NOAA Technical Memorandum
NMFS-SEFC-69

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 V

REDFISH BIOASSAYS

(Part B) Brine Avoidance/ Attraction

JANUARY 1982

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

NOAA Technical Memorandum NMFS-SEFC-69

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

VOL. V(B) BRINE AVOIDANCE/ATTRACTION BIOASSAYS ON REDFISH

By
D. W. Owens, Ph.D.
K. A. Jones, Ph.D.
Texas A&M University
and
B. J. Gallaway, Ph.D.
LGL Ecological Research Associates, Inc.

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

EDITOR

William B. Jackson
Fishery Biologist

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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Owens, D. W., K. A. Jones and B. J. Gallaway. 1981. Brine avoidance/attraction bioassays on redfish. Vol V (Part B). In: Jackson, W. B. (editor). Shrimp and redfish studies; Bryan Mound brine disposal sites off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-69. p. NOAA, NTIS Accession No. ________.

I. PROJECT ADMINISTRATION SECTION

Volume V(B) - BRINE AVOIDANCE/ATTRACTION

TABLE OF CONTENTS

PROJECT ADMINISTRATION

<u>NOAA</u>

Program Manager

Fredric A. Godshall, Ph.D.
Environmental Data and Information Service
Center for Environmental Assessment Services
Marine Environmental Assessment Division
Washington, D.C.

<u>NMFS</u>

Contracting Officer's Technical Representative

Edward F. Klima, Ph.D.
Director
Galveston Laboratory
Southeast Fisheries Center

Project Manager

Charles W. Caillouet, Ph.D.
Chief, Environmental Research Division

Project Staff (Environmental Research Division)

William B. Jackson
Senior Advisor
Contracts and Deliverables

Gregg R. Gitschlag
Senior Advisor
Field Operations and Logistics

Terry Roberts, Ph.D.
Oceanographer

Maurice L. Renaud, Ph.D.
Oceanographer

Dennis Koi
Computer Programmer

Petronila C. Prado
Clerk Stenographer

Beatrice Richardson
Clerk Typist

LIST OF VOLUMES

This Final Report is printed in six separate volumes:

Volume I(A) - SHRIMPING SUCCESS

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

Science Applications, Inc.

C. E. Comiskey, Ph.D., et al.

Volume I(B) - SHRIMP CATCH-EFFORT ANALYSIS

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

Science Applications, Inc.

C. E. Comiskey, Ph.D., et al.

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, Ph.D.

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(A) - Brine Toxicity Bioassays on Redfish

Texas A&M University

J. M. Neff, Ph.D.
M. P. Coglianese
W. McCulloch

and

LGL Ecological Research Associates, Inc.

L. A. Reitsema, Ph.D.
S. Anderson

Work Unit 7(B) - Brine Avoidance/Attraction Bioassays on Redfish

Texas A&M University

D. W. Owens, Ph.D.
K. A. Jones

and

LGL Ecological Research Associates, Inc.

B. J. Gallaway, Ph.D.

Volume VI - SHRIMP BIOASSAYS

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

University of Houston

N. R. Howe, Ph.D.

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 near shore 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.

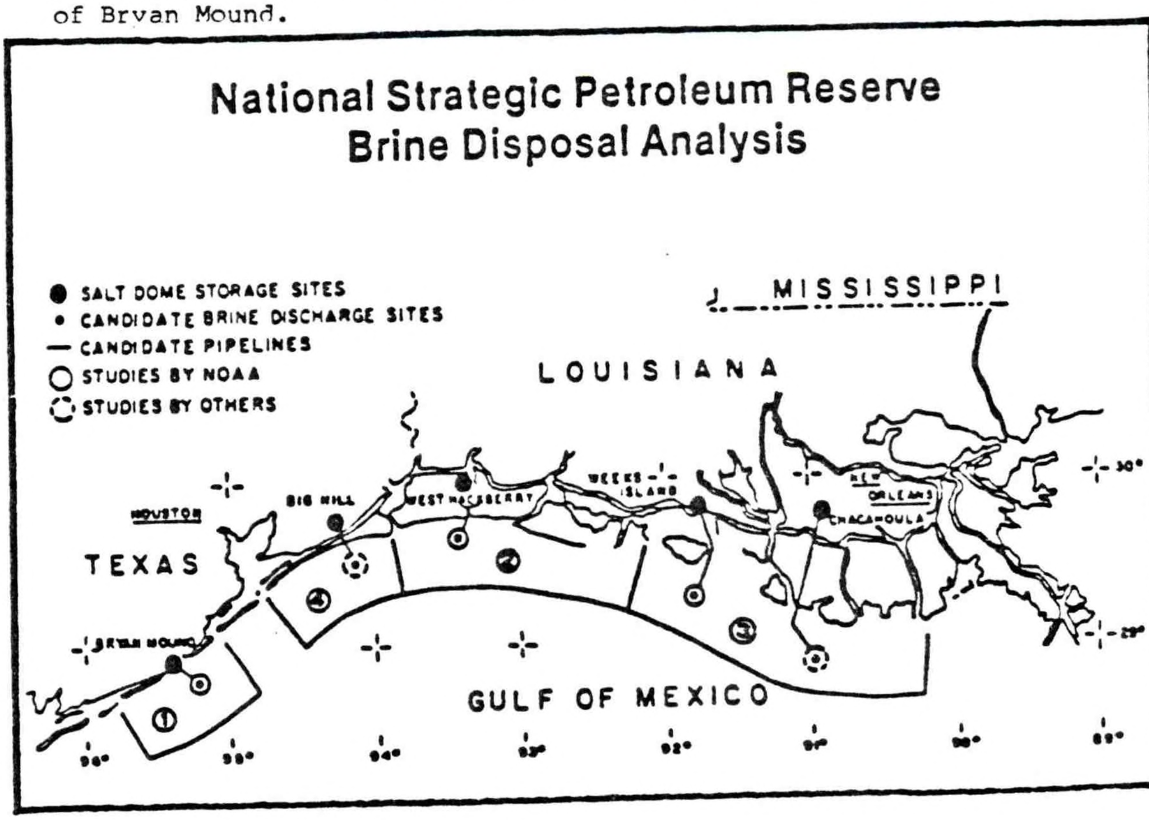

Figure 1. Regions of Study for Brine Disposal Assessment-DOE/NOAA Interagency Agreement (adapted from Environmental Data and Information Service, DOC/NOAA).

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 complement the site-specific oceanographic and biological monitoring of brine disposal conducted by Texas A&M University, a regional assessment of important commercial and recreational fisheries was initiatee in August, 1979. The objectives of this assessment were (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), 5285 Port Royal Road, Springfield, Virginia, 22161; 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.

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, Virgina 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., R. McCord, D. Bozworth, S. Grady, C. Hall, C. Brandt and T. Farmer. 1981. Analyses of data on shrimping success, shrimp recruitment and associated environmental variables. Vol. I(A). In: Klima, E. F. (Contracting Officer's Technical Representative). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-65. ___ p. NOAA, NTIS, Accession No. _______________.

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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. NOAA, NTIS, Accession No. PB81-249591.

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. NOAA, NTIS Accession No. PB81-249609

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. NOAA, NTIS Accession No. PB81-249583.

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Neff, J. M., M. P. Coglianse, L. A. Reitsema, S. Anderson and W. McCulloch. 1981. Brine toxicity bioassays on redfish. Vol. V (Part A). In: Jackson, W. B. (editor). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-69. p. NOAA, NTIS Accession No. ________________.

Owens, D. W., K. A. Jones and B. J. Gallaway. 1981. Brine avoidance/attraction bioassays on redfish. Vol. V (Part B). In: Jackson, W. B. (editor). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-69. p. NOAA, NTIS Accession No. ________________.

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 hydrocarbon 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, 164 p. NOAA, NTIS Accession No. PB81-174971.

Brooks, J. M. 1980. Determine seasonal variations in inorganic nutrient compostion 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, 55 p. NOAA, NTIS Accession No. PB81-182685.

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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, 226 p. NOAA, NTIS Accession No. PB81-174955.

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, 335 p. NOAA, NTIS Accession No. PB81-133803.

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. NOAA, NTIS Accession No. PB81-133795.

Reitsema. L. A. 1980. Determine seasonal abundance, distributiona nd 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, 162 p. NOAA, NTIS Accession No. PB81-175838.

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Related Publications

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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. 1980. Relationship between ex-vessel value and size composition and annual landings of shrimp from the gulf and south Atlantic coasts. Marine Fisheries Review 42(12):28-33.

Caillouet, C. W. and D. B. Koi. 1980. 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 42(2):18-27.

Caillouet, C. W. and D. B. Koi. (1981). Trends in ex-vessel value and size composition of reported May-August catches of brown and white shrimp from the Texas, Louisiana, Mississippi and Alabama coasts, 1960-1978. Gulf Research Reports 7(1):(in press).

II. PRINCIPAL INVESTIGATORS' SECTION

WORK UNIT 7(B) - BRINE AVOIDANCE/ATTRACTION BIOASSAYS ON REDFISH

D. W. OWENS, Ph.D.
K. A. JONES, Ph.D.

TEXAS A&M UNIVESITY

AND

B. J. GALLAWAY, Ph.D.

LGL ECOLOGICAL RESEARCH ASSOCIATES, INC.

ABSTRACT

A circular tank with a water depth of 77 cm and diameter of 5.49 m was used to monitor the behavior of the redfish or red drum (*Sciaenops ocellata*) in response to various temperature and salinity gradients. Photoelectric gates separated eight peripheral compartments from the open center of the tank and monitored fish entering and leaving the compartments. The experimentation was based on a free choice, non-conditioning design. The 100 fish used had a standard length of 42.75 $\pm$ 1.44 cm ($\bar{X} \pm SE$) and generated an average of 83.12 $\pm$ 24.66 compartment entries per hour of monitoring. Redfish were determined to be most active in our system during the first six hours of the dark phase, though a minimum adjustment period of three hours was necessary for this species to begin normal exploratory behavior.

A comparison of various brines made from salt dome salt and artificial sea salts (Instant Ocean) indicated that redfish did not readily discriminate among these solutions as determined by the activity measures employed. In addition, brines made with Brazos River water as a diluent were not distinguished from brines made from tap water as a diluent. Redfish neither avoided nor were attracted to the 2 ppt brine gradients, as compared to control compartments receiving no brine.

In separate experiments, attraction/avoidance for brines at each of three different gradient strengths (0.5, 1.0 and 2.0 ppt) were tested on fish acclimated at three different ambient temperatures (15°, 20°, 25° C). In addition, in the same protocol, brine heated to 2°C above background was compared to unheated brine using separate compartments. Redfish at colder acclimations of 15° and 20° C appeared attracted to heated brine only if the saline gradient in the test compartments was low (0.5 ppt above background). At an acclimation temperature of 25° C during fall, the unheated brine appeared attractive compared to both the heated brine and the appropriate controls. A separate experiment conducted at 25° C acclimation during spring indicated a similar trend, but the results were not statistically significant. We hypothesize that redfish may use saline gradients as part of their cueing system for movements from estuaries to the open gulf, as well as for localized movements as required for behavioral thermoregulation.

Direct observations also indicated that the redfish responded to slight saline gradients under certain conditions. However, the diffuser's maximum near-field concentration of approximately 5 ppt above ambient reported by Randall (1981) is not of sufficient magnitude to harm any life stage of this euryhaline species. In addition, even if redfish are attracted to the brine gradients, it is not likely that they would remain long in the area without further reinforcement of the behavior (e.g. cool water or food resources). None of the present monitoring programs involving sampling near the diffuser are using methods which will result in capture of large redfish which would enable confirmation or rejection of the hypothesis that redfish may aggregate around the brine diffuser under certain conditions.

LIST OF FIGURES

LIST OF TABLES

APPENDIX I

APPENDIX II

APPENDIX III

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

We would like to thank Robin Krenek for his daily perserverance in conducting much of the experimentation. He developed the important techniques for reversing the water flow in the monitoring tank as well as many other innovations which proved very useful. Jay McGovern was instrumental in refitting the tank complex and Steven Sparagana assisted with several experiments. We extend our thanks to both of these people. We also thank Nancy Scott, Jane Versyp, Karen White and Tammie Knight for assistance in manuscript preparation. LGL Ecological Research Associates, Inc. provided the fish and Brazos River water used in the experiments, as well as considerable advice with statistical evaluation. We thank Benny J. Gallaway for providing valuable editorial assistance in the preparation of this report.

INTRODUCTION

Because of the need to store large quantities of oil, the Strategic Petroleum Reserve (SPR) Program is utilizing solution mining to evacuate salt domes located along the coast of the Gulf of Mexico (Davis 1981). In the solution mining process, large quantities of brines are disposed of at certain coastal sites. An understanding of the possible effect these brines might have on the behavior of selected marine organisms is an important consideration.

The redfish or red drum (Sciaenops ocellata Linnaeus) is an important species along the gulf coast utilized by recreational and commercial fisherman (Pearson 1929; Perret et al. 1980). Redfish may be present at times in the area of the Bryan Mound brine disposal site (Chittenden et al. 1981, Chittenden and McEachran 1976). Although a complete understanding of the behavioral biology of this species is not available, they do spend their early life history in the estuaries and appear to move as adults into the Gulf where they are commonly taken near passes and in the surf during the fall spawning season (Perret et al. 1980). The redfish is known to be both euryhaline and eurythermal (Simmons 1957, 1969; Simmons and Breuer 1962), thus their ability to endure slight variation in water temperature and salinity is quite good.

In our laboratory, during unusual occurrances such as power failures, fresh water treatments for parasites and accidental dropping during weighing, we did witness this species under acute physiological stress conditions. Behaviorally, the results of such stresses were easily recognized by solitary movement (this species nearly always schools), surface gulping, disorientation and excessive gilling. When field-collected redfish were subjected to 5 ppt salinity and 5°C temperature changes, no visible signs of a comparable physiological stress were observed even immediately after a 240 km truck ride from the coast. Schooling, respiratory rates and swimming behavior all appeared normal.

In contrast to the physiological effects of slight salinity and temperature gradients, the behavioral effects are much less readily predicted. We have been unable to locate a single behavioral study on this or any other bottom dwelling, schooling species that would assist us in making predictions regarding redfish. Since redfish are to some degree migratory, it is not unreasonable to consider salinity and temperature gradients as potential orientation cues for this species. Most estuaries and coastal oceanic provinces are, in fact, characterized by a series of natural gradients. It would, therefore, not be surprising if species such as the redfish had evolved very sensitive orientation systems to regularly utilize such gradients for directed movements in a euryhaline and eurythermal environment. An orientation system such as this would be particularly useful in moving between estuaries and the Gulf of Mexico.

Given the relative certainty that redfish would be able to detect subtle salinity gradients (e.g., Hasler 1957, Hara 1971) and the likelihood that such gradients are important to seasonal movements of preadults and adult fish, we addressed the questions (1) would preadult and adult redfish respond to the expected salinity gradient (plume) from Bryan Mound brine disposal, and , if so (2) would they be attracted to or avoid the area of discharge? In the case of an attraction response, there was concern that if spawning aggregations developed in the vicinity of the discharge, a potential result might be the exposure of embryos, larvae and juveniles to toxic conditions. The concern with respect to potential avoidance of the brine plume impact area and/or its toxicity was related mainly to the differences in inorganic ionic ratio of the SPR brines as compared to natural seawater, the poor quality of the Brazos River water being used for solution mining (for a characterization of the Bryan Mound brine see Neff et al. 1981), and the original concern that the brine would be discharged at slightly elevated temperatures (SPR-FEA 76/77-6, 1977).

RATIONALE AND OBJECTIVES

The study was approached from an experimental basis using a quantitative and objective animal behavior monitoring tank specifically designed for the automatic evaluation of the behavior of large marine species (Kleerekoper 1978). As with most behavioral experimental apparatus, the system is dependent upon the experimental subject (in this case redfish) being able to adjust to the monitoring tank environment in terms of exhibiting regular movements as well as demonstrating a level of activity sufficient to generate an adequate sample size with regard to choosing among the different environmental conditions offered. In addition, a major part of the experiment was the development of the procedures and modifications of the experimental apparatus enabling us to present to the redfish a stable array of different environmental conditions during the course of a given experiment. As the redfish had never been used in experimental studies of this nature, the initial objectives of the study were to:

(1) modify the experimental apparatus as necessary to produce and maintain a stable array of defined environmental conditions for testing redfish responses and;

(2) determine optimum periods of activity of redfish for such experimental purposes.

Given that objectives (1) and (2) were met, the objectives of the remaining experiments were to:

(3) determine if redfish responded in a different fashion to four different types of brine solutions including (a) Bryan Mound salt (Ranch House Stock Salt)/Brazos River water, (b) Ranch House Stock Salt/tap water, (c) Instant Ocean Salt/Brazos River water and (d) Instant Ocean/tap water,

(4) determine if redfish acclimated to 15°, 20°, and 25° C and 35 ppt exhibited avoidance or attraction to simulated discharge conditions including three different brine concentrations (0.5, 1.0 and 2.0 ppt above ambient) at two temperatures (ambient and 2° C above ambient) and;

(5) verify significant avoidance/attraction responses with additional experiments.

In addition, we attempted to evaluate other behavioral responses to the brines (coughing, gilling, etc.) based upon direct visual observations of the fish in our monitoring tank as they first encountered the brines. Similarly, animals were acclimated in 190ℓ aquaria and observed both before and after exposure to brines.

SUMMARY AND CONCLUSIONS

- In our laboratory system, redfish demonstrated highest activity levels during the first six hours of darkness. They were less active during the light phase. Compartment entries averaged 83.12 $\pm$ 24.66 ($\bar{X} \pm$ SE) per fish per hour for the 100 animals which produced usable experimental records. Although redfish are good experimental subjects, their activity levels were often quite variable.

- Although redfish clearly sensed the presence of brines as low as 2 ppt above ambient, they tolerated extended exposure to salinity increases of at least 5 ppt without visible signs of difficulty.

- Redfish did not appear to differentiate among salt dome and control brines or among Brazos River water and control water brines.

- In none of our experiments was there any indication that redfish would avoid brines at concentrations of 2 ppt or less above an ambient salinity of 35 ppt.

- Under warm water acclimation conditions (25° C) there was strong evidence that redfish would be initially attracted to a brine gradient of between 1 and 2 ppt above an ambient salinity of 35 ppt. Attraction to brine alone was not indicated when fish were acclimated to 20° or 15° C.

