NOAA Technical Memorandum
NMFS-SEFC-70

NOAA/NMFS FINAL
REPORT TO DOE

Shrimp and Redfish Studies, Bryan Mound Brine Disposal Site Off Freeport, Texas
1979-1981

A report to the Department of Energy on work conducted under provisions of Interagency Agreement DE-A10178US07146 during 1979-1981.

Volume VI
SHRIMP
BIOASSAYS

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Center
Galveston Laboratory
Galveston, Texas 77550

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NOAA Technical Memorandum
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VOL. VI -BRINE TOXICITY AND AVOIDANCE/ ATTRACTION BIOASSAYS ON SHRIMP

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I. EDITORS' SECTION

Volume VI - SHRIMP BIOASSAYS

TABLE OF CONTENTS

PROJECT ADMINISTRATION

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LIST OF VOLUMES

This Final Report is printed in six separate volumes:

Volume I - SHRIMP RECRUITMENT AND CATCH-EFFORT ANALYSIS

Work Unit 2 - Analysis of Data on Shrimping Success, Shrimp Recruitment and Associated Environmental Variables

Science Applications, Inc.

C. E. Comiskey

Work Unit 3 - Texas Coast Shrimp Catch and Effort Data Analysis

Science Applications, Inc.

C. E. Comiskey

Volume II - SHRIMP MARK-RELEASE

Work Unit 4 - Shrimp Mark-Release Investigations

LGL Ecological Research Associates, Inc.

M. F. Johnson, Ph.D.

Volume III - SHRIMP SPAWNING SITE SURVEY

Work Unit 5 - Shrimp Spawning Site Survey

LGL Ecological Research Associates, Inc.

B. J. Gallaway, Ph.D.
L. A. Reitsema

Volume IV - CATCH-EFFORT SAMPLING SURVEY

Work Unit 6 - Interview Sampling Survey of Shrimp Catch and Effort

LGL Ecological Research Associates, Inc.

M. F. Johnson, Ph.D.

Volume V - REDFISH BIOASSAYS

Work Unit 7 - Brine Toxicity and Avoidance/Attraction Bioassays on Redfish

LGL Ecological Research Associates, Inc.
and
Texas A&M University

D. W. Owens, Ph.D.
J. M. Neff, Ph.D.

Volume VI - SHRIMP BIOASSAYS

Work Unit 8 - Brine Toxicity and Avoidance/Attraction Bioassays on Shrimp

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INTRODUCTION

In compliance with the Energy Policy and Conservation Act of 1975, Title 1, Part B (Public Law 94-163), the Department of Energy (DOE) implemented the Strategic Petroleum Reserve (SPR) with the goal of storing a minimum of one billion barrels of crude oil. After evaluating several physical storage possibilities, DOE determined that storage in commercially developed salt dome cavities through solution-mining processes was the most economically and environmentally advantageous option.

Four coastal areas along the northwestern Gulf of Mexico were assessed for brine discharge into nearshore waters (Figure 1). This project, "Shrimp and Redfish Studies; Bryan Mound Brine Disposal Site off Freeport, Texas", deals with potential impacts of brine disposal from the Bryan Mound site. Under permit from the Environmental Protection Agency (EPA), this brine discharge site (Latitude 28° 44.28'N; Longitude 95° 14.64'W) was selected about 12.5 miles directly offshore of Bryan Mound.

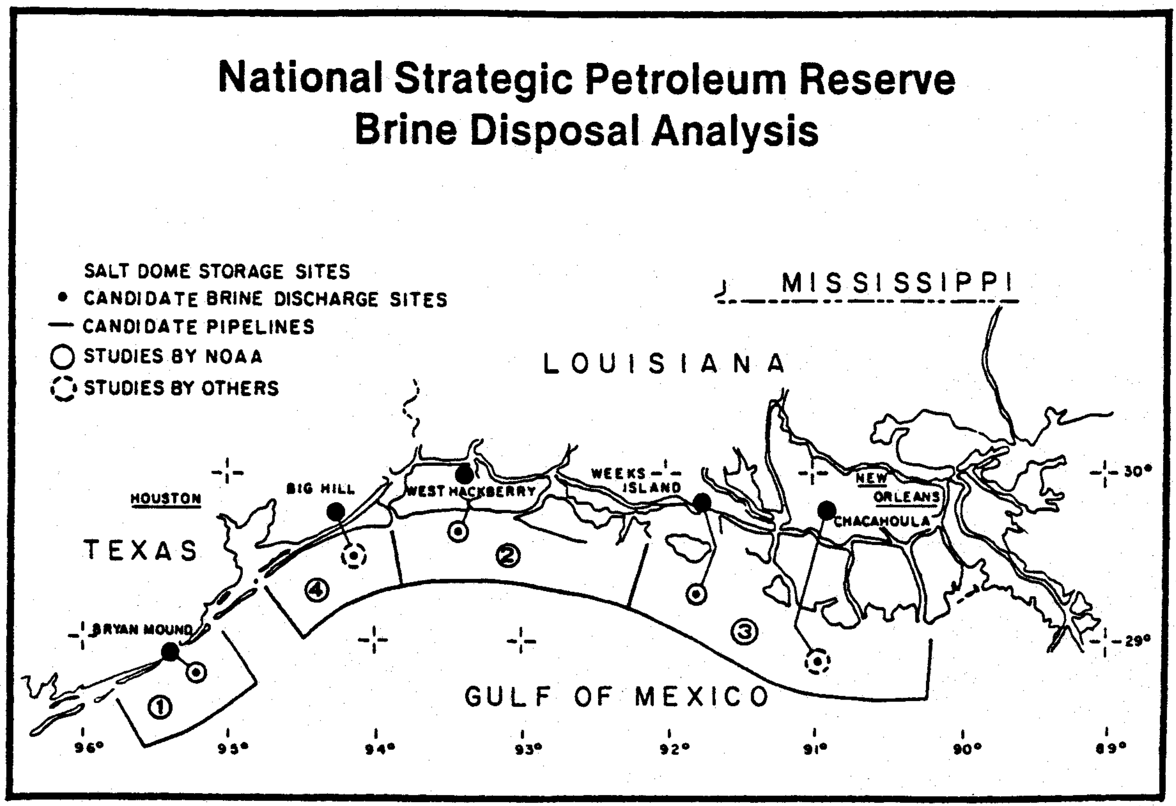

Figure 1. Regions of Study for Brine Disposal Assessment-DOE/NOAA Interagency Agreement (adapted from Environmental Data and Information Service, DOC/NOAA).

The process of creating a storage cavern within a salt dome involves dissolving the solid salts with raw water. The water source for leaching of the Bryan Mound salt dome is the Brazos River. Water from the Brazos River is piped under pressure into the dome. The resultant brine (dissolved salts) is discharged, at variable rates (over 100,000 barrels/ day) into the Gulf of Mexico.

To compliment site-specific oceanographic monitoring of brine disposal, a regional source assessment of important commercial and recreational fisheries was initiated in August, 1979. The objectives of this assessment are (1) to conduct a pre-discharge/post-discharge assessment of shrimp populations in relation to the Bryan Mound salt dome brine disposal site and (2) to determine acute toxicity and avoidance/attraction responses of shrimp and redfish to Bryan Mound brine. These objectives were achieved through field and laboratory investigations and through statistical analysis of the data. Specific studies included (1) analysis of data on shrimping success, shrimp recruitment and associated environmental variables, (2) analysis of Texas coast shrimp catch and effort data, (3) shrimp mark-release investigations, (4) shrimp spawning site survey, (5) interview sampling survey of shrimp catch and effort, (6) brine toxicity and avoidance/attraction bioassays on redfish and (7) brine toxicity and avoidance/attraction bioassays on shrimp.

The major products of the Shrimp and Redfish Studies are: Final Reports available through the National Technical Information Service (NTIS), Springfield, Virginia; data files available through the Environmental Data and Information Service (EDIS), Washington, D.C., and any publications that may be written by participating principal investigators and submitted to scientific or technical journals. Preliminary results have been made available through DOE/NOAA/NMFS project reviews and workshops attended by project participants and various governmental, private and public user groups.

Additional environmental data on the Bryan Mound site are available. The DOE has developed comprehensive Environmental Impact Statements listed below:

1. Strategic Petroleum Reserve - Seaway Group Salt Domes, June 1978, Final EIS, DOE/EIS-0021.

2. Strategic Petroleum Reserve - Bryan Mound Salt Domes, January 1977, Final EIS, FES 76/77-6.

3. Strategic Petroleum Reserve - Expansion of Reserve, January 1979, Final Supplement to Final EIS, FEA-FES-76-2.

All three reports are available from the U.S. Department of Commerce, National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, Virginia 22161.

Texas A&M University (TAMU) has conducted studies of physical oceanography, sediments, water quality, benthos and nekton at the Bryan Mound brine disposal site from September, 1977 to February, 1979. In addition, TAMU has developed a towed sensing system for tracking the brine plume. Results of this research are available in:

> Metzbower, H. T., S. S. Curry and F. A. Godshall. 1980. Handbook of the Marine Environment - Bryan Mound. NOAA Report to DOE Strategic Petroleum Reserve Program, Salt Dome Storage/Brine. 92 p.

The Massachusetts Institute of Technology (MIT) has developed a mathematical, 3-dimensional, hydrodynamic simulation model of the brine plume dispersion. The model and test-tank simulations have the capacity to evaluate effects of varying effluent discharge rates and currents and to identify various plume configurations and densities. Salinity dispersion was modeled showing that a dilution rate of 100:1 can be expected within 100 feet of the diffuser head. The MIT analyses are available in DOE's final Bryan Mound EIS (FES 76/77-6) listed earlier.

LIST OF REPORTS AND PUBLICATIONS

Shrimp and Redfish Studies, Bryan Mound Brine Disposal Site off Freeport, Texas 1979-1981

Comiskey, C. E. 1981. Analyses of data on shrimping success, shrimp recruitment and associated environmental variables. Vol. I. *In*: Jackson, W. B. and E. Peter Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-65. (In preparation).

Comiskey, C. E. 1981. Texas coast shrimp catch and effort data analysis. Vol. I. *In*: Jackson, W. B. and E. P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-65. (In preparation).

Gallaway, B. J. and L. A. Reitsema. 1981. Shrimp spawning site survey. Vol. III. *In*: Jackson, W. B. and E. P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-67, 84 p. Available from: NTIS, Springfield, Virginia.

Howe, N. R. 1981. Brine toxicity and avoidance/attraction bioassays on shrimp. Vol. VI. *In*: Jackson, W. B. and E. P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-70, 60 p. Available from: NTIS, Springfield, Virginia.

Johnson, M. F. 1981. Shrimp mark-release investigations. Vol. II. *In*: Jackson, W. B. and E. P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-66, 110 p. Available from: NTIS, Springfield, Virginia.

Johnson, M. F. 1981. Interview sampling survey of shrimp catch and effort. Vol. IV. *In*: Jackson, W. B. and E. P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-68. (In preparation).

Owens, D. W. and J. M. Neff. 1981. Brine toxicity and avoidance/attraction bioassays on redfish. Vol. V. *In*: Jackson, W. B. and

E. P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-69. (In preparation).

Biological/Chemical Survey of Texoma and Capline Sector Salt Dome Brine Disposal Sites off Louisiana, 1978-1979

Boehm, P. D. and D. L. Fiest. 1980. Determine hydrocarbons composition and concentration in major components of the marine ecosystem. Vol. VI. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-30, 136 p. Available from: NTIS, Springfield, Virginia.

Brooks, J. M. 1980. Determine seasonal variations in inorganic nutrient composition and concentration of the water column. Vol. VIII. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-32, 31 p. Available from: NTIS, Springfield, Virginia.

Hausknecht, K. A. 1980. Describe surficial sediments and suspended particulate matter. Vol. V. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-29, 56 p. Available from: NTIS, Springfield, Virginia.

Landry, A. M. and H. W. Armstrong. 1980. Determine seasonal abundance, distribution and community composition of demersal finfishes and macro-crustaceans. Vol. IV. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-28, 180 p. Available from: NTIS, Springfield, Virginia.

Margraf, F. J. 1980. Analysis of Variance of Gulf Coast shrimp data. Vol. IX. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-33, 293 p. Available from: NTIS, Springfield, Virginia.

Parker, R. H., A. L. Crowe and L. S. Bohme. 1980. Describe living and dead benthic (macro- and meio-) communities. Vol. I. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-25, 103 p. Available from: NTIS, Springfield, Virginia.

Reitsema, L. A. 1980. Determine seasonal abundance, distribution and community composition of zooplankton. Vol. II. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-26, 133 p. Available from: NTIS, Springfield, Virginia.

Schwarz, J. R., S. K. Alexander, A. J. Schropp and V. L. Carpenter. 1980. Describe bacterial communities. Vol. III. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-27, 48 p. Available from: NTIS, Springfield, Virginia.

Tillery, J. B. 1980. Determine trace metal composition and concentration in major components of the marine ecosystem. Vol. VII. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-31, 72 p. Available from: NTIS, Springfield, Virginia.

Related Pulications

Caillouet, C. W., F. J. Patella and W. B. Jackson. 1979. Relationship between marketing category (count) composition and ex-vessel value of reported annual catches of shrimp in the eastern Gulf of Mexico. Marine Fisheries Review 41(5-6):1-7.

Caillouet, C. W., F. J. Patella and W. B. Jackson. 1980. Trends toward decreasing size of brown shrimp, *Penaeus aztecus*, and white shrimp, *Penaeus setiferus*, in reported annual catches from Texas and Louisiana. NOAA/NMFS Fishery Bulletin 77(4):985-989.

Caillouet, C. W., D. B. Koi and W. B. Jackson. (1981). Relationship between ex-vessel value and size composition of annual landings of shrimp from the Gulf and south Atlantic Coasts. Marine Fisheries Review (in press).

Caillouet, C. W. and D. B. Koi. (1981). Trends in ex-vessel value and size composition of annual landings of brown, pink and white shrimp from the Gulf and south Atlantic coasts of the United States. Marine Fisheries Review (in press).

II. PRINCIPAL INVESTIGATORS' SECTION

WORK UNIT 8 - SHRIMP BIOASSAYS

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University of Houston

FOREWORD

This report summarizes the experiments comprising Work Unit 8, Contract No. NA79-GA-C-00031. All experiments were completed and all required data archived by December 8, 1980. The report is organized in manuscript form as required, in this case two manuscripts that deal with different sorts of experiments. Certain departures from normal manuscript style, such as placing legends on figures and inserting figures in the text, were incorporated to make the report more readable. Manuscripts are an excellent format idea, since they are likely to be more concise, more critically reviewed and, eventually, more public. The principal investigator will submit Chapters I and II for publication in *Environmental Pollution* by May, 1981. Information that should be in the report but does not warrant inclusion in a publishable manuscript is placed in this foreword, in a general conclusion and recommendation section and in appendices to the report. The only experiment treated in that fashion is an *in situ* bioassay, an experiment that was performed in good faith but that for reasons beyond the investigator's control was not definitive. No attempt was made in the report to present an exhaustive catalog of the data furnished to the National Marine Fisheries Service, rather, selective analyses that contributed directly to conclusions were discussed.

Without exception, where the cooperation of the Galveston Laboratory, NMFS, was either stipulated in advance or humbly requested at a later time, that assistance was rendered in a timely, friendly, and thoroughly professional way. Without that cooperation, some of the experiments described would doubtless still be in progress.

ABSTRACT

Salt brines mixed from salt dome salt (Ranch House Coarse Salt, United Salt Co.) or Instant Ocean (Aquarium Systems, Inc.) and Brazos River water or deionized water were tested for toxicity and for behavioral and physiological effects on large white shrimp (*Penaeus setiferus*) and large brown shrimp (*P. aztecus*). Results indicate that half of a group of animals at 25 C^{O}can be expected to die 48 hr after an addition of enough brine to raise salinity by about 22^{o}/oo or after 96 hr when salinity is increased by 18^{0}/oo. Sensitivity to brine was greater at 30^{0}C in both species, and white shrimp survived brine better as temperature was lowered to 15^{0}C. Animal size did not affect brine survival, nor were there consistent differences in the effects of different brine types. The latter finding suggests that the principal lethal effect of brine is osmotic stress rather than toxicity of trace constituents. The relationship between mean time to death and salinity increase indicates that large animals of either species can be expected to survive well over 500 hr at the worst-case salinity predictions (6.5^{0}/oo above ambient) for the diffuser site. Heart rate and animal color were significant signs of incipient brine distress.

