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 A) Brine Toxicity

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

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NOAA Technical Memorandum NMFS-SEFC-69

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

VOL. V (A) BRINE TOXICITY BIOASSAYS ON REDFISH

By

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and

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

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ERRATA

Page 18, paragraph 4:

Survival was generally greater in the mid and low salinities tested and had a greater effect on the survival of redfish embryos than did temperature. Significant temperature and salinity interactions existed and were more pronounced at high test salinities. Redfish larvae differed from embryos in that temperature was a more important factor than salinity for their survival. No precise temperature-salinity optimum was found.

ADDENDUM

Following page 20:

Based upon the objectives of this study, the hypotheses which were tested and the resultant findings are:

1. That there is an optimum combination of salinity and temperature for the normal embryo-larvel survival of *Sciaenops ocellata*, outside this optimum survival will be diminished:

   This study has demonstrated that both salinity and, to a lesser extent, temperature and their interactions are important determinants of the survival of redfish embryos and larvae. Survival is generally greater at the lower salinities tested (34ppt), but no generalizations can be made relating survival to temperature. Thus, the ranges of both temperature and salinity were not of sufficient magnitude to delineate optimal conditions.

2. That the survival of early life stages of *S. ocellata* will be different in brine prepared with dome salt than in brine prepared with artificial sea salt:

   Survival of early life stages of redfish was generally reduced in brine prepared with dome salt as compared to brine prepared with artificial sea salts, particulary at the higher tested salinities (40ppt).

3. That the survival of early life stages of *S. ocellata* will be different in brine prepared with Brazos River water than in brine prepared from distilled water:

   Brine prepared with Brazos River water appeared to be more toxic than brine prepared with distilled water, although the effect of dilution water was not as great as that of the salt source.

4. That elevated temperatures will influence the survival of early life stages of *S. ocellata* to the different brine treatments:

Temperatures tested in this study (23-30 C) provided no clear evidence that redfish embryo-larval survival was influenced by elevated temperatures.

While temperatures tested in this study (23-20 C) significantly affected the survival of the early life stages of redfish, in only one experiment of four was a significant temperature-brine interaction present. Although there was some indication of reduced redfish survival in the highest tested temperature, no major consistent temperature effect was obtained.

DISCLAIMER

This document is a Final Report. It has been reviewed by the National Marine Fisheries Service and the National Oceanic and Atmospheric Administration and approved for printing. Such approval does not signify that the contents necessarily reflect the views and policies of the U.S. Department of Energy, NOAA or NMFS. This Report has not been formally released by the DOE. Mention of trade names or commerical products herein does not constitute endorsement or recommendation for use.

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This volume should be cited as follows:

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 Bound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-69. p. NOAA, NTIS Accession No. ________________.

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Volume V(A) - BRINE TOXICITY

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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.

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Volume II - SHRIMP MARK-RELEASE

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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.

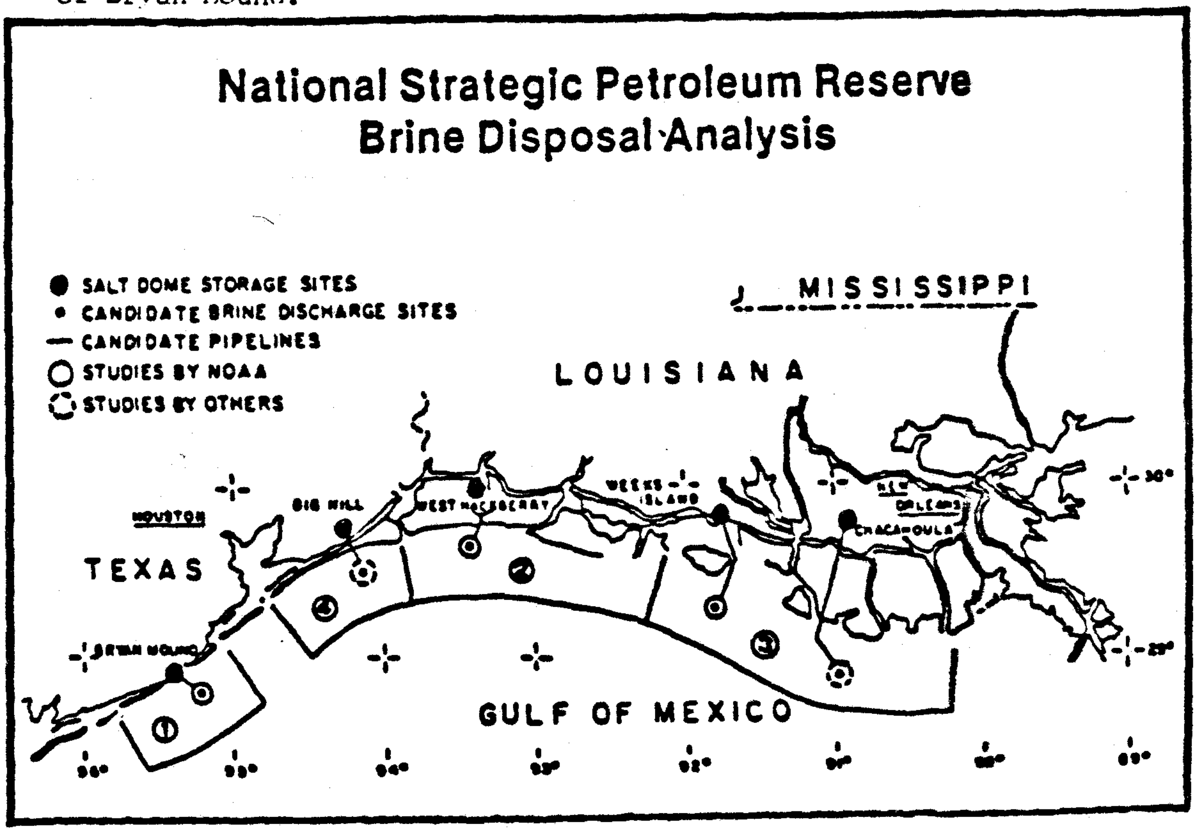

Figure 1. Regions of Study for Brine Disposal Assessment-DOE/NOAA Interagency Agreement (adapted from Environmental Data and Information Servic , DOC/NOAA).

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. ______________.

Comiskey, C., R. McCord, D. Bozworth, S. Grady, C. Hall, C. Brandt and T. Farmer. 1981. Texas coast shrimp catch and effort data analysis. Vol. I(B). 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. 234 p. NOAA, NTIS Accession No. ______________

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.

Johnson, M. F. 1981. Interview sampling survey of shrimp catch and effort. Vol. IV. In: Jackson, W. B. and J. R. Bennett (eds.). Shrimp and redfish studies; Bryan Mound brine disposal site off Freeport, Texas, 1979-1981. NOAA Technical Memorandum NMFS-SEFC-68, 38 p. NOAA, NTIS Accession No. PB82-116062.

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. 75 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.

Hausknecht, K. A. 1980. Describe surficial sediments and suspended particulate matter. Vol. V. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 19798-1979. NOAA Technical Memorandum NMFS-SEFC-29, 83 p. NOAA, NTIS, Accession No. PB81-174963.

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.

Schwarz, J. R., S. K. Alexander, A. J. Schropp and V. L. Carpenter. 1980. Describe bacterial communities. Vol. III. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-27, 74 p. NOAA, NTIS Accession No. PB81-174948.

Tillery, J. B. 1980. Determine trace metal composition and concentration in major components of the marine ecosystem. Vol. VII. In: Jackson, W. B. and G. M. Faw (eds.). Biological/chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. NOAA Technical Memorandum NMFS-SEFC-31, 100 p. NOAA, NTIS Accession No., PB81-174989.

Related Publications

Caillouet, C. W. and F. J. Patella. 1978. Relationship between size composition and ex-vessel value of reported shrimp catches from two gulf coast states with different harvesting strategies. Marine Fisheries Review 40(2):14-18.

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(A) - BRINE TOXICITY BIOASSAYS ON REDFISH

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ABSTRACT

Redfish or red drum, *Sciaenops ocellata* (Linneaus), embryos, larvae and juveniles were exposed to four test brine solutions: artificial sea salts in distilled water, dome salt in Brazos River water, dome salt in distilled water and artificial sea salt in Brazos River water, at various combinations of temperature and salinity. Embryos were exposed for 72 h, and larvae for 120 h, to four brine types, four salinities (34, 36, 38 and 40 ppt) and three temperatures (23°, 26°and 30°C), resulting in a 4x4x3 factorial design. Juveniles were exposed for up to two weeks to acute concentrations of dome salt/Brazos River water brine. Embryos were the most sensitive life stage and juvenile redfish the most resistant to brine concentrations. Response surface methodology indicated that in general, salinity effects were more dominant than temperature effects. Salinities above 38 ppt adversely affected embryonic survival in all brine types, whereas temperature had no consistent effect upon embryonic survival. Brine prepared with dome salt and Brazos River water represented the most toxic exposure condition for redfish embryos. The optimal conditions for hatching success and embryonic survival of redfish were combinations of mid-range salinities (34-36.5 ppt) and temperatures (23°-26°C) in all brine types. Embryonic survival decreased markedly in all brine exposures after 72 h exposure, presumably due to difficulty in the transition from endogenous to exogenous nourishment. Redfish larvae (2 weeks old) were more resistant to brine exposure than embryos and did not respond differentially to different brine types until after 96 h exposure when slightly greater survival occurred in artificial sea salt/Brazos River water brine relative to the other three brine types. Survival of juvenile redfish was affected by concentrations of dome salt/Brazos River water brine exceeding 5 ppt in seawater.