- At the colder acclimation temperatures (15° and 20° C), redfish appeared attracted to the warmed brine, but only if the saline gradient was low (0.5 ppt above ambient).

- There appears to be no additive effect of the observed attractions to heated water and to brines since these attractions occur at opposite ends of the temperature acclimation scale.

Based upon the results of our studies it is our opinion that aggregations of subadult and adult redfish could develop around the diffuser under certain conditions. Given the results of the bioassay studies of brines and early life stages of redfish (Neff et al. 1981) and our work, we foresee no direct deleterious effects of the brines *per se* on redfish, unless salinity concentrations exceed 45 to 50 ppt. These levels are included in the tolerance range of subadult and adult redfish (Perret et al. 1980), but are toxic to embryos and larvae (Neff et al. 1981).

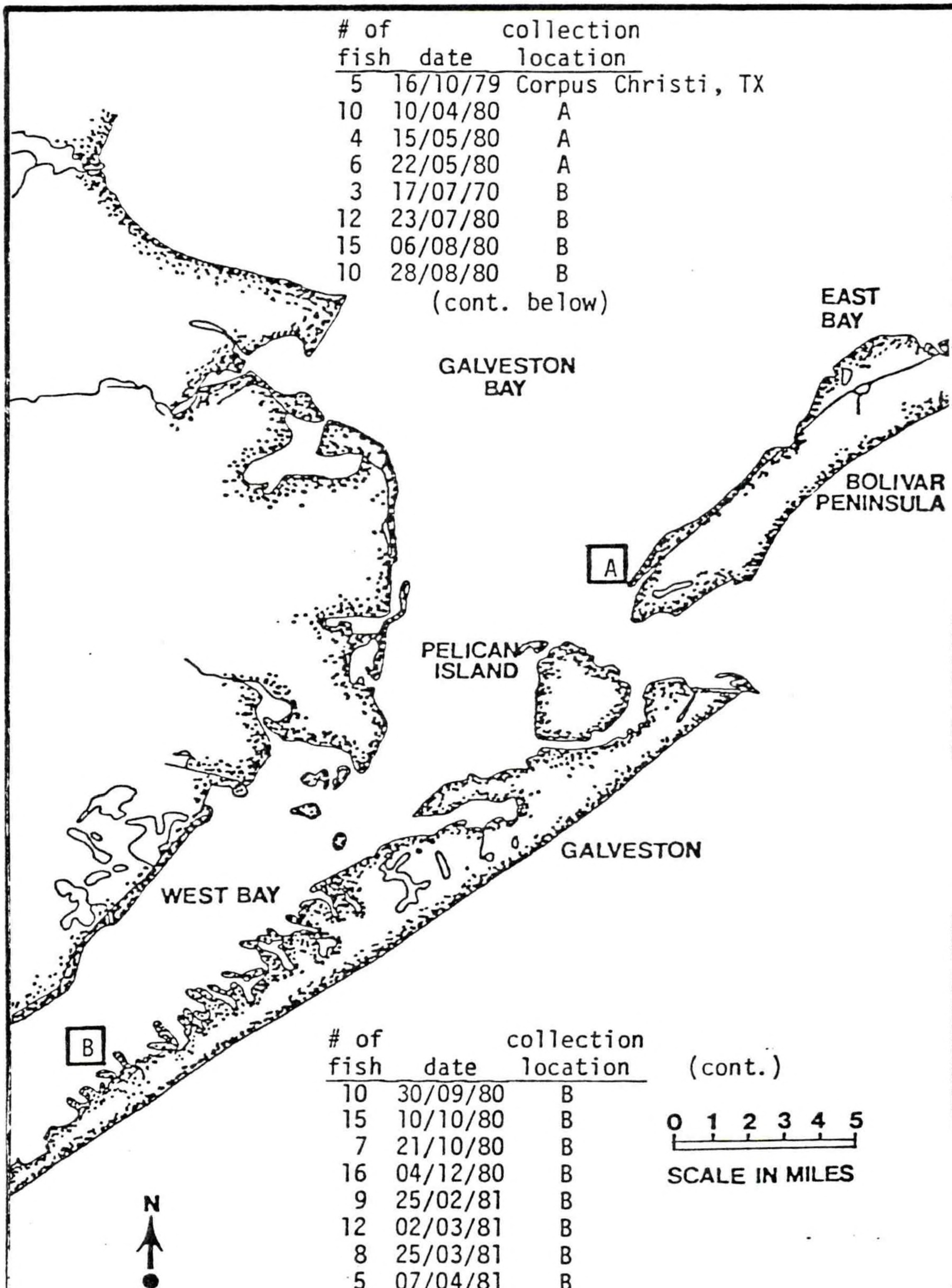
MATERIALS AND METHODS

The conduct of this study was dependent upon being able to collect, transport and maintain subadult and adult redfish in good condition in the laboratory holding tank. In the experiments, the fish were taken from the holding tank and placed in a circular monitoring tank where they were exposed to different water quality conditions within cells or compartments located around the periphery of the tank. The number of entries into cells of different water quality and the amount of time spent in a cell were used as the response variables indicating either avoidance or attraction behavior. Thus, the experiments were also dependent upon the activity level of the fish and the fish being able to "adjust" to the experimental system as determined by their exhibiting regular and apparently normal locomotory behavior. Although variable from species to species, in this system the fishes which have been used commonly show low activity levels for the first four hours, followed by a period of high activity for up to three days in what has been called the exploratory period (e.g. Kapoor and Kleerekoper, 1970). Lastly, the avoidance/attraction nature of the experiments required that we be able to maintain an environmental gradient (cell water quality) over the period of an experiment.

EXPERIMENTAL SUBJECTS

A single collection of 5 redfish was obtained by hook and line from the Corpus Christi area while 142 fish were obtained from the lower Galveston Bay region during 1980-81 (Fig. 1) using a bottom-set long line. The average standard length of the 100 fish which generated usable results was 42.75 cm with a standard error (SE) of 1.44 cm. Average weight of the fish was 1.25 $\pm$ 0.12 kg. The fish were transported to our laboratory facility near Bryan, Texas where they were maintained in a 15,000 ℓ holding tank equipped with a sand flilter. Fish were acclimated in the holding tank for a minimum period of two weeks prior to each experiment. The level of acclimation temperature (15°, 20°, and 25°C) depended upon the experiment to be performed; the acclimation salinity was 35 ppt in all experiments. Temperature and salinity were monitored and adjusted daily such that they were always within 1°C and 1 ppt, respectively, of the test conditions.

The fish were held on a 10h:14h light-dark cycle (approximately the cycle that occurs in the field during the spawning season). They were maintained in a light-controlled room on a partially reversed photoperiod to facilitate experimentation on the animals during their scotophase. Initially, the lights over the fish were turned off at 0200 h C.S.T. or C.D.T. depending on the time of year. Following the initial experiments, lights off was changed to 1100 h in order to permit us to move animals from the holding tank to the experimental tank under light conditions but still run the experiments during the fish's scotophase. The subjects were fed a diet of shrimp, squid, and fish during each photophase except on weekends. We fed during the day so that we could use the feeding response both as a means of evaluating the health of the animals and as an index of the acclimation status. It normally took two or more weeks for



# of fish	date	collection location
5	16/10/79	Corpus Christi, TX
10	10/04/80	A
4	15/05/80	A
6	22/05/80	A
3	17/07/70	B
12	23/07/80	B
15	06/08/80	B
10	28/08/80	B

(cont. below)

# of fish	date	collection location
10	30/09/80	B
15	10/10/80	B
7	21/10/80	B
16	04/12/80	B
9	25/02/81	B
12	02/03/81	B
8	25/03/81	B
5	07/04/81	B

(cont.)

Fig. 1. Capture location and date of capture of redfish utilized in this study.

the fish to begin to feed. An individual fish was not fed on the day it was utilized. In most cases the fish were used only once in the monitoring tank. There were, however, three fish which were used three times each in different treatment groups. These fish showed no evidence of conditioning in repeated experimental runs (run = monitoring period).

EXPERIMENTAL APPARATUS

A fiberglass-coated steel tank 549 cm in diameter x 103 cm deep (Kleerkeoper 1978) was used to monitor locomotory behavior of the redfish. The tank, filter, electronics and plumbing of this tank were redesigned and rebuilt especially for accommodating redfish and maintaining brine gradients. The fiberglass surfaces of the tank were painted with black epoxy paint. Sixteen dividers extended radially toward the center of the tank, partitioning the periphery into 16 compartments or cells and leaving an open field in the center (Figs. 2 and 3). Alternate dividers contained light sources whose beams passed through red filters (of a wavelength of approximately 520 nm which is beyond the range of visual sensitivity of most fish) and fell onto bands of phototransistors contained in adjacent dividers on both sides. Thus, a photoelectric "gate" which was activated by the passage of the redfish, monitored the entrance or exit of the fish into or out of every compartment. With each gate activation, the compartment identity and the binary time was translated, via a logic interface, onto paper tape by means of a paper tape punch located outside the testing area (Fig. 4). The information was then stored for processing by computer.

For these studies, artificial seawater (Instant Ocean) maintained at 35 $\pm$ 1 ppt salinity was recirculated between the monitoring tank and a reservoir of 23,500 ℓ capacity. The water was passed through a large diatomaceous earth filter prior to delivery to the monitoring tank. Two methods were used to deliver water to the tank. Initially, water was diffused into a channel surrounding the periphery of the monitoring tank and communicating with the tank through three 2.5 cm holes located in the rear wall of each compartment. In this arrangement the water flowed, essentially, out of the compartments and toward the center where it exited through a Venturi pipe. This flow pattern was only used in the preliminary experiment on diurnal activity. In the eventual brine studies, a reversed flow pattern was used; i.e., the water was introduced in the center of the tank, flowed outward into each compartment and exited into the peripheral channel. The channel then drained by a swivelarm standing pipe. The water in the tank was maintained at a depth of 77 cm while the flow rate into the tank was 150 ℓ/min. No rheotactic responses to this flow rate were observed except in the case of fish in very poor physical condition.

The monitoring tank was illuminated by an overhead matrix of 30 incandescent lamps. A layer of white plastic beneath this matrix served to diffuse the light. During the scotophase the intensity of the lamps was maintained at 1.6 lux as measured at the surface of the water.

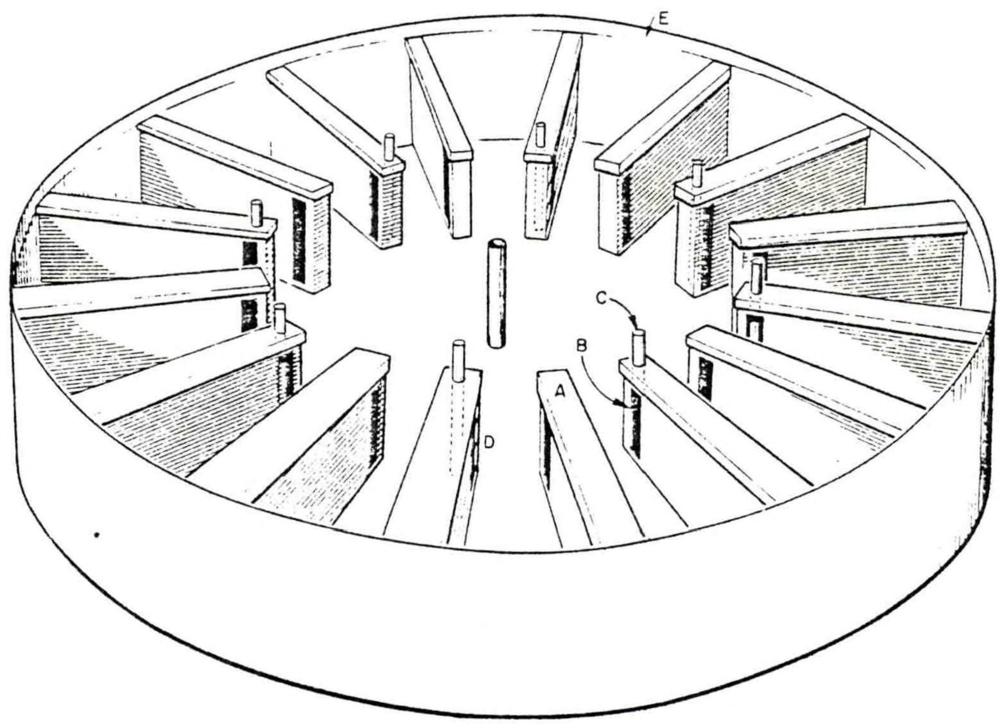


Fig. 2. Animal monitoring tank with open central area and radially oriented dividers (A) and vertical windows (B), behind which are either incandescent lamps and red filters (C) or banks of photoconductive cells (D); and peripheral channel (E).

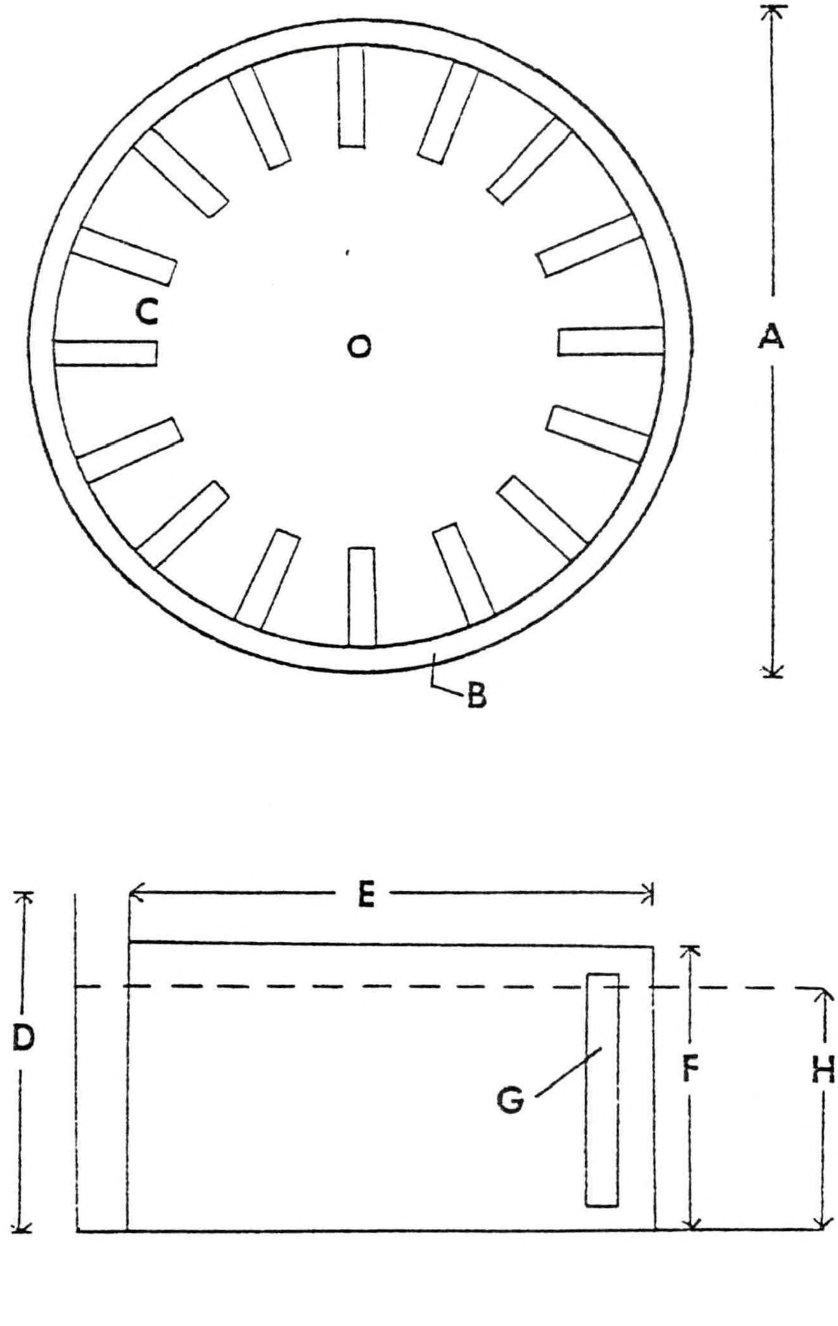


Fig. 3. TOP. Overhead view of the monitoring tank. A) 549 cm dia; B) peripheral channel, 15 cm wide; C) entrances to compartments 50 cm; D) 103 cm; E) 138 cm; F) 97 cm; G) rectangular window (5 x 89 cm); H) water level 93 cm. BOTTOM. Side view of a divider and the peripheral channel.

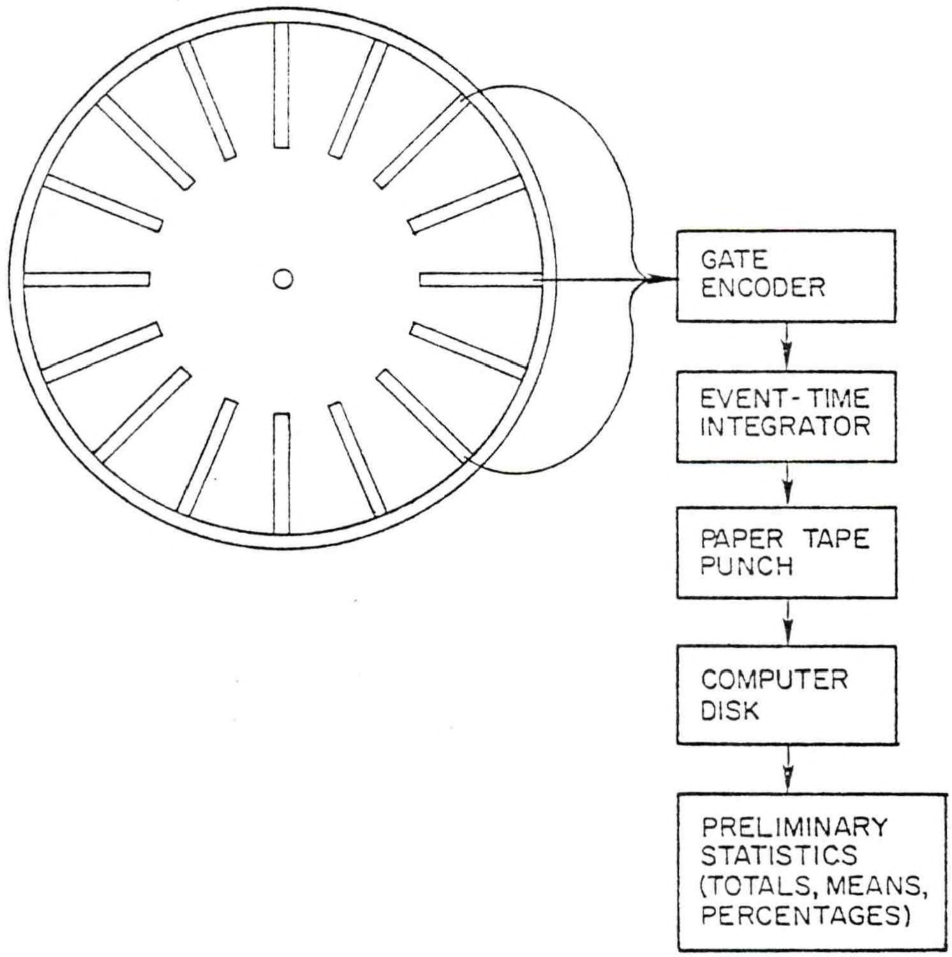


Fig. 4. Flow diagram showing how the information from gate triggerings in the monitoring tank was collected, interpreted, stored, and processed into a form amenable to statistical analysis. The gate encoder identified the gate triggered by the passage of the fish. The event/time integrator combined the gate identity with time of day and relayed both pieces of information to the paper tape punch.