Behavior experiments were conducted by exposing animals to brine plumes in still water or by offering animals a choice between brine-sea water mixtures and sea water. Results showed an increase in activity for animals that were subjected to brine-sea water mixtures 10^{0}/oo or more above their previous environmental salinities. In choice experiments, animals were somewhat repelled from toxic brine concentrations (20^{0}/oo) but not from lower tested concentrations. Neither behavioral effect is likely under field conditions where worst-case salinity increases are expected to be much lower than 10^{0}/oo.

TABLE OF CONTENTS

CHAPTER I.

Lethal and Sublethal Effects of a Simulated Salt-Brine Effluent and its Components on Adults and Subadults of Two Species of Penaeid Shrimp

INTRODUCTION

In March, 1980, the Department of Energy began to discharge brine, consisting of Bryan Mound Salt dome salt dissolved in Brazos River water, at rates exceeding 100,000 barrels per day from a diffuser located 20 km offshore from Freeport, Texas. One recurrent public concern has been that the brine discharge may adversely affect the commercial penaeid shrimp fishery, whether by direct toxic effects or by physiological or behavioral perturbations. Information of several kinds must be integrated to adequately assess those potentially adverse impacts:

1. Hydrographic profiles of the potentially affected area.
2. Estimates of the brine plume's behavior in space and time under a suitable range of environmental parameters.
3. Natural history (including abundance) of penaeid shrimp in the potentially affected area.
4. Toxic and sublethal effects of brine and its components on penaeid shrimp.

Reasonably good information is presently available for the first three items (NOAA, 1977; Texas A & M, 1979). An addition to our data on the final item is the goal of the present study.

The diffuser is located in water approximately 20 m deep at the bottom of a water column characterized by considerable vertical stability (Texas A & M, 1979). In the latter report, bottom salinities measured for one year at the sampling station nearest the diffuser site or at depths greater than 15 m near the diffuser averaged slightly more than $34^{o}/oo$ (range $32.4 - 37.0^{o}/oo$). Even during periods of abnormally high freshwater run-off, i.e. June, 1979, Dr. Donald Harper (personal communication) reports the bottom salinity at the diffuser site to be $33^{o}/oo$. Since the high density of the brine should confine its major effects to within 2 m of the bottom (NOAA, 1978), it is reasonable to assume that any effects of brine will occur in water with a normal ambient salinity of 34^{o}/oo. Temperatures of water deeper than 15 m near the site ranged from a summer high of 30^{o}C to a winter low of 10^{o}C.

Brine plume behavior has been modeled for a variety of projected current regimes (NOAA, 1977). Near field (0-100 feet) effects are considered to be dominated by diffuser jet mixing and, based on a worst-cast estimate of 1:45 dilution, should increase salinity by about 6.5^{o}/oo in the presence of little or no current. Intermediate field (to about 1000 feet) is dominated by lateral spreading of the brine. Far field dilution is produced by advection. The transient plume model has been used (NOAA, 1978) to generate durations of Eulerian (fixed with respect to the sea floor) exposure to various levels of increased salinity at selected locations near the diffuser for a simulated 90-day discharge. That simulation indicates that locations 2,000 feet parallel to the shore from the diffuser are exposed to an increased salinity of 2^{o}/oo for no more than 24 hours at a time.

Though penaeid shrimp are the dominant nekton species in the diffuser area (Texas A & M, 1979), it is likely that most penaeid shrimp spawn well inshore (white shrimp) or offshore (brown shrimp) from the expected far field limits (Galloway and Reitsema, 1981).

Whether brine at the projected concentrations will kill or disturb large penaeid shrimp in the area has not been explored, though some inferences can be drawn from related studies. Potential impact from brine consists of two components, elevated salinity and possible river water contamination. Further, the mode of action for impact from elevated salinity is far from clear. As Neff, et al. (1978) have pointed out, salt dome salt may be toxic because of toxic trace metals, because of altered major ion ratios (esp. Ca^{++}/Mg^{++}) or because it imposes an additional osmotic stress. Few studies have considered more than the last of these possibilities. The most comprehensive and applicable study of salinity effects on larger animals of either species in question is that of Venkataramiah, *et al*. (1974). Those results based on static bioassays suggest that the expected increase in salinity near the diffuser is well within the tolerance limits of juvenile brown shrimp, *Penaeus aztecus* (up to 75 mm), but that tolerance to extremes of salinity may decrease with increasing size up to 75 mm. Adults were not examined. The toxicity of a chemical feedstock (Dow Chemical) derived from Bryan Mound salt for early stages of white shrimp, *Penaeus setiferus*, has also been examined (Wilson, *et al*., 1979). Those results, though rather difficult to interpret, suggest that lethal limits for postlarvae may be as low as 38^{o}/oo (LD50-96) and that brine tolerance limits may decrease with increasing temperature above 28^{o}C.

The present study examines lethal and certain sublethal effects of a diluted simulated brine effluent and of a series of three control brines on adults and subadults of *P. setiferus* and *P. aztecus*.

MATERIALS AND METHODS

Test animals

Specimens of *P. setiferus* and *P. aztecus* from 6-11 cm in abdomen length were captured by commercial shrimp trawl offshore from Freeport, Texas, then maintained in a 15,000 ℓ outdoor holding tank equipped with sand substratum and subgravel filters at the laboratory in Galveston, Texas. Sea water was continually replaced in the holding tank at 0.3 tank volumes/day, and animals were fed approximately 20% of their volume of chopped, frozen squid daily during holding. Animals were held for at least five days before experiments. Mortality, salinity and temperature in the holding tank were monitored daily. Holding tank temperatures ranged from 15^{o}C to 31^{o}C during the experiments.

Acute toxicity experiments

For toxicity bioassays animals were transferred to glass 40 ℓ aquaria subdivided with plastic screen to accommodate five animals each. Tanks were placed in a constant-environment room and fitted with constant-level siphons adjusted to maintain water volumes of 25 ℓ. Aeration was provided in each chamber by a full-length perforated plastic air diffuser in each tank. Animals were exposed to 650 lx of fluorescent illumination for 14 hr daily, preceeded and followed by 1/2 hr of 200 lx simulated dawn and dusk. Air temperature in the aquarium room was controlled to within $1^{o}C$ of $25^{o}C$ in most experiments and inlet water temperature was controlled to within $0.1^{o}C$ of $25^{o}C$ by recirculating sea water from an insulated head tank through a sea water heat exchanger (Neslab SWHX + PBC-2). Temperatures were continuously monitored in room air and in five of 18 test tanks (Analog Devices multi-channel digital thermometer + Gould printer).

Four test brines were mixed 800 ℓ at a time by adding either Instant Ocean Synthetic Sea Salts without trace element mix (IO) (Aquarium Systems, Inc.) or Ranch House Coarse Salt (SD) (United Salt Co., Inc.) at approximately 320 g/ℓ to deionized, charcoal filtered water (DI) (<1μmho/cm) or to water (BR), collected in plastic carboys from the Brazos River at the intake structure for the DOE solution mining program. Instant Ocean was selected as a control salt to test potential effects of Brazos River water in brine, and Ranch House Salt, mined from a salt dome at Hockley, Texas, was selected as a substitute for Bryan Mound salt which was unavailable in crystalline form. Both salts, both diluents and a sample of natural sea water from the laboratory's supply were analyzed for major ions and certain trace elements by a commercial laboratory, Galveston Laboratories (Table 1). This analysis confirmed the similarity in major ion concentrations between salts from the Hockley and Bryan Mound domes. Copper and cadmium concentrations in seawater were measured by Dr. Paul Boothe (Texas A & M University), using flameless atomic absorption spectrometry after co-precipitation (Fe) with APDC. Salt and diluent suspensions were continually agitated (and heated to 35°C during cold weather) until the specific gravity of the clear supernatant liquid reached 1.20, at which time the supernatant brine was pumped into reservoirs above the aquarium room.

Table 1. Chemical analyses of test salts, test diluents, and Galveston laboratory seawater.

Ion	Units	Deionized Water	Brazos River Water[a]	Sea-Water	Ranch House Salt	Instant Ocean	Bryan Mound[b]
Na^+	ppm	17	844 (4000)	9300	461000	414000	373800
Cl^-	ppm	<5	4780 (6200)	19990	617300	537000	617000
K^+	ppm	3	190 (200)	750	5600	18100	940
Mg^{++}	ppm	5	247 (380)	958	30	82500	29
Ca^{++}	ppm	1	55 (200)	325	9750	16000	2290
SO_4^{--}	ppm	12	576 (890)	2100	23650	56000	6230
Cu^{++}	ppb	<10	12	1.05[c]	<10	<10	<30
Cd^{++}	ppb	<50	<50	0.085[c]	<50	<50	<6
Zn^{++}	ppb	<10	----------	----------	----------	<10	254
Ag^+	ppb	<10	----------	----------	----------	<10	<30

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[a] Values in parentheses provided by Neff, personal communication.

[b] Values for Bryan Mound salt are recalculated from measurements of a 317^{o}/oo brine reported by Texas A&M University (James, 1977).

[c] Measured by Dr. Paul Boothe, Dept. of Oceanography, Texas A & M University

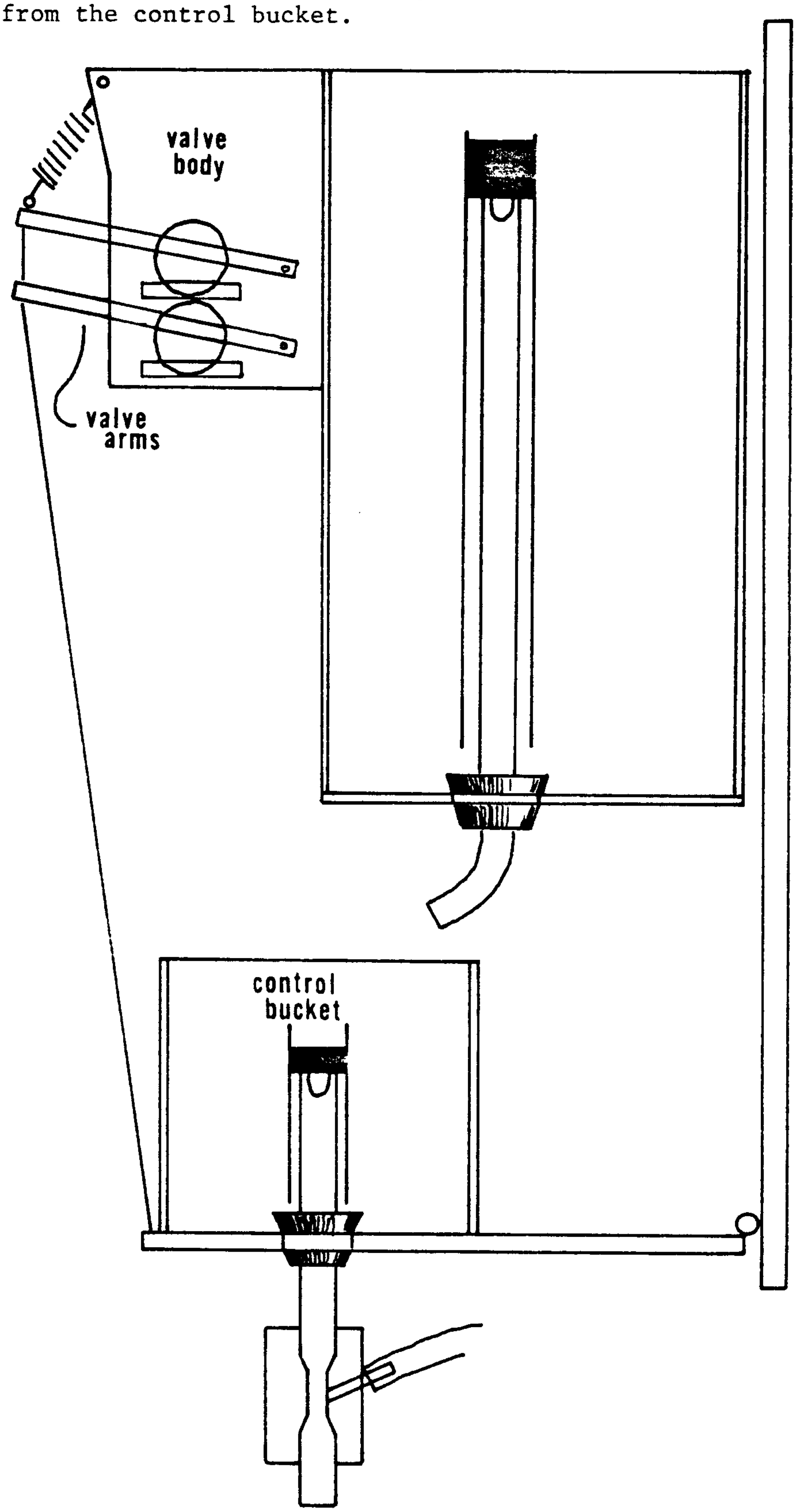
Brine flowed by gravity through a float valve into a head tank in the aquarium room from which it was diluted with sea water from the temperature-controlled head tank in a modified proportional diluter (Mount and Brungs, 1967). Modifications included elimination of the toxicant-mixing cell to accommodate proportions of brine up to 10%, replacement of the standard solenoid (or needle) water and toxicant valves with a newly designed tubing valve (Figure 1) that functioned well with saturated, therefore crystallizing, salt brine, and construction with acrylic sheet instead of glass. Outputs from each of five dose chambers and the single control chamber were divided into three equal streams that were passed into the three test tanks for each dose, yielding eighteen test tanks, containing a total of 90 animals for each test. The diluter was adjusted to exchange the water in each test tank five times each day.

Animals without obvious lesions or missing appendages were placed in the chambers of the test tanks filled with water at holding tank temperature. Chamber assignments were randomized by lots. Test tank temperature was then gradually brought to 25°C over 24 hours, if holding tank temperature was within 5° of 25°C, or over 48 hours if holding tank temperature was from 15-20°C. Animals were not fed during this acclimation period or during the subsequent bioassay.

A dose series was selected in a range-finding static bioassay. For each experiment brine concentrations ranged from approximately 40°/oo to 50°/oo, with adjacent doses differing by a factor of 1.17 in total osmolality. Since brines prepared from synthetic sea salts and salt dome salt differed in the relationship between salinity (as grams of salt per liter of solution) and osmolality (as milliosmols per liter), brine doses were expressed as milliosmols above control sea water osmolality, and salinities of all brine sea water solutions were measured with a vapor-pressure osmometer (Wescor 5100B).

At the conclusion of the 24 or 48 hour acclimation period (1000 to 1200 local time), toxicity experiments were begun by turning on brine flow. This method of initiating brine delivery, one of several recommended by EPA (1975),

Figure 1. Schematic diagram of a mechanical valve used to interrupt brine and sea water flows into the proportional diluter. The moving part of the valve is operated by the weight of water flowing from the control bucket.



provided gradual exposure of test animals to final toxicant concentration. At five exchanges per day, salt concentration reached 75% of its final value within about seven hours and 90% after about 12 hours. Observations were made then and 0.75, 1.5, 3, 6, 12, 24, 48 72 and 96 hours later. The use of this modified logarithmic time series as recommended by Sprague (1969) permitted estimates of mortality proportion at fixed times and of mean time to death as a function of dose. Observations included mortality, molting, behavior and color for all animals and heart beat rate (time for 10 beats) for five quiescent animals at each dose. Death was defined as lack of movement, heart beat and scaphognathite (gill bailer) movement and failure to move in response to repeated touches with a probe. Behavior was scored as quiescent for animals oriented normally on the tank bottom or clinging to a chamber divider without pleopod or periopod movement, active for swimming animals or animals that oriented normally and showed pleopod movement, and moribund for live animals that failed to orient normally. Color was scored on a subjective scale from 0 (translucent) to 4 (very opaque with marked cuticular mottling). Dead animals were removed, measured and sexed and exuvia were removed at each observation. After the 96 hour observation, five animals from the survivors in each dose (fewer if fewer than five survived) were selected by lots, then blotted for 30 seconds, weighed, measured and sexed. The cuticle over the pericardium was then removed, the pericardium slit and hemolymph (about 5 µl) transferred to a paper disc to determine hemolymph osmolality. Carcasses were then dried to constant weight at 90 C and reweighed to determine body water content.