The present investigation has indicated that dome salt is the most toxic element of the brines which were tested and that Brazos River water was more toxic to redfish in early life stages than distilled water. The most critical factor influencing early development of redfish appears to be successful transition from endogenous nutrition to exogenous feeding (i.e. 72-80 h). Redfish larvae which successfully make this transition would be expected to survive in low concentrations of brine in a field situation.

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ACKNOWLEDGEMENTS

The cooperation of Mr. Bob Colura and the Texas Parks and Wildlife Department, and of Dr. C. Arnold at the Port Aransas Marine Lab (University of Texas), who provided the redfish used in this study is especially appreciated. Various personnel of the National Marine Fisheries Service (NMFS), the Environmental Data and Information Service (EDIS) and the Department of Energy (DOE) provided useful criticisms.

We would like to thank Ms. Jean Erwin of LGL Ecological Research Associates, Inc. (LGL) for her invaluable editorial assistance and for preparation of this report. The contributions of Ms. Pat Oliver and Ms. Bonnie Bower of LGL for assistance in typing of this report and for drafting the figures which were utilized are greatly appreciated. The clerical assistance of Ms. Jean Rierson and Ms. Becky Schult, Texas A&M University, during the early phases of this project is also appreciated. We thank Mr. Kent Retzer for his technical assistance.

INTRODUCTION

The Bryan Mound salt dome near Freeport, Texas has been selected as one of the several storage sites for the Strategic Petroleum Reserve (SPR) program. The salt dome caverns have been and are being prepared for petroleum storage by solution mining through pressurized injection of Brazos River water into the caverns. The resulting brine is then discharged at rates which may exceed 100,000 barrels/day into the Gulf of Mexico at a site 12.5 mi (20.1 km) offshore, south of Bryan Mound.

Concern about the potential adverse environmental impact of offshore disposal of the brine is based upon numerous considerations. The brine contains approximately ten times more total dissolved solids than natural seawater at the disposal site. Although the major component of Bryan Mound salt domes is sodium chloride (NaCl, 99%), other inorganic components of the brine differ from those of seawater. Potassium, calcium, magnesium and sulfate are present at relative concentrations much lower than those in seawater. Additionally, in natural seawater the calcium/magnesium ratio is approximately 0.32, while in Bryan Mound brine it is 78.3 (SPR-FEA 76/77-6, 1977). These ions are important in the osmoregulatory processes of marine organisms and any alteration of their relative concentration may adversely affect an organism's osmoregulatory abilities (see Prosser 1973).

Additionally, the Brazos River flows through several industrial and agricultural areas and its waters contain relatively high concentrations of Cd, Cr, Pb, Hg, Ni and Zn (SPR-FEA 76/77-6, 1977 [Table 23, pp. 3-32]). Offshore discharge of the brine affords a mechanism for rapid transfer of these metals to the relatively pristine offshore area. Temperature also represents a source of potential danger for marine life as the brine is discharged at a temperature somewhat above ambient (SPR-FEA 76/77-6, 1977). The temperature within about 15 m of the diffuser is up to 1°F (0.6°C) above ambient (F. Godshall, personal communication, EDIS/CEAS/MEAD). Therefore, discharge of Bryan Mound brine into the ocean off Freeport, Texas alters the temperature and salinity profile of the surrounding area as well as the inorganic ion concentration ratios of the seawater in the immediate vicinity of the diffuser head. A model of the brine diffusion characteristics (U.S. Department of Commerce 1977) predicts an area of 500 acres (200 ha) in which salinities could be one part per thousand (ppt) greater than ambient. The area covers 2,000 acres (800 ha) under stagnant conditions. Field observations have shown that these predictions are relatively accurate (F. Godshall, pers. comm.).

The redfish or red drum, *Sciaenops ocellata* (Linneaus), is one of several important species of fin fish that might be adversely affected by the brine discharge. In the Gulf of Mexico, they are most abundant along the coasts of Mississippi, Florida and Texas (Yokel 1966), where they have supported a large commercial and sport fishery. Although the behavioral biology is not completely understood, the redfish larvae and juveniles are found primarily in estuaries and inshore waters, while adults are more frequently found offshore. However, during the spawning season, which occurs from mid-August to December in the Gulf of Mexico (Boothby and Avault 1971), they are commonly taken near passes and in the surf. 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.

Nevertheless, redfish may be present at times in the area of the Bryan Mound disposal site (Chittenden et al. 1981, Chittenden and McEachran 1976); further, the expected salinity gradient (plume) from the disposal site may act as an attractor to spawning redfish (Owens and Jones 1981) with the subsequent exposure of embryos, larvae and juveniles to potentially toxic conditions since it is generally agreed that the early life stages of fish are most sensitive to stress (Rosenthal and Alderdice 1976, McKim 1977, Macek and Sleight 1978). Therefore, this report addresses the effects of the brine (Brazos River water, elevated temperature and salinity) on the survival of early life stages of redfish, while the response of large redfish (> 45 cm standard length) to the brine is discussed in Owens and Jones (1981).

In all further references in this paper, embryo will refer to development stages prior to hatching and up to two weeks after hatching; larvae to free swimming stages less than one month and juvenile to fish greater than one month of age.

OBJECTIVES

The objectives of the present investigation were to determine the interactive effects of elevated salinity and temperature (resulting from the mixture of warm brine with seawater) on the survival of early stages of redfish. Through a multi-factorial approach, the following working hypotheses were tested.

1. That there is an optimum combination of salinity and temperature for the normal embryo-larvel survival of *Sciaenops ocellata*, outside this optimum survival will be diminished.

2. That the survival of early life stages of *S. ocellata* will be different in brine prepared with dome salt than in brine prepared with artificial sea salt.

3. That the survival of early life stages of *S. ocellata* will be different in brine prepared with Brazos River water than in brine prepared from distilled water.

4. That elevated temperatures will influence the survival of early life stages of *S. ocellata* to the different brine treatments.

MATERIALS AND METHODS

BRINE PREPARATION AND ANALYSIS

Brine solutions were prepared from a commercially available artificial sea salt (Instant Ocean, Aquarium Systems, Inc., Eastlake, Ohio) and from Gulf Coast salt dome salt (United Salt Co., Hockley, Texas). The salts were dissolved in either distilled water, or in Brazos River water. The Brazos River water was obtained between mile 2 and 3 (3.2-4.8 km) on the Brazos River Diversion Canal, near the site of the Bryan Mound water intake.

Four brines were used so that it was possible to investigate the differences among redfish responses due to salt type, brine chemical composition, brine concentration (salinity) and possible contaminants introduced with Brazos River water. The four test brines were: 1) artificial sea salt in distilled water; 2) dome salt in Brazos River water; 3) dome salt in distilled water; and 4) artificial sea salt in Brazos River water.

Stock brines were prepared by dissolving 315-320 g of the test salt per liter of the respective water types. Moderate heat (to 38 °C) was applied to aid dissolution. Insoluble fractions and suspended solids were removed by settling. Salinities of all stock brines were between 293 and 312 ppt. The brines were stored at 4 °C in glass carboys in the dark until used, and fresh brines were prepared for each bioassay. Artificial seawater (Instant Ocean in distilled water) at a salinity of 34 ppt was utilized to dilute the brines to test salinities. Thus, all experiments at 34 ppt contained only artificial seawater; i.e. no dome salt or Brazos River water was present in test solutions at 34 ppt. This was representative of field conditions in that brine was being added to natural seawater, resulting in increased salinities at the disposal site. Artificial seawater at 34 ppt thus represented a control condition relative to brine content, and was a better control than seawater since it can be consistently prepared to known specifications at any time or place.

The four stock brines and two water types were characterized chemically, and the concentrations of the following inorganic ions were determined: sodium, potassium, magnesium, calcium, chloride, sulfate, copper, cadmium, zinc and silver. Cation concentrations were determined by atomic emission (Na, K), or absorption spectrophotometry (Mg, Ca, Cu, Cd, Zn, Ag) with a Perkin Elmer atomic absorption spectrophotometer (Model No. 370A).

Chloride was determined with a Buchler-Cotlove chloridometer, and sulfate was measured by a turbidimetric method adapted for seawater analysis (B.J. Presley, pers. comm., Texas A&M). The salinity of stock brines and all exposure media was monitored with an optical refractometer (Environmental Devices Corp.).