DETERMINATION OF ACTIVITY LEVELS AND TEST PROCEDURES

Obtaining an improved knowledge of the daily activity patterns of redfish was important in order to determine the optimum time to monitor activity. Animals which exhibit low activity levels in our experimental apparatus produce records with much reduced statistical power. Our purpose was to test the animals at a time of day when they would normally be making the greatest number of behavior decisions. Although there were some reports available which indicated that redfish might be nocturnal (e.g. Simmons and Breuer 1962), there were no definitive data describing the daily activity patterns of redfish nor, even if there had been, that the natural patterns would be representative of activity levels of captive fish under laboratory conditions. Thus, preliminary experiments were performed in order to:

(1) determine general activity levels, rates of adjustment to the monitoring tank, and other requirements for monitoring the behavior of redfish in our system.

(2) determine when during a normal photoperiod, a redfish was more likely to exhibit the highest activity levels.

(3) determine if a light/dark activity difference could be detected for this species in our system.

For this initial experiment, water flow was from the periphery to the center of the tank and all 16 compartments were accessible to the fish. Starting at various times of the day, but with an effort to equally represent all 24 h, a fish was netted from the holding tank and immediately placed in the center of the animal monitoring tank. All fish were then monitored for the next 26 h with the exception of one fish which was monitored for two days. The first hour of monitoring was considered as an adjustment period and data collected during this period were deleted from the statistics. Therefore, we considered the true record to begin on the second hour; the 26th hour served to overlap into a second day and was not used in the analysis. Ten fish were monitored. They averaged 39.1 ± 1.18 cm ($\bar{X}$ ± SE) in standard length and 0.96 ± 0.08 kg in weight. Three fish had incomplete records because they were so active that the system ran out of paper tape toward the end of the runs. Unfortunately time did not permit running each fish for several days as would have been required for biological rhythm studies.

The parameter used to determine activity was the number of compartment entries per unit time. Each record was partitioned into half-hour segments. The number of entries per segment was converted to a percentage of the total number of entries obtained for the entire period of monitoring. For those experiments with incomplete records, an approximation of the total number of entries for the 24 h was computed by substituting the mean number of entries for each missing half-hour period. The percentages were then computed as before. The periods with the substituted mean entries were not used in other analyses. The percentages were used in a one-way, two treatment ANOVA contrasting the activity between night and daytime hours. In addition, the percent data also served to produce a combined activity plot for all fish.

The ten fish used in this experiment generated 3,797 ± 685 ($\overline{X}$ ± SE) entries per animal during the 24-h monitoring period. The mean activity of the ten individuals plotted against time is shown by Fig. 5. Above and beyond what effects light and dark may have had on the activity of the redfish, there was a strong increase in activity the longer the fish remained in the tank. This was particularly obvious after the first three hours (Fig. 6). Redfish exhibited this temporal increase in activity regardless of whether the experimental run was begun under light or dark conditions. Since this effect confounded the resolution of any light/dark activity differences, it was removed by fitting the data with a linear regression for time (slope = 0.049; F = 98.78; PR > F < 0.0001). The residuals were then used in an analysis of variance (ANOVA) contrasting the light/dark activities (Table 1).

TABLE 1. ANOVA TABLE FOR LIGHT VS. DARK MEAN PERCENT HALF-HOURLY ACTIVITY LEVELS

Source	DF	SS	MS	F	PR
Light vs. Dark	1	16.20	16.20	8.52	0.0037
Residual	442	840.55	1.90		
TOTAL	443	856.75			

The light and dark mean activity levels were found to be significantly different, with light activity at 2.06 ± 0.1% per 1/2 h ($\overline{X}$ ± SE) and dark at 2.35 ± 0.1% per 1/2 h. This significant difference was primarily attributable to a very high level of activity during the first 6 h of darkness. Figure 7 is a plot for a single animal which was continued in the tank for 48 h.

Given the above results, we determined that:

(1) the minimum adjustment time for redfish in our tank system appeared to be about 3 h; and

(2) that redfish in our system exhibited maximum activity levels during the first 6 h of the dark phase of the day, which was considered to represent the best time to conduct the subsequent experiments.

We also learned during this phase of the work that there was considerable variability in the activity levels of individual redfish and that the act of turning the overhead lights on or off caused a marked but transient reduction (about 1 h) in activity levels.

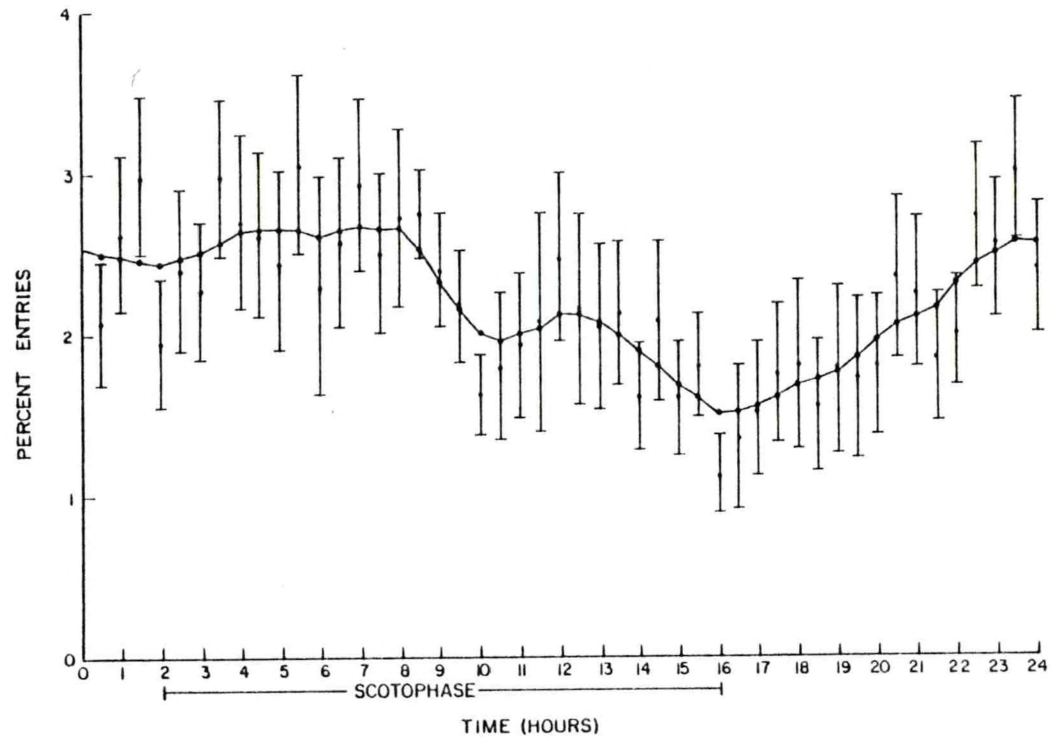


Fig. 5. The mean (+ SE) activity of ten redfish vs. the time of day. The period of darkness (scotophase) has been indicated on the time scale. Activity is expressed in terms of percent entries calculated for each fish by converting the number of entries during each half-hour period to a percentage of its total entries. The smooth line running through the data represents a weighted average of one half-hour period and the three periods to either side.

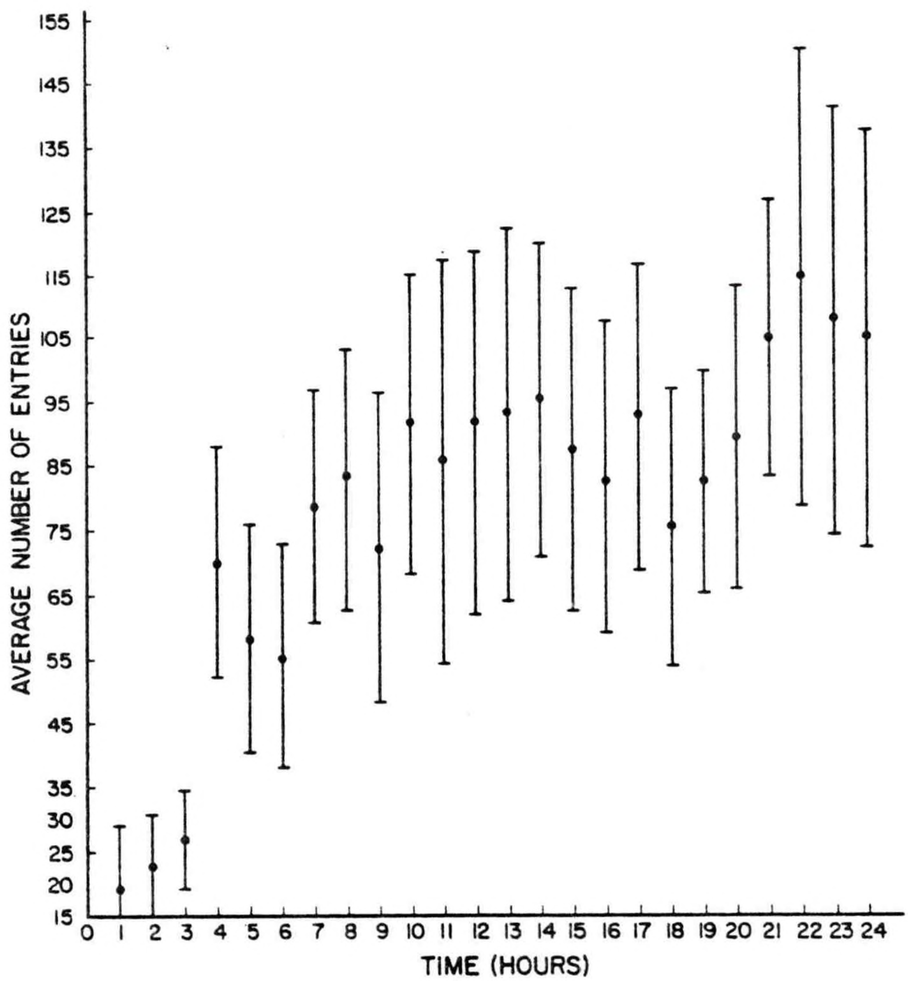


Fig. 6. Mean activity (entries) of ten redfish versus the length of time spent in the monitoring tank. Only the data for the second 1/2 hour period of each hour are shown.

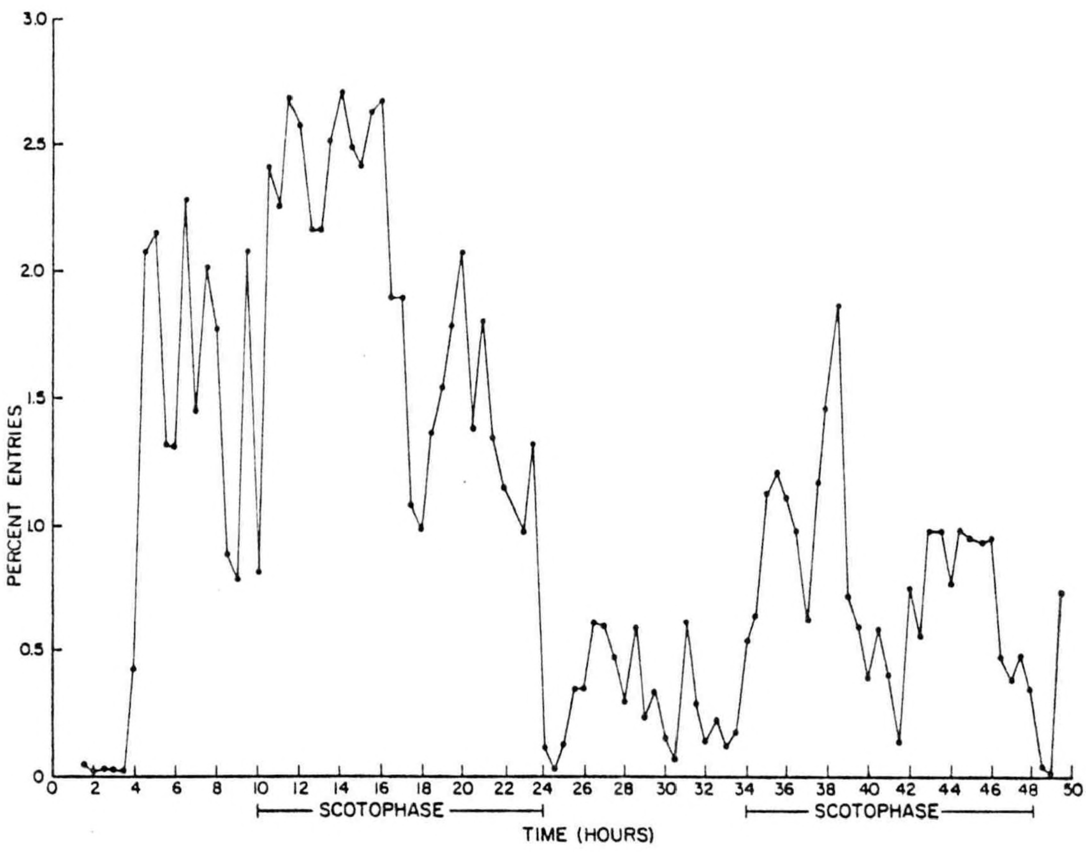


Fig. 7. Activity of one redfish monitored for 48 hrs. The periods of darkness are indicated.

In general, we considered the activity levels exhibited by redfish during the first few hours in our system to be low. Therefore, to enhance the amount of information that could be obtained, we decided to block-off every other compartment leaving a total of eight functional gates. This modification served to increase the amount of activity in those compartments available to the fish as well as enable us to use relatively short (2 h) monitoring periods. We decided this short monitoring period was also important since we were unable to maintain distinct gradients for longer periods in our system. We decided that a minimum of five compartment entries would represent the threshold level of activity to include the data generated by a particular specimen in the quantitative analysis. Through experience we observed that fish which demonstrated less than five entries were usually in poor physical condition.

The general testing procedure which evolved from the preliminary studies and that was used in subsequent experiments is described in the following account. A fish was removed from its holding tank 1 h before the beginning of the scheduled scotophase and placed in the monitoring tank. Both tanks were programmed for a 10:14 LD cycle with lights off at 1100 h, and both had the same temperature and salinity. The fish was allowed to adjust to the new surroundings for the next 3 h; i.e. 1 h in light and 2 h in darkness. This adjustment time was derived from the above investigations where we determined that the switching off of the lights immediately induced cessation of activity, requiring 1 h for normal activity to resume. The 3 h total acclimation was also derived from the same investigations, as it appeared to take this long for maximal activity to begin after introduction of the fish into the monitoring tank. Fifteen minutes before the end of the adjustment period we began introduction of the brine solutions into the tank so that the salinity gradients were established by the beginning of the monitoring period which followed. The activity of the redfish was recorded for the next 2 h, after which the fish was tagged, measured and returned to the holding tank. If the fish did not exhibit at least five compartment entries he was not tagged etc., but was returned to the holding tank for an additional 2-4 weeks of acclimation. In nearly all cases this additional acclimation time correlated positively with an increased level of activity upon retesting. The specific test conditions are explained below with each subsequent experiment.

Although the monitoring tank is located in a light-tight room in which a great effort has been made to remove any possible external cueing systems, it is difficult to eliminate the possibility of the animals orienting to unknown factors. In order to remove this potential bias from our research, we changed the experimental compartments (i.e. compartments receiving brines) on a regular and predetermined basis.

TEST BRINES

Salt for brines was obtained from the United Salt Company salt dome near Hockley, Texas. This salt is very similar in composition to that comprising the Bryan Mound salt domes (Neff et al. 1981). Control brines were made with artificial sea salts (Instant Ocean) from Aquarium Systems, Inc., Eastlake, Ohio. To prepare brines, an excess of salt was placed in a 120 ℓ plastic bucket with either Brazos River water obtained between mile 2 and 3 on the Brazos Diversion Channel near Bryan Mound or local tap water. A submersible pump was placed in the mixture and allowed to run vigorously for at least 6 h. The pump also gradually heated the water up to about 35°C. The mixture was then allowed to settle overnight prior to decanting into a separate 120 ℓ plastic bucket. The chemical composition of these brines has been reported by Neff et al. (1981).

The brines were introduced into the monitoring tank by first pumping the solutions up to buckets suspended from the side of the tank and then siphoning the solutions into the rear of the test or "treatment" compartments (Fig. 8). A standpipe in each bucket maintained a constant water level and ensured a steady siphon rate. The rate was adjustable by hose clamps. Air stones were placed at the rear of each functional compartment (including all controls) to thoroughly mix the incoming brine. Because of difficulty in generating the required brine gradients, we partially closed off part of each gate opening in order to reduce back-flow out of the brine compartments (Fig. 9). The remaining open area at the lower right side of each gate was a 40 x 25 cm rectangle through which the fish had to pass in order to enter a compartment. This modification enabled us to develop the desired brine gradients without causing too great a reduction in fish activity.

