box tests demonstrated that sustained concentrations of mud at toxic levels did not occur in the field situation.

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APPENDIX 1

SEAWATER PUMP PROBLEMS

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Due to the rigorous conditions (currents, wind, etc.) around the OCEAN RANGER, maintaining a system for pumping seawater to the laboratory became a formidable task. To avoid exposing a pump to these conditions, it would have been desirable to place the pump on the main deck and draw water from sea level to the laboratory. This, however, was not possible because of the 80-ft plus head (sea level to deck level). A staged pumping system might have worked, but would have been very difficult to install. Therefore, it was suggested that a submersible pump be used as the only practical alternative. Unfortunately, repeated pump failures occurred due to saltwater seepage into electrical connections, physical damage to the pump during storms, and burned out motors. This resulted in either: 1) holding test organisms in nonflowing water until the pump was repaired; or 2) exposing them to the OCEAN RANGER's saltwater system.

Temperature control was difficult when holding organisms in the nonrunning water. However, it was fairly well maintained by placing bagged ice into the troughs. Some shrimp mortalities in the holding tanks may have been directly attributable to the temperature fluctuations. Also, the salmon fry did show signs of problems (hemorrhaging, blue irridescence, etc.) following a relatively long period (2-3 days) with no fresh seawater.

In holding organisms in the seawater from the OCEAN RANGER's system, there was some concern whether exposure to trace metals and antifouling compounds associated with the plumbing system might affect the organisms. There were never any obvious mortalities in either fish or shrimp stocks when exposed to the rig's seawater for short periods (< 12 hr). However, heavy mortalities occurred in shrimp tanks after being exposed to the OCEAN RANGER's seawater for a period of 4 days. These delayed mortalities were probably due to the initial temperature rise when the pump failed. There were no salmon fry in the holding tanks at this time. All other organisms appeared unaffected by either temperature rises or exposure to the rig's seawater.

With the exception of bioassay no. 22, no test solutions were made with the rig's seawater, although there were instances where test organisms had been exposed to it previous to the start of the bioassay. During bioassay no. 11, water was hoisted over the side in 30-gal plastic buckets for mixing test solutions.

Although the pump problems did cause delays in setting up tests and complications in holding test organisms, the test conditions (temperature, salinity, etc.) for any particular bioassay were carefully controlled so that all results were valid.

The following account describes the status of the seawater pump through the course of the field studies:

Date

6/30/77 - FRI personnel arrive on OCEAN RANGER. Pump not installed.

7/6/77 - Submersible pump operational; however, it failed later in the day.

7/7/77 - Pump repaired and operational, with no shrimp mortalities occurring. Shrimp were held in nonrunning water, artificially aerated and cooled.

7/13/77 - Submersible pump failure. The OCEAN RANGER's seawater system was used for cooling bioassay tanks; however, test organisms were held in artificially aerated and cooled nonrunning seawater.

7/15/77 - Submersible pump operational.

7/17/77 - Submersible pump detached from anchor cable. The pump was operational again after repairs.

8/3/77 - Submersible pump failure.

8/4/77 - Submersible pump operational with no significant loss of test organisms.

8/5/77 - Submersible pump failure. Organisms maintained on artificial aeration and cooling. OCEAN RANGER's saltwater was used in the cooling troughs.

8/7/77 - Bioassay no. 11 began with ambient seawater hoisted over the side in 30-gal plastic buckets.

8/10/77 - Submersible pump operational.

8/12/77 - Submersible pump failure.

8/14/77 - Submersible pump operational.

8/16/77 - Submersible pump failure.

8/17/77 - Submersible pump operational, with only occasional shrimp mortalities over the past week.

9/5/77 - Submersible pump failure. All organisms placed on the rig's seawater system.

9/6/77 - Submersible pump operational.

9/9/77 - Submersible pump failure. Heavy shrimp mortalities incurred. The OCEAN RANGER's seawater hooked up permanently.

9/12/77 - Heavy shrimp mortalities which remove 60 percent of shrimp stock in 3 days.

9/17/77 - FRI personnel leave OCEAN RANGER.

Approximate total number of days pump down = 35

Total number of days FRI personnel on board OCEAN RANGER = 80

APPENDIX 2

CUMULATIVE MORTALITIES FOR ALL BIOASSAYS DONE ONBOARD THE OCEAN RANGER

CUMULATIVE MORTALITIES

Test No: 1
Start Date: July 8, 1977
Species: Pandalus hypsinotus-coonstripe shrimp
No. Test Tanks: 14
Total No. Animals: 140

Mean Lengths: 28.1 mm
Mean Wet Weights: 14.8 g
Start Time: 1715 (6.7% ¢ 10% started 2215)
Temp. at Start: 11.9°C
Conditions: 60ℓ total volume.
Aerated only.
Mud obtained July 3, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)						
		1	1.5	15	24	48	72	96
Control A	10	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0
0.025% A	10	0	0	0	0	0	0	0
0.025% B	10	0	0	0	0	0	0	0
0.1% A	10	0	0	0	0	0	0	0
0.1% B	10	0	0	0	0	0	0	0
0.5% A	10	0	0	0	0	0	1	1
0.5% B	10	0	0	0	0	0	0	0
1% A	10	0	0	0	0	0	0	0
1% B	10	0	0	0	0	0	0	0
5% A	10	0	0	0	0	0	0	0
5% B	10	0	0	0	0	0	0	0
6.7%	10	0	0	0	0	0	0	0
10%	10	0	0	0	0	0	0	0

CUMULATIVE MORTALITIES

Test No: 2
Start Date: July 10, 1977
Species: *Anisogammarus confervicolous* - amphipods
No. Test Tanks: 12
Total No. Animals: 120

Mean Wet Weights: 0.07 g
Start Time: 1600
Temp. at Start: 12.7°C
Conditions: 900 ml total volume.
Done in 1000 ml beakers with 600 ml liners.
Aerated. mud collected July 9, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)								
		1	2	3.5	4.5	5.5	6.5	16	24	48
Control A	10	0	0	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0	0	0
0.5% A	10	0	0	0	0	0	0	0	0	0
0.5% B	10	0	0	0	0	0	0	0	0	0
1% A	10	0	0	0	0	0	0	0	0	0
1% B	10	0	0	0	0	0	0	0	0	1
5% A	10	0	0	2	5	8	9	9	9	9
5% B	10	0	0	0	5	7	10	10	10	10
10% A	10	0	7	8	10	10	10	10	10	10
10% B	10	0	9	10	10	10	10	10	10	10
20% A	10	0	10	10	10	10	10	10	10	10
20% B	10	0	10	10	10	10	10	10	10	10

CUMULATIVE MORTALITIES

Test No: 3
Start Date: July 11, 1977
Species: Anisogammarus confervicolous - amphipods
No. Test Tanks: 12
Total No. Animals: 120

Mean Wet Weights: 0.07 g
Start Time: 1030
Temp. at Start: 12.2°C
Conditions: 1000 ml total volume. In 1000 ml beakers with 600 ml liners. No aeration, mud collected July 9, 1977.