SOURCE OF REDFISH

Redfish embryos, larvae and juveniles were obtained from two sources. Mr. Bob Colura of the Texas Parks and Wildlife Department (TPW) facility, Palacios, Texas provided the embryos utilized in Embryo Bioassay 1 (see EXPERIMENTAL DESIGN below). All other embryos, larvae and juveniles were obtained from Dr. C. Arnold of the University of Texas, Marine Sciences Institute, Port Aransas, Texas.

For transportation to the laboratory, redfish embryos or larvae were placed in plastic bags one-third filled with filtered, sterilized seawater of ambient temperature and salinity. The bags were then filled with oxygen, sealed, and placed in styrofoam coolers during the 6 h of vehicle transport back to College Station, Texas.

Embryonic assays were initiated immediately upon arrival at the lab due to the short (about 20 h) embryonic period. Larvae were transferred to aquaria and observed for one to two days prior to the initiation of bioassays in order to be sure that fish injured in transport were not utilized in the experiments. The aquaria were provided with light aeration and filter fiber filtration.

A continuous culture of the rotifer *Brachionus plicatilis* was maintained and served as the food source for the newly hatched redfish larvae. The rotifer cultures were maintained on the algae *Tetraselmis chuii*.

EXPERIMENTAL DESIGN

Embryo Bioassay 1

Bioassay 1 was designed to investigate the response of embryos to two brine types and to facilitate the design of the remaining bioassays. The two brine types were dome salt/Brazos River water and artificial sea salt/distilled water. These brine types represent the "worst" and "best" cases of brine, respectively, related to the ionic composition of natural seawater. Salinities utilized for both brines were 34, 36, 38 and 40 ppt, and test temperatures of 23°, 26° and 30°C (± 0.5°C) were maintained in constant temperature incubators (Environator, Calumet Scientific). The experimental design of Bioassay 1, therefore, was a 2x4x3 factorial (2 brine types x 4 brine concentrations x 3 temperatures). Photoperiod was maintained at 10 h light:14 h dark (fall spawning conditions) during the bioassay.

Embryos were placed in freshly prepared exposure media immediately upon return to the laboratory, when the embryos were approximately 6-10 h old after fertilization. Exposure chambers were 10 cm glass finger bowls containing 50 ml exposure media. Twenty embryos were placed in each bowl and, except where noted (see Tables 2-4), there were four replicate bowls per factor combination. Embryonic and newly hatched larval (the embryos will hatch approximately at an age of 24 h) survival was monitored and recorded at 24, 48 and 72 h exposure, at which times the remaining live organisms were transferred to freshly prepared exposure media to prevent an undesirable buildup at metabolic by-products. Rotifers were provided daily *ad libitum* as food for newly hatched redfish larvae. Due to high mortality in artificial sea salt/distilled water brine at 72 h, the bioassay was terminated at this point.

Embryo Bioassay 2

Bioassay 2 utilized redfish embryos of approximately the same age as those used in Bioassay 1 (6-10 h). However, all four brine types were used in this bioassay: artificial sea salt/distilled water, dome salt/ Brazos River water, dome salt/distilled water and artificial sea salt/ distilled water. As in Bioassay 1, the four brine concentrations (salinities) were 34, 36, 38 and 40 ppt and temperatures were 23°, 26° and 30°C, resulting in a 4x4x3 factorial design. There were four replicate bowls of 20 embryos per bowl at each factor combination. Embryos were exposed in the same manner as in Bioassay 1 and embryonic/larval survival was monitored and recorded after 12, 24, 48 and 72 h of exposure. The remaining live embryos and newly hatched larvae were transferred to freshly prepared exposure media at 24 h intervals, at which time rotifers were supplied as food. The experiment was terminated after 72 h due to high mortalities.

Embryo Bioassay 3

Bioassay 3 utilized redfish embryos and exposure was initiated when the embryos were the same age as those utilized in Bioassays 1 and 2. The experimental protocol for Bioassay 3 was identical to that of Bioassay 2.

Larval Bioassay

The larval bioassay utilized 2-week-old redfish larvae in an experimental design similar to that of the embryonic bioassays 2 and 3. Larvae were exposed to the four brine types at four salinities (34, 36, 38 and 40 ppt) and three temperatures (23°, 26° and 30°C) again resulting in a 4x4x3 factorial design. However, in this bioassay there were four replicates of five larvae per bowl at each factor combination resulting in 20 animals per treatment. Survival was monitored and recorded every 24 h with the survivors transferred to a new media. The larvae were fed *Artemia salina* nauplii every two days. Due to increased survival in the larval experiments, the bioassays continued for 120 h.

Juvenile Bioassays

Two bioassays were conducted with redfish juveniles (approximately two months old) to determine the acute toxicity of various dilutions of dome salt/Brazos River water brine in artificial seawater to this life stage. In the first experiment, juveniles were exposed to brine concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 percent volume of stock brine to artificial seawater. Corresponding salinities were 35.3-46.6 ppt (Table 37). Five fish were placed in each 20 cm finger bowl containing 1,000 ml of exposure medium. Four replicate bowls were utilized for each brine concentration. Survival was monitored and mortalities recorded every 48 h with the survivors transferred to a new media and fed *Artemia salina* larvae.

In the first juvenile bioassay there was appreciable mortality only at the highest brine concentration (50% mortality at 5% brine), so a second juvenile bioassay was initiated incorporating higher brine concentrations. Juvenile redfish were exposed in all glass aquaria containing 4 ℓ of test media at concentrations of dome salt/Brazos River water brine of 1, 2, 3, 4, 5, 7.5 and 10 percent of stock brine to artificial seawater (37.0-59.6 ppt, see Table 37). Seven fish were added to each aquarium, and three replicate aquaria were utilized per brine dilution. Juveniles were fed *Artemia salina* larvae daily and the test media were renewed every 48 h. In both juvenile bioassays, the test organisms were transferred directly from 34 ppt artificial seawater to the appropriate test medium. Both juvenile bioassays continued for 14 days.

STATISTICAL ANALYSES

<u>Analysis of Variance</u> (Repeated Measures)

The analysis was conducted on all embryo and larval bioassays upon the arcsine square-root transform of survival percentages. The purpose of the analysis is to detect any significant effects (salinity, temperature, brine type and exposure duration) and their interaction upon survival.

Observing the same experimental unit over different treatments or observing the effect of a treatment on an experimental unit through time has two main consequences. It introduces correlation between observations and has the advantage of having each experimental unit act as its own control. Analysis of variances using repeated measures is a linear model's technique specifically designed to handle experiments with the above variance-covariance structure. Analysis of the main effects is handled in the usual way with the repeated measure observations (i.e. each bowl has a measured survival for each time interval) collapsed. Analysis of the repeated measures and their interaction with the main effects is handled through the use of orthogonal polynomials. In other words, repeated measures involving time are analyzed by partitioning the time-of-measurement sum of squares into various components in order to examine the shape of the time response and by examination of its interactions with the main effects. For a more detailed description and explanation of the repeated measure design and analysis the reader is referred to Winer (1971). The BMDP-79 program P2V was used to analyze the data and is described in Dixon (1977).

The model utilized in the repeated measures design was:

$$Y_{ijk\ell m} = \mu + S_i + T_j + B_k + (ST)_{ij} + (SB)_{ik} + (TB)_{jk} + (STB)_{ijk} + R_\ell + (SR)_{i\ell} + (TR)_{j\ell} + (BR)_{k\ell} + (STR)_{ij\ell} + (SBR)_{ik\ell} + (TBR)_{jk\ell} + (STBR)_{ijk\ell} + e_{m(ijk\ell)}$$

where:

$y_{ijk\ell m}$ = arcsine square-root transform of percent survival

μ = overall mean

S_i = salinity of the i'th treatment (i = 1 to 4)

T_j = temperature of the j'th treatment (j = 1 to 3)

B_k = brine type of the k'th treatment (bioassay dependent)

R_ℓ = exposure duration of the ℓ'th recording period

$e_{m(ijk\ell)}$ = error within the treatment combination

The analysis of variance was broken into two component tables, the first of which analyzed the treatments and their interactions (i.e. brine type, salinity, temperature) and the second analyzed the time response and its interactions with the treatment combinations. Additionally, the second analysis of variance was decomposed into linear and quadratic polynomials which help to show the time response pattern of survival with the treatment combinations.

The raw percent survival mean for each treatment combination are also tabulated to allow accessibility to the data. Note that extreme caution must be exercised in the interpretation of this (untransformed) data as percentages are notorious for the skewed distributions they generate.