BRINE COMPARISONS

One of the major objectives of the study was to determine whether redfish responded in a different fashion to brines of different types, including one having the same composition as that to be discharged. This test was conducted under acclimation conditions of 25° C and 35 ppt. The flow of ambient water was directed from the center of the monitoring tank towards the periphery. Each of four compartments spaced at 90° intervals received one or the brines as described above; the remaining four functional compartments served as controls. As the brines were introduced to the compartments, near-bottom levels of temperature and salinity were monitored using a Yellow Springs Temperature-Salinity-Conductivity Meter. At 30 min after initiation of the composite 96 ℓ/h brine flow, water in the four brine compartments were 2 $\pm$ 0.02 ppt higher in salinity than the water in the center of the tank and in the control compartments, and an experiment was initiated. We were able to maintain a relative difference in compartment salinity over the course of the tests, each of which required 2 h and 15 min. The absolute salinity level in the tank increased on the average from 35 ppt in the control compartments and center of the tank and 37 ppt in the treatment compartments to approximately 37 and 38 ppt, respectively (Appendix Fig. I-1). Thus, under the worst case condition as determined at the end of the experiment, at least a 1 ppt difference was maintained under a water quality regime characterized by gradually increasing salinity and constant temperature.

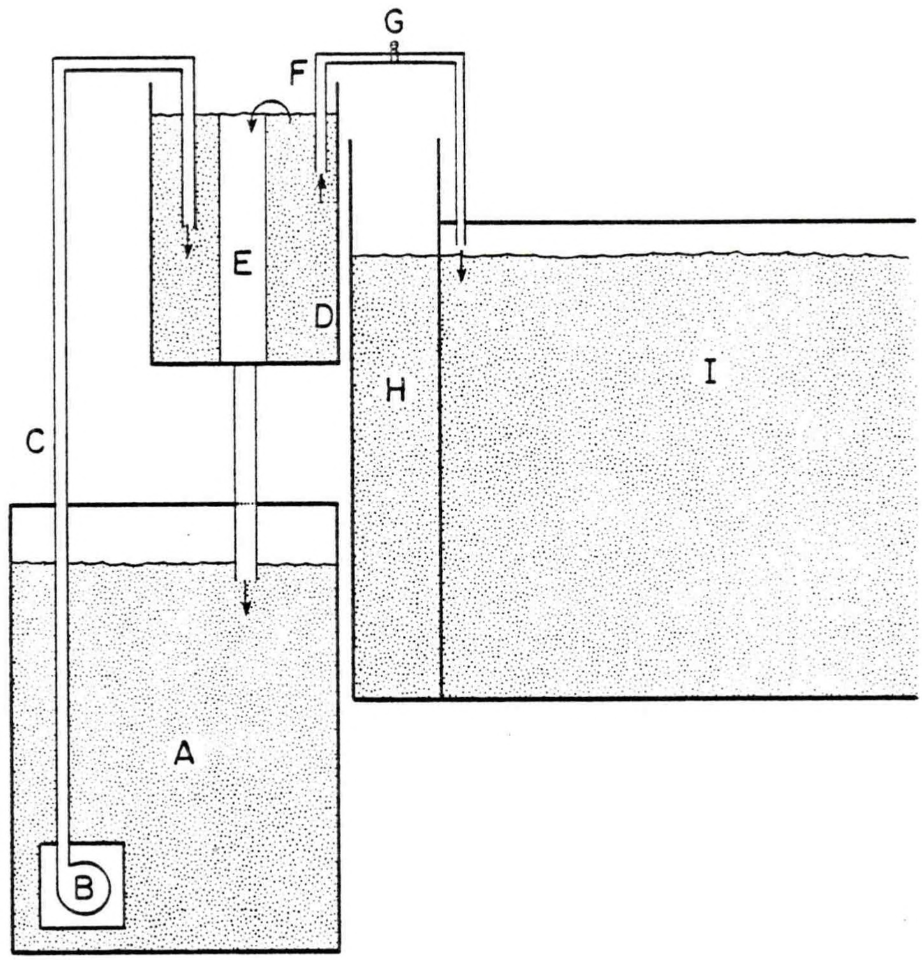


Fig. 8. Constant head apparatus for the steady introduction of the brine into a compartment. Brine was pumped (B) from a plastic reservoir (A) to a container (D) attached to the side of the monitoring tank. It was then siphoned (F) from the container into the rear of the recipient compartment (I). The siphon rate was fixed by a hose clamp (G). Excess brine returned to the reservoir via a standpipe (E) fitted to the suspended container: (C) vinyl tubing; (H) peripheral channel.

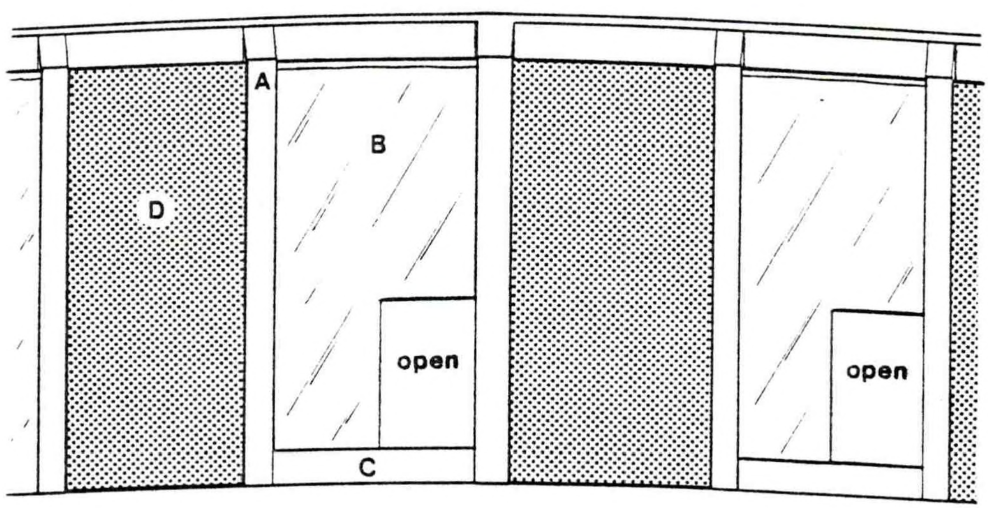


Fig. 9. View of the entrances to four compartments. (A) radial divider; (B) plexiglass shield to reduce the escapement of brine (and heated water) into the central area of the tank; (C) PVC threshold (this was used only in the four-brine protocol and was removed for the remaining experiments) opening size was 25 x 35 cm with threshold in place and 25 x 40 cm without threshold; (D) plastic screen mesh to prevent entry into that compartment.

A total of 21 redfish were subjected to the above test. The 11 fish which produced useable records averaged 42 cm in standard length (SE = 1.1 cm) and 1.17 kg in weight (SE = 0.07 kg). The percent variables (entries and time spent) for each fish were calculated by dividing the individual compartment values by the totals for all eight compartments and multiplying times 100. These data were evaluated for homogeneity of variance using Bartlett's test (Sokal and Rohlf 1969) and were found to require a logarithmic as opposed to an arcsine transformation in order to achieve homogeneity of variance. The transformed data were then subjected to a one-way analysis of variance to compare compartmental differences at the 0.05 level of significance. If significantly different, orthogonal contrasts were to have been used to compare compartments receiving brines with control compartments, as well as the various salt (Salt Dome vs. Instant Ocean) and water (tap vs. Brazos River) type combinations.

TEMPERATURE AND BRINE STRENGTH INTERACTIONS

In these tests, we again used eight functional compartments with two opposite compartments receiving Salt Dome-Brazos River brine. Because we were interested in the possible interaction of heat and brine, one of the two compartments receiving brine was also heated by 2° ± 0.2° C above ambient. Heating the water was done by recirculating compartment water through a heating bath in a 120ℓ plastic bucket adjacent to the compartment. The heated water was introduced into the compartment across the bottom through a diffuser pipe 1.7 m in length connected to a small pump in the heating bath. Recirculating water was removed from the same compartment by a siphon tube going to the heating bath. Because of the possible effect of this extra plumbing on the behavior of the fish, both the non-heated brine compartment and two 90° control compartments were fitted with the same recirculating plumbing system (Fig. 10). These "plumbing controls" were considered the most appropriate controls for this experiment.

Fish acclimated to three different ambient temperatures were tested in three different salinity gradients,

Acclimation or Ambient Temperature (°C): Brine Concentration Above Ambient Salinity (ppt)	15°C	20°C	25°C
0.5	a	d	g
1.0	b	e	h
2.0	c	f	i

yielding a total of nine separate experiments.

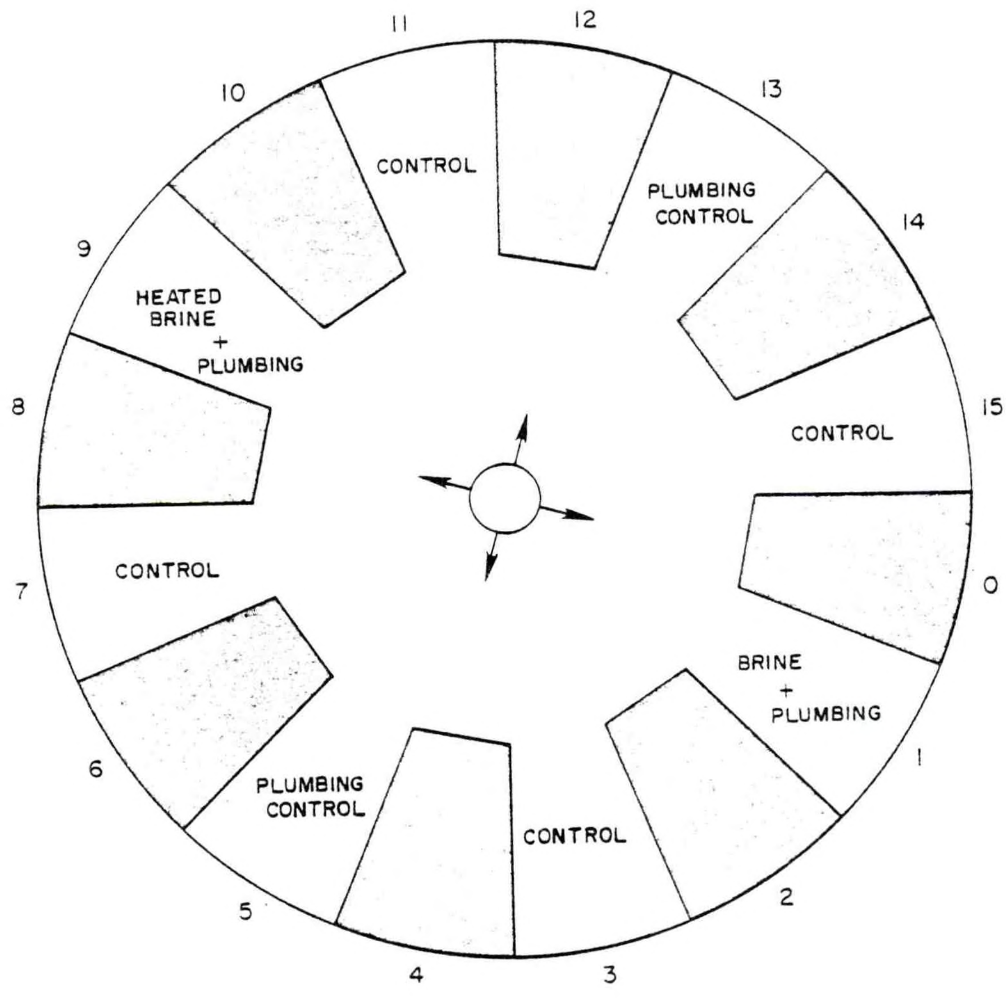


Fig. 10. Overhead schematic of the monitoring tank showing the spatial arrangement of the treatment and control compartments in the temperature and brine strength comparisons.

Our success at maintaining the temperature-salinity differences among compartments is shown by Appendix Figs. I-2-5. In general, a difference was maintained between treatment and control compartments in terms of relative temperature and salinity but the absolute levels increased somewhat by the end of each 2 h 15 min experiment.

This protocol required 90 redfish to obtain a minimum of seven replicates for each of the nine experiments. The 69 fish which generated useable records averaged 45 cm in standard length (SE = 0.6) and 1.45 kg in total weight (SE = 0.07). In all cases, a logarithmic transformation was required to achieve homogeneity of variance prior to conducting the nine ANOVA's. Orthogonal contrasts were used to evaluate significant differences ($p = 0.05$) among compartments. Control compartments without the extra plumbing were contrasted with the other treatments, including controls with plumbing; control compartments with plumbing were compared to compartments receiving heated and ambient brines and the compartment receiving heated brine was contrasted to the compartment receiving brine of ambient temperature.

As described in RESULTS, redfish acclimated at 25° C in fall 1980 demonstrated what we considered a significant attraction response to a brine concentration 2.0 ppt in excess of ambient salinity. To provide confirmation of this result, 15 fish were acclimated at 25° C during spring 1981 and tested in an experimental array in which six compartments served as controls and two opposite compartments received brine concentrations 2 ppt above ambient salinity as described above. The 10 fish which generated useable records in this experiment averaged 44 ± 1.7 cm in standard length and 1.38 ± 0.13 kg in weight. The data produced by these fish required an arcsin transformation to achieve homogeneity of variance, and, following transformation were subjected to ANOVA. An orthogonal contrast was used to compare compartments receiving salt dome/Brazos River water brine to the control compartments.

OTHER OBSERVATIONS

Two direct observational techniques were evaluated upon initiation of the project. In the first study, fish were released in the circular monitoring tank and allowed to adjust for 2 h. Brine was then introduced into four compartments as described above. Three fish were watched in this manner by three observers using one-way mirrors at the edge of the tank. This direct observation technique required increasing the light level to 30 lux. An attempt was made to quantify actions such as coughing, gilling, erratic swimming and surfacing.

In a second preliminary study, fish were acclimated in a 50 gallon aquarium and given a direct dose of full-strength brine. The brine was introduced near the animals head via a small piece of tubing. Their behavior was observed both before and after this procedure.

RESULTS

BRINE COMPARISONS

Of the 21 fish acclimated at 25° C and 35 ppt salinity in the summer of 1980 and used to determine avoidance or attraction to the various brines, 11 produced useable records. Reasons for not including all records included mechanical failure, loss of salinity gradient or a lack of activity by the fish during the monitoring period (less than five total entries). Fish with very low activity levels were nearly always in poor physical condition. The 11 fish used generated an average of 170 entries (SE = 54) per animal during a 2-h run.

Results of ANOVA's performed on the log transformed data for percent entries and time spent (Appendix Table II-1) indicated that the differences among the compartments (Appendix Fig. III-1) were not significant at the 5% level. Given an array of brine types, redfish acclimated to 25°C and 35 ppt were apparently neither attracted to nor did they avoid brine concentrations 2 ppt above ambient salinity, regardless of the composition of the brines and type of water used.

TEMPERATURE AND BRINE STRENGTH INTERACTIONS

Of the 90 fish tested in this fall and winter 1980-1981 experiment, 69 produced useable records, averaging 106 $\pm$ 10 entries per fish over the 2 h runs. Results of the nine ANOVA's for each of the two response variables are shown in Appendix Tables II-2-7; the mean responses for each compartment are graphed in Appendix Figs. III-2-7.

Summaries of the respective ANOVA's are presented in Tables 2 and 3. For each of the response variables (log of percent entries and time spent), significant differences were observed for compartments at acclimation temperatures of 20° and 25°C, but only in the presence of a 2 ppt brine gradient. These are interpreted as an attraction response to the higher brine gradient.

At 20°C acclimation, redfish exhibited no significant differences in terms of entering treatment compartments (including plumbing controls) versus entering control compartments without brine, heat and/or extra plumbing. Additionally they entered compartments with brine and/or heat as readily as they entered control compartments with extra plumbing but no heat or brine imputs. With respect to the brine treatments, the redfish entered the compartment containing brine having ambient temperature significantly more frequently than the compartment containing warmed brine (Table 2, Appendix Fig. III-3). With respect to time spent in a compartment, redfish acclimated at 20°C were evaluated to have spent a significantly greater amount of time in the treatment compartments than in the control compartments, but no significant differences were observed among the more meaningful brine and plumbing control treatments (Table 3; Appendix Fig. III-6).

Table 2. Summary of log percent entries ANOVA and treatment contrasts for determining avoidance/attraction responses to brine gradients. Complete ANOVA tables are found in Apendix II-2-7. A graphical representation of these data is found in Appendix Figs. III-1-7. Treatments (Trts) = Brine (BR), Heated Brine (HBR) and Plumbing Controls (PC). Controls - C1-C4 of Appendix Figs. III-1-7.

Experiment (No. of Fish Used)	Ambient Temperature (C°)	Brine Above Ambient (ppt)	Contrast	F	PR>F
a (7)	15	0.5	Compartments	1.65	.15
			Trts vs. Controls	.01	.93
			Brines vs. PC	.93	.34
			BR vs. HBR	5.80	.02*
b (7)	15	1.0	Compartments	.90	.52
			Trts vs. Controls	2.82	.10
			Brines vs. PC	.00	.99
			BR vs. HBR	1.56	.22
c (9)	15	2.0	Compartments	.79	.60
			Trts vs. Controls	.30	.58
			Brines vs. PC	.05	.82
			BR vs. HBR	.02	.88
d (7)	20	0.5	Compartments	.95	.48
			Trts vs. Controls	.02	.89
			Brines vs. PC	1.17	.29
			BR vs. HBR	4.33	.04*
e (7)	20	1.0	Compartments	.96	.47
			Trts vs. Controls	1.82	.18
			Brines vs. PC	.27	.61
			BR vs. HBR	.71	.40
f (7)	20	2.0	Compartments	3.37	.01**
			Trts vs. Controls	2.46	.12
			Brines vs. PC	.20	.66
			BR vs. HBR	4.29	.04*
g (7)	25	0.5	Compartments	.56	.79
			Trts vs. Controls	2.13	.15
			Brines vs. PC	.83	.37
			BR vs. HBR	.02	.90
h (7)	25	1.0	Compartments	1.87	.10
			Trts vs. Controls	3.04	.09
			Brines vs. PC	1.84	.18
			BR vs. HBR	2.73	.11
i (11)	25	2.0	Compartments	2.22	.05*
			Trts vs. Controls	1.68	.20
			Brines vs. PC	8.40	.01**
			BR vs. HRB	3.09	.08

* Indicates 0.05 level of significance

** Indicates 0.01 level of significance

Table 3. Summary of log time spent ANOVA and treatment contrasts for determining avoidance/attraction responses to brine gradients. Complete ANOVA tables are found in Appendix II-2-7. A graphical representation of these data are found in Appendix Figs. III-1-7. Treatments (Trts) = Brine (BR), Heated Brine (HBR) and Plumbing Controls (PC). Controls = C1-C4 of Appendix Figs. III-1-7.