		Observation Times (Hours)															
Concentrations (Volume:Volume)	Animals/ Tank	1	4	24	48												
Control A	10	0	0	0	0												
Control B	10	0	0	0	0												
0.5% A	10	0	0	0	0												
0.5% B	10	0	0	0	0												
1% A	10	0	0	0	0												
1% B	10	0	0	0	0												
5% A	10	0	10	10	10												
5% B	10	0	10	10	10												
10% A	10	0	10	10	10												
10% B	10	0	10	10	10												
20% A	10	0	10	10	10												
20% B	10	0	10	10	10												

CUMULATIVE MORTALITIES

Test No: 4
Start Date: July 13, 1977
Species: Pandalus hypsinotus - coonstripe shrimp
No. Test Tanks: 12
Total No. Animals: 120

Mean Lengths: 28.1 mm
Mean Wet Weights: 14.4 g
Start Time: 1600
Temp. at Start: 13.3°C
Conditions: 60ℓ total volume. Aeration only.
Mud collected July 13, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)									
		2	4	7	12	16	20	24	48	72	96
Control A	10	0	0	0	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0	0	0	0
0.5% A	10	0	0	0	0	0	0	0	0	0	0
0.5% B	10	0	0	0	0	0	0	0	0	0	1
1% A	10	0	0	0	0	0	0	0	0	0	0
% B	10	0	0	0	0	0	0	0	0	0	0
5% A	10	0	0	3	4	4	4	5	5	5	5
5% B	10	0	1	2	4	6	6	7	7	7	7
10% A	10	0	9	10	10	10	10	10	10	10	10
10% B	10	0	4	8	10	10	10	10	10	10	10
20% A	10	9	10	10	10	10	10	10	10	10	10
20% B	10	6	10	10	10	10	10	10	10	10	10

CUMULATIVE MORTALITIES

Test No: 5
Start Date: July 18, 1977
Species: Oncorhynchus gorbuscha - pink salmon
No. Test Tanks: 12
Total No. Animals: 119

Mean Lengths: 60.5 mm
Mean Wet Weights: 2.0 g
Start Time: 1645
Temp. at Start:
Conditions: 60ℓ total volume. Aerated Stirred with Dynaflo II. Fish in 3-gallon buckets with screened holes in bottom and sides. Mud collected July 18, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)															
		1	4	6	8	11	16	20	24	28	32	44	48	72	96		
Control A	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Control B	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0.5% A	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0.5% B	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
1% A	10	0	0	0	0	0	0	0	0	0	0	0	0	0	1		
1% B	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
3% A	8	0	0	0	0	0	8	8	8	8	8	8	8	8	8		
3% B	9	0	0	0	7	7	7	8	8	8	9	9	9	9	9		
5% A	10	0	0	2	3	10	10	10	10	10	10	10	10	10	10		
7% A	10	0	0	2	3	10	10	10	10	10	10	10	10	10	10		
7% B	11	0	0	7	11	11	11	11	11	11	11	11	11	11	11		

CUMULATIVE MORTALITIES

Test No: 6
Start Date: July 18, 1977
Species: Gnorimosphaeroma oregonensis
No. Test Tanks: 12
Total No. Animals: 120

Mean Wet Weights: 0.02 g
Start Time: 1645
Temp. at Start: 10.0°C
Conditions: Run simultaneously with No. 5 in 600 ml screened beakers

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)													
		1	2	4	6	8	11	16	20	24	48	72	96		
Control A	10	0	0	0	0	0	0	0	0	0	0	0	0		
Control B	10	0	0	0	0	0	0	0	0	0	0	0	0		
.5% A	10	0	0	0	0	0	0	0	0	0	0	0	0		
.5% B	10	0	0	0	0	0	0	0.	0	0	0	0	0		
1% A	10	0	0	0	0	0	0	0	0	0	0	0	0		
1% B	10	0	0	0	0	0	0	0	0	0	0	0	0		
3% A	10	0	0	0	0	0	0	0	0	0	0	0	0		
3% B	10	0	0	0	0	0	0	0	0	0	0	0	0		
5% A	10	0	0	0	0	0	0	0	0	0	0	0	0		
5% B	10	0	0	0	0	0	0	0	0	0	0	0	0		
7% A	10	0	0	0	0	0	0	0	0	0	0	0	1		
7% B	10	0	0	0	0	0	0	0	0	0	0	0	0		

CUMULATIVE MORTALITIES

Test No: 7
Start Date: July 18, 1977
Species: Anisogammarus confervicolous
No. Test Tanks: 12
Total No. Animals: 120

Mean Wet Weights: 0.07 g
Start Time: 1645
Temp. at Start: 10.0°C
Conditions: Run simultaneously with no. 5 in 600 ml screened beakers

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)										
		1	2	4	8	11	16	20	24	48	72	96
Control A	10	0	0	0	0	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0	0	0	0	0
.5% A	10	0	0	0	0	0	0	0	0	0	0	0
.5% B	10	0	0	0	0	0	0	0	0	0	0	0
1% A	10	0	0	0	0	0	0	0	0	0	0	0
1% B	10	0	0	0	0	0	0	0	0	0	0	0
3% A	10	0	0	0	0	0	0	0	0	0	1	1
3% B	10	0	0	0	0	0	0	0	0	0	0	0
5% A	10	0	0	0	0	0	0	0	0	0	0	0
5% B	10	0	0	0	0	0	0	0	0	0	0	0
7% A	10	0	0	0	0	0	0	0	0	1	1	1
7% B	10	0	0	0	0	0	0	0	0	0	0	0