Orthogonal Polynomial Regression (Response Surface Methodology)

In order to predict and display optimal and delterious combinations of salinity and temperature for each brine type at the various observation periods, response surface methodology was utilized for the embryo and larval bioassays (see Alderdice 1972). Again, an arcsine square-root transform was used on the survival percentages. This procedure utilized a model for regression analysis of the form:

$$Y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{12}x_1x_2 + b_{22}x_2^2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{33}x_3^2$$

where:

Y = arcsine square-root of survival percent

x_1 = salinity

x_2 = temperature

x_3 = duration time

and the b's are constants fitted by the regression. Thus, a prediction equation and resulting response surface diagram, was generated for each time period of each brine type utilizing the SAS Response Surface Regression procedure (SAS Institute Inc. 1979, 1981). The resultant contour plots are labeled with the appropriate percent survival rather than the transformed value in order to enhance clarity. This analysis, and resultant output, allows statistical evaluation of the utility of various model factors as viable predictors of changes in embryonic survival, and the response surface isopleths afford visual interpretations of survival trends within various combinations of temperature and salinity for each brine type. Through these analyses, a prediction of best and worst conditions for embryonic survival can be made.

Probit Analysis

The probit procedure of SAS (SAS Institute Inc. 1979) was utilized in the analysis of juvenile bioassays. Note that a data transformation was not required as probit analysis only requires a cummulative count of the mortalities as input. This procedure calculates the maximum likelihood estimates of the intercept, slope, and natural (threshold) mortality rate of the acute assay data. The maximum likelihood estimates were calculated for the parameters A and $B > 0$ in the probit equation:

$$M = A + Bx$$

where:

M = probit mortality (5 plus normal equivalent deviates of cummulative portion of dead juveniles)

x = dose (i.e. percentage by volume of stock brine to artificial seawater)

Additionally, this procedure derives the estimated dose and 95% fiducial limits for probability levels 0.01-0.10, 0.15, 0.20, 0.25, 0.85 and 0.90-0.99. A plot of points computed as raw probability minus the estimated threshold response rate at each level of stimulus, superimposed on the normal probability sigmoid curve was also produced. Since this methodology does not lend itself to an examination of the confounding effect of exposure time the analysis was performed at 96 h, 7 days and 14 days in experiment 1 and 24 h, 48 h and 96 h in experiment 2 as a check on the consistency of the results. For a more detailed statistical description of probit analysis, see Finney (1971).

RESULTS

BRINE ANALYSIS

The results of the brine analyses are presented in Table 1. Cadmium, copper, zinc and silver, if present, were below detectable levels for the analytical equipment utilized. The brines consisting of dome salt had markedly lower concentrations of potassium, magnesium and sulfate than those brines prepared with artificial sea salt.

EMBRYO BIOASSAY 1 - (Two brine types)

Mean percent survival of redfish embryos/larvae under the different exposure regimes of Bioassay 1 at 24, 48 and 72 h is summarized in Tables 2-4, respectively. Although there was considerable variability in the data, some trends emerge. At 24 h (about the time of first hatching), survival was high in both brines at all combinations of salinity and temperature, and there was a trend toward increased survival with increasing temperature. The dome salt/Brazos River water brine, particularly at the highest salinity (40 ppt) and lowest temperature (23° C) tested, appeared to be toxic. By 72 h, survival was poor at all factor combinations. Survival was lowest at combinations of high salinity and high temperature. The dome salt/Brazos River water brine produced lower survival under most salinity/temperature combinations than did the artificial sea salt/distilled water brine.

Table 5 presents the analysis of variance table for repeated measures analyses performed on the transformed survival data. In the analysis of main and interactive treatment effects, salinity (SAL), brine type (BRINE), salinity x temperature (ST) and salinity x brine (SB) were significant at $P < 0.05$. The analysis of exposure duration and its interaction with treatment effects indicated that exposure duration (R), exposure duration x temperature (RT), salinity x temperature (ST) and exposure x temperature x salinity (RTS) effects were significant at $P < 0.05$. There were significant linear and quadratic interactions between exposure duration and treatment effects. Overall, exposure duration exhibited the most pronounced effect upon embryonic survival, and salinity and brine type exhibited a significant effect. However, interpretation of these main treatment effects was difficult due to the significant interactions which occurred.

Response surface diagrams resulting from regression analyses provide a visual representation of temperature x salinity interactions (Figs. 1-2). Table 6 is a summary of the linear and quadratic effects of single variables and combinations of variables (i.e., salinity, temperature, exposure duration) on arcsine square-root of percent survival of embryos exposed to artificial sea salt/distilled water brine. The linear effects of temperature and exposure duration, temperature and temperature x exposure duration effects were all significant at $P < 0.05$, indicating that these terms were good predictors of percent embryonic survival.

Clarification of the interactive effects between combinations of salinity and temperature and exposure duration was provided by the response surface diagrams generated for data from the artificial sea salts/distilled water series (Fig. 1). The contours illustrate the high survival at all factor combinations after 24 h exposure, with optimal survival predicted at higher salinities at all temperatures, and the lowest survival predicted at combinations of low salinities and low temperatures. The pattern of the isopleths was similar at 48 h except that the lowest survival was predicted at the highest salinities. Optimal survival was predicted at intermediate salinities across all temperatures. There was a sharp decrease in the percent of embryonic survival at all factor combinations after 72 h of exposure. Highest survival, although far from optimal, was predicted at intermediate salinities, in agreement with the predictions at 48 h of exposure.

A summary of the linear and quadratic effects of single variables and combinations of variables from the regression analysis on transformed embryonic survival of organisms exposed to dome salt/Brazos River water brine is displayed in Table 7. Exposure duration, temperature x exposure duration and exposure duration effects were significant at $P < 0.05$, indicating that these factors were statistically significant as predictors of percent embryonic survival. Clearly, exposure duration appeared to have the most significant effect upon embryonic survival.

Response surface diagrams generated for survival data of organisms exposed to dome salt/Brazos River water brine are represented in Fig. 2. Survival was predicted to be high at all factor combinations after 24 h exposure, with optimal survival predicted at low-intermediate salinities across all temperatures. The lowest survival was predicted for combinations of high salinities x low temperatures. At 48 h, highest embryonic survival was predicted at combinations of low salinities and low temperatures, with high salinities being most deleterious to survival. After 72 h of exposure, survival was low in all factor combinations, and the overall pattern and direction of the isopleths was similar to that observed for the 48 h time period.

Although the results were somewhat variable, the overall trend was similar with both brine types in Bioassay 1. That is, high temperatures and, particularly, high salinities, were the most deleterious factor combinations. Additionally, as indicated by the shapes of the response surface isopleths, the salinity effect was more pronounced than the temperature effect.

EMBRYO BIOASSAY 2 - (Four brine types)

The results of the second embryonic bioassay are summarized in Tables 8-11, which list mean percent survival of redfish embryos/larvae under all temperature x salinity combinations for the four brine types. It should be re-emphasized that the conditions of the 34 ppt series for all four brine types were identical. That is, replicate series of artificial sea salts/distilled water exposure bowls were run for each temperature of each brine type at 34 ppt. These replicates represented "zero" brine exposure for each brine type.

Survival was high at all factor combinations after 12 h of exposure to all four brine types (Table 8). At 24 h of exposure, there appeared to be greater survival at the higher temperatures at most factor combinations of all test brines, with the highest survival occurring in the 34 ppt factor combinations (Table 9). Although there was some variability in the data, the poorest survival was generally noted in dome salt/Brazos River water brine, with progressively decreasing survival at salinities greater than 34 ppt. Overall, high salinities and low temperatures appeared to exhibit the greatest toxicity in all brines after 24 h of brine exposure. After 48 h of exposure, survival markedly decreased for all test brine exposure at all factor combinations, although the percent survival was relatively high at 34 ppt (Table 10). Artificial sea salt/distilled water brine appeared to be the least toxic, whereas dome salt/Brazos River water brine was the most toxic. Once again, there was a sharp decrease in survival with an increase in salinity from 34-36 ppt in all test brines. After 72 h, survival had decreased at all factor combinations in all brines (Table 11). Survival was lowest at the higher salinities and at the higher temperatures within these salinities. Overall survival was particularly low in dome salt/Brazos River water brine.

A summary of the results of repeated measures analysis on the transformed survival data from Bioassay 2 is presented in Table 12. All single and interactive treatment effects were significant at $P < 0.05$ (Table 12). Exposure duration and all exposure duration x treatment effects, except exposure duration x temperature x brine (RTB), were significant at $P < 0.01$. The linear component of exposure duration (R(1)) was significant, while the quadratic exposure duration component (R(2)) was not ($P < 0.001$, $P < 0.746$, respectively). As in Bioassay 1, the effect of exposure duration had the most pronounced effect upon embryonic survival. Salinity, temperature and brine type also exhibited pronounced effects on embryonic survival through various significant interactions.

The interactive effects of salinity and temperature at each observation period for each brine type are shown in Tables 13-16 and Figs. 3-6.

Table 13 is a summary of response surface analyses on transformed survival data of embryos exposed to artificial sea salt/distilled water brine. Only salinity x exposure duration and temperature x exposure duration were significant ($P < 0.05$) and can be considered viable predictors of changes in embryonic survival. The contours indicated that salinity exhibited a more pronounced effect than temperature at all time periods (Fig. 3). Survival at all factor combinations in all brines was optimal after 12 h of exposure, whereas survival at all factor combinations after 72 h was very low. The highest survival of embryos at the 48 h exposure period was predicted at low salinities, while high salinities in combination with low to moderate temperatures markedly reduce survival.