Experiment (No. of Fish Used)	Ambient Temperature (C°)	Brine Above Ambient (ppt)	Contrast	F	PR>F
a (7)	15	0.5	Compartments	.74	.64
			Trts vs. Controls	.15	.70
			Brines vs. PC	2.60	.11
			BR vs. HBR	.01	.91
b (7)	15	1.0	Compartments	.95	.48
			Trts vs. Controls	1.32	.26
			Brines vs. PC	.10	.75
			BR vs. HBR	.96	.33
c (9)	15	2.0	Compartments	.84	.56
			Trts vs. Controls	.05	.83
			Brines vs. PC	.11	.75
			BR vs. HBR	1.01	.32
d (7)	20	0.5	Compartments	1.19	.33
			Trts vs. Controls	1.88	.18
			Brines vs. PC	1.56	.22
			BR vs. HBR	4.47	.04*
e (7)	20	1.0	Compartments	.59	.76
			Trts vs. Controls	.04	.84
			Brines vs. PC	.01	.92
			BR vs. HBR	.71	.41
f (7)	20	2.0	Compartments	3.51	.01**
			Trts vs. Controls	6.51	.02*
			Brines vs. PC	.85	.36
			BR vs. HBR	.75	.39
g (7)	25	0.5	Compartments	1.07	.40
			Trts vs. Controls	.00	.96
			Brines vs. PC	5.61	.02*
			BR vs. HBR	.62	.43
h (7)	25	1.0	Compartments	1.19	.34
			Trts vs. Controls	5.03	.03*
			Brines vs. PC	1.10	.30
			BR vs. HBR	.59	.45
i (11)	25	2.0	Compartments	3.15	.01**
			Trts vs. Controls	.59	.45
			Brines vs. Pc	4.52	.04*
			BR vs. HBR	6.01	.02*

* Indicates 0.05 level of significance

** Indicates 0.01 level of significance

Redfish acclimated to 25° C and 35 ppt water and exposed to a 2 ppt salinity gradient among the treatments also generated data indicating significant differences in the response variables among compartments (Tables 2 and 3). They entered brine compartments significantly more frequently than the appropriate plumbing control compartments, and demonstrated no significant preference in terms of entries for either heated or ambient-temperature brine (Table 3; Appendix Fig. III-4). In terms of time spent in a compartment, significantly more time was spent in brine-receiving compartments than in appropriate control compartments, and more time was spent in the ambient-temperature brine compartment than in the heated-brine compartment (Table 4; Appendix Fig. III-7).

Collectively, we interpreted the above results to indicate an attraction of redfish to slightly higher salinity levels when acclimated to waters of 25°C temperature and 33 ppt salinity (Fig. 11). When acclimated to 20°C and 35 ppt salinity water, redfish showed little in the way of a significant response, except when the brine-receiving compartments were 2 ppt higher than ambient conditions. Under these conditions, the fish entered the unheated brine compartments more frequently than the compartments receiving heated brines; and, on a collective basis, the fish spent more time in the treatment compartments than in the unmodified control compartments. Although overall compartment differences were not significant, there appeared to be a trend towards attraction to the heated brine at the lower acclimation temperatures (Tables 2 and 3, Appendices II and III).

Of the 15 redfish acclimated to 25°C and 35 ppt salinity water in spring 1981, 10 produced usable records. The number of entries per fish averaged 86 $\pm$ 16 for each 2 h monitoring period. In contrast to the results obtained in the similar experiment performed during fall 1980, significant differences among all compartments were not indicated (Appendix Table II-8; Appendix Fig. III-8). However, it should be noted that one of the compartments receiving brine input was characterized by the highest average number of entries as well as by the highest average time spent in a compartment (Appendix Fig. III-8). In addition, the Brines versus Controls comparison for time spent was highly significant. It appears as if these fish may have developed a preference for one side of the tank.

OTHER OBSERVATIONS

Three redfish were observed individually as they initially encountered the brine gradients produced in the experiment comparing the different brine types. A 15 min control observation period was followed by 15 min of observation while the brines were running. The following are the actual laboratory notes compiled from the records of the three independent observers located on different sides of the tank. The term "run" refers to the experimental monitoring period for a fish on a specific day.

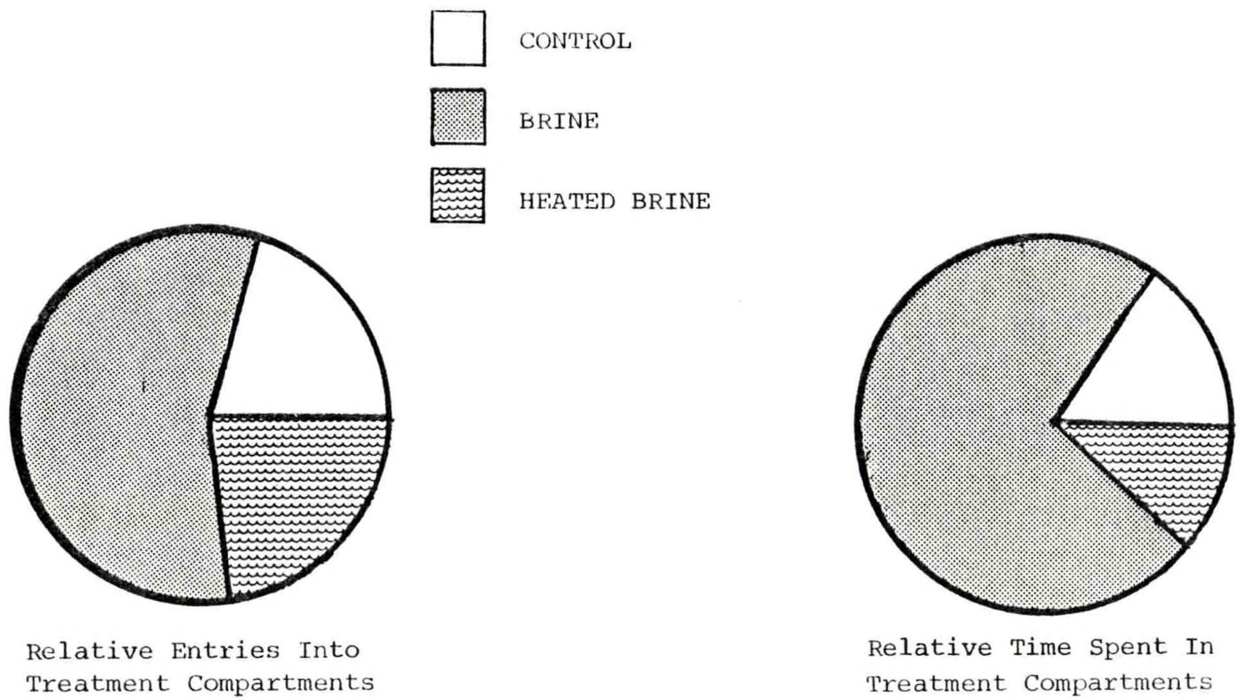


Fig. 11. Evidence for an attraction response of redfish acclimated to 25°C and 35 ppt to brine + 2 ppt above ambient salinity.

Run #23

This animal did not enter compartments but rather swam in a regular circle in the center of the tank for the first 15 min control period. It would occasionally veer toward one of the closed compartments which are covered with black netting. However, after brine flow was initiated, very striking differences in locomotory patterns were noted. Instead of circling, the fish would often double back on itself or abruptly cross the middle of the tank. It generally increased its swimming speed and would often accelerate suddenly. It seemed to become "Excited" and exhibited many more straight paths and sharp turns. Once, the fish stopped at the opening to a brine compartment and shook laterally very vigorously for about one second while its opercula were opened. It then moved on in the center of the tank.

Run #25

This animal swam very slowly and occasionally stopped briefly during the control run. During the 15 min brine observation it turned toward the center of the tank more often. Occassionally it would make sharp turns near a compartment containing brine. It continued to swim rather slowly around the center of the tank.

Run #26

This animal was inactive. It rested in a control compartment during the first 15 min, then just prior to turning on the brine it moved to a brine compartment where it remained for the rest of the experiment being bathed in the brine facing the center of the tank. This animal behaved as if it were in poor physical condition in that it appeared sluggish, was slow to avoid the dip net and did not exhibit the usual exploratory behavior seen in healthy animals.

Two of the three fish clearly altered behavior in response to the influx of brines into the tank. These two individuals appeared to become more active and alert in response to the brine. We could not differentiate responses to the different types of brine. The third animal was inactive during the entire experimental run.

After completing these three runs we abandoned this observational technique. We realized that leaving the lights on or switching the lights off at the end of the direct observation period was reducing activity levels during the subsequent electronic monitoring (see METHODS). Further complicating such observation using our monitoring tank was both our inability to see the animal well enough to describe the behaviors or to know exactly what quantity of brine the animal was encountering. Thus, quantification of these results was not possible since the technique was discontinued after only three animals.

Our second attempt at developing a direct observation technique yielded little useful information. The three animals given a direct dose of brine over their heads gave us no detectable indications of sensing the brine. There was no incease in gilling, no coughing, etc. We abandoned this methodology in the belief that the 50 gallon aquarium was too small for the animal to reach a suitable level of acclimation.

DISCUSSION

Although any laboratory apparatus designed to test the responses of fish to environmental gradients represents an alien environment and results must be used with caution when applied to natural situations, we believe that the Kleerekoper-designed monitoring tank was adequate for addressing the questions posed in this study focusing on the redfish. The apparatus enabled us to present an array of environmental choices, including simulated discharge conditions, to the fish, and to monitor their response electronically without the possible bias of the presence of a human observer. Of the eleven species of fishes which have been tested in this type of apparatus all (including redfish) have exhibited a characteristic "exploratory period" after 2-4 h of acclimation. It is during this "exploratory period" prior to any conditioning, that important environmental cueing can be evaluated.

The different environmental conditions available to the fish were represented in rather restricted chambers located around the edges of a circular tank. As response variables indicating avoidance or attraction of the fish to the environments offered, we used the relative number of entries into a chamber supplemented by the relative amount of time spent in a chamber having defined environmental characteristics. The approach thus required that the fish not only be able to behaviorally adjust to the monitoring tank, but also that they would enter the peripheral cells. The possibility of inadvertant orientations to azimuth factors was controlled for by regular changes of treatment compartments around the tank during each experiment. The redfish proved a reasonable experimental subject in that (1) they appeared to adjust to the circular tank in terms of exhibiting regular and normal locomotory behavior and (2) they showed little hesitation in entering the peripheral chambers, even given the extremely restrictive nature of these cells in contrast to the openness of thier natural environment. Given these attributes, we believe the results obtained provide an index of the response of redfish to the various salinity conditions per se. This is not to say that the experiments were ideal and without problems, but rather that they were adequate to address the questions posed within the limits of the resources committed for the effort.

The maximum gradients of brine concentration and temperature that we offered to the fish were 2 ppt and 2° C, respectively. These levels were selected based upon projections from water quality models developed for preliminary assessments of environmental impacts that might be expected to result from the project. Randall (1981) has reported that, during March and April 1980, actual discharge of 247 ppt brine from the Bryan Mound solution mining facility at a rate of 330,000 barrels/day resulted in brine concentrations in the immediate vicinity of the diffuser as much as + 5 ppt above the ambient salinity contour (0.2 km^2). Some 7.4 km^2 of bottom were covered by waters + 2 ppt above the ambient salinity contour. Water temperature levels did not appear to have been measurably affected by the discharge. In our experiments, significant orientations to the brine were detected under some conditions at + 2 ppt, but not at lesser gradients (+ 0.5 and 1.0 ppt).

One of the original concerns with respect to the potential impact of the solution mining was that redfish might either be attracted to or avoid the area characterized by the presence of the brine plume because of its peculiar ionic composition and water quality. Our results provided no substantial evidence that subadult and adult redfish differentiated among an array of different brine types at concentrations of 2 ppt salinity above ambient. The tests included brine of similar make-up to that being discharged. Redfish did not exhibit what we considered a significant avoidance or attraction response to any of four brine types to which they were simultaneously exposed in our tests under salinity conditions 2 ppt in excess of acclimation conditions (25°C and 35 ppt).

Using simulated discharge brines in a simpler array of conditions, we did obtain evidence of a significant attraction response to brine at 2 ppt over ambient salinity during the fall season, particularly when the fish were acclimated to 25°C water. A similar attraction was suggested for the spring season, but the tendency was not always statistically significant. Fall represents the spawning season, during which redfish characteristically move from estuaries to the open Gulf. Redfish acclimated to the warm summer water temperatures of estuaries may indeed use increasing salinity gradients as predictive cues for directed movements from estuarine to oceanic habitats. Although redfish are euryhaline, Simmons and Breuer (1962) reported the optimum salinity range to be 30 to 35 ppt, and Perret et al. (1980) state that large redfish prefer higher salinity than do small fish. Redfish in the area of the diffuser may be primarily large fish (Chittenden et al. 1981), and could be attracted to the area of brine disposal, particularly during periods characterized by ambient salinities of less than 33 ppt. In addition, redfish may use slight brine gradients as behavioral cues for locating cooler strata during warm water temperature stress. Thus, our data appears consistent with the hypothesis that redfish may use one variable cue (salinity) to assist in orientation relative to a second variable cue (temperature).

Should such an aggregation of large redfish develop around the area of discharge, we can see little, if any, deleterious effects resulting directly from the brine discharge. In our work, we routinely exposed field-collected redfish to salinity differences of as much as 5 ppt during transportation and transfer to the laboratory, and saw no deleterious effects. Perret et al. (1980) reported that redfish occur in salinities ranging from 0 to 50 ppt. With respect to embryos, larvae and juveniles, Neff et al. (1981) have reported that a brine concentration of about 45-50 ppt represents the most conservative 7-day LC50--level (the concentration resulting in 50% mortality) for any stage. With respect to low salinities, freshwater rearing of redfish is practiced (Crocker 1981) and freshwater baths are a recommended treatment for certain parasites of redfish (Lawler 1977). The salinity regimes associated with the brine discharge should not prove detrimental to redfish populations. The indirect consequences, if any, of a possible aggregation of large redfish near the diffuser are difficult to envision. It is perhaps possible that aggregations might be subject to increased fishing mortality or increased parasite transmission. On the other hand, the lack of a meaningful reward at the diffuser area (e.g.

food or temperature) suggests that aggregations, if they did occur, would be very transient in nature, thus reducing the probability of adverse consequence to the population.

Although not always statistically significant, the response data for redfish acclimated to low temperatures (15° and 20°C) indicated a tendency towards attraction to the heated brines when gradients were weak (0.5 or 1.0 ppt). Similar responses have been demonstrated by field studies in which fish have been shown to be attracted to warm water discharges during cooler seasons (Neill and Magnuson 1974; Gallaway and Strawn 1974, 1975). However, results from the initial field monitoring studies of the brine plume do not indicate that environmental temperatures are significantly altered by the brine plume, suggesting that warmed water will not be available to serve as a attractant during the cooler seasons.

To summarize our experimental results as related to responses of subadult and adult redfish to temperature and salinity gradients, when acclimation was to warm water (25°C), redfish were attracted to increased salinity levels in the range of those which are present around the diffuser. When acclimation was to cooler water (15° and 20° C, the fish appeared to us to preferentially respond to warmed brine as compared to brines of ambient temperature. These data suggest that redfish may aggregate around the diffuser during late summer, early fall and perhaps other seasons. None of the present fish distribution studies are using methods adequate to determine whether, in fact, such aggregations develop. Nevertheless, impact on redfish resulting from such aggregations (if they occur) and the brine per se are expected to be minimal based upon the results of this study in conjunction with the results of the bioassay studies reported by Neff et al. (1981).

Other sciaenid fishes (spot, Leiostomus xanthurus and croaker, Micropogon undulatus) have been demonstrated to respond to salinity gradients (Perez 1969). Acclimated to 17° C, these species demonstrated what was interpreted as an avoidance response when exposed to salinity gradients of ± 5 and 10 ppt. At ± 1 ppt no avoidance or attraction was observed. Although not directly comparable to results of this program, these data would be interpreted to suggest that redfish might avoid the area in the immediate vicinity of brine discharge characterized by a similar gradient (+ 5 ppt) and stronger than any we tested. Results of the plume monitoring studies (Randall 1981) shows that the area thus far affected by + 5 ppt salinity has been small ($\leq 0.2\ km^2$).

The results of our light vs. dark activity evaluation supports the common belief among fisherman that the redfish is primarily nocturnal in its activity patterns. It is important to emphasize, however, that the fish were not inactive in our tank during the day, but merely showed reduced activity. Since it was our practice to feed the fish during the day in order to evaluate their acclimation status, it is possible that this procedure may have increased day activity levels by conditioning the fish for periodic light period activity.

The data also clearly shows that each time the lights went on or off there was an obvious reduction in activity. It is possible that this observation may be a laboratory artifact due to the abruptness of these changes in our experimental facility. Because of the requirement of our phototransistor-based system, we were unable to approximate the more gradual light changes which occur in nature.

During the second half of the dark phase there was a gradual decrease in activity and a fairly predictable dip at between 0800 and 1000 h (real time). Although the fish were in a separate room with black-out curtains as well as considerable background noise in the form of pumps, it is possible that this reduction in activity during the fishes scotophase was due to our daily activities commencing in the other parts of the building.