CUMULATIVE MORTALITIES

Test No: 8
Start Date: July 24, 1977
Species: Pandalus hypsinotus - coonstripe shrimp
No. Test Tanks: 10
Total No. Animals: 60

Mean Lengths: 30.9 mm
Mean Wet Weights: 20.2 g
Start Time: 1400
Temp. at Start: 10.1°C
Conditions: 60ℓ total volume. Aerated only
Mud collected July 24, 1977. Shrimp in
vexar lining.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)											
		2	4	6	8	10	12	16	20	24	48	72	96
Control A	6	0	0	0	0	0	0	0	0	0	0	0	0
Control B	6	0	0	0	0	0	0	0	0	0	0	0	0
0.5% A	6	0	0	0	0	0	0	0	0	0	0	1	1
0.5% B	6	0	0	0	0	0	0	0	0	0	1	1	1
1% A	6	0	0	0	0	0	0	0	0	0	0	0	0
1% B	6	0	0	0	0	0	0	0	0	0	0	0	0
5% A	6	0	0	0	0	0	0	0	0	0	0	1	1
5% B	6	0	0	0	0	0	0	0	0	0	1	2	2
6.7%	6	0	0	0	0	0	0	0	0	0	0	0	0
10%	6	0	0	0	0	0	0	0	0	0	0	0	0

CUMULATIVE MORTALITIES

Test No: 9
Start Date: July 30, 1977
Species: <u>Pandalus hypsonotis</u> - coonstripe shrimp
No. Test Tanks: 12
Total No. Animals: 120

Mean Lengths: 30.2 mm
Mean Wet Weights: 19.8 g
Start Time: 1820
Temp. at Start:
Conditions: 2 aerators per tank. 60ℓ total volume. Shrimp in vexar lining. Mud collected July 30, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)											
		1	2	3	4	8	12	16	20	24	48	72	96
Control A	10	0	0	0	0	0	0	0	0	0	0	0	1
Control B	10	0	0	0	0	0	0	0	0	0	0	0	0
2.5% A	10	0	0	0	0	0	0	0	0	0	0	0	0
2.5% B	10	0	0	0	0	0	1	1	1	1	1	1	1
5% A	10	0	0	0	0	0	0	0	0	0	0	0	0
5% B	10	0	0	0	0	0	0	0	0	0	0	0	0
10% A	10	0	0	0	0	1	3	3	6	6	6	6	6
10% B	10	0	0	0	0	0	0	0	3	4	5	5	5
15% A	10	0	1	3	5	7	8	10	10	10	10	10	10
15% B	10	0	4	5	8	8	8	8	9	10	10	10	10
20% A	10	8	10	10	10	10	10	10	10	10	10	10	10
20% B	10	7	8	10	10	10	10	10	10	10	10	10	10

CUMULATIVE MORTALITIES

Test No: 10
Start Date: July 30, 1977
Species: *Anisogrammarus confervicolous* - amphipods
No. Test Tanks: 12
Total No. Animals: 120

Mean Wet Weights: 0.07 g
Start Time: 1820
Temp. at Start:
Conditions: Aerated only. In 600 ml screened beakers with aquaria of bioassay 9. Mud collected July 30, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)											
		1	2	3	4	8	12	16	20	24	48	72	96
Control A	10	0	0	0	0	0	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0	0	0	0	0	0
2.5% A	10	0	0	0	0	0	0	0	0	0	0	0	0
5% A	10	0	0	0	0	0	0	0	0	0	0	0	0
5% B	10	0	0	0	0	0	0	0	0	0	0	0	0
10% A	10	0	0	0	0	0	0	0	0	0	0	0	0
10% B	10	0	0	0	0	0	0	0	0	0	0	0	0
15% A	10	0	0	0	0	0	0	0	0	0	0	0	0
15% B	10	0	0	0	0	0	0	0	0	0	1	1	1
20% A	10	0	0	0	0	1	1	2	4	4	4	4	4
20% B	10	0	0	0	0	0	0	0	2	2	5	5	5

CUMULATIVE MORTALITIES

Test No: 11 stirred
Start Date: August 7, 1977
Species: Oncorhynchus gorbuscha - pink salmon
No. Test Tanks: 12
Total No. Animals: 118

Mean Lengths: 66.0 mm
Mean Wet Weights: 2.3 g
Start Time: 1700
Temp. at Start:
Conditions: 60ℓ total volume. Aerated. Stirred with paddles. Run at same time as bioassay 11 unstirred. In vexar screens. Mud collected Aug.11, 1977

Concentrations (Volume:Volume)	*Animals/ Tank	Observation Times (Hours)							
		4	8	16	24	36	48	72	96
Control A	10	0	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0	0
0.1% A	7	0	0	0	0	0	0	0	0
0.1% B	8	0	0	0	1	1	1	1	1
0.5% A	4	0	0	0	0	1	1	1	1
0.5% B	10	0	1	8	9	9	10	10	10
1% A	9	0	0	0	0	1	1	2	3
1% B	10	1	7	10	10	10	10	10	10
2% A	10	0	0	2	4	8	8	9	9
2% B	10	0	8	10	10	10	10	10	10
3% A	10	3	10	10	10	10	10	10	10
3% B	10	6	10	10	10	10	10	10	10

* 10 originally introduced. Low numbers indicate loss due to jumping out of tank.

CUMULATIVE MORTALITIES

Test No: 11 unstirred
Start Date: August 7, 1977
Species: Oncorhynchus gorbuscha - pink salmon
No. Test Tanks: 12
Total No. Animals: 120

Mean Lengths: 66.0 mm
Mean Wet Weights: 2.3 g
Start Time: 1700
Temp. at Start:
Conditions: 60ℓ total volume. Aerated, unstirred. Run at same time as bioassay 11 stirred. In 3-gallon buckets with screened holes.