Median lethal time (LT 50) was computed for doses in which significant mortality occurred by graphic approximation to probit analysis (Litchfield and Wilcoxon, 1949). Median lethal brine concentration (LC50) was computed at 48 and 96 hours by the trimmed Spearman-Karber method (Hamilton, et al., 1977). Analyses of variance were performed with program P2V of the BMDP series (UCLA, 1977). 96-hour experiments were performed twice for each combination of species, salt type, and diluent type, once during the period from April through August (spring) and once from September through December (fall). In addition, the effects of temperature on brine toxicity were investigated in a series of shorter (48 hr) experiments at 15, 20, and 30°C performed with Brazos River water and salt dome salt with two doses plus control and ten animals per dose.

RESULTS

Acute brine toxicity

Dose-mortality data and lethal times are summarized in Tables 2 and 3. Each experiment furnished between one and five estimates of LT50, depending on the rate of mortality caused by the five doses in each experiment. Log (LT50) was strongly negatively correlated with log(dose) for both *P. setiferus* ($r=0.81$, $p<0.01$) and *P. aztecus* ($r=0.81$, $p<0.01$). Those relationships are shown graphically in Figure 2 with least squares regression lines fitted for each species. Species regression lines did not differ significantly ($p>0.5$). The design of the test (96 hr duration and gradual brine onset) did not allow for meaningful LT50 estimates of less than 20 hr or more than about 400 hr. LT50 values were subjected to analysis of variance to detect potential effects of species, salt type, diluent type, or animal size (log length) on LT50 adjusted for covariance with log(dose). Log length was used to correct skew in length measurements and to provide a parameter linearly related to animal weight, since the latter was unobtainable for dead animals. That analysis (Table 4) revealed no significant main effects of species, salt type, diluent type or season, no significant covariance of LT50 with animal size, and, as has been described above (Figure 2), a highly significant covariance of log (dose) with LT50. The only other significant variance term, the salt type-season interaction term is the result of three anomalously low LT50 experimental means (adjusted for log(dose) covariance) for which there is no ready explanation.

Median lethal concentrations at 48 (LC50-48) and 96 hr (LC50-96) appear

Table 2. Summary of doses, mortality, and median lethal times for 96-hr experiments with *P. setiferus*.

Salt	Water	Run	Dose #	mOsm/kg	Less control	# of animals	Deaths @ 48 hr	Deaths @ 96 hr	LT50 (hr)[e]	Salt	Water	Run	Dose #	mOsm/kg	Less control	# of animals	Deaths @ 48 hr	Deaths @ 96 hr	LT50 (hr)
RS[a]	DI[b]	1	0	745	0	14	1	1	--	RS	DI	2	0	629	0	15	0	0	--
			1	1186	441	15	0	1	--				1	1017	388	15	0	3	--
			2	1306	561	15	5	8	84				2	1236	607	15	7	8	--
			3	1406	661	15	5	14	60				3	1349	720	15	13	15	86
			4	1487	742	15	12	15	31				4	1426	797	15	15	15	31
			5	1567	822	15	12	15	31				5	1595	966	15	15	15	--
RS	BR[c]	1	0	713	0	15	0	0	--	RS	BR	2	0	644	0	15	0	0	--
			1	1209	496	15	0	4	120				1	1316	672	15	13	14	25
			2	1278	565	15	1	4	140				2	1314	670	15	15	15	25
			3	1355	642	15	3	13	57				3	1373	731	15	15	15	--
			4	1477	764	15	10	14	39				4	1527	883	15	15	15	--
			5	1565	852	15	15	15	28				5	+2000	1356	15	15	15	--
IO[d]	DI	1	0	746	0	14	0	1	--	IO	DI	2	0	1088	0	10	1	1	--
			1	1123	377	15	0	1	--				1	1303	215	10	1	1	--
			2	1238	492	15	3	4	210				2	1454	366	15	2	4	260
			3	1380	634	15	0	5	210				3	1535	447	15	3	8	120
			4	1406	660	14	13	13	40				4	1652	564	15	6	12	56
			5	1531	785	15	9	13	35				5	1820	732	15	13	14	23
IO	BR	1	0	754	0	15	0	0	--	IO	BR	2	0	812	0	15	0	0	--
			1	1103	349	15	0	1	--				1	989	177	15	0	0	--
			2	1236	482	15	1	2	215				2	1144	332	15	0	0	--
			3	1362	608	15	1	2	150				3	1281	469	15	0	0	--
			4	1423	669	15	5	9	64				4	1374	562	15	0	1	--
			5	1506	752	15	9	11	72				5	1522	710	15	6	11	56

[a] RS, Ranch House salt. [b] DI, deionized water. [c] BR, Brazos River water. [d] IO, Instant Ocean.

[e] LT50 is listed for each dose for which it could be calculated.

Table 3. Summary of doses, mortality, and median lethal times for 96-hr experiments with *P. aztecus*.

Salt	Water	Run	Dose #	mOsm/kg	Less control	# of animals	Deaths @ 48 hr	Deaths @ 96 hr	LT50 (hr)[e]	Salt	Water	Run	Dose #	mOsm/kg	Less control	# of animals	Deaths @ 48 hr	Deaths @ 96 hr	LT50 (hr)
RS[a]	DI[b]	1	0	790	0	14	0	0	--	RS	DI	2	0	615	0	15	0	0	--
			1	1184	394	15	0	1	--				1	810	195	15	0	2	--
			2	1283	493	15	0	1	--				2	1138	523	15	3	5	190
			3	1390	600	15	2	12	66				3	1318	703	15	12	15	26
			4	1446	656	15	12	15	38				4	1756	1141	15	15	15	--
			5	1553	763	15	14	15	32				5	1574	959	15	15	15	--
RS	BR[c]	1	0	807	0	14	0	0	--	RS	BR	2	0	641	0	15	0	0	--
			1	1169	362	15	1	2	--				1	1080	436	15	2	3	380
			2	1248	441	15	4	7	90				2	1116	472	15	2	4	240
			3	1372	565	15	7	11	51				3	1267	623	15	6	12	52
			4	1402	595	15	12	13	39				4	1348	704	15	11	13	36
			5	1488	681	15	15	15	--				5	1522	878	15	13	15	26
IO[d]	DI	1	0	823	0	15	0	0	--	IO	DI	2	0	1007	0	15	0	0	--
			1	1142	319	15	0	0	--				1	1238	223	15	0	0	--
			2	1238	415	15	2	6	140				2	1328	321	15	1	2	490
			3	1380	557	15	5	10	74				3	1459	452	15	1	4	175
			4	1406	583	15	7	15	49				4	1507	500	15	3	6	130
			5	1531	708	15	13	14	34				5	1683	676	15	7	13	45
IO	BR	1	0	732	0	15	1	1	--	IO	DI	2	0	1036	0	15	1	1	--
			1	1164	432	15	2	2	310				1	1247	211	15	0	1	--
			2	1257	525	15	2	4	170				2	1339	303	15	1	4	165
			3	1352	620	15	1	7	98				3	1468	432	15	0	7	100
			4	1410	678	15	5	9	72				4	1558	522	15	1	7	90
			5	1466	734	15	3	10	70				5	1705	669	15	10	13	43

[a] RS, Ranch House salt. [b] DI, deionized water. [c] BR, Brazos River Water [d] IO, Instant Ocean

[e] LT50 is listed for each dose for which it could be calculated.

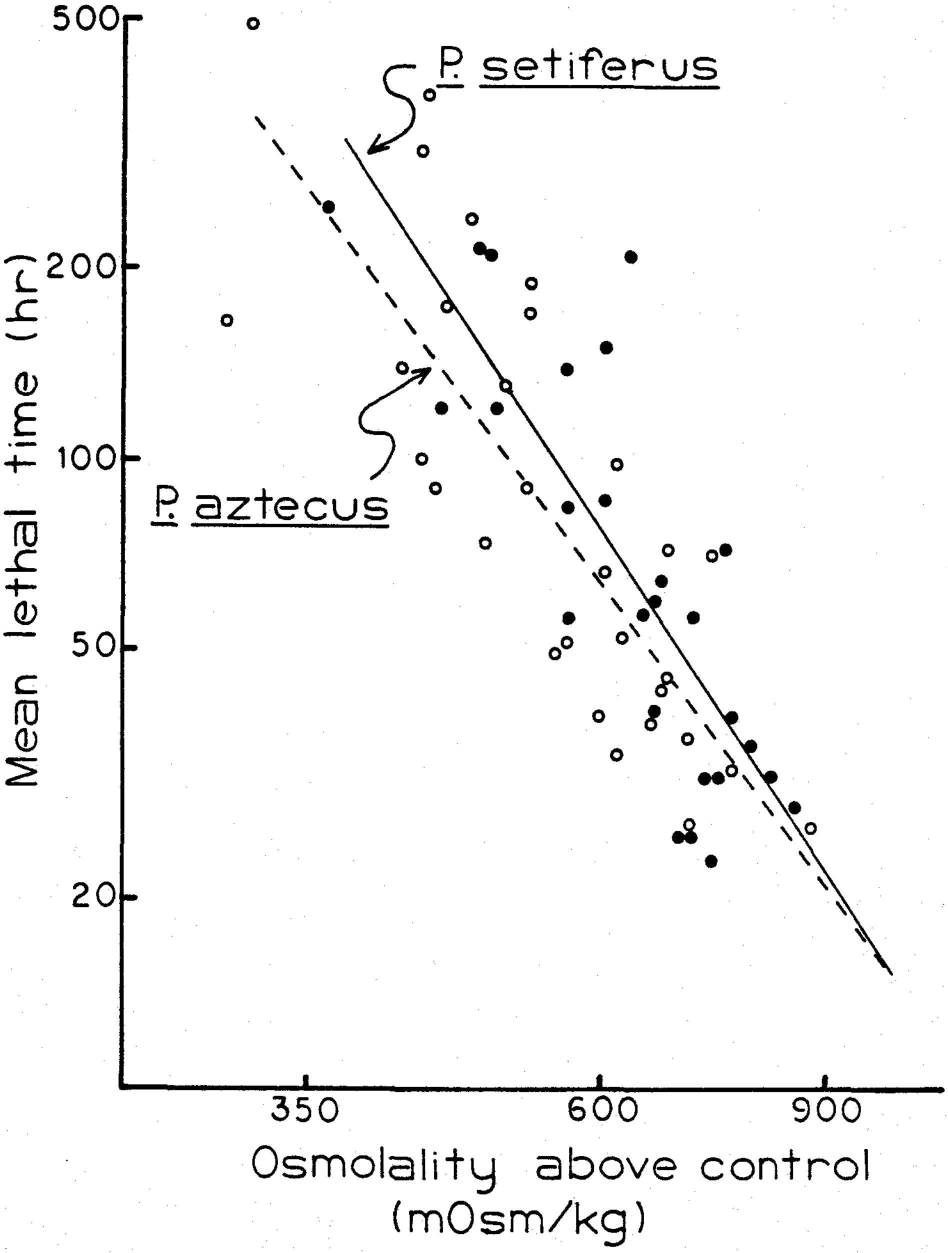


Figure 2. Median lethal time (LT50) as a function of dose for P. setiferus (closed circles) and P. aztecus (open circles) with least squares regression lines for each species. Both axes are logarithmic.

Table 4. Means of median lethal time (LT50) estimates for each experiment adjusted for covariance with dose.

Species	Instant Ocean				Salt Dome			
	Deionized Water		River Water		Deionized Water		River Water	
Species	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring
P. setiferus	149	94	120	92	95	135	105	94
P. aztecus	14	124	108	6	70	134	17	120

Analysis of variance for LT50 values

Source	Degrees of Freedom	Mean Square	F
Species	1	4706	1.56
Salt type	1	669	0.22
Diluent type	1	2126	0.71
Season	1	2929	0.97
Species x salt type	1	2017	0.67
Species x diluent type	1	117	0.04
Salt type x diluent type	1	311	0.10
Species x season	1	3124	1.04
Salt type x season	1	14103	4.68*
Diluent type x season	1	5435	1.81
First covariate (log osmo)	1	202913	75.16*
Second covariate (log · length)	1	1157	0.43
All covariates	1	116922	43.31
Error	48	3009	

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* F statistics with significant sources of variance, $p<0.05$

Table 5. Median lethal concentrations (mOsm/kg above control) calculated for each experiment at 48 and 96 hr.

Species	Instant Ocean				Salt Dome			
	Deionized Water		River Water		Deionized Water		River Water	
	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring
P. setiferus								
Control	746	1088	754	812	745	629	713	644
LC50-48	641	779	726	741[a]	679	582	712	586[b]
LC50-96	615	432	656	657	563	551	597	465[b]
P. aztecus	323	1007	732	1036	790	615	807	641
	541	659[a]	794[b]	625	634	593	532	637
	439	518	632	460	555	506	462	533

Note: *a* = estimate from probit plot, *b* = estimate from LT50 regression

Analysis of variance for LC50 values

Source	Degrees of Freedom	48 hour		96 hour	
		Mean Square	F	Mean Square	F
Species	1	3335	0.18	11610	0.41
Salt	1	7700	0.42	1958	0.07
Water	1	12376	0.68	5005	0.18
Run	1	4128	0.23	9850	0.35
Species x salt	1	564	0.03	2280	0.08
Species x water	1	945	0.05	1314	0.05
Salt x water	1	14823	0.82	16835	0.59
Species x run	1	5005	0.28	4064	0.14
Salt x run	1	232	0.01	1463	0.05
Water x run	1	540	0.03	280	0.01
Species x salt x water	1	280	0.02	855	0.03
Species x salt x run	1	5292	0.29	370	0.01
Species x water x run	1	2232	0.12	2376	0.08
Salt x water x run	1	6683	0.37	280	0.01
Error	1	18157		28476	

in Table 5. In all cases except five, those values could be calculated by the trimmed Spearman-Karber method. For the exceptions, excessive or insufficient mortality resulting from misaligned dose series required estimation of LC50 by extrapolation from log dose-probability plots (alternate method 1) or by extrapolation to 48 and 96 hours from LT50 - vs log dose plots (alternate method 2). Those estimates are shown in parenthesis, with numbers to show the appropriate alternate method used, in Table 5. Since control sea water osmolality ranged from a low of 615 mOsm/kg to a high of 1088 mOsm/kg, it is to be expected that LC50, expressed as absolute osmolality, varied in response to control osmolality. That prediction was borne out by the results. Figure 3 shows LC50-96 as a function of control (=acclimation) osmolality. Least squares regression indicates that LC50-96 rises at the rate of approximately 0.8 mOsm/kg for each mOsm/kg or acclimation oxmolality. That correspondence justified the expression of LC50 as the difference between control and lethal osmolalities (osmolality).

LC50-48 and -96 were examined for effects of species, salt type, diluent type and season by analysis of variance (Model I, full factorial design). That analysis, appearing in the lower half of Table 5, showed no significant variance contribution (by F-test) by any main effect or interaction between and among main effects. Grand means across all treatments indicate best estimates for median lethal concentration of 654±42 (95% Confidence Interval) mOsm/kg at 48 hr and 540±41 (95% CI) mOsm/kg at 96 hr, values that correspond to salinity increases of 22°/oo and 18°/oo respectively, assuming that dome brine is pure NaCl.