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APPENDIX I

GRADIENT SCHEMATICS

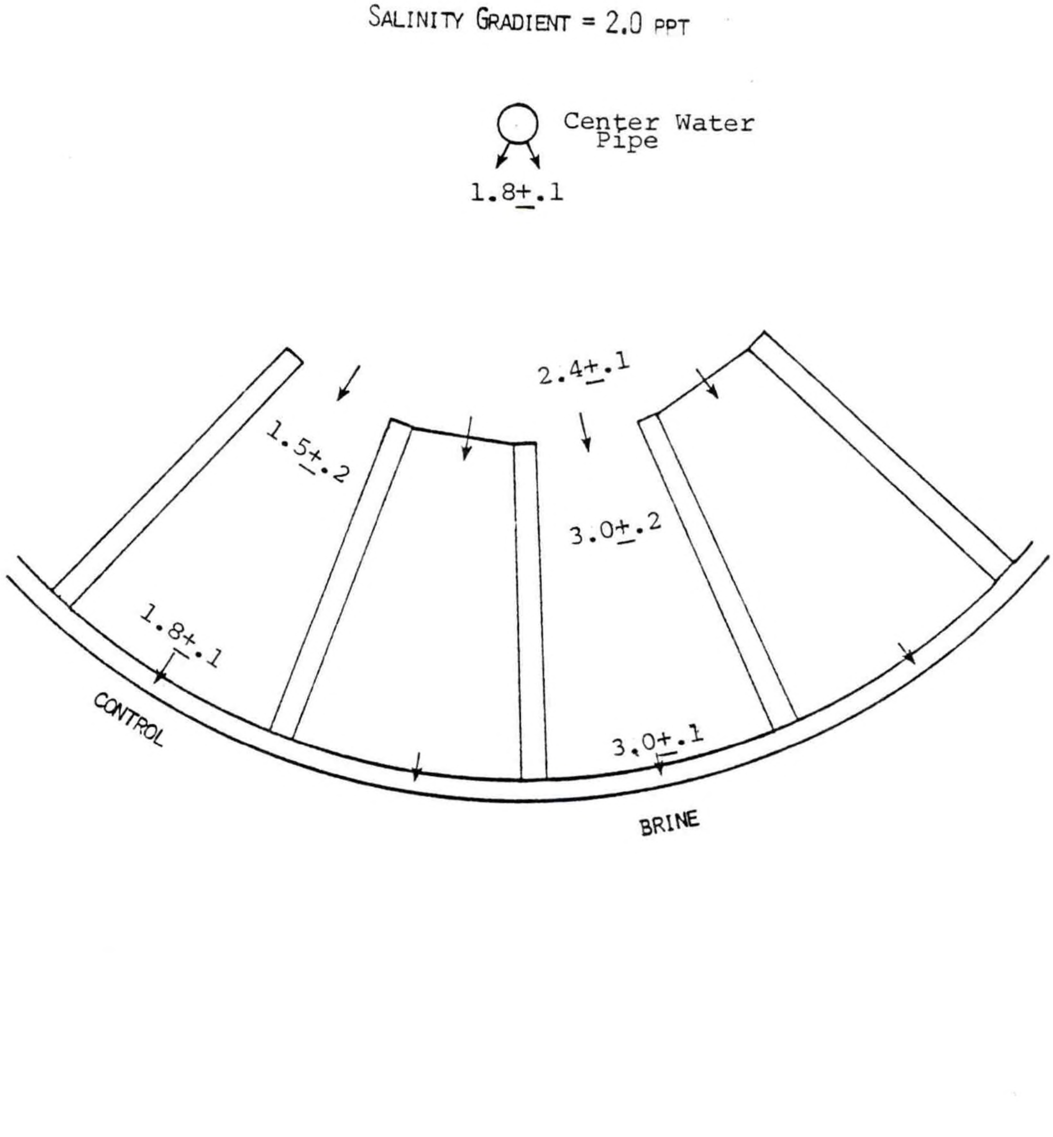

Fig. I-1. Schematic showing the mean (± SE) increases over the starting ambient salinity evident at completion of the brine comparison experiments. The means were calculated from salinity readings taken five min after the end of every run. The arrows indicate the direction of ambient water flow.

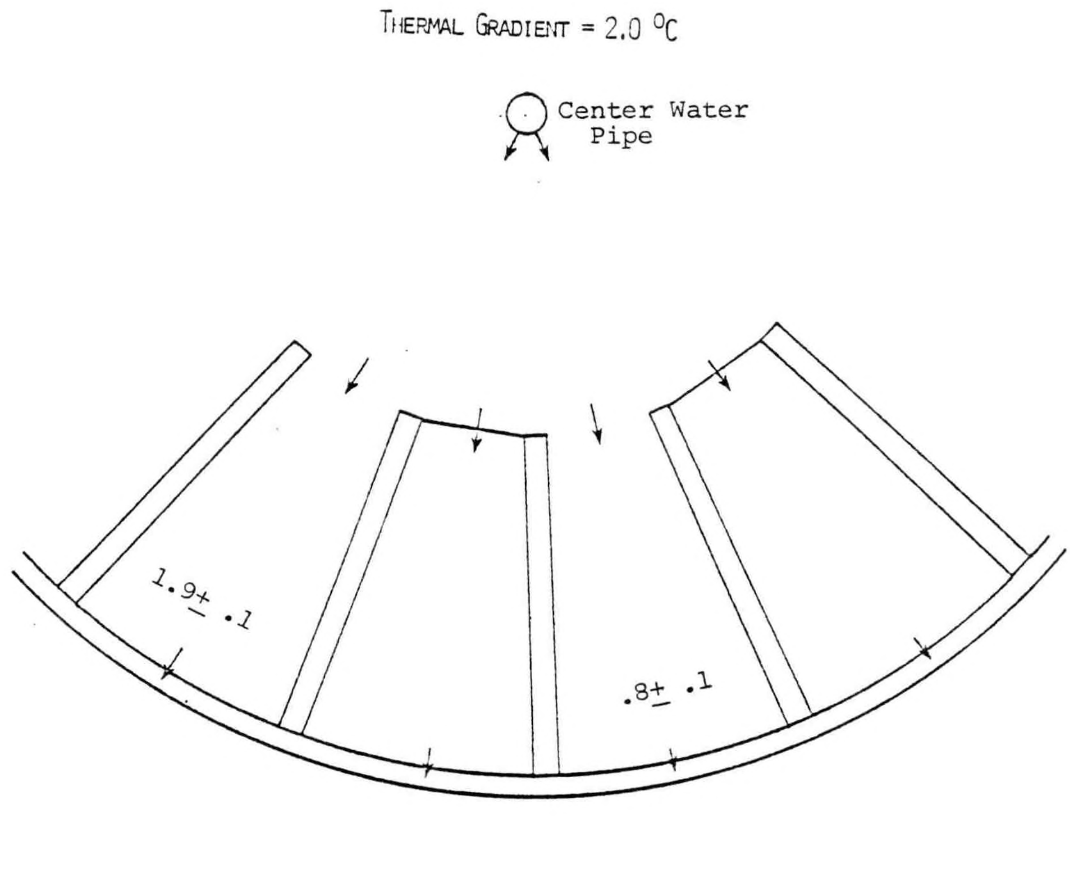

Fig. I-2. Schematic showing the mean (± SE) increases over the starting ambient temperature evident upon completion of the temperature-brine strength experiments. The means were calculated from temperature readings taken five min after the end of every run. The compartment with the higher mean received the heated water. The arrows indicate the direction of flow.

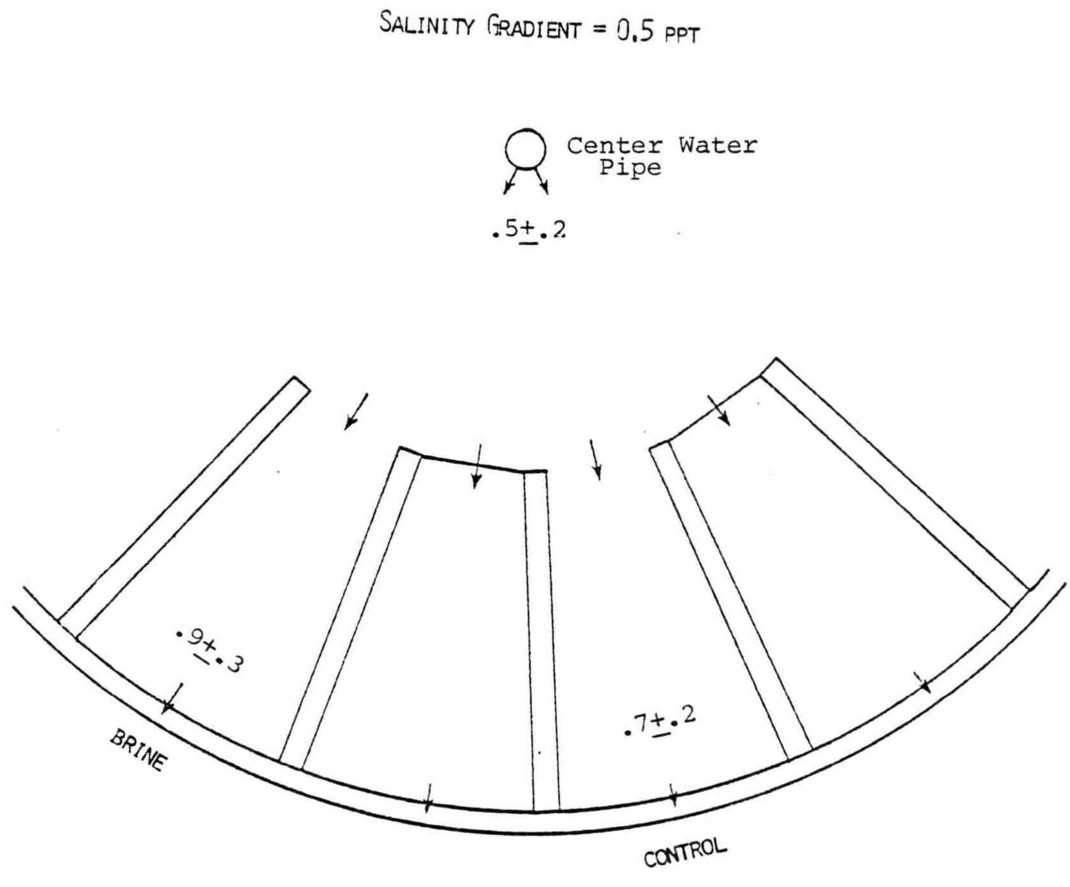

Fig. I-3. Schematic showing the mean (± SE) increases over the starting ambient salinity with the proposed salinity gradient of 0.5 ppt. The means were calculated from readings taken five min after the end of every run. Brine and control compartments are labeled. The arrows indicate the direction of water flow.

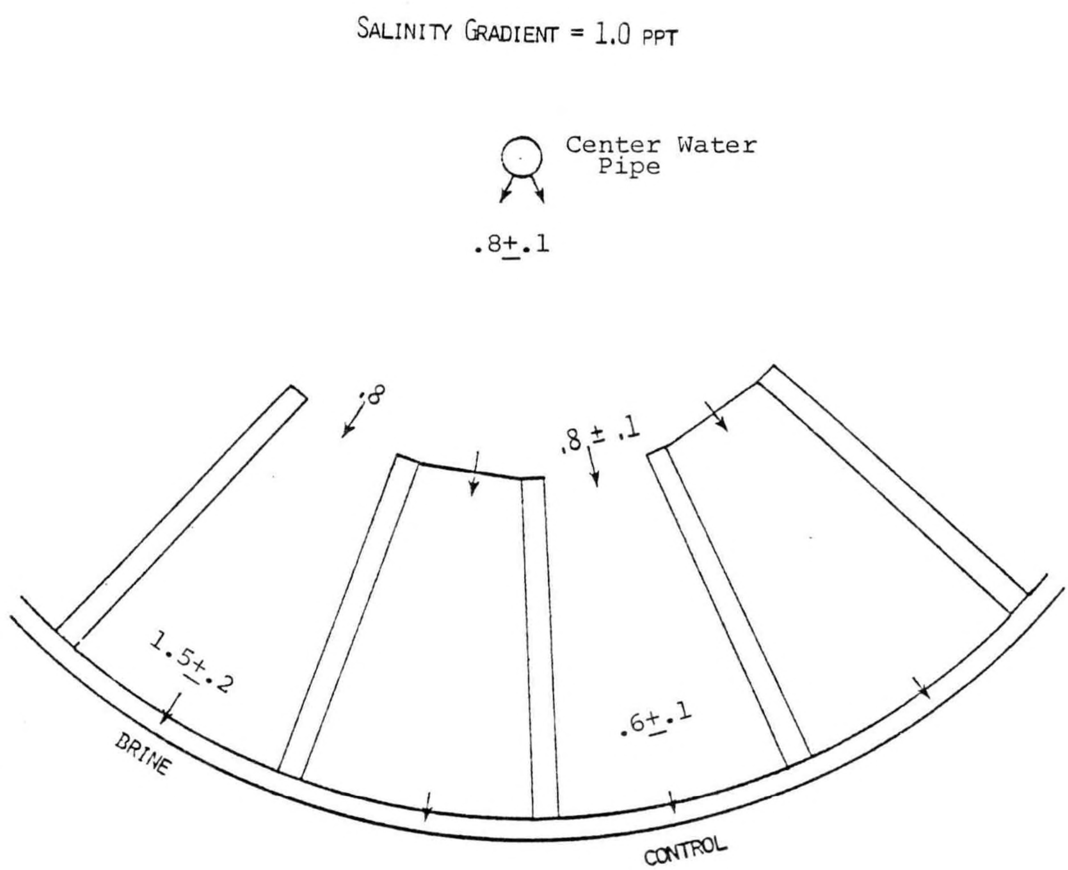

Fig. I-4. Schematic showing the mean (± SE) increases over the starting ambient salinity with the proposed salinity gradient of 1.0 ppt. The means were calculated from readings taken five min after the end of every run. Brine and control compartments are labeled. The arrows indicate the direction of water flow.

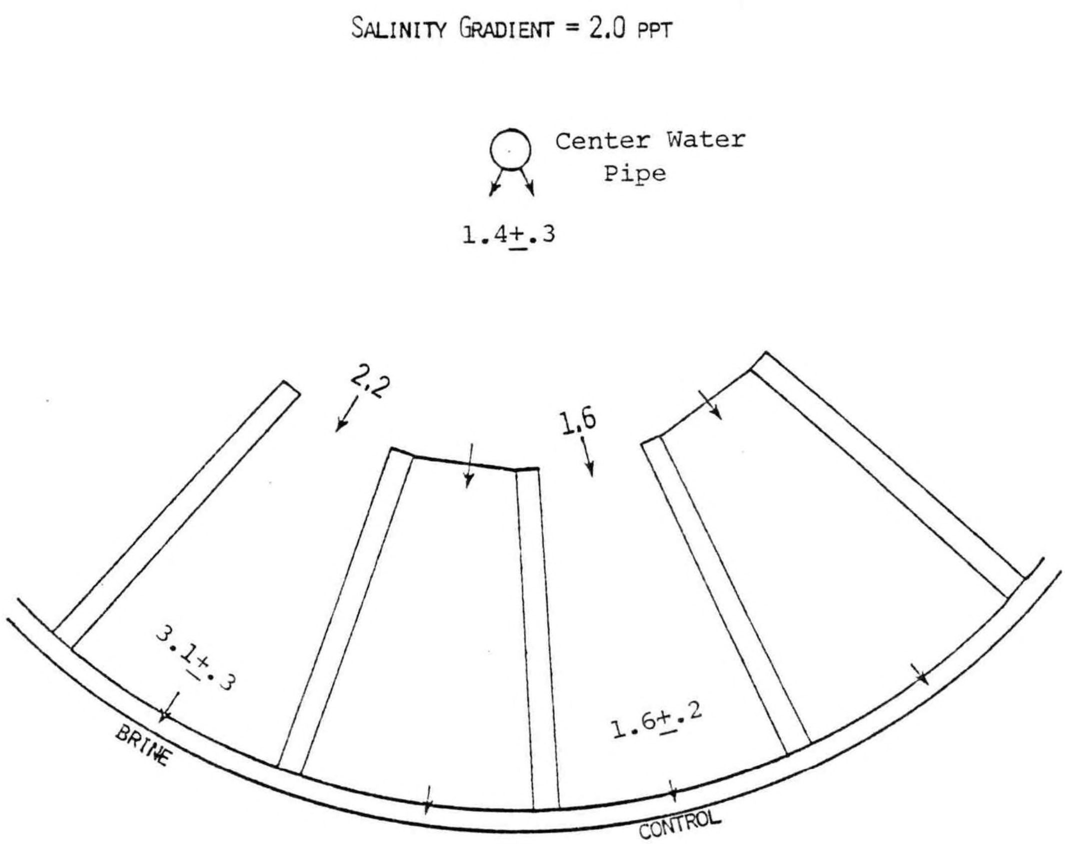

Fig. I-5. Schematic showing the mean (± SE) increases over the starting ambient salinity with the proposed salinity gradient of 2.0 ppt. The means were calculated from readings taken five min after the end of every run. Brine and control compartments are labeled. The arrows indicate the direction of water flow.

APPENDIX II

ANOVA TABLES

Table II-1. Results of the ANOVAs using the log-transformed: A) percent entries data; B) percent time spent data. The compartments term has been further partitioned by orthogonal contrasts. The brines term consists of the salt/water combinations (Instate Ocean/tap water; Instant Ocean/Brazos River water; Ranch House stock salt/tap water; stock salt/Brazos River water).

A. Percent Entries

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	3.6010	.5144	1.26	.28
Brines vs Control	1	.1489		.37	.54
Salt types	1	.1832		.45	.50
Water types	1	.0262		.06	.80
Residual	75	30.5443	.4073		
Total	82	34.1453			

B. Percent Time Spent

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	17.5722	2.5103	1.55	.16
Brines vs Control	1	1.6860		1.04	.31
Salt types	1	2.3524		1.45	.23
Water types	1	1.8939		1.17	.28
Residual	75	129.9374	1.6242		
Total	82	147.5094			

Table II-2. Results of the ANOVAs using the log-transformed percent entries data gathered at 15°C. The compartment term has been partitioned further by orthogonal contrasts. The salinity gradient level is indicated above each ANOVA. The treatments (Trts) term consists of the brine compartment (BR), the heated brine compartment (HBR) and the two plumbing controls (PC).

0.5 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.8696	.1242	1.65	.15
Trts vs Controls	1	.0005		.01	.93
Brines vs PC	1	.0702		.93	.34
BR vs HBR	1	.4354		5.80	.02
Residual	44	3.3034	.0751		
Total	51	4.1730			

1.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.5267	.0752	.90	.52
Trts vs Controls	1	.2351		2.82	.10
Brines vs PC	1	.0000		.00	.99
BR vs HBR	1	.1305		1.56	.22
Residual	31	2.5878	.0835		
Total	38	3.1145			

2.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.0444	.0653	.79	.60
Trts vs Controls	1	.0243		.30	.58
Brines vs PC	1	.0042		.05	.82
BR vs HBR	1	.0017		.02	.88
Residual	52	4.1702	.0802		
Total	59	4.6146			

Table II-3. Results of the ANOVAs using the log-transformed percent entries data gathered at 20°C. The compartment term has been partitioned further by orthogonal contrasts. The salinity gradient level is indicated above each ANOVA. The treatments (Trts) term consists of the brine compartment (BR), the heated brine compartment (HBR) and the two plumbing controls (PC).