Concentrations (Volume:Volume)	*Animals/ Tank	Observation Times (Hours)															
		4	8	16	24	36	48	72	96								
Control A	8	0	0	0	0	0	0	0	0								
Control B	8	0	0	0	0	0	0	0	0								
0.1% A	8	0	0	0	0	0	0	0	0								
0.1% B	5	0	0	0	0	0	0	0	0								
0.5% A	5	0	0	0	0	0	0	0	0								
0.5% B	8	0	0	0	0	0	0	0	0								
1% A	5	0	0	0	0	0	0	1	1								
1% B	8	0	0	0	0	0	0	0	0								
2% A	7	0	0	1	1	1	1	1	1								
2% B	9	0	0	0	1	1	1	1	1								
3% A	8	1	1	6	7	7	7	7	7								
3% B	8	0	0	2	3	3	3	3	3								

* 8 originally introduced. Low numbers indicate loss due to jumping out of tank.

CUMULATIVE MORTALITIES

Test No: 12
Start Date: August 22, 1977
Species: Neomysis integer
No. Test Tanks: 7
Total No. Animals: 70

Start Time: 1600
Conditions: 1000 ml total volume. Done in 1000 ml beakers with 600 ml liners. No aeration or stirring. Mud collected Aug. 22, 1977.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours)															
		1	2.5	5	8	20	24	48									
Control	10	0	0	0	0	0	0	0									
1%	10	0	0	0	0	0	0	0									
5%	10	0	0	0	0	0	0	0									
10%	10	1	1	3	4	4	4	4									
15%	10	1	5	5	5	6	6	8									
20% A	10	10	10	10	10	10	10	10									

CUMULATIVE MORTALITIES

Test No: 13 unstirred
Start Date: 8-29-77
Species: Pandalus hypsinotus
No. Test Tanks: 12
Total No. Animals 120

Mean Lengths: 28.4 mm
Mean Wet Weights: 16.7 g
Start Time: 1645
Temp. at Start: 13.2°C
Conditions: 60 ℓ total volume.
Aerated only, no stirring.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours) 2	4	18	24	48	72	96
Control A	10	0	0	0	0	0	0	0
Control B	10	0	0	0	0	0	0	0
5% A	10	0	0	0	0	0	1	1
5% B	10	0	0	0	0	0	0	1
7.5% A	10	0	0	0	0	0	1	1
7.5% B	10	0	0	0	0	1	1	1
10% A	10	0	0	0	0	0	0	0
10% B	10	1	1	1	3	3	4	4
12.5% A	10	1	2	2	2	2	2	2
12.5% B	10	0	0	0	0	1	1	1
15% A	10	4	8	9	9	10	10	10
15% B	10	5	5	5	5	5	5	5

CUMULATIVE MORTALITIES

Test No: 14
Start Date: 8-29-77
Species: Neomysis integer
No. Test Tanks: 24
Total No. Animals : 240

Start Time: 1645
Temp. at Start: 13.2°C
Conditions: Run simultaneously with Bioassay No. 13.

Concentrations (Volume:Volume)	* Animals/ Tank	Observation Times (Hours)						
		2	16	24	48	72	96	
Control A-stirred	10	0	0	0	0	0	0	
Control B- "	5	0	0	0	0	0	0	
0.1% A- "	9	0	0	0	0	0	0	
0.1% B- "	10	0	0	0	0	0	0	
1% A- "	5	0	0	0	0	0	0	
1% B- "	7	0	0	0	0	0	1	
2.5% A- "	5	0	0	0	0	1	1	
2.5% B- "	8	0	0	0	1	1	8	
5% A- "	10	0	0	0	6	6	6	
5% B- "	6	0	2	2	2	3	3	
10% A- "	10	1	3	4	9	9	10	
10% B- "	6	0	2	3	6	6	6	
Control A-unstirred	9	0	0	0	1	1	1	
Control B- "	10	0	0	0	1	1	1	
5% A- "	10	0	0	0	0	0	0	
5% B- "	9	0	0	0	0	0	0	
7.5% A- "	7	0	0	0	0	0	2	
7.5% B- "	9	0	0	0	1	1	1	
10% A- "	8	0	0	0	0	0	0	
10% B- "	8	0	0	0	1	1	1	
12.5% A- "	9	0	0	0	0	0	0	
12.5% B- "	7	0	0	0	0	0	0	
15% A- "	10	5	8	8	8	10	10	
Δ15% B- "	10							

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* 10 animals were originally introduced in each tank. These numbers are low due to loss of organisms through screen.

Δ Test solution lost at 1600 hour observation.

CUMULATIVE MORTALITIES

Test No: 16
Start Date: 9-3-77
Species: Neomysis sp.
No. Test Tanks: 20
Total No. Animals 200

Start Time: 1330
Temp. at Start: 12.9°C
Conditions: 1000 mls total volume.
Stirring done by aeration.

Concentrations (Volume:Volume)	Animals/ Tank	Observation Times (Hours) 24	48
Control A	10	1	1
Control B	10	0	0
1% A	10	0	0
1% B	10	0	0
2.5% A	10	0	0
2.5% B	10	3	4
5% A	10	0	3
5% B	10	0	2
5% C	10	3	5
5% D	10	0	1
7.5% A	10	2	3
7.5% B	10	3	6
10% A	10	2	7
10% B	10	2	3
10% C	10	3	5
10% D	10	6	8
12.5% A	10	2	8
12.5% B	10	1	6
15% A	10	5	10
15% B	10	6	10

CUMULATIVE MORTALITIES

Test No: 17
Start Date: 9-3-77
Species: <u>Pandalus hypsinotus</u>
No. Test Tanks: 15
Total No. Animals: 120

Mean Lengths: 29.2 mm
Mean Wet Weights: 19.0 g
Start Time: 2000
Temp. at Start: 14.20°C
Conditions: All stirred, aerated

Concentrations (Volume:Volume)	Animals/ Tank	Observation Time					
		3	9	18	24	48	
Control A	10	0	0	0	0	0	
Control B	10	0	0	0	0	0	
5% A whole mud	10	0	1	2	2	4	
5% B whole mud	10	0	1	2	2	3	
10% A whole mud	10	0	7	9	9	9	
10% B whole mud	10	7	7	9	9	9	
5% A pH at ambient	10	0	0	0	0	3	
5% B pH at ambient	10	1	1	1	1	3	
10% A pH at ambient	10	9	10	10	10	10	
10% B pH at ambient	10	6	6	6	6	6	
5% A Barite only	10	0	0	0	0	0	
5% B Barite only	10	0	0	0	0	0	
10% A Barite only	10	0	0	0	0	1	
10% B Barite only	10	0	0	0	0	0	
20 Barite only	10	0	0	0	0	0	