The predicted response of embryos to combinations of dome salt/ Brazos River water is presented in Table 14, Fig. 4. The coefficients for the linear and quadratic effects of salinity and temperature and the quadratic effect of exposure duration were significantly different from 0 at $P < 0.01$, demonstrating the predictive utility of the equations. The patterns of the isopleths in the response surface diagram generated for dome/salt Brazos River water brine data were very similar to those observed for artificial sea salt/distilled water brine data, again indicating a greater effect of salinity than of temperature. Best survival was predicted at lower salinities across the range of temperatures. Although the direction of the isopleths was similar with both brines, it should be noted that survival at all temperature x salinity combinations in the dome salt/Brazos River water series was much lower than corresponding combinations in the artificial sea salt/distilled water series.

Regression coefficients (Table 15) and response surface contours (Fig. 5) show the predicted response of redfish embryos to dome salt/ distilled water. Many of the linear and quadratic treatment effects as well as the salinity-exposure duration interaction were significant at $P < 0.01$, indicating that predictions of effects upon embryonic survival elicited by treatments was somewhat complex in this brine exposure. Illustration of treatment interactions is provided by the response surface diagrams (Fig. 5), and exhibit a pattern similar to those for the other brine types. That is, the horizontal direction of the isopleths at all time points indicated a stronger salinity than temperature effect. As was true for the other brine types, the best embryonic survival was predicted at lower salinities across a range of temperatures, whereas lowest survival was predicted at high salinities. Overall survival was again low after 72 h, with predicted survival similar to that predicted for artificial sea salts/distilled water and higher than that predicted for embryos exposed to the dome salt/Brazos River water series at corresponding factor combinations.

Regression coefficients (Table 16) and response surface contours (Fig. 6) show the survival data of embryos exposed to artificial sea salts/Brazos River water. The linear effect of salinity, the quadratic effect of salinity, temperature and exposure duration, and the salinity-exposure duration interaction was significant at $P < 0.05$. The horizontal direction of the response isopleths indicate a strong salinity effect. Predicted survival was optimal at all factor combinations after exposure for 12 h.

Although survival decreased with exposure duration, optimal combinations were not apparent in the isopleth contours as there had been for the other brine types (Fig. 6). That is, survival was similar at all factor combinations and decreased with the duration of exposure. After exposure for 48 h, there was a trend toward higher predicted survival at lower salinities. This trend was consistent throughout the data for all brine types. Overall survival was higher than that of dome salt/Brazos River water brine, but lower than that in the artificial sea salts/distilled water or the dome salt/distilled water brine series.

EMBRYO BIOASSAY 3 - (Four brine types)

The mean embryonic survival data of Bioassay 3 is summarized in Tables 17-20. Again, it should be noted that all brines at 34 ppt are actually artificial seawater. The mean survival data of Bioassay 3, which had the same design as Bioassay 2, were very similar to those of Bioassay 2, with high survival at all factor combinations for all brines after 12 h of brine exposure (Table 17). After 24 h of exposure, survival decreased at all factor combinations in all brines, with the most dramatic decrease in embryonic survival noted in dome salt/Brazos River water brine and dome salt/distilled water brine. The smallest change in embryonic survival was noted in organisms exposed to artificial sea salt/ distilled water combinations (Table 18). The lowest survival occurred in all brine types at the highest salinities. These same patterns were evident after 48 h of exposure, with the lowest overall survival noted in embryos subjected to dome salt/Brazos River water exposure. As in Bioassay 2, low overall survival at 72 h necessitated the termination of the bioassay (Table 20).

Table 21 is a summary of the results of repeated measures analysis of transformed survival data for Bioassay 3. All treatment effects were significant at $P < 0.05$, except for the salinity x temperature x brine type (STB) interaction and the temperature x brine type (TB) interaction. Again, a large portion of the variability appeared to be due to the effect of salinity. Also, as in Bioassay 2, all exposure duration main and interactive effects were highly significant ($P < 0.001$, Table 21). The linear components of all exposure duration x treatment interactions were significant ($P < 0.05$), and most quadratic components were also significant.

Response surface diagrams and the results of the regression analyses for the third embryonic experiment are shown in Figs. 7-10 and Tables 22-25.

Multiple regression analysis of embryonic survival in the artificial sea salt/distilled water series (Table 22) was significant for both linear and quadratic effects ($P < 0.005$). However, only the quadratic effect of exposure duration could be assigned as significant ($P = 0.0001$) in the polynomial coefficients. The response surface (Fig. 7) revealed that optimal survival was predicted at all temperature and salinity combinations after 12 or 24 h of brine exposure. At the 48 h observation point, the direction and location of the isopleths was similar to those obtained from

Bioassays 1 and 2, with the highest survival predicted to occur at lower salinities. However, relatively high survival was also predicted at the extremes, that is, high salinity and high or low temperature. It should be remembered, however, that predictions below 23°C were extrapolations beyond actual data points obtained in this study. The vertically ellipsoid shape of the contours also demonstrated the interaction between temperature and salinity. The direction and location of the isopleths were similar after exposure for 72 h, but overall survival at all factor combinations was low. Temperature appeared to be more significant than salinity in this series, contrary to the results obtained through response surface analysis for the artificial sea salt/distilled water of Bioassays 1 and 2.

Regression coefficients and response contours of embryos to dome salt/Brazos River water in Bioassay 3 are shown in Table 23 and Fig. 8. The linear and quadratic effect of salinity, the quadratic exposure duration effect, and the salinity-exposure duration interaction were all significant ($P < 0.001$). Response surface contours again approximated the pattern exhibited by those from Bioassays 1 and 2, with the strong horizontal component of the isopleths indicating the stronger effect of salinity than of temperature (Fig. 8). Optimal survival at all time points was predicted at low salinities across the range of temperatures, whereas the lowest survival was predicted at high salinities. Predicted survival at comparable combinations of temperature and salinity was much lower in the dome salt/Brazos River water brine series than in the artificial sea salts/distilled water brine series.

Regression coefficients and predicted response patterns of embryos exposed to the dome salt/distilled water brine are shown in Table 24 and Fig. 9. Effects of all main factors and combinations of factors except the linear exposure duration and quadratic temperature effects were significant at $P < 0.005$. The shape and direction of the isopleths were similar to the general pattern observed in other brine types, with salinity being the most dominant physical factor influencing predicted survival. The conditions in which the highest and lowest survival were predicted to occur were identical to those for the dome salt/Brazos River water series, although there was a slight increase in predicted survival at high temperatures and high salinities after 72 h of exposure. Overall predicted survival at comparable factor combinations closely approximated that which was predicted for embryos exposed to the dome salt/Brazos River water brine series.

The predicted response of redfish embryos and the regression coefficients of the transformed survival data for embryos exposed to artificial sea salts/Brazos River water brine are shown in Fig. 10 and Table 25. The linear and quadratic effects of exposure duration and salinity significantly influenced embryonic survival ($P < 0.05$) (Table 25). The shape and direction of the response surface isopleths clearly demonstrated the salinity effect, with high salinities being the most deleterious. Embryonic survival decreased with time and was low after 72 h of brine exposure. Overall survival (at comparable factor combinations) was greater than that for embryos exposed to either dome salt/Brazos River water or dome salt/distilled water brines, but was lower than the predicted survival of embryos exposed to artificial sea salts/distilled water.

LARVAL BIOASSAY (Four brine types)

The mean survival data from the larval bioassay (Bioassay 4) are summarized in Tables 26-30. After exposure for 48 h, larval survival was high in all factor combinations, with the greatest percent survival exhibited in the artificial sea salt/distilled water. Lowest survival (65.0-83.5%) occurred in the 38 ppt (26°C) exposure condition in all four brine types at 48 h.

Percent larval survival decreased in most factor combinations after 72 h and 96 h. Again, the lowest larval survival among all brine types was at the 38 ppt, 26°C factor combination. A difference in the toxicity of the brine types became apparent in the data obtained after the larvae had been exposed for 96 h, with lower larval survival observed in the artificial sea salt/Brazos River water and dome salt/distilled water brines. This pattern was sustained at the 120 h observation point, although overall changes in larval survival were not large. Generally, the data for the entire experiment indicated that larval survival was greatest in the absence of brine, and was least in all brine types at the 38 ppt, 26°C salinity/temperature combination. Additionally, regardless of the 38 ppt, 26°C condition, there was a trend toward decreased larval survival in all brines as the exposure salinity increased. The effect of temperature was variable and overall larval survival in all temperature combinations in all brines was relatively high (> 65%).