0.5 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.3671	0.0524	.95	.48
Trts vs Controls	1	.0010		.02	.89
Brines vs PC	1	.0649		1.17	.29
BR vs HBR	1	.2403		4.33	.04
Residual	39	2.1623	.0554		
Total	46	2.5294			

1.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.3334	.0476	.96	.47
Trts vs Controls	1	.0906		1.82	.18
Brines vs PC	1	.0133		.27	.61
BR vs HBR	1	.0354		.71	.40
Residual	41	2.0402	.0498		
Total	48	2.3737			

2.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.9814	.1402	3.37	.006
Trts vs Control	1	.1026		2.46	.12
Brines vs PC	1	.0081		.20	.66
BR vs. HBR	1	.1788		4.29	.04
Residual	41	1.7916	.0417		
Total	48	2.7730			

Table II-4. Results of the ANOVAs using the log-transformed percent entries data gathered at 25°C. The compartment term has been partitioned further by orthogonal contrasts. The salinity gradient level is indicated above each ANOVA. The treatments (Trts) term consists of the brine compartment (BR), the heated brine compartment (HRB) and the two plumbing controls (PC).

0.5 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartment	7	.1089	.0156	.56	.79
Trts vs Controls	1	.0596		2.13	.15
Brines vs PC	1	.0232		.83	.37
BR vs HBR	1	.0004		.02	.90
Residual	38	1.0649	.0280		
Total	45	1.1738			

1.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartment	7	.7987	.1141	1.87	.10
Trts vs Control	1	.1860		3.04	.09
Brines vs PC	1	.1124		1.84	.18
BR vs HBR	1	.1669		2.73	.11
Residual	40	2.4471	.0612		
Total	47	3.2458			

2.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartment	7	1.2106	.1729	2.22	.05
Trts vs Control	1	.1310		1.68	.20
Brines vs PC	1	.6548		8.40	<.01
BR vs HBR	1	.2406		3.09	.08
Residual	52	4.0521	.0779		
Total	59	5.2627			

Table II-5. Results of the ANOVAs using the log-transformed percent time spent data gathered at 15°C. The compartment term has been partitioned further by orthogonal contrasts. The salinity gradient level is indicated above each ANOVA. The treatments (Trts) term consists of the brine compartment (BR), the heated brine compartment (HBR) and the two plumbing controls (PC).

0.5 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.9265	.1324	.74	.64
Trts vs Controls	1	.0275		.15	.70
Brines vs PC	1	.4649		2.60	.11
BR vs HBR	1	.0022		.01	.91
Residual	39	6.9612	.1785		
Total	46	7.8877			

1.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.6014	.0859	.95	.4830
Trts vs Controls	1	.1193		1.32	.26
Brines vs PC	1	.0090		.10	.75
BR vs HBR	1	.0869		.96	.33
Residual	28	2.5231	.0901		
Total	35	3.1254			

2.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	1.2688	.1813	.84	.56
Trts vs Controls	1	.0103		.05	.83
Brines vs PC	1	.0226		.11	.75
BR vs HBR	1	.2163		1.81	.32
Residual	46	9.8782	.2147		
Total	53	11.1470			

Table II-6. Results of the ANOVAs using the log-transformed percent time spent data gathered at 20°C. The compartment term has been partitioned further by orthogonal contrasts. The salinity gradient level is indicated above each ANOVA. The treatments (Trts) term consists of the brine compartment (BR), the heated brine compartment (HBR) and the two plumbing controls (PC).

0.5 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	1.5283	.2183	1.19	.33
Trts vs Control	1	.3446		1.88	.18
Brines vs PC	1	.2853		1.56	.22
BR vs HBR	1	.8180		4.47	.04
Residual	38	6.9592	.1831		
Total	45	8.4875			

1.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.7401	.1057	.59	.76
Trts vs Control	1	.0071		.04	.84
Brines vs PC	1	.0018		.01	.92
BR vs HBR	1	.1268		.71	.41
Residual	40	7.1768	.1794		
Total	45	7.9169			

2.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	2.4940	.3563	3.51	.01
Trts vs Control	1	.6608		6.51	.02
Brines vs PC	1	.0806		.85	.36
BR vs HBR	1	.0766		.75	.39
Residual	37	3.7565	.1015		
Total	44	6.2505			

Table II-7. Results of the ANOVAs using the log-transformed percent time spent data gothered at 25°C. The compartment term has been partitioned further by orthogonal contrasts. The salinity gradient level is indicated above each ANOVA. The treatments (Trts) term consists of the brine compartment (BR), the heated brine compartment (HBR) and the two plumbing controls (PC).

0.5 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.6970	.0996	1.07	.40
Trts vs Control	1	.0003		.00	.95
Brines vs PC	1	.5242		6.61	.02
BR vs HBR	1	.0583		.62	.43
Residual	38	3.5521	.0935		
Total	45	4.2491			

1.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	1.5857	.2265	1.19	.34
Trts vs Control	1	.9621		5.03	.03
Brines vs PC	1	.2104		1.10	.30
BR vs HBR	1	.1130		.59	.45
Residual	36	6.8821	.1912		
Total	43	8.4678			

2.0 ppt

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	5.9129	.8447	3.15	.01
Trts vs Control	1	.1575		.59	.45
Brines vs PC	1	1.2122		4.52	.04
BR vs HBR	1	1.6095		6.01	.02
Residual	45	12.0605	.2680		
Total	52	17.9734			

Table II-8. Results of the ANOVAs using the arcsin-transformed: A) percent entries data; B) percent time spent data. The compartments term has been further partitioned by orthogonal contrasts.

A. Percent Entries

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	.6801	.0972	.89	.52
Brine vs Control	1	.1570		1.44	.23
Br 1 vs Br 2	1	.3096		2.84	.10
Residual	72	7.8434	.1089		
Total	79	8.5235			

B. Percent Time Spent

SOURCE	DF	SS	MS	F	PR>F
Compartments	7	3.3412	.4773	1.88	.09
Brine vs Control	1	1.6020		6.29	.01
Br 1 vs Br 2	1	1.0820		4.25	.04
Residual	72	18.3274	.2545		
Total	79	21.6686			

APPENDIX III

RESULTS GRAPHS

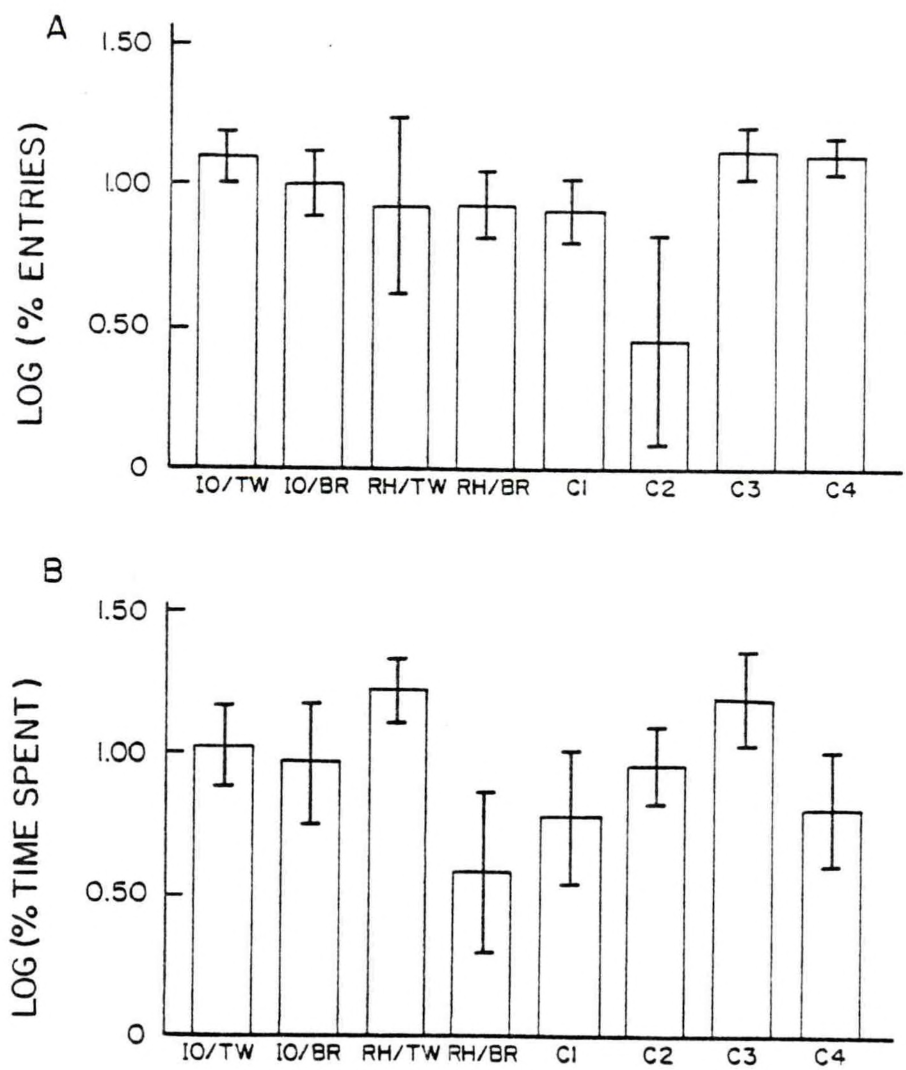

Fig. III-1. Mean responses of 11 redfish to the four brine compartments and the control compartments. Brackets indicate one standard error to either side: A) log-transformed percent entries data; B) log-transformed percent time spent data. IO=instant ocean; TW=tap water; BR=Brazos River water; RH=Ranch House stock salt. C1-C4 are the four control compartments.

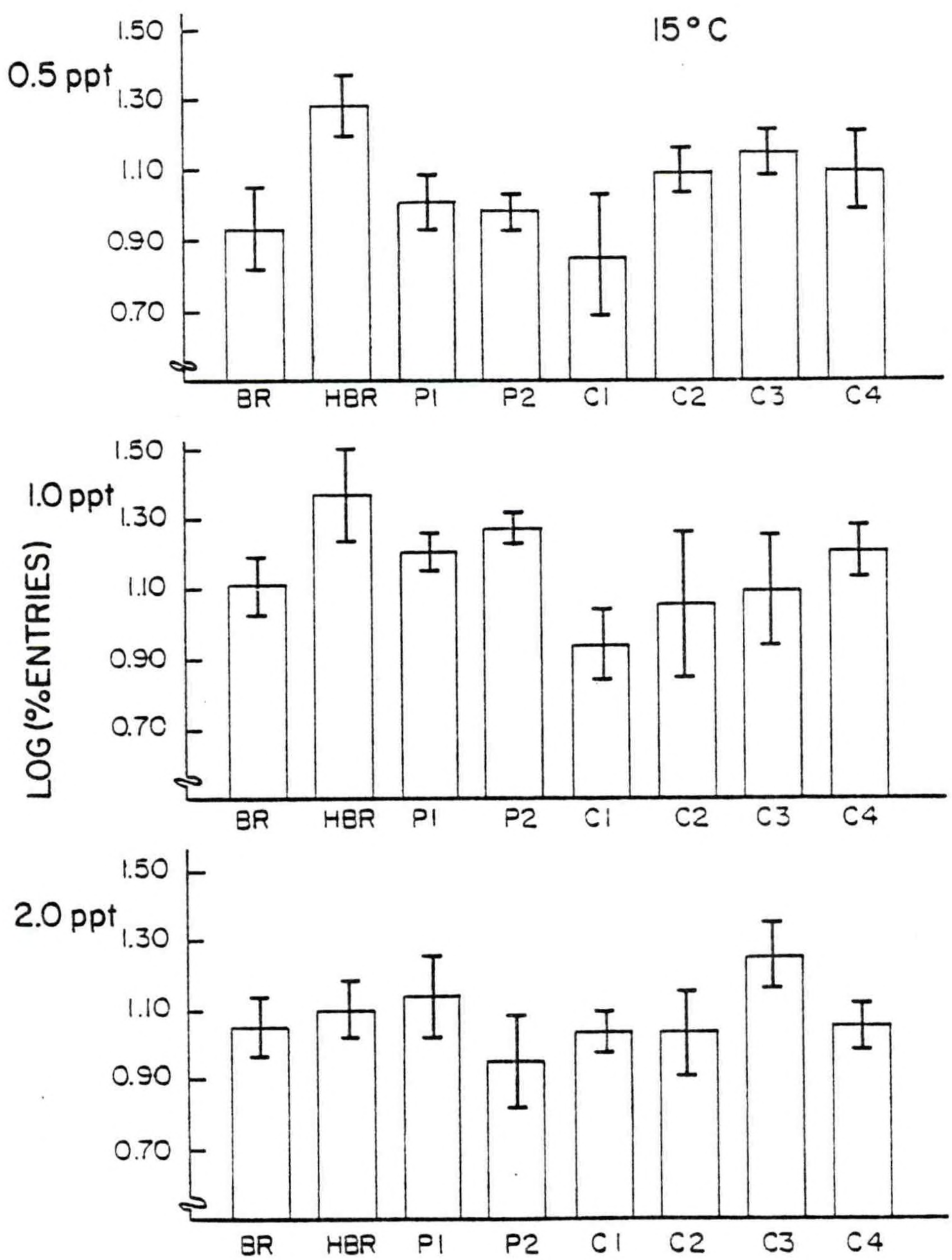

Fig. III-2. Mean ($\pm$SE) responses of redfish to brine (BR), heated water plus brine (HBR), plumbing controls (PC1 and PC2), and control compartments (C1-C4), using the log-transformed percent entries gathered at 15°C.

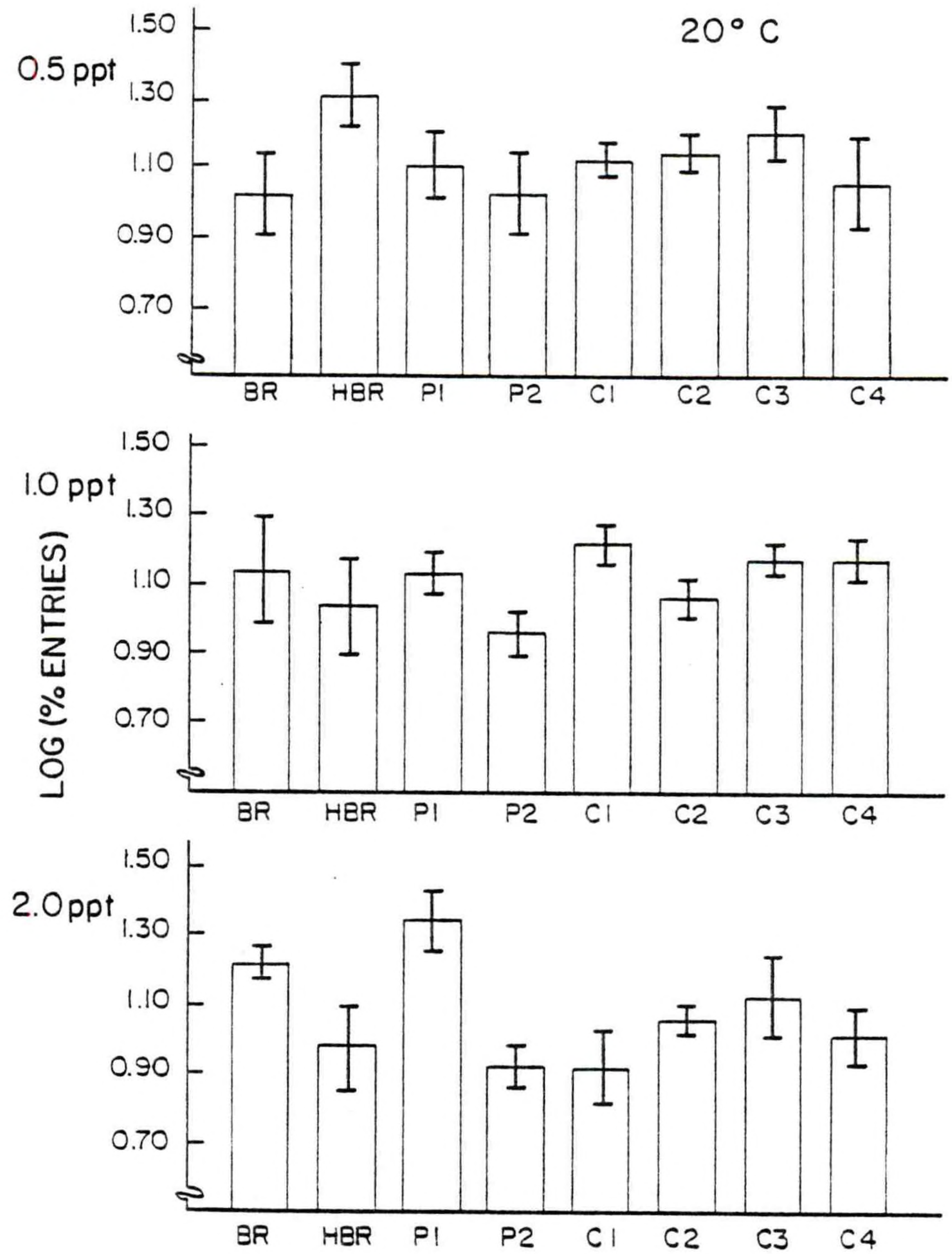

Fig. III-3. Mean (±SE) responses of redfish to brine (BR), heated water plus brine (HBR), plumbing controls (PC1 and PC2), and control compartments (C1-C4), using the log-transformed percent entries gathered at 20°C.