CUMULATIVE MORTALITIES

Test No: 19	Mean Lengths: 28.4 mm
Start Date: 9-6-77	Mean Wet Weights: 21.3 g
Species: Pandalus hypsinotus	Start Time: 1400
No. Test Tanks: 6	Temp. at Start: 12.5°C
Total No. Animals: 60	Conditions: Gel only

		Observation Times (hours)				
Concentrations (Volume:Volume)	Animals/ Tank	6	12	19	24	
Control A	10	0	0	0	0	
Control B	10	0	0	0	0	
5% A	10	0	0	0	0	
5% B	10	0	0	0	0	
10% A	10	0	0	0	0	
10% B	10	0	0	0	0	

CUMULATIVE MORTALITIES

Test No: 20
Start Date: 9-8-77
Species: Neomysis integer
No. Test Tanks: 12
Total No. Animals: 120

Start Time: 1100
Temp. at Start: 12.7°C
Conditions: Mysids placed in beakers containing solutions of supernatant, no stirring.

Concentrations (Volume:Volume)	Animals/ Tank	24	48 hour observation
Control A	10	0	0
Control B	10	0	1
1% A	10	0	0
1% B	10	1	1
2.5% A	10	0	1
2.5% B	10	0	0
5.0% A	10	1	2
5.0% B	10	0	0
7.5% A	10	0	2
7.5% B	10	1	3
10% A	10	1	1
10% B	10	1	2

CUMULATIVE MORTALITIES

Test No: 21
Start Date: 9-9-77
Species: Artemia salina
No. Test Tanks: 12
Total No. Animals: 120

Start Time: 1415
Temp. at Start: 19.3°C
Conditions: Artemia placed in petri dish at room temp., no stirring or aeration

Concentrations (Volume:Volume)	Animals/ Tank	Observation (48 hours)
Control A	10	0
Control B	10	1
1% A	10	0
1% B	10	0
2.5% A	10	0
2.5% B	10	1
5.0% A	10	1
5.0% B	10	0
7.5% A	10	1
7.5% B	10	1
10% A	10	0
10% B	10	1

CUMULATIVE MORTALITIES

Test No: 22
Start Date: 9-16-77
Species: Leptocottus armatus
No. Test Tanks: 8
Total No. Animals: 48

Mean Lengths: 14.3 cm
Start Time: 1400
Temp. at Start: 12.6°C
Conditions: stirring with mechanical stirrers, 60 liters total volume, aeration by airstones.

Concentrations (Volume:Volume)	Animals/ Tank	Observations (hours)						
		1	2	4	8	24	32	48
Control A	6	0	0	0	0	0	0	0
Control B	6	0	0	0	0	0	0	0
5% A	6	0	0	0	0	0	0	0
5% B	6	0	0	0	0	0	0	0
10% A	6	0	0	0	0	0	0	6*
10% B	6	0	0	0	0	0	0	0
20% A	6	0	0	0	0	5	6	6
20% B	6	0	0	0	0	6	6	6

* Loss of aeration - low D.O.

APPENDIX 3

WATER QUALITY MEANS AND RANGES FOR BIOASSAY TEST TANKS

BIOASSAYS 13, 14 - stirred

Treatment	Control A	Control B	.1% A	.1% B	1% A	1% B	2.5% A	2.5% B	5% A	5% B	10% A	10% B
Temp. mean	12.95	13.08	13.02	12.96	13.05	13.08	13.10	13.10	13.15	13.08	13.15	13.40
range	12.6/13.4	12.9/13.2	12.9/13.2	12.6/13.3	12.8/13.4	12.9/13.2	12.9/13.3	12.8/13.4	12.3/13.6	12.7/13.7	12.7/14.0	13.2/13.8
pH mean	7.80	7.85	7.75	7.78	7.90	7.68	7.80	7.80	8.03	8.08	8.25	8.10
range	7.6/7.9	7.8/7.9	7.5/8.0	7.6/8.0	7.8/8.0	7.5/7.8	7.6/8.1	7.7/8.0	7.9/8.1	8.0/8.2	8.2/8.3	7.8/8.3
D. O. mean	10.53	10.36	10.36	10.29	8.94	9.79	8.54	9.88	7.86	9.49	9.11	9.44
range	10.3/10.8	10.2/10.8	10.2/10.8	10.1/10.6	6.3/10.2	9.4/10.5	5.6/10.6	9.5/10.5	3.1/10.3	8.6/10.1	8.0/10.2	8.9/10.2
Salinity mean	26.23	26.30	26.28	26.23	26.38	26.28	26.00	25.98	25.50	25.08	23.60	24.80
range	26.0/26.9	26.0/26.5	26.1/26.4	26.0/26.8	26.4/26.8	25.9/26.7	25.7/26.5	25.6/26.4	25.2/25.9	24.9/25.4	23.3/24.0	24.5/25.4

BIOASSAY 13, 14 - unstirred

Treatment	Control A	Control B	5% A	5% B	7.5% A	7.5% B	10% A	10% B	12.5% A	12.5% B	15% A	15% B
Temp. mean	13.53	13.50	13.70	13.63	13.75	13.83	13.50	13.60	13.63	13.60	14.18	13.83
range	13.0/13.8	13.3/13.7	13.1/14.1	14.1/13.6	9.6/10.3	13.6/14.1	12.8/14.2	13.0/14.1	12.8/14.3	12.8/14.1	13.4/14.8	12.8/14.7
pH mean	7.73	7.78	7.85	7.83	7.93	7.90	7.88	7.88	7.95	8.00	8.15	8.13
range	7.6/7.8	7.7/7.8	7.8/8.0	7.6/8.0	7.8/8.1	7.7/8.1	7.8/8.0	7.7/8.0	7.9/8.1	7.9/8.1	7.9/8.5	8.0/8.3
D. O. mean	9.96	10.08	9.14	9.29	9.43	9.24	9.64	9.48	9.88	9.66	8.70	9.60
range	9.7/10.4	10.0/10.2	8.3/10.3	8.2/10.7	8.0/10.3	9.3/10.4	9.1/10.4	8.8/10.5	9.6/10.4	9.4/10.4	8.0/9.5	9.1/10.1
Salinity mean	26.43	26.88	26.90	26.78	26.08	26.60	25.78	25.73	25.30	25.13	24.48	24.70
range	26.2/26.5	26.8/26.9	26.5/26.9	26.4/27.2	25.7/26.9	26.2/27.6	25.2/26.1	24.2/26.4	23.2/26.1	22.8/26.0	24.0/24.9	23.9/25.2