The effects of temperature of lethal concentrations measured at 48 hours for both species with salt dome-rive water brine is shown in Figure 4. Data for these estimates were sparse, with ten animals per dose at temperatures other than 25°C. Open circles in Figure 4 are interpolations from mortality dose plots with one or more points above and below 50% mortality; closed circles are extrapolations from plots that did not meet that requirement. In general, Figure 4 indicates that sensitivity to brine is increased at 30°C in both species and that *P. setiferus*

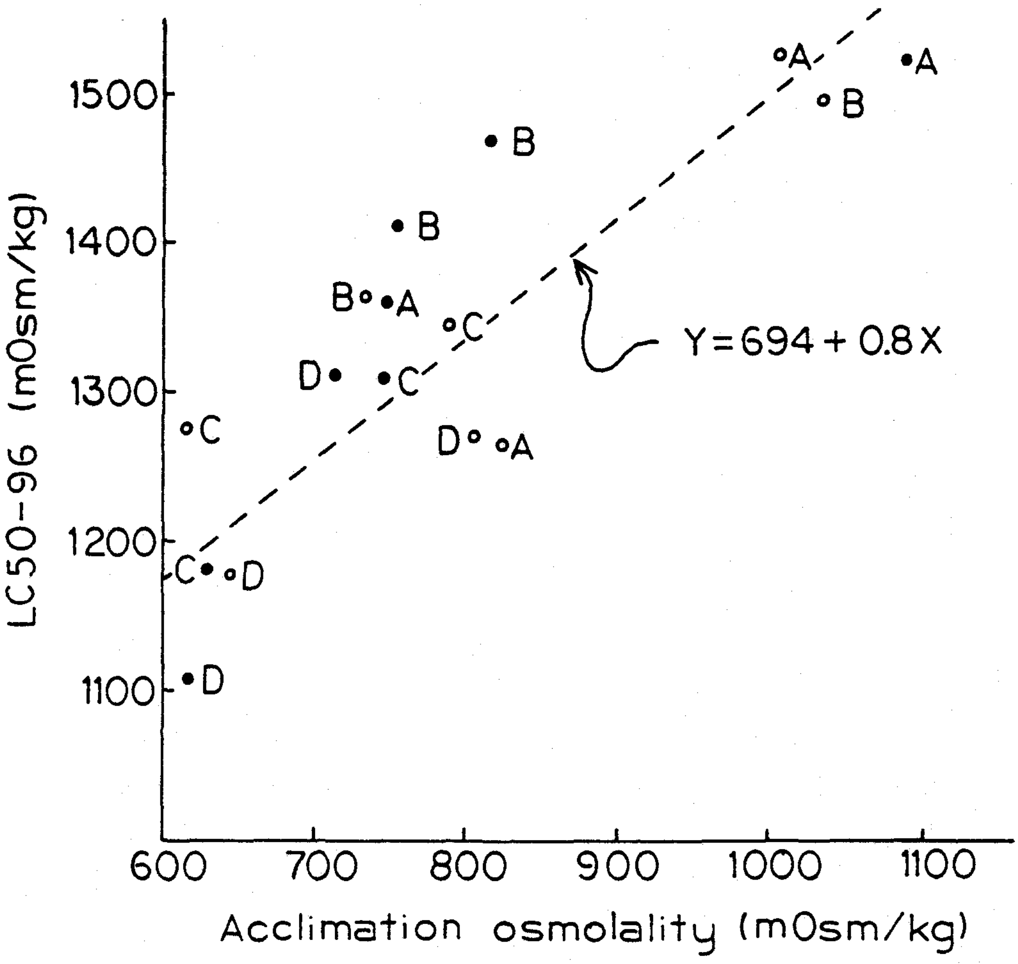

Figure 3. Median lethal concentration at 96 hr as a function of control (= acclimation) osmolality with least squares regression line. A is Instant Ocean in deionized water, B is Instant Ocean in river water, C is salt dome salt in deionized water, and D is salt dome salt in river water. Closed symbols show P. *setiferus* and open symbols, P. *aztecus*.

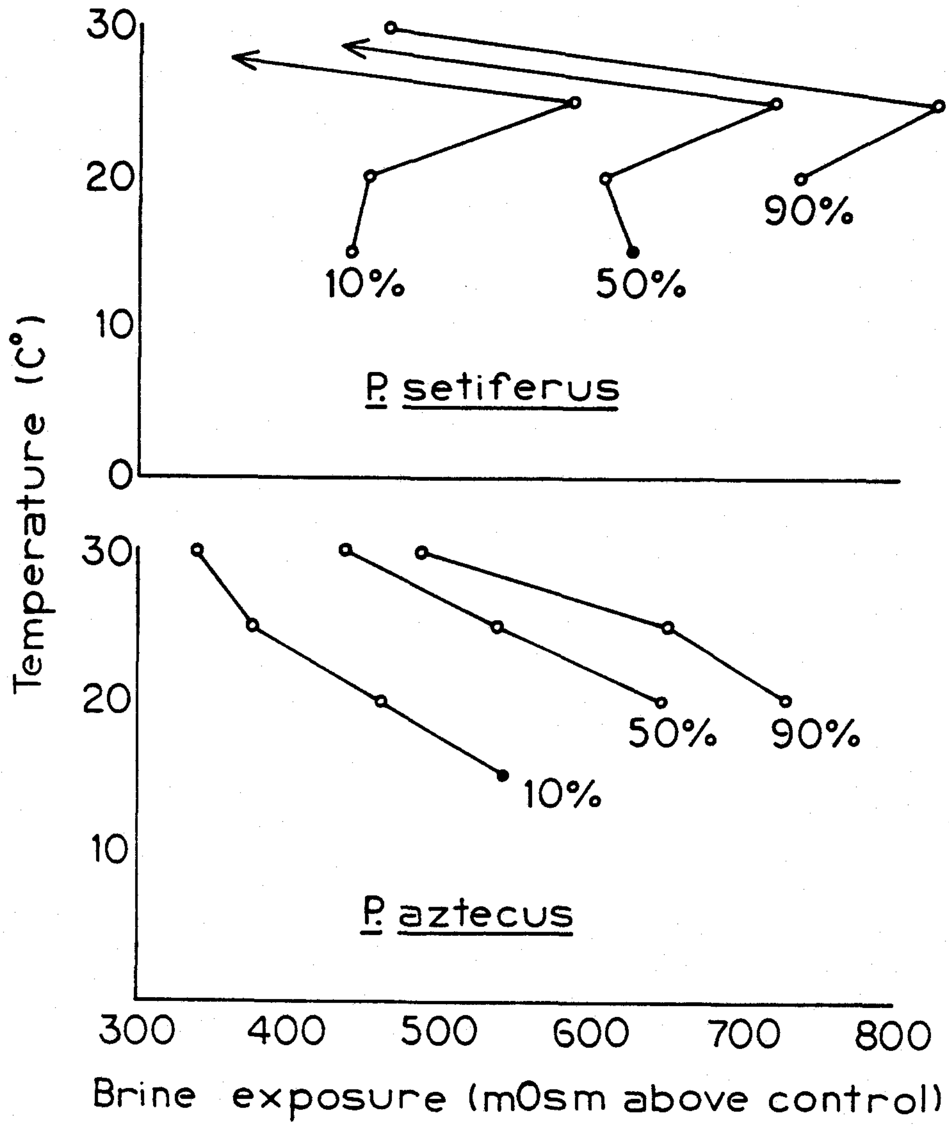

Figure 4. Temperature-salinity response surfaces for 3 mortality levels. Closed circles are extrapolations (see text).

survives high salinity better as temperature is reduced, at least to 15°C.

Sublethal effects

Animal color scores and heart rates provided early evidence of brine stress in acute experiments. Color scores were subjective and variable with approximately 60% concordance in replicate measures by different observers. To make doses across different experiments comparable, doses were compared to the LC50-96 for each experiment. The lowest dose that was higher than the LC50 was assigned a score of +1, and higher doses were given progressively higher scores. Similarly, doses lower than the LC50 were assigned negative scores. Since adjacent doses differed on the average by 17% in osmolality in all experiments, the difference between doses scored -1 and -2 (for example) should have been comparable across experiments. Mean color scores at each observation time were averaged within an experiment, then across all 16 experiments. The results shown in Figure 5 indicate that while even animals at doses well below LC50-96 became noticeably more opaque during the course of the experiments, animals exposed to higher doses became markedly more opaque than those at low doses by as early as six hours after the beginning of the experiment, even before brine doses had reached their eventual values.

Results with heart rates were similar. For each group of five readings at each dose the median value was selected. Doses in each experiment were then divided into those above and those below LC50-96 for that experiment. Median values were then averaged within the high and low groups of doses in each experiment for each observation period, then across experiments for each species. The results are shown in Figure 6, with open circles for doses lower than the LC50-96, closed circles for high doses and vertical bars to show 95% confidence limits for those means. By 6 hours for _P. setiferus_ and by 12 hours for _P. aztecus_ the hearts of animals at high doses beat significantly faster (lower beat period) than those

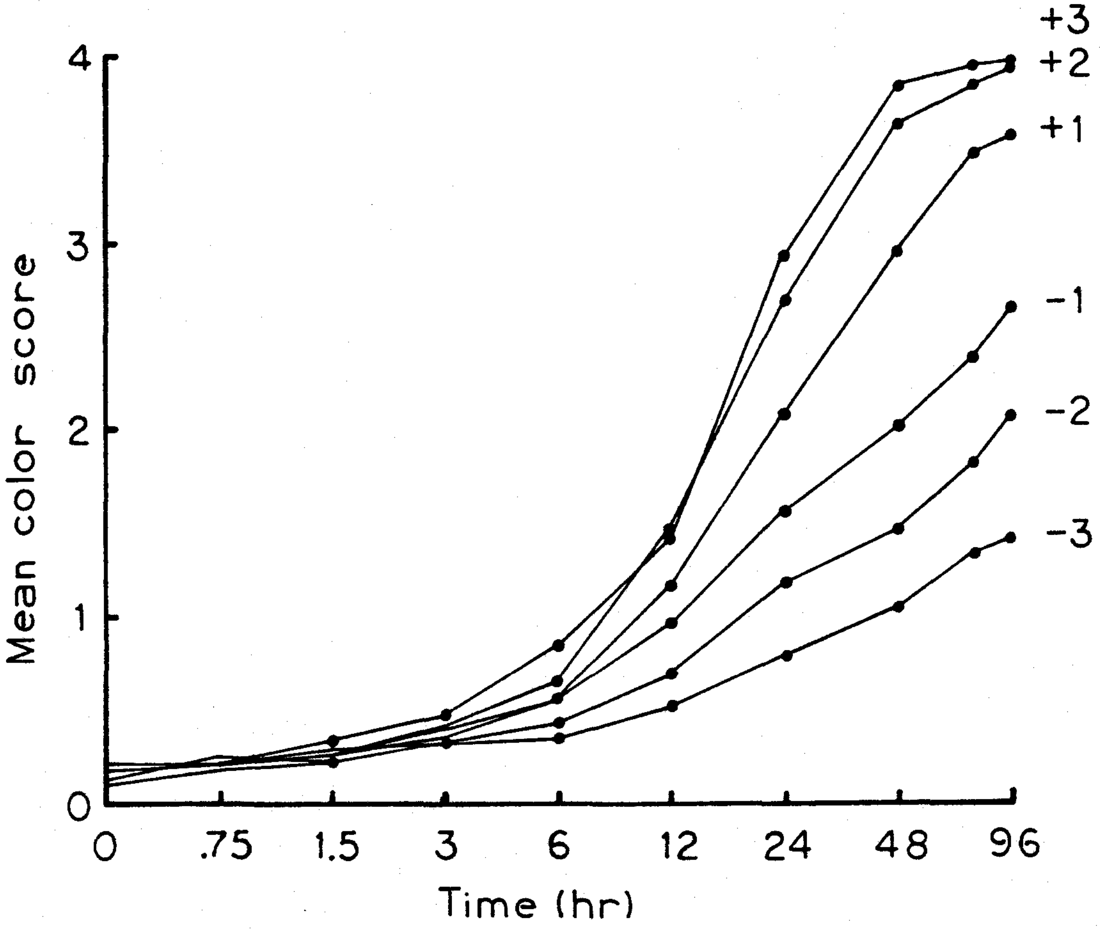

Figure 5. Effect of duration of exposure on mean subjective color score for all experiments. Positive numbers at the extreme right identify doses that were progressively higher than the LC50-96, and negative numbers denote progressively lower doses.

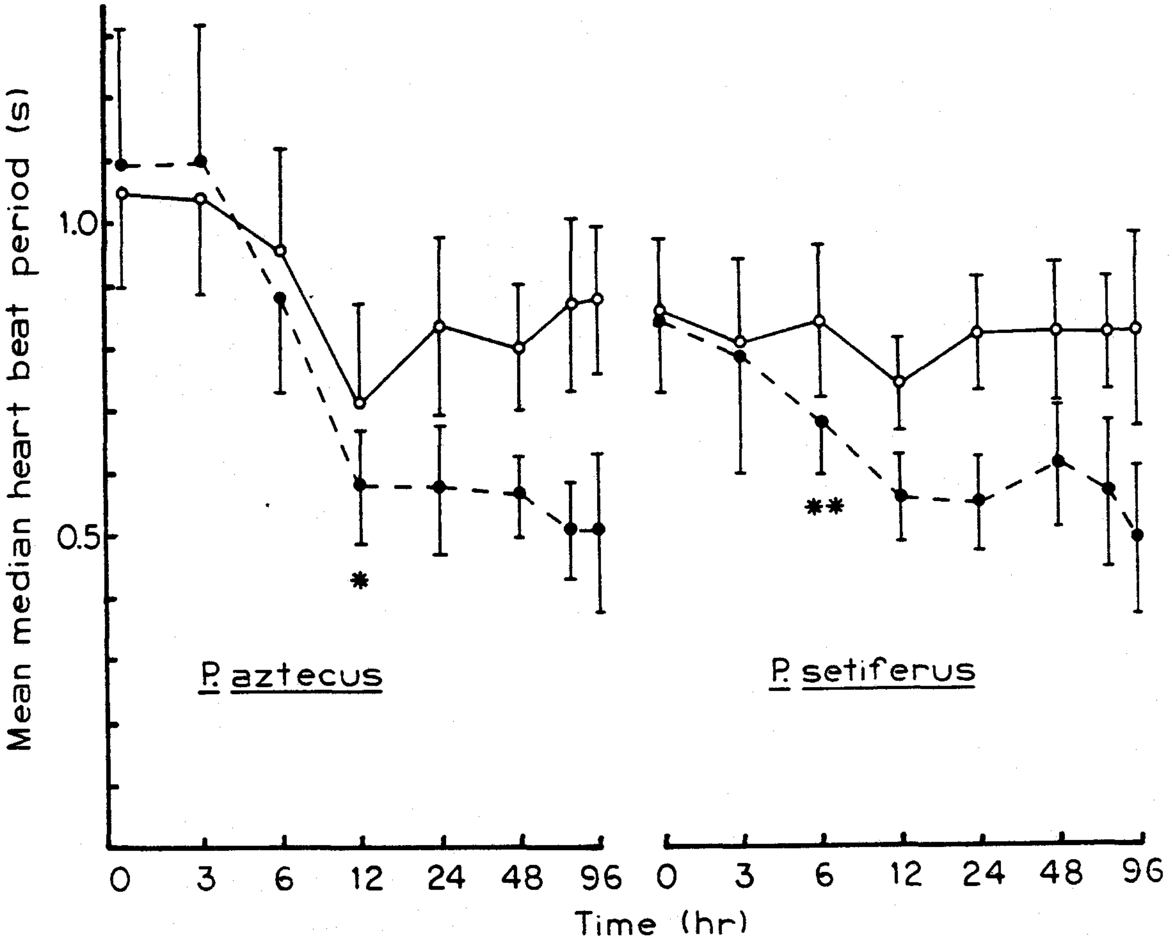

Figure 6. Means for all experiments of the median heart beat period recorded for doses higher (filled symbols) and lower (open symbols) than LC50-96 as functions of duration of exposure for each species. Asterisks denote earliest time at which values for high and low doses differed significantly (*: $p < 0.05$, **: $p < 0.01$).

at low doses. In both species heart beat period dips at the 12 hour observation, more noticeably for P. aztecus than for P. setiferus. This drop probably reflects the fact that the 12 hour reading was the only reading made during the dark period. P. aztecus is a nocturnally active species, and specimens of P. setiferus, though usually more active during the day, were disturbed by the lights that had to be turned on to make observations.

Behavior observations appear to reflect primarily the difference in diurnal activity patterns between the two species (Table 6). The percent of observations in which P. setiferus was active was significantly greater than that for P. aztecus ($t=7.9$, $p<0.001$), with the opposite pattern for percent of observations of quiescent animals. This finding is consistent with the fact that 9 of 10 observations were made during the day. Analysis of variance for all three behavioral categories document the species difference, but fail to show any other significant main effects. Frequency of observations of moribund animals failed to show a significant dose effect, a reflection of the low frequency of those observations and that they preceeded death by a relatively short time. Molting frequency was not affected by any fixed treatment (see analysis of variance, Appendix A).

Osmotic control appears to have been the only parameter affected differently by salt types, diluent types or season, but the results are difficult to interpret. Figure 7 shows the relationship between tank osmolality and hemolymph osmolality in all experiments. Over the range of test osmolalities neither species regulated well, with hemolymph osmolality rising about 0.75 mOsm/kg for each 1 mOsm/kg rise in external osmolality. Table VII shows cell means for both species adjusted for external osmolality covariance and a factorial analysis of variance. There was no significant difference between species in adjusted means, but salt type, diluent type and season each had significant main effects and interactions. There were tendencies for fall adjusted means to be higher than those for spring ($\bar{D}=48$ mOsm/kg, paired $t=2.1$, d.f.$=7$, $0.05<p<0.10$) and for differences between

Table 6. Behavioral observation means for fall (run 1) and spring (run 2) runs of 96-hr experiments.