The results of repeated measures analysis of Bioassay 4 are summarized in Table 31. Salinity, temperature and the salinity and temperature interaction exhibited a significant effect upon larval survival ($P < 0.001$). In the analysis of the interaction between the trial factor (exposure duration) and treatment factors, exposure duration (R) and all exposure duration x treatment interactions, except exposure duration x salinity (RS), exposure duration x salinity x brine (RSB) and exposure duration x salinity x temperature x brine (RSTB), were significant at $P < 0.03$. The analysis indicates that as in the embryonic assays, exposure duration was the factor which most affected larval survival and interacted significantly with treatment effects. Salinity and temperature elicited interactive effects upon larval survival, although brine type did not have a significant effect.

Response surface diagrams and regression coefficients of Bioassay 4 are shown in Fig. 11 and Tables 32-35.

Larvae exposed to artificial sea salt/distilled water brine indicate that salinity (both the linear and quadratic effects) was the only treatment significantly affecting the pattern of larval survival (Table 32). Response surface analysis of temperature/salinity combinations of artificial sea salts/distilled water indicated that high survival was predicted at all combinations of temperature and salinity.

Regression coefficients and the response surface diagrams of redfish larvae exposed to dome salt/Brazos River water are shown in Table 33 and Fig. 11. The linear and quadratic effects of salinity, the linear effect of temperature and the salinity-temperature interaction combinations were useful as predictors of larval survival ($P < 0.05$). Figure 11 (Part A) illustrates the predicted survival of redfish larvae exposed to dome salt/ Brazos River water brine. The contours indicate that optimal survival was predicted at low salinities. The strong horizontal direction of the isopleths described a pronounced salinity effect. However, temperature appeared to exhibit a more pronounced deleterious effect on larval survival than salinity at the higher temperatures.

The predicted response of redfish larvae and regression coefficients of transformed survival data of larvae exposed to dome salt/distilled water brine are shown in Fig. 11 and Table 34. Temperature and exposure duration effects were significant ($P < 0.05$) in predicting larval survival. Temperature exerted a more significant effect upon larval survival than salinity, as evidenced by the vertically ellipsoid shape and direction of the response surface isopleths (Fig. 11, Part B). Survival was optimal in shorter exposure durations (up to 72 h) at all factor combinations. After longer exposure periods, however, lower survival was predicted at combinations of mid-range temperatures and either low or high salinities. Optimal larval survival was predicted at high temperatures at all salinities. Larvae exposed to artificial sea salts/Brazos River water are represented in Table 35 and Fig. 11, Part C. Only the salinity-temperature interaction was significant in this case ($P < 0.03$), indicating that all other main and interactive affects were not useful in predicting larval surval (Table 35). The horizontal direction of the isopleths indicated a dominant salinity effect, and the elliptical shape of the contours reflected the temperature x salinity interaction. The lowest survival of larvae exposed to the artificial sea salt/Brazos River water brine was predicted at combinations of high temperatures and low salinities, with optimal larval survival predicted at all other factor combinations. These results were similar to those for larvae exposed to the other brine types in the larval bioassay.

Overall, artificial sea salt/distilled water conditions allowed the highest survival of redfish larvae. There was no consistent difference in the relative toxicities of dome salt/Brazos River water, artificial sea salt/Brazos River water and dome salt/distilled water brines. It should be noted that overall survival was much higher in the larval experiment than in the embryonic experiments and that in general, redfish larvae survival was similar among brine types and factor combinations.

JUVENILE BIOASSAYS

Results of probit analysis on the mortality data are presented in lethal concentrations at which 10, 50 and 90% of the sample dies (LC_{10}, LC_{50} and LC_{90}, respectively) in Table 36. The two acute bioassays show that redfish juveniles in the second bioassay were more sensitive to dome salt/Brazos River water than were those in the first bioassay (Table 36).

Ninety-six h LC_{50}'s for the first and second experiments were 6.63 and 3.65% dome salt/Brazos River water, respectively. There are several possible reasons for this discrepancy: 1) the brine was stronger in the second bioassay (297 *vs* 285 ppt), resulting in slightly higher salinities for similar brine dilutions (Table 37); 2) the juveniles in the second bioassay had been held in the lab approximately three weeks longer than the first group and, as a result, may have been more susceptible to salinity stress than the first group; and 3) in the first experiment, there was 50% mortality in the highest brine concentration tested (5% brine = 46.6 ppt) which is in reasonably close agreement with the LC_{50} of the second experiment (3.65% brine = 43.8 ppt).

In the first juvenile bioassay, mortality was highest during the first 96 h. In the ensuing 10 days, there was very little mortality, as evidenced by the minor decrease in the LC_{50}, which dropped from 6.63 at four days to 6.40 at 14 days. Highly variable mortality in the lower brine concentrations after 96 h caused higher chi-square (X^2) values and prohibited the calculation of 95% confidence intervals for the seven and 14 day time periods. Probit plots for the two acute bioassays are presented in Figs. 12-17. Note that lethal dose (LD) was, in this case, equivalent to lethal concentration.

DISCUSSION

The present investigation indicates that the embryonic and larval stages of redfish, *Sciaenops ocellata*, were more sensitive to exposure to increased brine concentrations than juvenile stages. This agrees with the general observation that the earlier developmental stages of fish are more sensitive to environmental factors than are later forms (Rosenthal and Alderdice 1976, McKim 1977).

The most critical age in the early development of redfish larvae appears to be at about 72 h, which is the time that they begin exogenous feeding. If they are unable to feed properly and obtain adequate nourishment, high mortality ensues. This was the most likely cause of the low survival observed in Bioassays 1-3, which had to be terminated after 72 h due to the low embryonic survival. This was also a major reason that the exposure duration appeared to be so significant in these bioassays. Other factors such as temperature or salinity could magnify this nutritive stress.

The results indicate that once the critical period in redfish embryonic development is passed, resistance to brine exposure dramatically increases. This was apparent in the larval bioassay in which two-week-old larvae exhibited relatively high survival (≥ 65%) after 120 h of exposure to the most toxic brine (dome salt/Brazos River water) even at salinity and temperature extremes.

Optimal survival of redfish embryos and larvae occurred at mid-range salinities (34-36.5 ppt) and temperatures (23-26° C) in all brine types. Although there were statistically significant temperature and salinity interactions in many of the brine exposures, the degree of this interaction was variable. The majority of this variability can be attributed to factor effects elicited at higher salinities, in which the lowest survival usually occurred in all brine types. The only exception to this was observed in the juvenile bioassay 4, in which juveniles were exposed rather than larvae.

Although temperature is an important factor affecting physiological systems, the range of temperatures tested in this investigation was not great enough to provide any evidence that temperature alone was a significant factor in redfish embryonic and larval survival. A trend was seen toward reduced survival at the highest tested temperature (30° C). In invertebrates, temperature has been shown to have a greater effect than salinity on respiration, which is representative of the metabolic processes of the individual (Laughlin and Neff 1979).

The higher salinities utilized in the present investigation surpass those to which redfish embryos and larvae are routinely exposed in the natural environment. At these test salinities metabolic energy may have been diverted from critical developmental processes to osmoregulation. If so, then this response could have led to a depletion of energy reserves and result in low survival in the test populations.

Brine prepared with dome salt and Brazos River water represented the most toxic exposure condition for redfish embryos and larvae, although variability existed among different experiments. The highest survival was consistently observed in artificial sea salts/distilled water rather than in the other brines. Additionally, dome salt in combination with the various diluent water types was more toxic than artificial sea salts, and Brazos River water was more toxic than distilled water as a diluent. The most pronounced effects on embryos and larval survival were due to the dome salt. These differences in brine toxicity may be due to differences in the ionic composition of the brines.

The effects of Bryan Mound brine on the survival of newly hatched spotted seatrout (*Cynoscion nebulosus*) was investigated by Johnson and Williams (1978), who found that the brine was no more deleterious to the larvae than artificially prepared brine, i.e. that salinity, and not brine composition, could be the cause of observed increases in mortality at higher test salinities. Their tests, however, exposed the larvae to the brine for only 1-2 h, as opposed to the continuous exposure approach utilized in this study, and they did report that exposure duration was positively correlated with survival, even for short time periods. Higher salinities were also more deleterious than lower salinities in the present investigation, but it was clear that brine prepared with dome salt was more deleterious than brine prepared with artificial sea salts at comparable salinities.

The brine plume dispersion model predicts that about nine acres (2.3 ha) will be impacted by salinity elevations of up to 3 ppt and temperature increases of up to 0.6 C (U.S. Department of Commerce 1977). These conditions would have little or no effect on redfish hatching success and embryonic and larval development as indicated by the present study. If the transition from an endogenous to an exogenous food supply represents the period of redfish development during which the greatest mortality occurs in the field, any possible deleterious effects of brine exposure would be most likely to occur at that time. Redfish larvae which are afforded food supplies adequate for productive metabolic processes would be expected to survive under normal brine exposure in a field situation. However, if salinities should increase to high levels (i.e. > 38 ppt) higher redfish mortality related to osmoregulatory adaptations should be anticipated. Also, the high concentrations of magnesium in Bryan Mound brine may represent another source of osmoregulatory stress by disrupting normal integumentary permeability at high brine-induced salinities (Prosser 1973).