BIOASSAY 11 - stirred

Treatment	Control A	Control B	.1% A	.1% B	.5% A	.5% B	1% A	1% B	2% A	2% B	3% A	3% B
Temp. mean	13.02	13.88	13.82	13.06	13.02	13.02	12.94	13.08	12.90	12.88	13.02	13.00
range	12.9/13.7	12.6/13.3	12.6/13.3	12.5/14.0	12.6/14.0	12.3/14.2	12.4/13.6	12.4/14.3	12.5/13.0	12.5/13.4	12.3/14.2	12.3/14.2
pH mean	7.48	7.50	7.58	7.60	7.64	7.66	7.76	7.78	7.98	8.04	8.14	8.08
range	7.3/7.7	7.4/7.6	7.3/7.8	7.3/7.8	7.4/7.9	7.4/7.9	7.5/8.0	7.6/8.0	7.7/8.3	7.8/8.3	8.0/8.4	8.0/8.4
D. O. mean	10.24	10.38	10.24	10.02	9.84	10.02	10.00	9.74	9.82	9.26	9.92	10.06
range	9.8/11.0	10.0/11.0	9.8/10.8	9.8/10.6	9.0/10.8	9.5/10.6	9.7/10.9	9.6/10.7	9.0/10.9	8.0/10.8	9.3/10.0	9.6/10.4
Salinity mean	28.82	28.66	28.68	28.44	28.74	28.68	28.48	28.60	28.16	28.20	28.16	28.18
range	28.6/29.2	28.5/29.0	28.5/29.1	28.8/29.4	28.5/29.0	28.2/28.9	28.2/28.8	28.3/28.9	28.0/28.5	28.1/28.5	27.9/28.4	28.0/28.4

BIOASSAY 11 - unstirred

Treatment	Control A	Control B	.1% A	.1% B	.5% A	.5% B	1% A	1% B	2% A	2% B	3% A	3% B
Temp. mean	13.48	12.68	13.48	13.28	13.52	13.52	13.42	13.00	13.52	12.90	12.46	13.22
range	12.5/14.2	12.5/13.4	12.5/14.5	12.6/14.0	12.4/14.6	12.5/14.5	12.6/14.5	12.5/13.6	12.3/14.7	12.3/13.5	12.5/14.5	12.5/14.0
pH mean	7.56	7.52	7.60	7.60	7.64	7.68	7.72	7.80	7.98	7.88	8.08	8.16
range	7.4/7.7	7.3/7.6	7.4/7.8	7.4/7.8	7.5/7.8	7.5/7.9	7.4/8.0	7.5/8.0	7.6/8.3	7.5/8.3	7.8/8.4	7.8/8.4
D. O. mean	10.00	10.08	9.88	10.06	9.90	9.58	9.42	9.92	9.14	9.08	9.78	9.04
range	9.6/10.6	9.6/10.6	9.4/10.2	9.4/10.08	9.5/10.7	8.7/10.6	7.4/10.7	9.3/10.6	8.7/10.6	8.4/10.6	9.5/10.6	8.8/10.6
Salinity mean	28.86	28.80	28.74	28.46	28.78	28.82	28.56	28.66	28.50	28.62	28.16	28.26
range	28.6/29.4	28.6/29.5	28.5/28.9	28.2/28.7	28.5/29.1	28.6/29.2	28.0/29.5	28.2/29.2	28.4/28.6	28.3/29.2	28.0/28.4	28.0/28.8

BIOASSAYS 9, 10

Treatment	Control A	Control B	2.5% A	2.5% B	5% A	5% B	10% A	10% B	15% A	15% B	20% A	20% B
Temp. mean	11.42	11.48	11.50	11.60	11.68	11.72	11.90	11.62	12.08	12.02	12.04	12.34
range	10.9/12.5	10.8/12.5	10.9/12.5	11.1/12.7	11.2/12.6	11.2/12.5	11.2/12.6	11.1/12.5	11.3/12.8	11.2/12.7	11.2/13.2	11.5/13.3
pH mean	7.46	7.42	7.64	7.66	7.88	7.96	8.14	8.10	8.30	8.36	8.26	8.28
range	7.1/7.8	7.1/7.7	7.4/7.9	7.3/7.8	7.7/8.0	7.9/8.2	7.9/8.4	7.8/8.4	7.9/8.8	8.0/8.8	7.9/8.6	7.7/8.7
D.O. Mean	10.06	10.44	10.40	10.28	10.52	10.28	10.16	10.20	10.26	10.10	10.16	9.94
range	9.4/10.8	9.8/10.9	10.2/10.6	10.1/10.5	10.2/10.9	9.9/11.1	10.2/9.8	9.9/10.4	9.7/10.6	9.6/10.5	9.9/10.7	9.5/10.2
Salinity mean	28.74	28.74	28.40	28.22	27.98	27.76	26.98	26.78	25.74	25.54	24.66	24.70
range	28.3/29.2	28.3/28.2	28.0/28.9	27.5/28.8	27.2/28.2	27.3/28.4	26.3/28.2	26.3/27.5	25.0/26.9	25.2/26.3	24.1/25.2	24.3/25.5

BIOASSAY 1

Treatment	Control A	Control B	.025% A	.025% B	.1% A	.1% B	.5% A	.5% B	1% A	1% B	5% A	5% B	6.7%	10%
Temp. mean	12.09	12.03	12.11	12.09	12.01	12.01	12.01	12.09	12.01	12.03	12.01	12.03	12.12	12.12
range	11.3 12.8	11.3/12.7	11.3/12.8	11.3/12.8	11.3/12.6	11.3/12.7	11.3/12.6	11.3/12.8	11.3/12.6	11.3/12.6	11.3/12.7	11.3/12.5	11.3/12.9	11.3/12.8
D.O. mean	10.5	10.6	10.42	10.50	10.56	10.46	10.00	10.42	10.04	10.32	9.28	9.94	10.18	10.18
range	10.2/11.0	10.0/11.3	9.8/11.2	10.4/11.1	10.0/11.3	10.1/11.2	9.9/10.4	9.5/11.2	8.9/10.8	9.6/11.3	6.6/10.2	9.5/10.4	9.6/10.8	9.8/10.8
Salinity mean	28.80	28.74	28.74	28.64	28.66	28.60	28.58	28.60	28.40	28.72	28.10	28.08	27.54	27.16
range	28.5/29.0	28.4/29.2	28.4/29.0	28.3/29.0	28.1/29.1	28.1/29.1	28.4/29.2	28.1/29.1	28.0/29.0	28.5/29.0	27.6/28.9	27.5/28.5	27.0/29.0	26.5/27.5