Species	Run	Quiescent (f x 100 ± SD)	Active (f x 100 ± SD)	Moribund (f x 100 ± SD)
P. setiferus	1	65.0 ± 4.2	30.6 ± 3.7	4.1 ± 2.5
	2	69.6 ± 8.5	28.0 ± 8.8	2.5 ± 1.9
P. aztecus	1	86.0 ± 3.0	10.8 ± 2.5	3.1 ± 3.1
	2	80.4 ± 6.8	17.0 ± 4.6	2.5 ± 2.6

Note: Values shown are numbers of observations expressed as percents of total observations (± standard errors of the means). See text (p. 9) for definition of behavior categories.

Analysis of variance for parameters

Source (all with 1 d.f.)	Quiescent Mean Square	Activity Mean Square	Moribund Mean Square
Species	2016.1*	1968.8*	2.0
Salt	144.5	75.0	21.1
Water	98.0	101.5	4.5
Run	2.0	19.5	10.1
Dose	162.0	3.8	98.0
Species x salt	3.1	11.3	0.5
Species x water	45.1	75.0	1.1
Salt x water	8.0	26.3	4.5
Species x run	210.1*	140.3*	2.0
Salt x run	50.0	22.8	6.1
Water x run	112.5	195.0*	4.5
Species x dose	0.1	7.0	3.1
Salt x dose	32.0	19.5	2.0
Water x dose	0	0.8	1.1
Run x dose	24.5	52.5	4.5
Species x salt x water	3.1	3.8	1.1
Species x salt x run	66.1	52.5	4.5
Species x salt x dose	10.1	3.8	6.1
Species x water x run	21.1	30.0	0.1
Species x water x dose	10.1	5.3	0.5
Salt x water x run	50.0	47.5	0.5
Salt x water x dose	4.5	26.3	1.1
Species x run x dose	28.1	26.3	1.1
Salt x run x dose	8.0	7.0	4.5
Water x run x dose	8.0	16.5	0.1
Species x salt x water x run	91.1	69.0	1.1
Species x salt x water x dose	15.1	5.3	2.0
Species x salt x run x dose	10.1	5.3	0.1
Species x water x run x dose	3.1	0.0	2.0
Salt x water x run x dose	8.0	5.3	1.1
Error	1.1	0.8	4.5

* Indicate significant ($p<0.05$) mean squares by F-test.

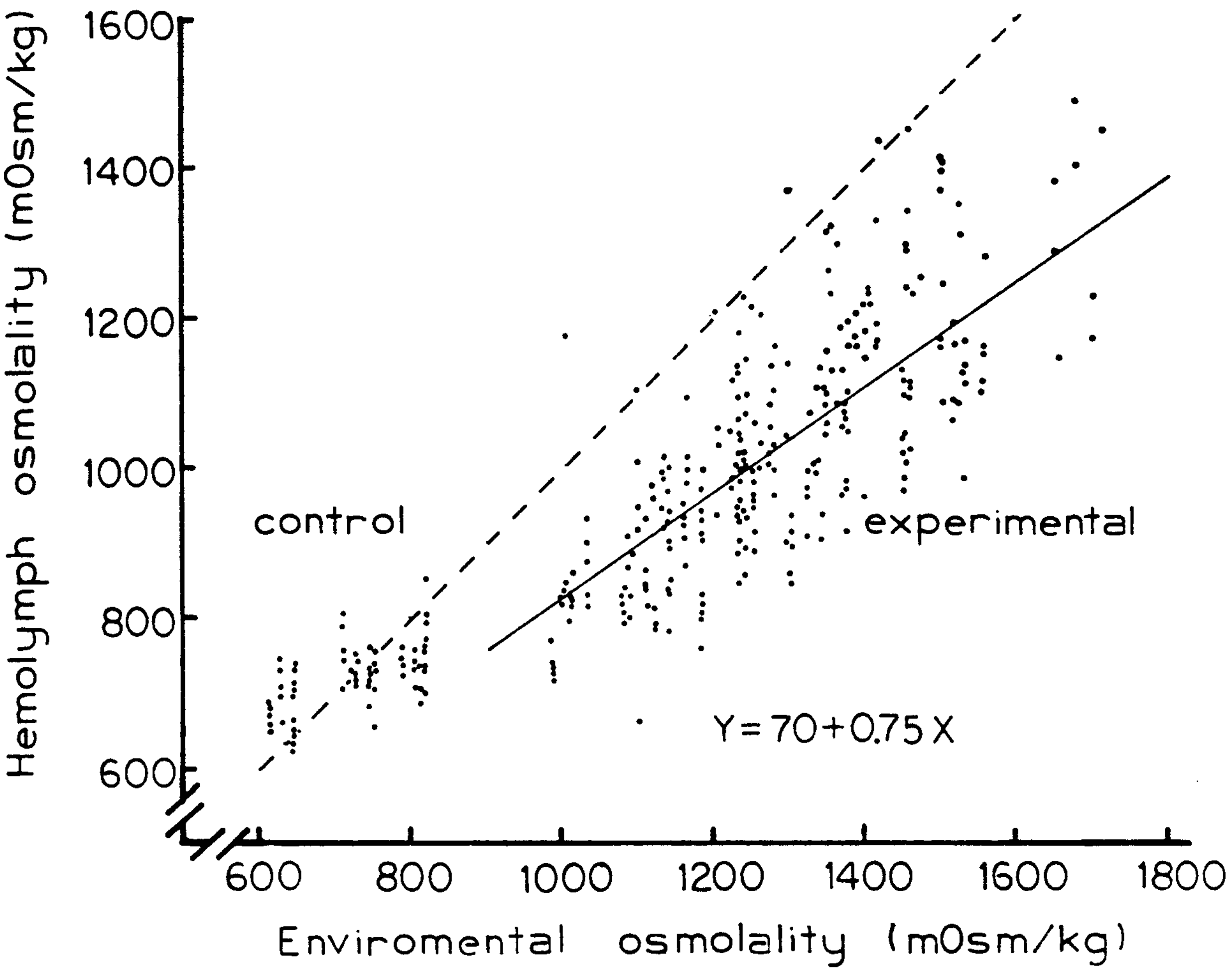

Figure 7. Scatter plot of hemolymph osmolality as a function of test tank osmolality. Dashed line is equiosmolar line. Solid line was fitted to all experimental points, except those from control tanks, the majority of which are at the extreme lower left corner of the graph.

Table 7. Hemolymph osmolality means adjusted for covariance with environmental covariance.

	Instant Ocean				Salt Dome			
	Deionized Water		River Water		Deionized Water		River Water	
Species	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring
P. setiferus	926	877	1051	892	944	984	1019	980
P. aztecus	981	951	1012	922	967	993	1022	938

Analysis of variance for hemolymph osmolality

Source	Degrees of Freedom	Mean Square	F
Species	1	13063	1.72
Salt type	1	47070	6.21*
Diluent type	1	48091	6.34*
Season	1	155937	20.56**
Species x salt	1	17409	2.29
Species x diluent	1	45102	5.95*
Salt x diluent	1	5265	0.69
Species x season	1	911	0.12
Salt x season	1	68031	8.97**
Diluent x season	1	136295	17.97**
Covariance, environ. osmol.	1	7000789	922.94**
Error	318	7585	

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* F statistics with significant sources of variance, $p<0.05$.

** F statistics with significant sources of variance, $p<0.01$.

fall and spring runs to be smaller for salt dome salt than instant ocean (paired t=2.72, 3 d.f., $0.05<p<0.10$). Differences between fall and spring runs were significantly larger for river water than for deionized water (paired t=7.2, 3 d.f., $p<0.01$). Though the appropriate variance contributions were significant, adjusted means did not differ according to brine type ($p>0.2$) or water type ($p>0.5$).

DISCUSSION

The preceeding results have shown that under the conditions of these experiments half of any group of exposed adult and subadult specimens of P. setiferus and P. aztecus can be expected to die when held 48 hours in media 654±42 (95% CI) mOsm/kg above their previous environmental osmolality or for 96 hours at 540±41 (95% CI) mOsm/kg above normal. These values correspond to transfers from normal seawater (1000 mOsm/kg)to water of 165% and 154% of seawater salinities, respectively, or salinity increases of approximately 22 and 18 ^{o}/oo. Though it is not surprising that LC50-96 values are lower than those for LC50-48, it is entirely possible that the difference represents a synergism between increased duration of exposure and such experimental rigors as close confinement, causing cuticular abrasions, and starvation. Control survival was uniformly excellent (never more than 1 death in 15), but mere survival may not have guaranteed the maintained ability to withstand salinity stress.

A logarithmic series of ten observations during toxicity experiments permitted estimates of mean lethal time as a function of brine dose. For both species LT50 appears to increase exponentially from about 20 hours at 400 mOsm/kg above ambient to more than 200 hours at 400 mOsm/kg. Estimates beyond this range were not practical, above 200 hours because of the brevity of experiments and below 20 hours because of the slow onset of brine exposure. Because of the internal consistency of LT50 estimates and their high sensitivities to dose, LT50 was used as the parameter to investigate potential effects of animal size on survival. In contrast to the finding of Venkatamariah, *et al*. (1974) that sensitivity to elevated salinity increases with increasing size in juvenile *P. aztecus*, there was no evidence of increasing sensitivity with increasing size in adults and subadults of either species tested.

Neither LT50 or LC50 appeared to differ according to salt type, diluent type or season. This finding suggests that the major toxic effect measured in all experiments was osmotic stress. If Brazos River water had contained toxic constituents with measureable effects on mortality at the doses administered (approximately 10% brine in seawater v/v, at the highest doses) then experiments with Brazos River brine should have resulted in relatively low LT50's and LC50's. Likewise, if seawater-salt dome brine mixtures were toxic by virtue of altered ion ratios then one might have expected lower LC50's and LT50's in these experiments than in experiments using Instant Ocean brines, the ion ratios of which would have more closely approximated those in seawater. Finally, that season did not have a significant effect would suggest that long-term changes in the sensitivity of large shrimp to salinity were not of large magnitude compared with experimental error.

In the relationship of LT50 to dose (Figure 2) there is no indication of a marked increase in slope with decreasing dose that would indicate a lethal threshold, that is, a maximum dose below which no detectable effect on mortality occurred. Even so, it is possible to extrapolate that relationship to worst-case predictions for salinity increases caused by brine discharge at the disposal site. If a 6.5^{o}/oo increase is a worst case estimate based on one volume of saturated brine diluted in 45 volumes of seawater (NOAA, 1977), then the corresponding increase in osmolality should produce an LT50 of 1275 hours with a lower 95% confidence limit of 558 hours for *P. aztecus*, the species that provides the lower of the two estimates. Even if there is indeed no lethal threshold for brine, and shrimp were confined in the worst case exposure for as long as 558 hours, both extremely unlikely events, it is probable that physiological adaptation to increased salinity would occur during so long an exposure, especially since 6.5^o/oo above normal seawater salinity is well within the range of normal environmental salinities for both species. The evidence for temperature-salinity interactions presented here suggests that temperatures above 25°C for both species and below 25°C for P. setiferus should increase sensitivity to brine. These data clearly do not establish those temperature effects with much certainty due to small sample sizes and a limited dose series at temperatures of other than 25°C. Even so, the data for P. aztecus, the principal species near the diffuser, suggest that the yearly maximum bottom temperature of 30°C (Texas A & M, 1979) would be associated with 10% mortality at 48 hours, providing the increased salinity reached 340 mOsm/kg (**Figure** 4) or about 12^o/oo, nearly twice the worst-case prediction for the near field, and six times the maximum 24 hour exposure predicted in the NOAA (1978) model of brine plume behavior.

Results with sublethal parameters suggest that a rise in heart rate may be a good candidate for an indicator of incipient osmotic stress lethality, since significant dose-related differences in heart rate appear as soon as 6 and 12 hours after the initiation of brine infusion, approximately as soon as full-strength dose was established in these experiments. On the other hand, heart rate was extremely variable in these experiments. Heart rate was clearly affected by general level of activity and doubtless by many unmeasured and uncontrolled biological and environmental parameters. Differences due to brine dose could only be detected by comparing the mean heart beat periods of groups of approximately 160 animals at frequent intervals, a requirement that may reduce the utility of bioassays based on heart beat.

Color score was likewise an early indicator of osmotic stress in these experiments (Figure 5). It suffers the same defect as heart rate for bioassays (i.e. low signal to noise ratio), and in addition suffers a defect that may limit its repeatability. In these experiments, observers of color were not blind to the treatment regime, and it is therefore possible that their expectations of a dose effect could have influenced their judgements about color score. Though improvements could be made to make judgements more objective, it would be difficult to entirely eliminate this sort of bias, since brine had several early effects on shrimp, including increased heart rate (see preceeding paragraph) and increased frequency of a behavior involving rapid medial flexures of the eye stalks, that could convey information about dose to even the most scrupulous observer.

Though it was generally impossible to distinguish the effects of different brine types or of season on dose-related parameters, osmotic control did provide an exception. Means of hemolymph osmolality for each experiment, adjusted for covariance with the tank osmolality from which individual animals were removed, showed significant variance components due to the main effects of season, brine type and diluent type, plus several significant interaction terms between these fixed treatments. The seasonal effect may admit of a simple explanation. Animals for fall runs had generally experienced long prior exposure to temperatures lower than the test temperature of 25°C. Results from the temperature effect experiments (Figure 4) suggest that cold-acclimated animals brought to 25°C within 24 or 48 hours for tests may have experienced a more severe osmotic challenge than animals previously exposed to warmer water temperatures. This hypothesis was not borne out in analyses of the lethality data: LC50 and LT50 were not sensitive to season. The discrepancy might be due to a better signal to noise ratio in osmolality measurements. Osmolality of fluid samples could be routinely measured to within 3 mOsm/kg of the true value, making that measurement far more precise, for example, than time to death, which was measured to the nearest 24 hour toward the end of each 96 hour experiment. There is at present no additional evidence that can be used to explain the other apparently significant treatment effects on hemolymph osmolality.

REFERENCES

APHA. 1975. Standard methods for the examination of water and wastewater. American Public Health Association. 14th ed. New York. 1193 pp.

Galloway, B.J. and L.A. Reitsema. 1981. Work Unit 5: Shrimp spawning site survey. Vol. III. In Jackson, W.B. and E.P. Wilkens (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum. NMFS-SEFC-67.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Env. Science and Technol. 11: 714-719.

James, P. 1977. Proceedings of the strategic petroleum reserve workshop: environmental considerations of brine disposal near Freeport, Texas. Center Mar. Resources, Texas A & M Univ. 77pp.

Litchfield, J. T. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Mount, D. I. and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Res. 1: 21-29.

Neff, J. M., L. G. L. Young, and J. L. Steed. 1979. Toxicity of brine effluents to benthic marine polychaete worms. Appendix D of NOAA(1978), this reference list.

NOAA, 1977. Analysis of brine disposal in the Gulf of Mexico: (1) Bryan Mound. National Oceanic and Atmospheric Administration. Washington, D.C., 165 pp.

NOAA, 1978. Analysis of brine disposal in the Gulf of Mexico: Bioassay results. National Oceanic and Atmospheric Administration. Washington, D.C.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

Texas A & M. 1979. Evaluation of brine disposal from the Bryan Mound site of the Strategic Petroleum Reserve Program. Progress Report, Predisposal Study. Feb. 1979.

UCLA. 1977. BMDP biomedical computer programs. P-series. Univ. of Calif. Press. Los Angeles. 880 pp.

Venkataramiah, A., G. J. Lakshmi, and G. Gunter. 1974. Studies on the effects of salinity and temperature on the commercial shrimp, *Penaeus aztecus* Ives, with special regard to survival limits, growth, oxygen consumption and ionic regulation. Contract report to U. S. Army Engineer Waterways Experiment Station by the Gulf Coast Research Laboratory, Ocean Springs, Miss. 134 pp.

Wilson, W. B., R. Harrison, A. Aldrich, D. Ramsey, H. Holcomb, and J. C. Parker. 1979. The toxicity of effluents from salt dome formation to early developmental stages of the white shrimp *Penaeus setiferus*. Appendix B of NOAA (1978), this reference list.