The effect of brine discharge on the early life stages of redfish is dependent upon the presence of these stages in the brine plume and upon the length of time spent in the plume. There is no direct evidence that adult redfish prefer to spawn in the vicinity of the brine discharge as opposed to other areas nor, in fact, that they spawn there at all. If the adults do spawn in the area (perhaps having been attracted to the area due to the brine discharge) then spawning products would be exposed to the brine. "Nearfield" conditions, within 50 ft (15 m) of the discharge ports, may exceed temperatures of 30°C and salinities of 30 ppt (F. Godshall, pers. comm. NOAA/EDIS/CEAS) which this study indicates would be detrimental to redfish embryonic survival after exposure durations of less than 24 h. Beyond this "nearfield" area, brine dilutions of 75-100:1 have taken place and temperatures and salinities would not be at particularly harmful levels in terms of redfish embryonic and larval survival. It is unlikely, however, that embryos or larvae would be present in the "nearfield" area for an extended period, and the resultant mortality would depend upon the extremes of temperature and salinity to which the fish was exposed. The duration of redfish embryonic or larval presence in the "midfield" area, up to 10,000 ft (3.0 km) down-current, could be considerably longer, but probably not as long as the 72 h exposure periods studied herein.

In conclusion, the effect of brine discharge upon redfish spawning products is not expected to be great enough to be detrimental to the population. If adult redfish are attracted to the brine discharge site and spawn there in preference to other areas, any increased redfish mortality would be limited to the small "nearfield" area. Actual fertilization success and long-term sublethal physiological effects of brine exposure on redfish were not investigated in this study, but no evidence exists which would indicate that these would be affected by any greater an extent than embryonic and larval survival.

LITERATURE CITED

Alderdice, D.F. 1972. Responses of marine poikilotherms to environmental factors acting in concert. p. 1659-1722. In: O. Kinne (ed), Marine Ecology. Vol. 1., Part 3. Wiley Interscience, New York.

Boothby, R.N. and J.N. Avault, Jr. 1971. Food habits, length-weight relationship and condition factor of the red drum (*Sciaenops ocellata*) in southeastern Louisiana. Trans. Am. Fish. Soc. 100:290-295.

Chittenden, M.E., Jr., J. Ross and J. Pauela. 1981. Nekton. In: R.W. Hann and R.E. Randall (eds.). Evaluation of Brine Disposal from the Bryan Mound Site of the Strategic Petroleum Reserve Program. Final Report of Predisposal Studies. Texas A&M University and Texas A&M Research Foundation. Contract No. DE-FC96-79P010114.

Chittenden, M.E., Jr. and J.D. McEachran. 1976. Composition, ecology and dynamics of demersal fish communities on the northwestern Gulf of Mexico continental shelf, with a synopsis for the entire gulf. Texas A&M Sea Grant College Program, 105 pp.

Dixon, W.J. Ed. 1977. BMD biomedical computer programs. 3rd ed., 4th printing. Los Angeles, University of Calif. Press.

Finney, D.J. 1971. Statistical methods in biological assey. Second edition. London, Griffin Press.

Johnson, A.G. and T. Williams. 1978. p. 17-40. In: Analysis of brine disposal in the Gulf of Mexico. U.S. Dept. Comm. NOAA Env. Data Sci. S/T 78-172.

Laughlin, R.B., Jr. and J.M. Neff. 1979. The respiratory response of larval mud crabs, *Rhythispanopeus harrisii* following exposure to factorial combinations of temperature, salinity and phenanthreme (a petroleum derived polynuclear aromatic hydrocarbon). Mar. Biol. (In Press).

Macek, K.J. and B.H. Sleight III. 1978. Utility of toxicity tests with embryos and fry of fish in evaluating hazards associated with chronic toxicity of chemicals to fishes. p. 137-146. In: F.J. Mayer and J.F. Hamelink (eds.), Aquatic toxicology and hazard evaluation. Amer. Soc. Test. Mat. Philadelphia, PA.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Bd. Canada 34: 1148-1154.

Prosser, C.L. 1973. Water: Osmotic balance; hormonal regulation. p. 1-78. In: C.L. Prosser (ed.), Comparative animal physiology. W.B. Saunders Company, Philadelphia. 966 p.

Rosenthal, H. and D.F. Alderdice. 1976. Sublethal effects of environmental stresses, natural and pollutional, on marine eggs and larvae. J. Fish. Res. Bd. Canada. 33:2047-2065.

SAS Institute, Inc. SAS user's guide 1979, 1981 editions. Raleigh, North Carolina.

Simmons, E.G. 1957. An ecological survey of the upper Laguna Madre of Texas. Publ. Inst. Mar. Sci. Univ. Tx. 4:156-202.

Simmons, E.G. 1969. Big red. Tex. Parks Wildl. May 27. 25-31.

Simmons, E.G. and J.R. Breuer. 1962. A study of redfish, *Sciaenops ocellata* Linnaeus and black drum, *Pegomias cromis* Linneaus. Publ. Inst. Mar. Sci. Univ. Tx. 8:184-211.

SPR-FEA 76/77-6. 1977. Strategic Petroleum Reserve draft supplement final environmental impact statement. Bryan Mound Salt Dome Federal Energy Administration. Washington, D.C.

U.S. Department of Commerce. 1977. Tech. Rept. PB-275-415. Analysis of brine disposal in the Gulf of Mexico (1) Bryan Mound. NOAA. Washington, D.C.

Winer, B.J. 1971. Statistical principles in experimental design. McGraw-Hill, New York.

Yokel, B.J. 1966. A contribution to the biology and distribution of the red drum, *Sciaenops ocellata*. M.S. Thesis. Univ. Miami. 160 p.

APPENDIX TABLES

Table 1. The concentration of ions in the four stock test brines, Brazos River water and distilled water (ppm).

	Salinity (ppt)	Metal Concentrations (mg/l)									
		Na	Cl	K	Cd	Cu	Zn	Ca	Mg	Ag	SO_4
Dome salt/Brazos River water	293	116,000	176,000	230	<0.05	<0.1	<0.05	630	4,800	0.01	1,380
Dome salt/Distilled water	295	118,000	178,000	230	<0.05	<0.1	<0.05	620	1.1	0.01	1,370
Artificial sea salt/ Brazos River water	298	109,000	183,000	5,400	<0.05	<0.1	<0.05	820	11,500	0.01	13,000
Artificial sea salt/ Distilled water	309	107,000	199,000	6,000	<0.05	<0.1	<0.05	530	16,000	0.01	16,200
Brazos River water	10	4,000	6,200	170	<0.05	<0.1	<0.05	200	380	0.01	890
Distilled water	-	32	-	0.33	<0.05	<0.1	<0.05	<0.1	<0.1	-	-

Table 2. Mean percent survival of redfish embryos and larvae at 24 hours during exposure to two types of brine at different combinations of salinity and temperature. Control condition (34 ppt salinity) in artificial seawater (Instant Ocean). (Embryo Bioassay 1)

Salinity ppt	Temperature °C	Replicates	Mean Survival, %±S.D. ASW/DW[1]	DS/BRW[1]
34	23	3	71.33±13.05	71.33±13.05*
34	26	4	78.75±17.95	78.75±17.95*
34	30	4	92.75± 5.62	92.75± 5.62*
36	23	3,4	81.67±27.54	63.75±22.87
36	26	3,4	93.67± 1.15	87.00± 6.48
36	30	2,3	90.00± 0	58.00± 2.00
38	23	4	66.25±34.97	66.25±34.97
38	26	3	69.13±17.39	69.33±17.39
38	30	4	87.75± 7.41	69.75±17.88
40	23	4,3	90.25± 4.57	35.67±34.08
40	26	4,4	66.50±10.41	56.50±30.71
40	30	4,2	90.25± 6.85	92.00± 4.24

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River water.

*Listed observations are repeated values from the ASW/DW exposure brine.