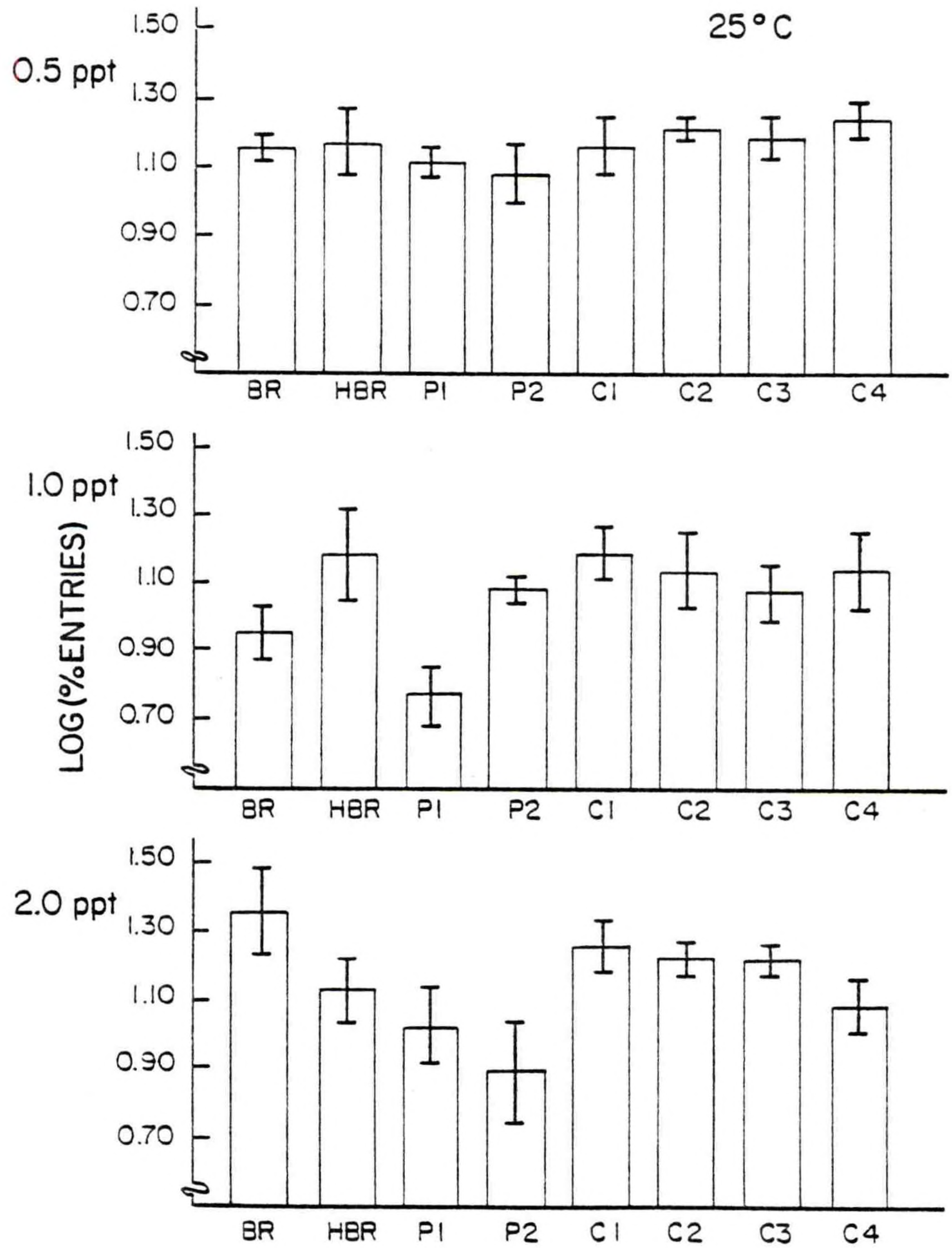

Fig. III-4. Mean ($\pm$SE) responses of redfish to brine (BR), heated water plus brine (HBR), plumbing controls (PC1 and PC2), and control compartments (C1-C4), using the log-transformed percent entries gathered at 25°C.

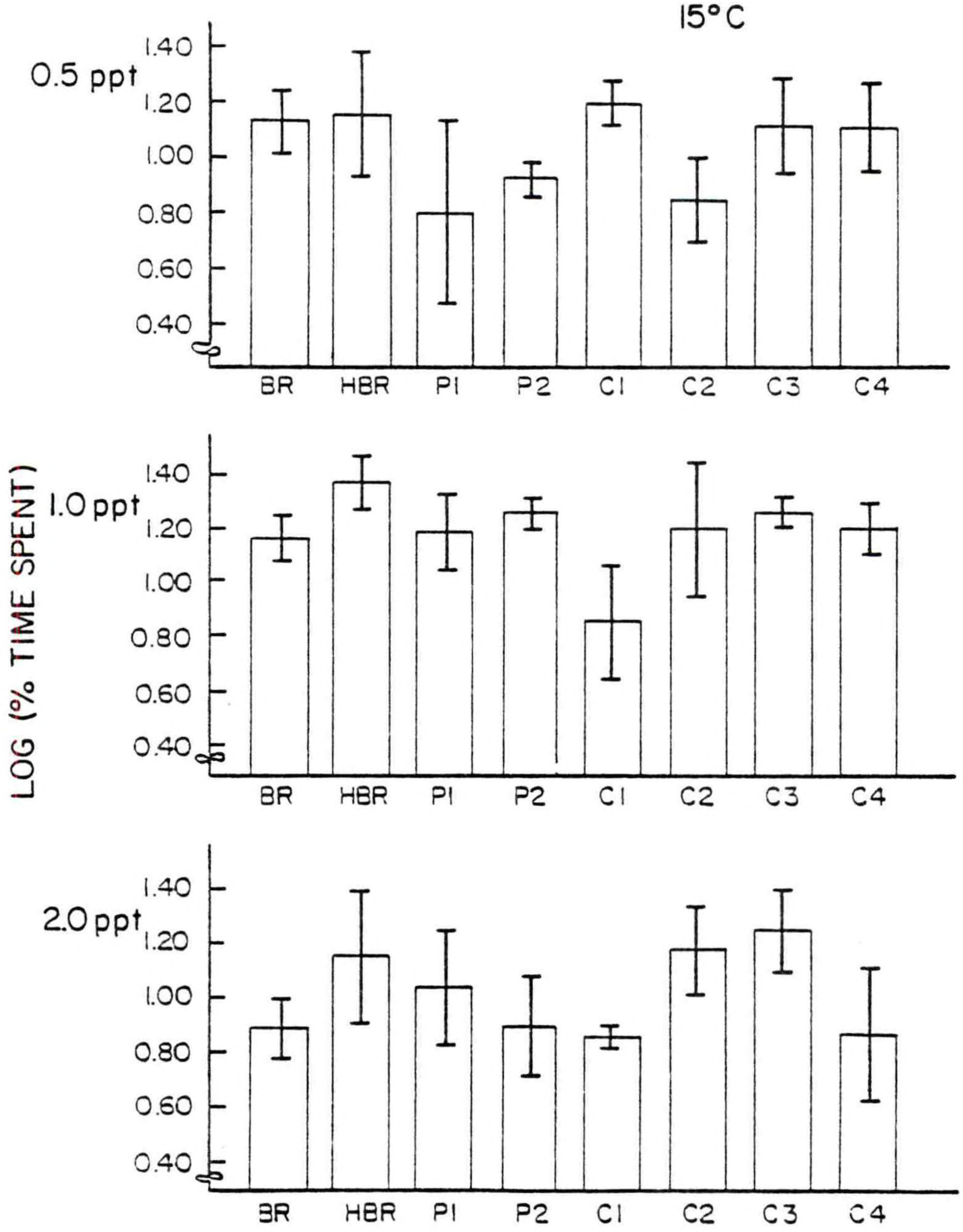

Fig. III-5. Mean (±SE) responses of redfish to brine (BR), heated water plus brine (HBR), plumbing controls (PC1 and PC2), and control compartments (C1-C4), using the log-transformed percent time spent gathered at 15°C.

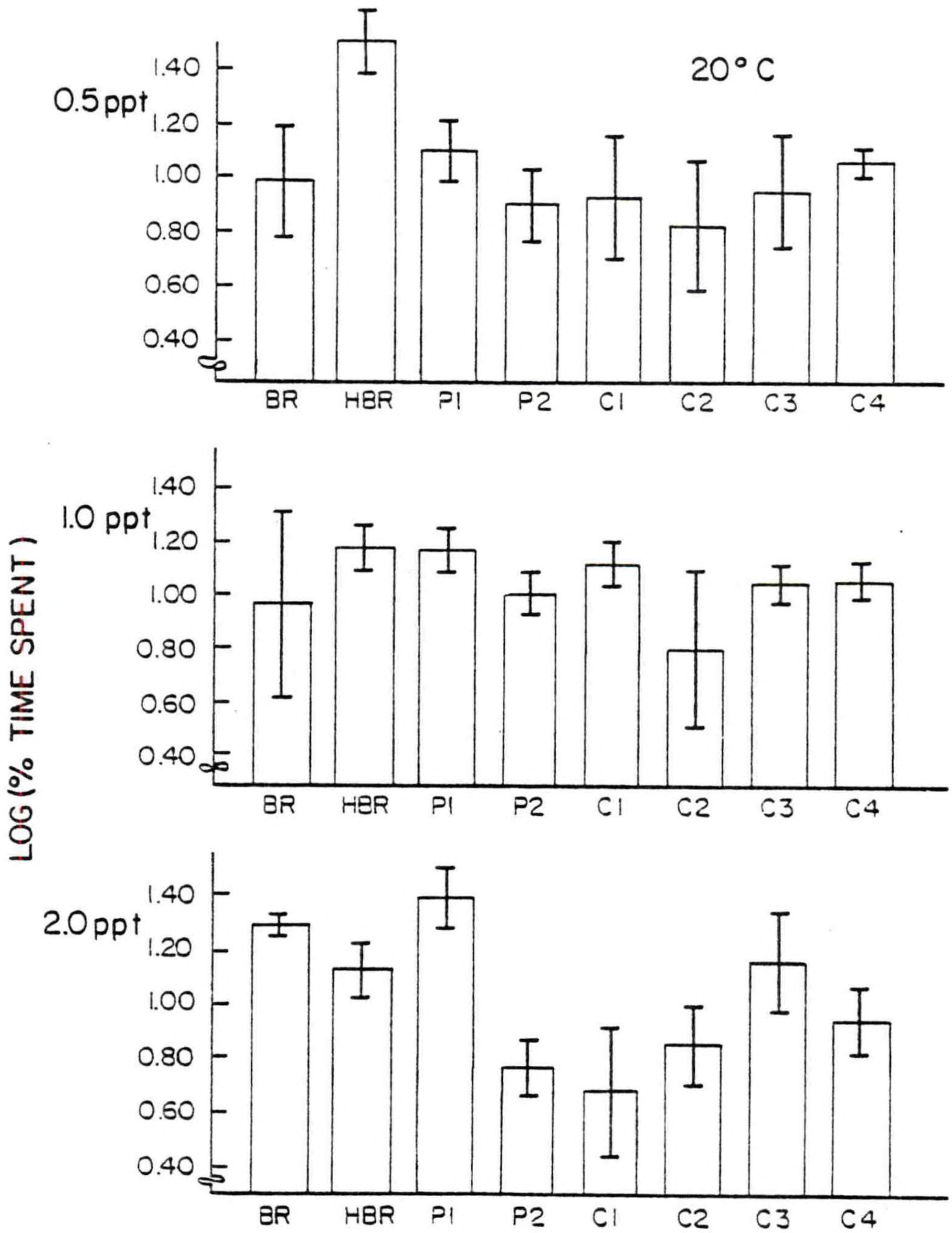

Fig. III-6. Mean (+SE) responses of redfish to brine (BR), heated water plus brine (HBR), plumbing controls (PC1 and PC2), and control compartments (C1-C4), using the log-transformed percent time spent gathered at 20°C.

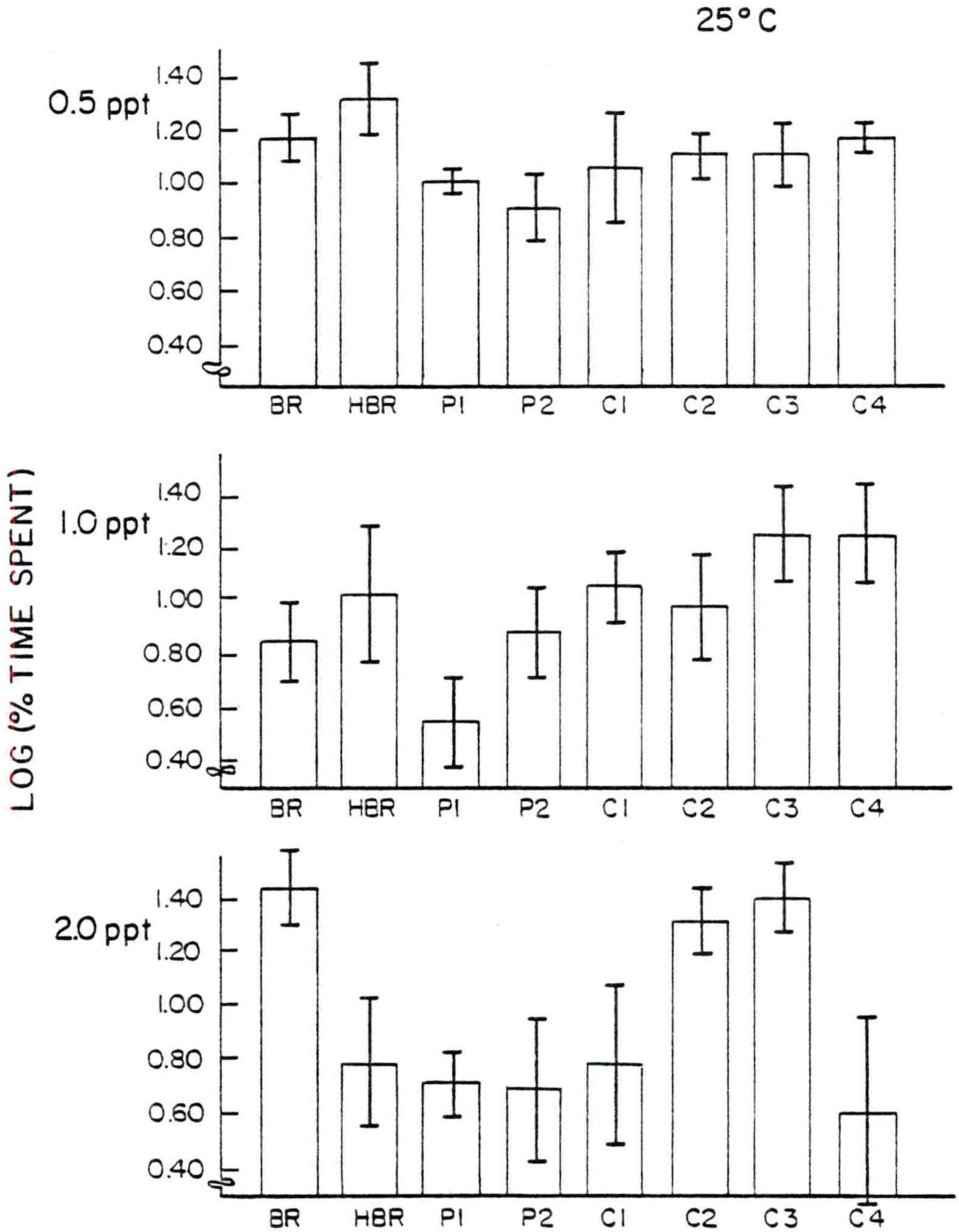

Fig. III-7. Mean (±SE) responses of redfish to brine (BR), heated water plus brine (HBR), plumbing controls (PC1 and PC2), and control compartments (C1-C4), using the log-transformed percent time spent gathered at 25°C.

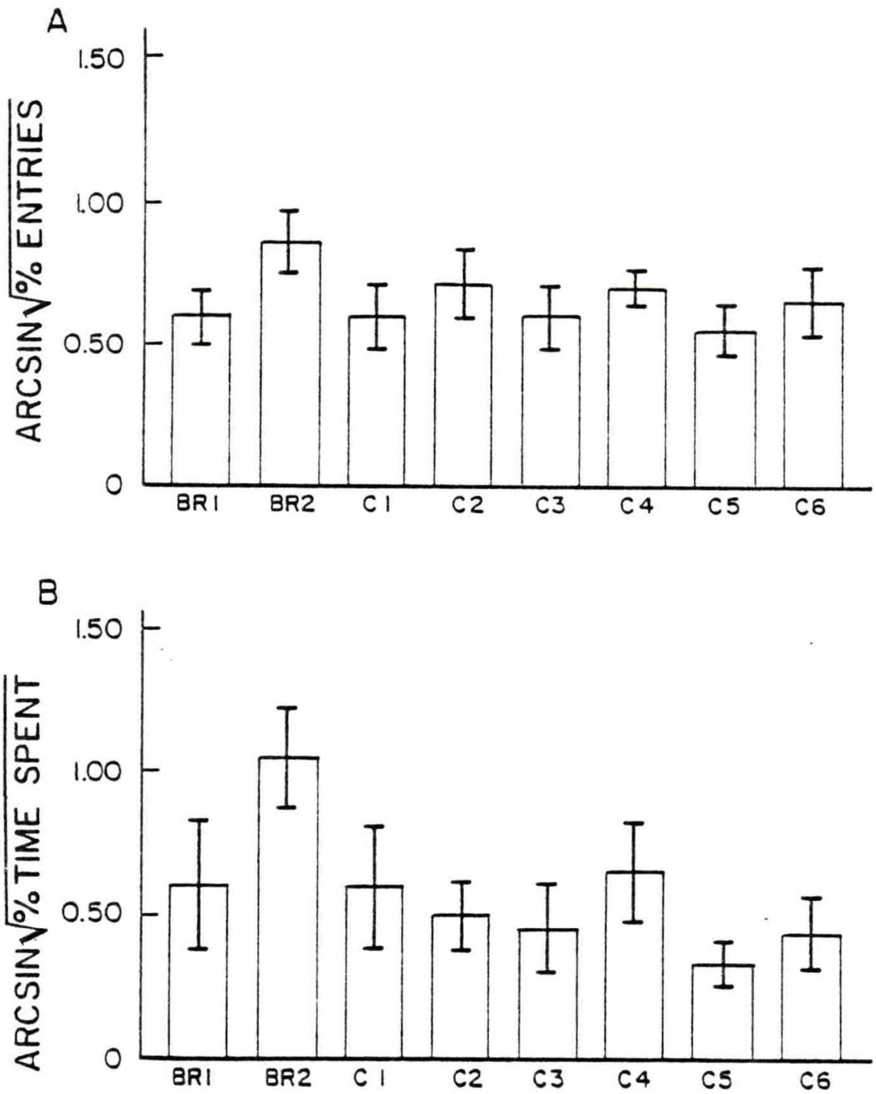

Fig. III-8. Mean (±SE) responses of 10 redfish to two brine compartments (BR1 and BR2) and six control compartments (C1-C6) using the arcsin-transformed: A) percent entries data; B) percent time spent data.