CHAPTER II.

Behavioral Effects
of a Simulated Salt-Brine Effluent
and its Components on Adults and Subadults
of Two Species of Penaeid Shrimp

INTRODUCTION

In March, 1980, the U.S. Department of Energy began to discharge brine, consisting of salt from the Bryan Mound salt dome in Freeport, Texas, dissolved in water from the Brazos River, at rates exceeding 100,000 barrels per day from a diffuser located approximately 20 km offshore from Freeport, Texas. A companion study (Chapter I) examined toxicity and certain sublethal effects of a simulated brine discharge on adult and subadult specimens of two penaeid shrimp species, _Penaeus setiferus_ and _P. aztecus_. Those results were developed from experiments in which test animals were confined in uniform concentrations of brine throughout exposure. Application of the results will depend on realistic estimates for duration of exposure under natural conditions. To make those estimates it will be necessary to predict how shrimp will behave in response to brine: attraction toward the brine source should increase and prolong exposure, whereas avoidance should reduce exposure. Further, demonstrated attraction or avoidance behavior could also help predict changes in shrimp population densities near the diffuser. The present study uses two experimental approaches to examine possible attraction or avoidance behavior in shrimp that encounter a simulated brine plume.

MATERIALS AND METHODS

Adult and subadult specimens of both species 6-11 cm in abdomen length were captured and maintained as previously described (Chapter I). Animals selected for tests had no obvious cuticular lesions or missing appendages. Where holding and experimental tank temperatures differed by more than $5^{\circ}C$, animals were brought to the laboratory and gradually exposed to the experimental temperature over a 24 hr period. Water and air temperatures were controlled as previously described (Chapter I). Four test brines, constituting all pairwise combinations of Instant Ocean synthetic sea salt of salt dome salt (United Salt Ranch House Coarse salt) with deionized water or Brazos River water, were prepared as before (Chapter I). Test animals were used once, then discarded.

Static bioassays

A 200 ℓ glass aquarium was divided into five equally sized compartments by cementing dividers of plastic mesh across the narrow dimension. The bottom of the aquarium was filled with coarse (3 mm) blasting sand to a depth of 5 cm. Beneath the sand substratum two lengths of perforated plastic pipe (1.25 cm inside diameter), each with a vertical inlet tube at one end and stoppered at the other end, served as brine diffusers. The sand substrate was distributed evenly, the tank filled with 100 ℓ of aerated sea water, and a single animal placed in each compartment. Animals were allowed to become accustomed to their compartments for 15 min before observations began. For the next 5 min each animal was observed for time spent active (pleopod movement, walking or swimming) and for the occurrence of escape responses, involving rapid ventroflexion of the abdomen or of "eye knocking", a behavior that consists of rapid, symmetrical, movements of the eyestalks toward the midline and has been considered a response to physiological stress (K.N. Baxter, personal communication).

After 5 min of observations a pair of valves was opened, allowing 20 ℓ of a brine-in-seawater solution to flow by gravity through the brine diffusers and into the observation tank. Brine solutions were prepared to yield increased salinities (by refractometer) of from 6-11^{o}/oo (low), 11-15^{o}/oo (medium) and 15-22^{o}/oo (high). The flow of brine solution lasted approximately 30 s. Because the brine solution was diffused at low pressure under the sand substratum little mixing occurred between brine solution and tank water. The denser brine mixture displaced the seawater upward and established a sharp, easily visible refractive boundary between the layers 2 cm above the sand surface. The moment that brine emerged from the sand in each compartment was recorded. Observations continued throughout brine infusion and for 5 min after brine appeared in the last compartment. Especially active animals accomplished considerable mixing of the brine and water layers, though a sharp salinity discontinuity was still visible at the end of all experiments. A water sample was carefully taken from just above the sand substrate at the conclusion of each experiment to determine dose levels. Observations, including changes in the activity of status of each animal, the appearance of specific behaviors and the time of arrival of the brine solution in each compartment, were recorded on tape. Tapes were replayed while a stopwatch ran to prepare an accurately-timed transcript of each animal's behavior. From those transcripts exact five-minute periods before and after the arrival of brine in each compartment were selected for analysis.

Experiments were performed once for each species, at each of the three dose levels of the four test brines at 25^{o}C and 15^{o}C for a total of 120 animals of each species. No animal escaped, died, or became moribund during the experiments. To minimize the possibility of introducing a temperature stimulus with the brine solution, the temperature of the brine solution was adjusted to within 0.2^{o}C of tank water temperature before each experiment. To minimize mixing of brine and water layers, the observation tank was not aerated during the observation period. After each experiment the observation tank was drained and the sand substratum rinsed with seawater.

Flow-through bioassays

The possibility that animals might select or avoid a brine plume was investigated in an I-maze constructed of plywood lined with grey polyvinyl-chloride sheet. Dimensions and design features for the maze are shown in a scale drawing in Figure 1. Water entered both ends of the maze with the flow rates matched and adjusted to 0.75 ℓ/min by a variable-speed, two-channel peristaltic tubing pump (Cole-Parmer Masterflex). Water from each channel of the pump passed through a perforated tubing diffuser into the bottom of a sand-filled diffusing chamber from which it flowed over a dam, then across the bottom of the maze through an 8-mm slit. In the center of the maze an 8-mm wide screen-covered slot led to an outlet chamber. Water flowed from the outlet chamber through two discharge lines and a single standpipe that established the water level in the maze at 20 cm. In operation a series of valves on the two discharge lines was adjusted to allow the slowest possible flow from the standpipe and balanced flows from both discharge lines. Those precautions were found to be necessary in preliminary trials with dyed brine solutions. Those trials confirmed that the flow of brine mixtures was laminar across the bottom of the maze and did not cross the outlet slot to the other arm of the maze. Though flows were laminar for brine mixtures differing in salinity from the surrounding water by as little as $2^{o}/oo$, flow was markedly turbulent when the inlet solution did not contain brine, suggesting that the density of the inlet solution strongly influenced its flow characteristics. Dye tests were also used to establish minimum rinsing times of 5 min. for removing residues of brine solutions from the maze. A moveable cage (open at the bottom) was used to confine animals over the outlet slot of the maze. The width of the cage (6 cm) forced animals to orient parallel to the outlet slot.

The maze was carefully levelled using the inlet dams as references and placed under uniform diffuse fluorescent illumination (500 lx). Backgrounds that could be seen from inside the maze were matched on both sides of the maze. Brine solutions were continuously prepared with a modified proportional diluter

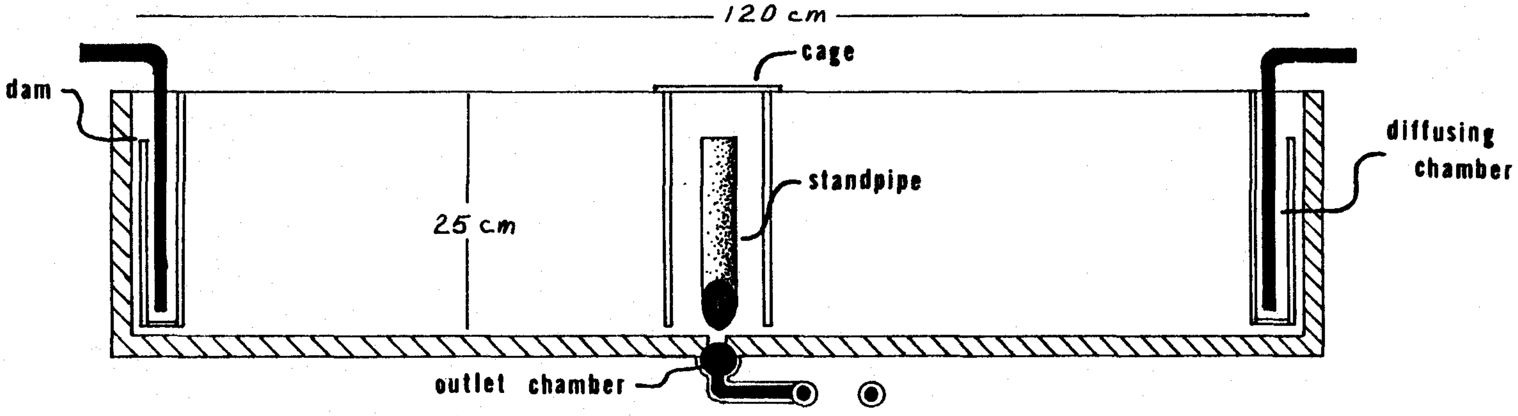

Figure 1. Scale drawing, side view, of I-maze. Certain details have been omitted for clarity.

(Mount and Brungs, 1967) as previously described (Chapter I).

At the beginning of tests an animal was placed in the starting cage for 5 min , after which time the cage was gently raised by a string and pulley arrangement. Animals were allowed to move freely for 1 min. The maze had been divided into unequal thirds by boundary marks along the top of the walls, with the central area consisting of a portion of maze length 12 cm to either side of the central outlet slot. Elapsed time in seconds was recorded each time an animal crossed a boundary between areas, so that for each 60-s run total time in each area and number of trips to each area could be computed.

Four animals of each species were tested for each of three dose levels of each of the four test brines: low (5^{o}/oo above ambient seawater salinity), medium (10^{o}/oo above ambient) and high (20^{o}/oo above ambient). Each animal was tested 20 times in succession. Ten trials were control trials, with seawater entering both arms of the maze. After five control runs the inlet tubes were switched to balance any uncontrolled differences in inlet plumbing. Ten experimental trials were completed, half with the brine solution entering the right arm of the maze and half left. Five minutes were allowed between sets of trials that required rinsing of residual brine from one side of the maze. The order of trials for each group of four animals at each dose level was completely balanced, that is, control trials were first for two animals, experimental trials first for the other two, and brine entered first from the left for two and first from the right for the other two. At the conclusion of each trial, animals were recaptured in a net as gently as possible to minimize turbulent mixing of the brine mixture into the seawater. Even so, that some animals were more active than others surely resulted in different degrees of such mixing from experiment to experiment. In approximately half of the experiments for each species the orientation of the animal in the starting cage (toward or away from the observer) was recorded at each trial. After the conclusion of all trials the sex and length of each animal were recorded.

RESULTS

Static bioassays

Three parameters were generated for each animal: time active, number of active episodes, and number of stress-related (alarm) behaviors (sum of escapes and eye knocks). For each parameter the before-brine value was then subtracted from the post-brine value. Table 1 shows those differences averaged over the five animals in each dose, the four test brines, and both species. The same values, separated by species, are plotted with standard errors in Figure 2. Though animals were generally more active by all measures when exposed to brine, that figure shows that the data were highly variable. Only values for number of active episodes by P. aztecus at high brine concentrations and 25°C and time active for P. setiferus at high brine concentrations and 15°C differed significantly from 0. Paired t tests on the means for each factor combination showed the following significant relationships:

1. Animals averaged over species and temperatures spent more time active after the administration of medium and high brine doses but not low brine doses.
2. Both species spend more time active after brine administration, regardless of brine dose or temperature, and P. aztecus exhibited more activity episodes after brine administration, regardless of dose or temperature.
3. All parameters increased significantly after the administration of brine, regardless of brine type, dose, or species, at 15°C, but not at 25°C.

These findings are summarized in tables of mean differences (Table 2). No significant differences by species or brine type could be demonstrated.

Table 1. Means and standard errors for five animals in each treatment cell of static behavior bioassays.

Species	Temperature (°C)	Behavior Parameter	Salt Dose		
			Low	Medium	High
1	15	Alarm	0.85 ± 0.55	0.25 ± 0.46	0.25 ± 0.51
		Activity	2.25 ± 1.00	0.65 ± 0.58	2.00 ± 0.94
		Time	21.35 ± 11.25	20.80 ± 20.70	32.25 ± 5.12
	25	Alarm	1.00 ± 1.01	0.90 ± 0.98	- 0.80 ± 0.84
		Activity	0.20 ± 0.16	- 0.05 ± 0.29	- 0.65 ± 0.66
		Time	- 1.55 ± 21.70	14.65 ± 11.36	4.95 ± 25.04
2	15	Alarm	- 0.13 ± 0.13	1.15 ± 0.72	0.64 ± 0.28
		Activity	0.47 ± 0.18	1.05 ± 0.92	1.88 ± 0.39
		Time	18.60 ± 12.70	15.25 ± 17.20	29.28 ± 9.53
	25	Alarm	0.05 ± 0.05	0.20 ± 0.67	0.80 ± 0.37
		Activity	1.45 ± 0.71	1.25 ± 0.79	0.40 ± 1.07
		Time	2.50 ± 8.40	28.10 ± 18.40	15.10 ± 23.15

Note: Mean values = Experimental minus control. See text (p.9) for explanations of the scored parameters.

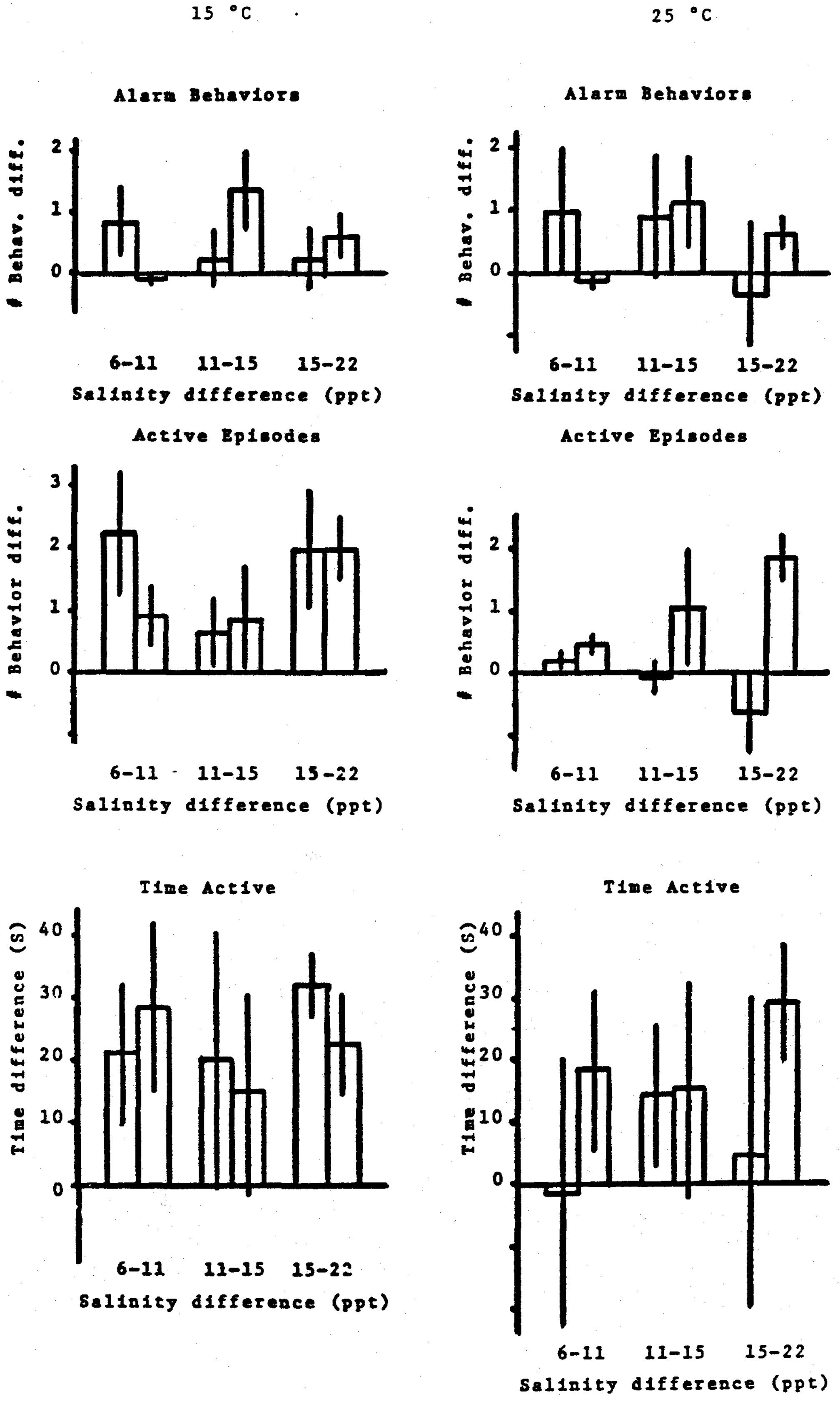

Figure 2. Cell means from Table I with standard errors indicated by vertical lines. Experiments performed at 15 °C are on the left, those at 25 °C on the right. In each pair of histogram bars, values for *P. setiferus* are on the left and *P. aztecus* on the right.