BIOASSAY 4

Treatment	Control A	Control B	.50% A	.5% B	1% A	1% B	5% A	5% B	10% A	10% B	20% A	20% B
Temp. mean	12.04	11.78	11.68	11.68	11.74	12.04	12.14	11.7	12.53	12.63	12.80	13.20
range	11.1/13.3	10.4/13.9	10.2/13.5	10.4/13.3	11.4/13.5	11.2/13.3	11.4/13.5	10.2/13.6	11.5/13.6	11.9/13.6	11.6/14.2	12.5/14.6
D.O. mean	10.54	10.52	10.56	10.50	10.42	10.56	10.80	10.40	10.80	10.73	10.30	10.20
range	9.8/10.9	9.9/10.8	9.8/11.2	10.0/11.2	10.0/10.8	9.8/11.2	9.9/11.8	10.0/10.6	10.6/11.0	10.6/10.9	9.6/11.2	9.6/11.1
Salinity mean	28.58	29.60	28.86	29.10	29.02	28.56	27.14	28.22	26.67	26.53	23.50	24.40
range	28.2/29.5	29.0/31.4	28.5/29.7	28.8/30.9	28.7/30.0	28.1/29.5	23.9/29.0	27.1/29.0	27.1/29.1	25.1/27.9	25.0/27.4	25.0/27.4

BIOASSAYS 5, 6, 7

Treatment	Control A	Control B	.5% A	.5% B	1% A	1% B	3% A	3% B	5% A	5% B	7% A	7% B
Temp. mean	10.02	9.90	9.88	10.12	9.94	9.96	10.08	10.10	10.10	10.10	10.10	10.22
range	9.9/10.2	9.7/10.1	9.6/10.1	9.9/10.7	9.8/10.2	9.7/10.5	9.8/10.9	9.8/11.0	9.9/10.6	10.0/10.9	9.9/10.8	10.0/11.0
pH mean	7.58	7.54	7.56	7.56	7.64	7.62	7.88	7.66	8.00	7.84	8.06	7.86
range	7.4/7.7	7.4/7.7	7.4/7.8	7.4/7.8	7.5/7.8	7.5/7.8	7.5/8.0	7.5/8.0	7.8/8.1	7.7/8.2	7.9/8.3	7.7/8.1
D.O. mean	10.70	10.66	10.72	10.70	10.72	10.76	10.62	10.68	10.54	10.54	10.42	10.62
range	10.6/10.9	10.4/11.0	10.6/10.8	10.5/10.9	10.7/10.8	10.7/10.8	10.6/10.8	10.6/10.8	10.5/10.6	10.4/10.6	10.2/10.6	10.6/10.7
Salinity mean	28.94	28.94	28.88	28.84	28.88	28.90	28.44	28.52	28.06	28.22	27.72	27.84
range	28.8/29.1	28.8/29.1	28.6/29.1	28.6/29.0	28.5/29.9	28.7/29.0	28.2/28.7	28.4/28.8	27.9/28.4	28.1/28.5	27.4/28.0	27.6/28.1

BIOASSAY 8

Treatment	Control A	Control B	.5% A	.5% B	1% A	1% B	5% A	5% B	6.7%	10%
Temp. mean	10.56	10.46	10.52	10.38	10.46	10.38	10.54	10.38	10.46	10.44
range	10.1/10.8	10.3/10.6	10.1/10.8	10.1/10.6	10.3/10.7	10.2/10.6	10.1/10.8	10.1/10.6	10.1/10.8	10.1/10.7
pH mean	7.34	7.40	7.48	7.42	7.40	7.44	7.49	7.44	7.60	7.71
range	7.2/7.5	7.3/7.6	7.4/7.5	7.3/7.6	7.3/7.6	7.4/7.6	7.5/7.7	7.3/7.6	7.5/7.8	7.5/8.1
D.O. mean	10.92	11.14	11.20	11.28	11.32	11.26	11.26	11.00	10.62	10.84
range	10.4/11.4	10.6/11.4	10.6/11.5	10.6/11.5	10.6/11.5	10.6/12.0	10.5/11.6	10.3/12.6	10.5/11.4	10.4/11.2
Salinity mean	29.92	30.12	29.84	29.94	29.78	29.78	29.14	29.32	29.08	29.10
range	29.8/30.1	29.8/30.5	29.4/30.0	29.6/30.2	27.1/30.2	29.1/30.1	28.9/29.4	28.9/29.9	28.5/29.0	27.4/28.5

BIOASSAY 16

Treatment	Control A	Control B	1% A	1% B	2.5% A	2.5% B	5% A	5% B	5% C	5% D
Temp. mean	14.95	15.00	14.90	14.85	15.00	15.15	15.00	15.00	15.05	15.00
range	13.0/16.9	13.0/17.0	13.0/16.8	13.0/16.7	13.0/17.0	13.3/17.0	13.2/16.8	13.1/16.9	13.1/17.0	13.0/17.0
D.O. mean	9.90	9.75	9.85	9.90	9.80	9.90	9.75	9.65	9.45	9.75
range	9.6/10.2	9.5/10.0	9.2/10.5	9.2/10.6	9.3/10.3	9.3/10.5	9.2/10.3	9.2/10.1	8.5/10.4	9.1/10.4

Treatment	7.5% A	7.5% B	10% A	10% B	10% C	10% D	12.5% A	12.5% B	15% A	15% B
Temp. mean	15.05	15.20	15.30	15.20	15.20	15.30	15.20	15.35	15.30	15.25
range	13.0/17.0	13.4/17.0	13.6/17.0	13.4/17.0	13.4/17.0	13.4/17.20	13.4/17.0	13.5/17.2	13.3/17.3	13.4/17.1
D.O. mean	9.85	9.75	9.65	9.75	9.45	9.90	9.60	9.35	9.50	9.55
range	9.1/10.6	9.0/10.5	8.9/10.5	8.9/10.6	9.0/9.9	9.0/10.8	8.9/10.3	8.9/9.8	9.2/9.8	9.1/10.0