Table 2. Two-factor tables for mean difference between experimental and control observation periods in static behavior bioassays.

A. Parameter by dose

parameter	low	medium	high
alarm behaviors	0.44(0.28)[a]	0.63(0.24)	0.22(0.36)
active episodes	1.09(0.47)	0.73(0.29)	0.91(0.63)
time active	10.23(5.72)	19.70(3.12)*	20.40(6.36)**

B. Parameter by species

parameter	*P. setiferus*	*P. aztecus*
alarm behaviors	0.41(0.28)	0.45(0.20)
active episodes	0.73(0.47)	1.08(0.23)*
time active	15.41(4.99)**	18.14(4.02)*

C. Parameter by temperature

parameters	15 °C	25 °C
alarm behaviors	0.50(0.19)**	0.36(0.28)
active episodes	1.38(0.31)**	0.43(0.33)
time active	22.92(2.66)*	10.63(4.43)

[a] Means of dependent variables are shown with standard errors in parentheses.

* Mean values significantly greater than 0, $p<0.01$.

** Mean values significantly greater than 0, $p<0.05$.

Flow-through bioassays

The results for the maze experiments were even more variable. Raw data summaries are included in Appendix B. Those data were used to generate the following three statistics for detecting attraction or avoidance:

1. Trips: For each animal the number of visits to the left arm of the maze was summed for each treatment (brine from the left or right arm) and for control trials, then divided by the sum of left and right visits for that treatment (or control), yielding a proportion of left visits. Each proportion was multiplied by 100 to yield a percentage. The percentage of left visits in the control trials was then subtracted from the percentage for each brine treatment to yield the statistic, trips, a measure of the preference for the left maze arm during brine flow, compared with control.
2. Time: Time was computed in an identical fashion. Percentage of time spend in the left arm (of the time spent in both arms) was computed for each of the two treatments plus control trials. Treatment percentage minus control percentage was a measure of preference for the left maze arm (by time) during the brine treatments compared with control.
3. Activity: The sum of visits to both arms of the maze during both sets of experimental trials less the same sum for control runs was divided by total visits in control trials to yield a statistic, activity, that expressed general increase in activity during the administration of brine compared with control activity.

Values for each of the three statistics, averaged over the four animals in each treatment cell, are shown in Table 3. No treatment mean (right column) for any statistic differs significantly from 0(i.e., no difference from control). However, the difference between treatment means (brine from the left or right)

Table 3. Summary of flow-through maze experiments.

			P. setiferus				*P. aztecus*				
			Inst. Ocean		Salt dome		Inst. Ocean		Salt Dome		
Parameter	treatment	dose	DI[a]	BR[b]	DI	BR	DI	BR	DI	BR	$\bar{x}$
Trips[c]	brine left	low	-3.5	2.0	24.3	3.0	-3.5	1.5	33.3	-11.0	5.76
		med	-10.8	4.3	5.0	-13.5	-15.3	-3.8	-7.8	6.3	-4.45
		high	-24.0	2.5	4.0	-1.3	-18.3	-16.8	13.5	-8.5	-6.11
	brine right	low	-4.0	-9.0	13.5	5.3	-5.5	7.3	2.0	-0.8	1.10
		med	-9.5	-37.5	-12.8	-10.8	13.5	2.8	9.8	11.0	-4.19
		high	-6.5	22.8	21.3	8.3	14.3	-13.5	-0.8	-5.8	5.01
Time[d]	brine left	low	-6.8	-3.5	21.0	12.8	-1.3	18.3	32.5	-14.3	7.34
		med	-10.5	4.5	3.3	-16.0	-16.8	2.8	-5.8	5.3	-4.15
		high	-32.3	-4.8	4.5	-11.3	-32.3	-21.5	12.8	-3.8	-11.09
	brine right	low	0.8	5.5	13.3	2.0	-7.5	9.0	10.3	-1.8	3.95
		med	-7.5	-36.3	-7.5	-13.8	15.3	7.0	6.5	11.8	-3.06
		high	-8.0	-10.5	27.5	-10.8	15.0	-22.0	-3.3	-5.3	-2.18
Activity[e]		low	-4.0	-11.0	25.5	4.5	14.3	-10.0	12.0	1.3	4.08
		med	11.3	-42.3	-5.8	-16.8	17.5	2.5	-9.0	-6.8	-6.18
		high	-0.8	-8.5	7.0	18.0	-18.8	9.5	-9.0	12.5	1.24

[a] DI, deionized water

[b] BR, Brazos River water

[c] Trip values are calculated as [((trips to left maze arm) ÷ (total trips)) - (control portion of left trips)] x 100.

[d] Time values are computed similarly to trip values, using time spent in the left maze arm.

[e] Activity values are [(total trips in experimental runs) ÷ (total trips in control runs)] x 100.

for both species shows a trend. If mean trips when brine came from the right (i.e. opposite to the left arm) is subtracted from the mean for brine from the left (same side) in each experiment, the difference should reflect net attraction to a source of brine. That difference, averaged across species and brines, is 4.79 (±4.57 SE) for low brine doses, -0.28 (±7.65) for medium doses and -12.91 (±5.40) for the highest doses. This last mean difference is significantly lower than 0 (t=2.39, 7 d.f., p 0.05). This trend suggests that as brine concentration increases, animals become increasingly repelled from the source. The trend is the same for the time statistic, though mean difference is not significantly different from 0 at any dose. The activity statistic was not significantly affected by the administration of brine in these experiments. An unexpected and interesting finding was that *P. aztecus* (but not *P. setiferus*) appears to prefer to move right in the apparent absence of stimuli. For those control trials in which initial animal orientation was noted, the maze arm visited first within 30 s of the beginning of the trial was noted (Table 4). Specimens of *P. aztecus* usually moved left (69 trips left to 38 right) when oriented with head towards the observer, and usually moved right (62 right to 42 left) when oriented with head away from the observer. This right-handed bias was significant (χ^2=4.2, 1 d.f., p 0.05).

DISCUSSION

The behavior of animals in both of the bioassays described here showed extreme variability; as a result, the demonstration of significant brine effects required many averaging steps. Whether any laboratory bioassay for brine based on overt behavior or large penaeid shrimps could significantly reduce this variability is not known. As it stands, it remains possible that averaging and the attendant loss in degrees of freedom may have obscured treatment-related behavior differences of biological significance.

Table 4. Contingency tables for independence of maze arm choice and initial orientation in flow-trough behavior bioassay.

P. setiferus

	Initial orientation		
Choice	Front	Back	For H_0 = independence,
Left	57	53	$X^2 = 0.03$, $p<0.75$
Right	39	60	

P. aztecus

	Initial orientation		
Choice	Front	Back	For H_o = independence,
Left	69	42	$X^2 = 4.2$, $p<0.05$
Right	38	62	

The data from statistic tests indicate that exposure of animals to brine-induced salinity increases of 10°/oo or more increases their general activity, measured by proportion of active time or by number of swimming or walking episodes per unit time. That effect, in these experiments at least, was more pronounced at 15°C than at 25°C and more easily detected for P. aztecus than for P. setiferus. These findings are consistent with the idea that shrimp encountering substantial salinity increases in nature may increase activity, swimming speed for instance, therefore decreasing residence time in the plume, regardless of their ability to detect salinity gradients. Avoidance of extreme environments by kinesis is a common adaptation in animals. Nonetheless, the low brine dose in the static experiments evoked no detectable kinesis, though that dose was comparable in salinity to the extreme worst-case predictions for the area nearest the DOE brine diffuser, 6.5°/oo (NOAA, 1977). If this worst case estimate is accurate, then extension of the laboratory results reported here suggests no marked increase in activity for shrimp exposed to Bryan Mound effluent.

The maze experiments indicate that test animals were capable of avoiding brine plumes at brine-induced salinity increases of about 20°/oo. That avoidance, however, was far from absolute and was detected as a slight decrease in the frequency of voluntary movements into such a brine plume. Since an increase in salinity of 20°/oo is lethal to both species, with a median lethal time of approximately 90 hr, brine avoidance behavior may be adaptive. These results suggest, however, that brine-induced avoidance behavior is unlikely for shrimp near the Bryan Mound diffuser, since the apparent threshold for avoidance is well above worst-case salinity predictions.

Though both types of experiments demonstrate the ability of P. setiferus and P. aztecus to alter behavior in response to abrupt salinity increases, the sensitivities of these animals were substantially lower than those of postlarval and juvenile pink shrimp, P. duorarum, for which behavioral responses to salinity changes as small as $1^{o}/oo$ could be demonstrated (Hughes, 1969). These latter results suggest that future efforts in exploring potential effects of brine on shrimp should focus on the behavior of early life stages rather than on the behavior of adults and subadults.

An intriguing finding in the flow-through bioassays was the demonstration that P. aztecus turned significantly toward the right when released from the starting cage in control trials. It is unlikely that this phenomenon could have altered any of the other results reported here, since initial orientation was randomly assigned in all trials. Even so, "right-handedness" could have been a significant contributor to the noise in choice experiments and has potential implications for other behavioral experiments with P. aztecus. Causes or adaptive significance for the bias are difficult to imagine, though it is possible that the response is a laboratory artifact produced by confinement in a round holding tank. Shrimp placed in our holding tank swim around the tank perimeter, usually in a direction opposite to the prevailing counterclockwise current established by the discharge lines from subgravel filters. Animals occasionally bump the tank sides with their head appendages. Though the tank wall is covered with a soft algal mat, animals that swam clockwise might have abraded appendages on the left side of the head more than those on the right. No overt signs of asymmetrical abrasion were apparent during the study, but the question clearly demands further investigation.

REFERENCES

Hughes, D.A. 1969. Responses to salinity as a tidal transport mechanism of pink shrimp, *Penaeus duorarum*. Biol. Bull. 136:43-53.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Res. 1:21-29.

NOAA. 1977. Analysis of brine disposal in the Gulf of Mexico: (1) Bryan Mound. National Oceanic and Atmospheric Administration. Washington, D.C. 165 pp.

APPENDICES

Appendix A. Factorial analysis of variance for molting frequency in 96 hr toxicity bioassays.

Source	Degrees of Freedom	Mean Square	F
Species	1	157.5	5.25
Salt	1	34.0	1.13
Water	1	413.3	13.76
Run	1	2000.3	66.61
Dose	1	344.5	11.47
Species x salt	1	9.0	0.30
Species x water	1	413.3	13.76
Salt x water	1	101.5	3.38
Species x run	1	520.0	17.32
Salt x run	1	69.0	2.30
Water x run	1	13.8	0.46
Species x dose	1	94.5	3.15
Salt x dose	1	9.0	0.30
Water x dose	1	0.3	0.01
Run x dose	1	81.3	2.71
Species x salt x water	1	1.5	0.05
Species x salt x run	1	26.3	0.88
Species x salt x dose	1	52.5	1.75
Species x water x run	1	810.0	26.97
Species x water x dose	1	3.8	0.13
Salt x water x run	1	108.8	3.62
Salt x water x dose	1	124.0	4.13
Species x run x dose	1	0.0	0.00
Salt x run x dose	1	19.5	0.65
Water x run x dose	1	5.3	0.18
Species x salt x water x run	1	16.5	0.55
Species x salt x water x dose	1	34.0	1.13
Species x salt x run x dose	1	81.3	2.71
Species x water x run x dose	1	0.0	0.00
Salt x water x run x dose	1	116.3	3.87
Error	1	30.0	

Appendix B. Flow-through attraction/avoidance bioassay data summary.

P. aztecus

				Animal 1				Animal 2				Animal 3				Animal 4			
Salt[a]	Diluent[b]	Dose	Treatment[c]	Left trips	Right trips	Time left	Time right	Left trips	Right trips	Time left	Time right	Left trips	Right trips	Time left	Time right	Left trips	Right trips	Time left	Time right
1	1	lo	c	5	8	185	269	9	1	396	57	7	7	248	261	4	5	194	276
			l	3	4	130	145	3	3	107	132	5	4	168	104	3	2	169	114
			r	3	3	127	145	2	3	127	109	5	5	93	174	3	2	126	112
		med	c	23	16	225	143	6	5	177	215	10	7	311	235	6	4	338	210
			l	14	14	122	136	4	2	56	70	3	7	50	209	1	3	56	61
			r	15	15	134	123	4	1	137	40	8	6	152	113	4	0	208	0
		hi	c	15	8	419	127	11	6	294	59	7	3	313	117	3	8	108	278
			l	9	6	116	139	0	3	0	164	2	3	93	166	1	1	56	57
			r	8	7	176	85	5	2	192	17	3	2	153	100	2	0	66	0
2	1	lo	c	7	9	231	302	4	7	142	309	7	3	369	146	6	8	214	334
			l	7	3	228	44	5	0	236	0	5	4	109	168	5	0	266	0
			r	6	2	176	37	1	4	53	193	5	4	219	52	2	2	111	111
		med	c	6	5	315	239	8	7	281	268	7	3	229	68	6	6	298	243
			l	2	3	115	126	4	3	193	72	2	3	112	166	3	2	148	116
			r	2	3	110	166	4	1	226	56	4	2	171	80	4	1	208	57
		hi	c	8	11	225	310	7	9	250	303	3	7	164	365	4	6	225	341
			l	3	2	152	110	4	1	206	57	3	3	134	120	1	4	58	228
			r	5	5	136	127	3	4	88	181	0	5	0	229	3	2	134	86
2	2	lo	c	4	5	199	237	8	4	336	141	7	4	327	116	8	5	344	189
			l	3	3	146	86	2	3	82	156	3	2	150	105	3	4	95	136
			r	1	4	55	220	4	1	171	55	3	3	131	105	5	1	265	9
		med	c	5	7	207	312	7	8	202	242	4	8	171	329	6	10	136	320
			l	2	3	86	102	4	3	115	102	3	4	81	156	4	5	91	157
			r	3	5	56	170	3	1	144	20	2	4	77	181	4	3	137	117
		hi	c	6	7	236	336	7	2	361	115	7	11	130	359	7	8	146	227
			l	4	3	193	82	1	4	55	219	4	6	83	162	7	5	110	148
			r	1	4	52	221	3	2	166	102	3	4	57	140	7	4	127	117
1	2	lo	c	10	10	158	304	8	2	415	105	6	11	160	382	9	7	249	224
			l	8	7	181	83	3	3	136	67	4	1	143	20	4	5	122	137
			r	5	1	271	9	4	2	178	111	1	1	45	59	3	3	66	153
		med	c	6	7	286	271	4	5	235	278	4	6	157	337	5	7	177	303
			l	4	2	198	78	3	3	155	118	1	4	56	230	1	4	57	143
			r	2	3	106	171	3	2	165	113	3	4	134	149	2	3	104	113
		hi	c	11	10	281	245	14	12	306	218	7	2	367	77	23	23	213	256
			l	3	5	109	153	4	4	114	167	2	4	79	188	13	15	96	141
			r	4	7	42	236	6	6	138	126	3	5	84	190	14	11	131	116

[a] 1, Instant Ocean; 2, Ranch House salt

[b] 1, deionized water; 2, Brazos River water

[c] c, control; l, left; r, right

Appendix B. Flow-through attraction-avoidance bioassay data summary.

P. *setiferus*

				Animal 1				Animal 2				Animal 3				Animal 4			
Salt[a]	Diluent[b]	Dose	Treatment[c]	Left trips	Right trips	Time left	Time right	Left trips	Right trips	Time left	Time right	Left trips	Right trips	Time left	Time right	Left trips	Right trips	Time left	Time right
1	1	lo	c	6	4	226	343	5	5	225	230	6	6	174	273	2	3	98	149
			l	3	2	170	108	2	4	85	160	3	4	105	134	2	2	8	63
			r	2	3	111	170	3	2	75	67	2	3	108	169	0	0	0	0
		med	c	7	2	387	88	8	11	281	248	7	6	165	176	3	6	169	254
			l	2	5	91	187	1	1	54	52	4	3	136	76	2	3	83	170
			r	5	3	207	84	3	4	79	120	3	7	61	108	2	2	78	94
		hi	c	5	8	167	392	5	3	280	169	5	4	248	176	9	2	469	22
			l	2	3	108	169	1	4	6	183	2	3	107	134	3	4	59	132
			r	1	4	58	227	3	1	173	54	2	1	68	52	3	3	141	88
2	1	lo	c	3	4	146	144	1	2	55	80	4	4	227	224	7	10	182	290
			l	3	2	171	89	1	0	10	0	4	4	135	126	6	5	121	136
			r	2	0	103	0	1	2	57	110	3	2	125	85	2	5	104	162
		med	c	9	7	275	206	5	7	202	293	5	5	61	198	3	4	73	89
			l	4	5	88	184	1	1	13	42	4	2	128	111	1	1	30	13
			r	4	1	185	39	4	6	81	205	0	3	0	64	1	4	29	77
		hi	c	5	9	132	319	4	5	184	256	6	3	327	168	4	6	228	316
			l	1	4	35	131	2	5	83	199	4	1	81	32	3	1	162	57
			r	4	1	148	17	5	3	162	90	2	2	110	108	4	1	230	41
2	2	lo	c	6	2	226	64	4	7	70	274	9	3	375	125	3	6	116	296
			l	3	1	166	58	5	1	293	47	2	3	109	170	2	4	109	152
			r	2	3	109	202	4	1	189	58	3	2	152	106	3	2	162	101
		med	c	6	6	290	261	6	5	293	206	5	1	156	57	3	1	137	10
			l	3	1	127	49	4	2	172	72	0	1	0	26	2	1	72	30
			r	3	2	135	109	3	2	163	81	0	1	0	57	2	0	21	0
		hi	c	7	4	334	191	3	3	115	39	3	5	83	238	4	4	158	65
			l	4	2	183	59	3	3	64	50	1	1	15	14	2	5	18	252
			r	3	3	128	126	2	1	80	36	1	1	10	53	4	2	126	87
1	2	lo	c	8	6	284	276	7	2	219	87	3	7	112	214	6	5	294	275
			l	4	5	127	133	2	1	33	44	4	2	134	100	3	3	96	117
			r	1	1	57	53	1	2	59	44	3	2	165	41	2	3	118	172
		med	c	7	3	227	7	6	2	271	76	6	3	231	152	8	2	332	83
			l	3	1	160	25	2	1	77	58	4	0	156	0	2	1	84	9
			r	0	1	0	45	1	3	54	115	0	0	0	0	1	1	32	9
		hi	c	5	3	163	107	0	2	0	116	4	4	181	154	4	6	143	112
			l	5	3	179	86	0	0	0	0	2	2	12	61	1	1	50	24
			r	3	0	126	0	1	0	6	0	3	4	70	176	0	2	0	63

[a] 1, Instant Ocean; 2, Ranch House salt

[b] 1, deionized water; 2, Brazos River water

[c] c, control; l, left; r, right

Appendix C. *In situ* bioassays for a salt brine effluent with adult and subadult specimens of two species of penaeid shrimp.

When this series of experiments was conceived, laboratory investigations of brine toxicity were to be validated by exposing shrimp to a brine plume near the diffuser site.

MATERIALS AND METHODS

Shrimp were confined at the diffuser site in cages. Cage frames (70 X 140 X 50 cm) were welded from 1 cm mild steel round stock, then covered with two layers of 3 mm mesh plastic screen (Vexar) bound to the frame with metal staples (Hill Shoat Rings), except at two corners to allow the addition of shrimp. The layers of plastic screen were held approximately 5 cm apart by sewn-in sections of plastic pipe, so that when shrimp held on to the inner mesh layer, predators outside the cages could not bite their walking legs off. The cage was subdivided across its width with plastic screen into two compartments, each with square bottom 0.5 m^2 in area. At one end of the cage 60 cm lengths of 1 cm polypropylene rope with a shackle at one end were spliced into the bottom corners of the cage to serve as anchor bridles. Rings were sewn into the top corners at the same end of the cage. Triangular anchors weighing approxilmately 60 kg were cast from concrete. The anchors had loops of 1 cm steel rod at each corner and were attached by a loop to buoys provided by the National Marine Fisheries Service with a shackle, a swivel and a 40 m length of 3 mm galvanized aircraft cable.

Shrimp were captured by commercial trawl and held aboard the vessel for at least 4 hr in well-aerated seawater so that stressed animals could be culled.

A cage was suspended in an empty holding tank, 20 healthy shrimp, each at least 6 cm in abdomen length, and approximately 150 g of gutted fish carcasses (golden croaker) were placed into each compartment of the cage, and the cage was fastened closed. The cage was attached to an anchor by passing the bridle lines through two of the loops and shackling them to the cage corner rings. This arrangement allowed the cage to rest with either its top or bottom surface on the sea floor. Cages were deployed and recovered by passing the buoy cable over a wench cat-head. Two experiments were planned, one in autumn of 1979 and one in spring of 1980.

Cage locations were chosen to correspond with expected dimensions of the near-field, intermediate-field and far-field (NOAA, 1977) according to the following scheme:

Station #	Location (from diffuser)	Characteristics
1	25-100' down current	near-field
2	250' down current	close intermediate-field
3	750' down current	far intermediate-field
4	1500' down current	close far-field
5	6000' down current	mid far-field
6	6000' up current	control

These test sites are placed relatively closely together and treat only a small portion of the area considered in the MIT Model (NOAA, 1977). There are theoretical and practical reasons for this choice. First, projections of Eulerian exposure under average conditions at the site (NOAA, 1978) suggest that no exposure more than 2000' down current from the diffuser is likely to exceed $2^{0}/00$ increased limits for adult and subadult penaeids (Chapter I). Sites were positioned by best-available navigational information, including LORAN C and depth sounding. Water samples were collected from near the bottom at cage deployment and retrieval for temperature and salinity determinations. It was planned to recover the cages after 72 hr of immersion.

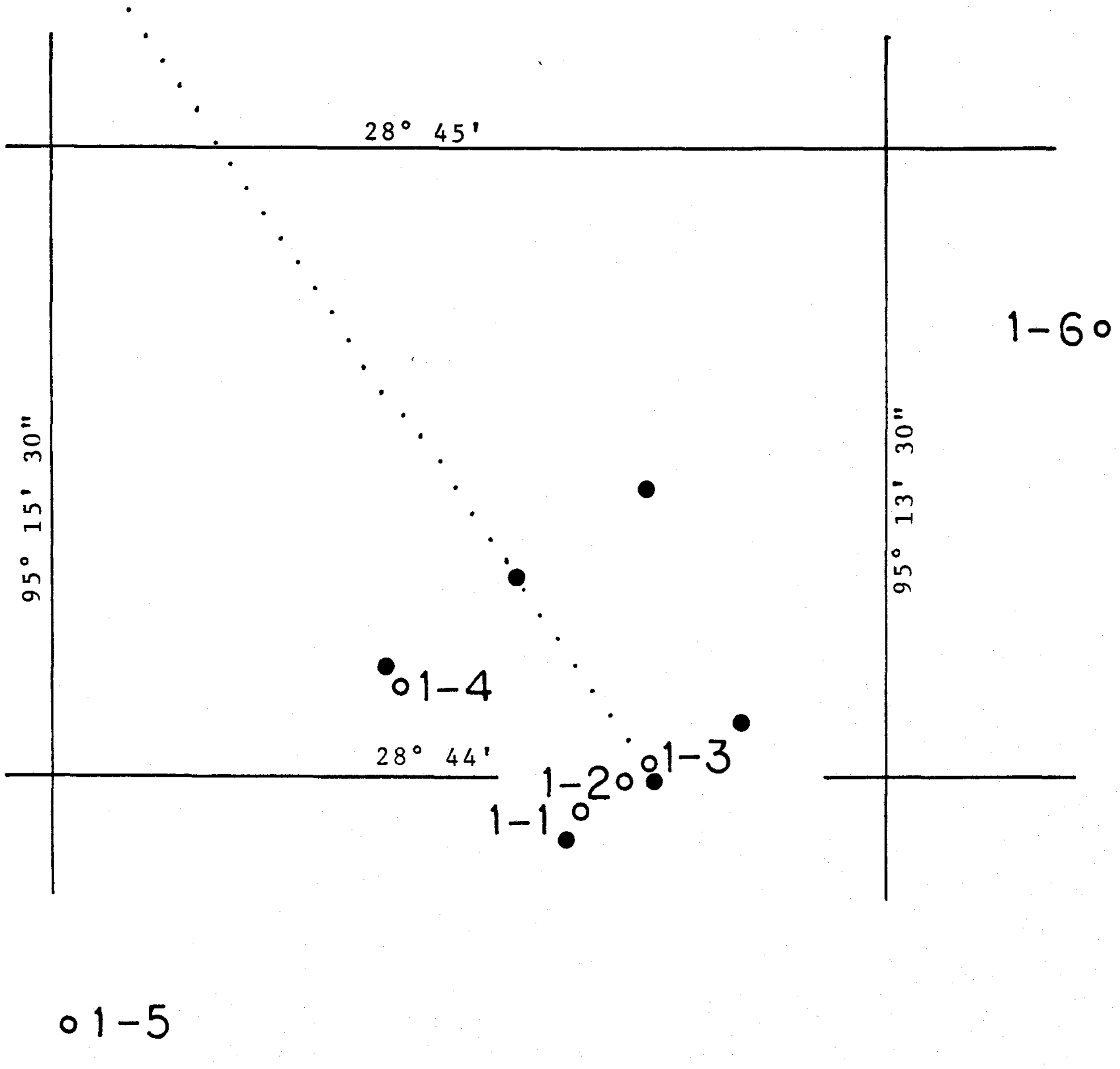

Figure 1. In situ cage sites for cruise 1. The cages are denoted by open circles. The closed circles are buoys at the diffuser area and the dotted line denotes the pipeline.

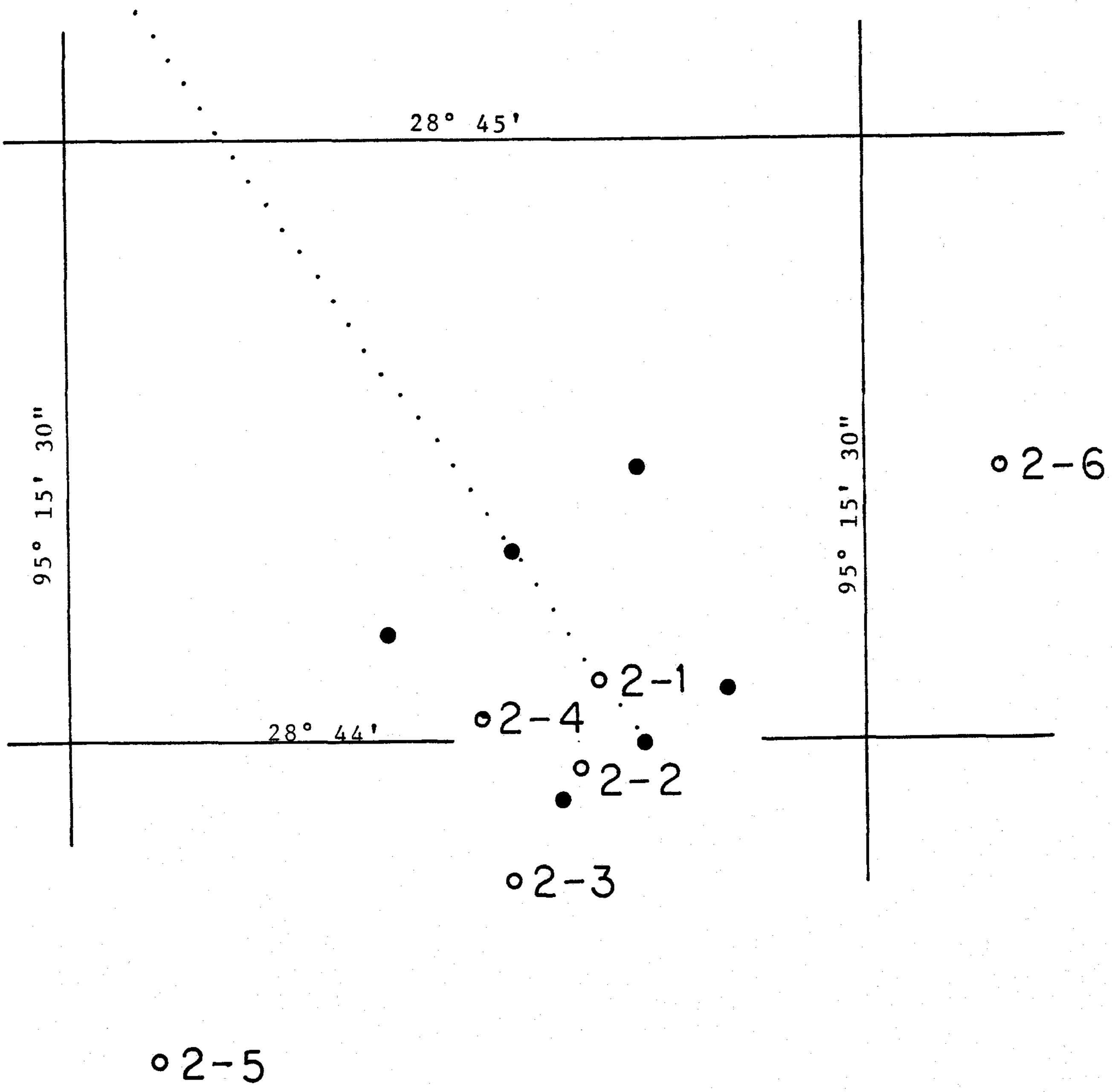

Figure 2. In situ cage sites for cruise 2. The cages are denoted by open circles. The closed circles are buoys at the diffuser area and the dotted line denotes the pipeline.

Table 1. *In situ* experiment data summary.

			Cage deployment				Cage retrieval			
			Water		Species		Water		Species	
Cruise	Duration (hr)	Station No.	Salinity	Temp.	*Penaeus setiferus*	*Penaeus aztecus*	Salinity	Temp.	*Penaeus setiferus*	*Penaeus aztecus*
1	144	1	37.0	28.5	20	20	37.0	29.4	10	16
		2	37.0	28.5	20	20	37.0	29.6	12	15
		3	37.0	28.5	20	20	----	----	--	--
		4	37.0	28.5	20	20	----	----	--	--
		5	37.0	28.5	20	20	----	----	--	--
		6	37.0	28.5	20	20	35.0	29.9	5	16
2	72	1	37.0	29.5	20	20	33.5	29.2	15	19
		2	36.0	29.5	20	20	35.0	29.2	16	19
		3	37.0	29.4	20	20	----	----	--	--
		4	36.0	29.4	0	41	35.0	29.2	0	40
		5	36.0	29.4	0	41	33.0	29.2	0	41
		6	34.0	29.4	0	40	35.0	29.2	0	32

RESULTS

The first experimental run (fall of 1979) was postponed due to delay in the start-up of the brine pumping schedule. Consequently, both experimental runs were performed in August of 1980 because of boat time demands. During the trial periods, brine flow at the diffuser site was intermittant.

In the first trial, six cages were deployed on August 1, 1980. Figure 1 shows the cage locations. Cage retrieval was postponed an additional 72 hr due to severe weather conditions resulting from a tropical storm in the Gulf of Mexico. When cage retrieval was finally accomplished, three buoys were lost. Numbers of surviving animals in the three cages retrieved are listed in Table 1. Temperature and salinity data for this run (Table 1) indicated no differences in the salinities among stations, despite their different locations in respect to the diffuser and to prevailing currents.

Cage deployment for the second run (Figure 2) was performed on August 26, 1980. Brown shrimp were substituted for white shrimp (Table 1) in three cages due to a scarcity of the latter. Table 1 lists the number of surviving animals and shows one cage missing.

DISCUSSION

In view of laboratory toxicity tests, no salinity-independent mortality could have been expected. In all likelihood, most deaths could have been due to the many handling steps in cage deployment. However, survival in the second run was favorable. The above experimental regime could have proven more beneficial, if the experiments had been performed at a period when a strong salinity gradient had existed in the diffuser area.

REFERENCES

NOAA. 1977. Analysis of brine disposal in the Gulf of Mexico: (1) Bryan Mound. National Oceanic and Atmospheric Administration. Washington, D.C. 165 pp.

NOAA. 1978. Analysis of brine disposal in the Gulf of Mexico: Bioassay results. National Oceanic and Atmospheric Administration. Washington D.C.