BIOASSAY 20

Treatment	Control A	Control B	1% A	1% B	2.5% A	2.5% B	5% A	5% B	7.5% A	7.5% B	10% A	10% B
Temp. mean	13.25	13.25	13.30	13.30	13.35	13.40	13.40	13.35	13.50	13.45	13.50	13.45
range	12.7/13.8	12.7/13.8	12.7/13.9	12.7/13.9	12.8/13.9	12.8/14.0	12.9/13.9	12.8/13.9	13.0/14.0	13.0/13.9	13.0/14.0	12.9/14.0
pH mean	7.70	7.65	7.85	7.80	7.90	7.95	8.15	8.05	8.30	8.30	8.35	8.40
range	7.6/7.8	7.6/7.7	7.8/7.9	7.8/7.8	7.9/7.9	7.9/8.0	8.0/8.3	8.0/8.1	8.1/8.5	8.1/8.5	8.1/8.6	8.2/8.6
D.O. mean	10.15	10.15	10.15	10.15	10.10	10.15	10.00	10.10	10.20	10.25	9.75	9.75
range	9.9/10.4	9.8/10.5	9.8/10.5	9.7/10.6	9.6/10.6	9.7/10.6	9.6/10.4	9.6/10.6	9.7/10.7	9.9/10.6	9.0/10.5	8.9/10.6

BIOASSAY 22

Treatment	Control A	Control B	5% A	5% B	10% A	10% B	20% A	20% B
Temp. mean	12.40	12.43	12.57	12.53	12.67	12.77	13.17	13.17
range	12.2/12.6	12.0/12.7	12.4/12.7	12.4/12.8	12.6/12.7	12.6/12.9	12.7/13.8	12.7/13.8
pH mean	7.83	7.83	7.93	7.97	8.23	8.20	8.63	8.50
range	7.8/7.9	7.7/7.9	7.9/8.0	7.9/8.0	8.2/8.3	8.2/8.2	8.6/8.7	8.5/8.5
D.O. mean	9.57	9.27	9.60	9.50	7.33	8.97	9.37	9.23
range	9.4/9.7	9.2/9.4	9.2/10.1	9.4/9.6	3.2/9.7	8.7/9.3	9.2/9.6	9.1/9.4

APPENDIX 4

SAMPLES SUBMITTED FOR HISTOPATHOLOGICAL ANALYSIS (RESULTS FROM DR. MARSHA LANDOLT, ASSISTANT PROFESSOR, COLLEGE OF FISHERIES, UNIVERSITY OF WASHINGTON)

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Sample no. 1 - Shrimp (control - test no. 13)

The gill sections are generally unremarkable with the exception of focal areas of sinusoidal dilation and congestion. The epithelium in all sections of the gill is unremarkable. Also the supporting stroma is as expected. No cellular debris or bacteria are noted in the interlamellar spaces.

Sample no. 2 - Shrimp (10 percent stirred - test no. 13)

Widespread disruption and distortion of gill lamellae are present throughout the section. In the apical region of the gill there is pronounced bilateral congestion and distortion of the lamellae. In the mid and basal sections the congestion is absent, however, the distortion persists. Large numbers of epithelial cells are frankly necrotic; others are somewhat pyknotic. The lamellar sinusoids are markedly distended but lacking in blood. Contained within the interlamellar spaces are prominent amounts of coarse granular birefringent debris. Also present in the interlamellar spaces are moderate amounts of an eosinophilic amorphous proteinaceous material.

Sample no. 3 - Shrimp (15 percent unstirred - test no. 13)

Widespread disruption of the gill lamellae is noted throughout the section. This destruction consists of a dilatation of the sinusoidal spaces, also frank necrosis of the lamellar epithelium and of some pyknosis of the lammelar epithelium. In some lamellae, the entire structure has been destroyed. Other lamellae show a sparing effect with only a minimal change present. The most striking change is the presence within the septal sinusoid of large numbers of spherical, brightly eosinophilic cells with eccentric small spherical nuclei. The identity of these cells is not known; they were not seen in either of the other shrimp sections. The cells appear to be proliferating and they completely fill the main sinusoidal channel. The cells are not seen in the sinusoids of the lamellae themselves, however. Contained within the interlamellar spaces are prominent amounts of coarse granular birefringent debris. Also contained within the interlamellar spaces are large numbers of bacteria. Most of these bacteria are long bacilli.

Sample no. 4 - Salmon (0.1 percent stirred - test no. 11)

The submitted specimen is essentially unremarkable. The gill lamellae appear to be intact with an intact epithelium and with an obvious vascular supply. No. lesions are noted.

Sample no. 5 - Salmon (3 percent stirred - test no. 11)

The gill filaments are generally intact with the exception of some shrinkage of the epithelial layer from the vascular supporting layer. The lamellae are as expected. The one remarkable finding within the lamellae, however, is a pronounced hypertrophy and also hyperplasia of the chloride cells at the bases of the secondary lamellae. These cells are quite pronounced, are spherical with a brightly eosinophilic cytoplasm and an eccentrically placed nucleus. Most of these cells appear to be contained within the tissue of the lamellae themselves. Contained within the interlamellar spaces of this specimen are prominent amounts of a coarsly granular birefringent debris.

Sample no. 6 - Salmon (3 percent unstirred - test no. 11)

Widespread destruction of the gill lamellae is present throughout the section. Most of the destruction appears to be concentrated at the basal ends of the lamellae and consists of necrosis of both epithelium and the underlying vascular support. As in case no. 5, there is pronounced hypertrophy and hyperplasia of the chloride cells at the bases of the lamellae. Many of these chloride cells, because of the destruction of the lamellae, are free within the interlamellar space. Some of them, however, are still contained. The cells are spherical with brightly eosinophilic cytoplasm and an eccentrically placed nucleus. Contained within the interlamellar spaces are prominent amounts of granular birefringent debris. Also noted within the section are focal areas of cartilage hyperplasia. This cartilagenous hyperplasia is of limited extent.