Table 3. Mean percent survival of redfish embryos and larvae at 48 hours during exposure to two types of brine at different combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 1)

Salinity ppt	Temperature °C	Replicates	Mean Survival, %±S.D. ASW/DW[1]	Mean Survival, %±S.D. DS/BRW[1]
34	23	3	49.33± 8.14	49.33± 8.14*
34	26	3	49.00±20.42	49.00±20.42*
34	30	4	72.25±15.09	72.25±15.09*
36	23	3,4	62.27±11.02	44.75±16.94
36	26	3,3	86.33± 9.07	55.67±12.50
36	30	2,3	69.50±13.44	20.33± 5.03
38	23	2,3	49.50±41.72	60.00± 5.06
38	26	3,4	31.33±36.89	50.75±18.59
38	30	4,3	51.75±12.55	37.33±10.97
40	23	4,2	53.00±15.10	10.00±14.14
40	26	4,4	20.25±13.91	36.50±32.02
40	30	4,2	52.50±10.60	48.50± 6.36

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 4. Mean percent survival of redfish embryos and larvae at 72 hours during exposure to two types of brine at different combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 1)

Salinity ppt	Temperature C	Replicates	Mean Survival, %+S.D. ASW/DW[1]	DS/BRW[1]
34	23	3	31.33±10.60	31.33±10.60*
34	26	3	6.33±10.97	6.33±10.97*
34	30	4	12.25± 9.11	12.25± 9.11*
36	23	3,4	27.67±26.65	16.25± 6.08
36	26	3,3	27.00±10.39	19.67± 6.11
36	30	2,3	12.50± 9.19	0.00± 0
38	23	2,3	21.00±29.70	38.33±10.41
38	26	3,4	0.00± 0	2.50± 5.00
38	30	4,4	6.75±10.44	1.50± 3.00
40	23	4,3	11.75±11.90	0.00± 0
40	26	4,4	0.00± 0	0.00± 0
40	30	4,2	0.00± 0	0.00± 0

[1] ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

* Listed observations are repeated values from the ASW/DW exposure brine

Table 5. Repeated measures analysis of variance on survival of redfish embryos exposed to artificial sea salts/distilled water and dome salt/Brazos River water brines at various combinations of temperature and salinity. (Embryo Assay 1)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
MEAN	109.109	1	109.109	1336.68	0.0000
SAL	1.816	3	0.605	7.41	0.0003
TEMP	0.241	2	0.121	1.48	0.2377
BRINE	0.496	1	0.496	6.08	0.0169
ST	1.655	6	0.276	3.38	0.0068
SB	0.894	3	0.298	3.65	0.0182
TB	0.192	2	0.096	1.18	0.3161
STB	0.747	6	0.125	1.53	0.1880
ERROR	4.326	53	0.082		
R(1)	26.249	1	26.249	1067.84	0.0000
R(1)S	0.297	3	0.099	4.02	0.0119
R(1)T	1.252	2	0.626	25.46	0.0000
R(1)B	0.071	1	0.071	2.90	0.0944
R(1)ST	0.402	6	0.067	2.72	0.0222
R(1)SB	0.030	3	0.010	0.41	0.7472
R(1)TB	0.042	2	0.021	0.86	0.4306
R(1)STB	0.038	6	0.006	0.26	0.9545
ERROR	1.302	53	0.025		
R(2)	0.411	1	0.411	30.34	0.0000
R(2)S	0.012	3	0.004	0.30	0.8283
R(2)T	0.151	2	0.075	5.58	0.0063
R(2)B	0.003	1	0.003	0.26	0.6152
R(2)ST	0.065	6	0.011	0.79	0.5790
R(2)SB	0.054	3	0.018	1.34	0.2720
R(2)TB	0.034	2	0.017	1.26	0.2910
R(2)STB	0.169	6	0.028	2.08	0.0714
ERROR	0.718	53	0.014		

...(Cont'd)

Table 5. (Cont'd)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
R	26.661	2	13.330	699.06	0.0000
RS	0.309	6	0.051	2.70	0.0177
RT	1.403	4	0.351	18.39	0.0000
RB	0.075	2	0.037	1.96	0.1458
RST	0.466	12	0.039	2.04	0.0277
RSB	0.085	6	0.014	0.74	0.6193
RTB	0.076	4	0.019	1.00	0.4105
RSTB	0.207	12	0.017	0.90	0.5459
ERROR	2.021	106	0.019		

Abbreviations:

SAL = Salinity
TEMP = Temperature
BRINE = Brine Type
ST = Salinity x Temperature
SB = Salinity x Brine
TB = Temperature x Brine
STB = Salinity x Temperature x Brine
R = Time of Exposure
R(1) = Linear Component of Time
R(2) = Quadratic Component of Time

Table 6. Multiple regression analysis on survival of redfish embryos exposed to artificial sea salts/distilled water brine at various combinations of temperature and salinity (Embryo Bioassay 1).

RESPONSE MEAN: 0.748 | R-SQUARE: 0.770

ROOT MSE: 0.229 | COEF. OF VARIATION: 0.307

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	17.264	0.704	109.29	0.0001
Quadratic	3	0.881	0.036	5.58	0.00015
Cross Product	3	0.727	0.030	4.60	0.0047
Total Regression	9	18.872	0.770	39.82	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	107	5.634	0.053

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	-6.718	8.596	-0.78	0.4362
Sal	1	0.716	0.414	1.73	0.0870
Temp	1	-0.463	0.233	-1.99	0.0495
Time	1	0.061	0.021	2.88	0.0048
Sal x Sal	1	-0.008	0.005	-1.54	0.1271
Sal x Temp	1	-0.003	0.003	-1.10	0.2726
Temp x Temp	1	0.012	0.004	3.20	0.0018
Sal x Time	1	-0.001	<0.001	-2.04	0.0437
Temp x Time	1	-0.001	<0.001	-2.91	0.0043
Time x Time	1	<-0.001	<0.001	-2.18	0.0316

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	1.117	0.279	5.31	0.0006
Temp	4	1.050	0.263	4.99	0.0010
Time	4	17.434	4.358	82.78	0.0001

df = degrees of freedom; SS - sums of squares; R-Square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 7. Multiple regression analysis on survival of redfish embryos exposed to dome salt/Brazos River water brine at various combinations of temperature and salinity (Embryo Bioassay 1)

RESPONSE MEAN: 0.663 R-SQUARE: 0.742

ROOT MSE: 0.220 COEF. OF VARIATION: 0.333

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	13.561	0.684	92.94	0.0001
Quadratic	3	0.301	0.015	2.06	0.1079
Cross Product	3	0.846	0.042	5.80	0.0012
Total Regression	9	14.708	0.742	33.60	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	105	5.107	0.049

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	-2.970	8.590	-0.35	0.7303
Sal	1	0.252	0.401	0.63	0.5307
Temp	1	-0.074	0.232	-0.32	0.7515
Time	1	0.054	0.021	2.48	0.0147
Sal x Sal	1	-0.005	0.005	-0.90	0.3685
Sal x Temp	1	0.002	0.003	0.73	0.4658
Temp x Temp	1	0.001	0.004	0.27	0.7864
Sal x Time	1	<-0.001	<0.001	-0.80	0.4256
Temp x Time	1	-0.001	<0.001	-4.08	0.0001
Time x Time	1	<-0.001	<0.001	-2.30	0.0234

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	1.240	0.310	6.37	0.0001
Temp	4	0.844	0.211	4.34	0.0028
Time	4	13.331	3.333	68.51	0.0001

df = degrees of freedom; SS - sums of squares; R-Square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 8. Mean percent survival of 4 replicates of redfish embryos and larvae at 12 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 2)

Salinity	Temperature	Mean Survival %+S.D.			
ppt	°C	ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	89.3± 5.5	92.4± 3.6*	86.8± 8.2*	88.8± 8.9*
34	26	79.2±19.1	83.2± 5.5*	82.1±11.4*	82.1± 4.4*
34	30	80.0±11.5	83.9± 9.1*	93.5± 2.9*	85.2± 5.9*
36	23	98.0± 3.4	85.8± 7.8	93.0± 3.0	79.8± 8.8
36	26	75.3±11.9	70.7±15.2	73.0± 6.7	73.9±27.2
36	30	87.6± 6.7	89.5± 4.4	87.2±16.9	85.5± 5.4
38	23	88.1± 5.5	60.7±11.3	87.3± 5.1	70.3±15.9
38	26	83.7± 3.9	83.6±10.1	83.7± 3.5	62.5±10.4
38	30	86.6± 9.0	69.7±15.5	97.3± 3.7	92.1± 3.4
40	23	86.7±13.1	67.8±18.4	73.9±11.1	90.2± 9.7
40	26	85.9± 3.2	56.8±15.0	65.9±15.0	76.6± 2.8
40	30	65.1± 4.0	75.6± 6.7	78.9±11.1	82.4±15.1

*Values represent survival in ASW/DW

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

Table 9. Mean percent survival of 4 replicates of redfish embryos and larvae at 24 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 2)

Salinity ppt	Temperature °C	Mean Survival %±S.D. ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	80.7± 6.3	71.8±24.0*	82.0± 5.9*	75.4±15.8*
34	26	64.5± 7.1	68.2± 5.6*	72.3±11.4*	70.0± 5.5*
34	30	68.6±20.1	82.6±14.8*	92.3± 7.4*	75.9± 9.0*
36	23	80.1± 8.7	57.5±13.5	80,7± 3.0	59.3±11.6
36	26	58.5±15.0	27.2± 8.6	47.9±12.7	43.8±24.9
36	30	77.1±13.6	88.4± 6.1	77.0±21.6	85.7± 3.6
38	23	58.7±13.9	57.7±15.5	50.5±14.1	46.3±12.5
38	26	58.3±18.9	35.8± 5.0	47.7±10.6	57.5±18.5
38	30	82.6±14.0	57.6±12.9	84.6± 5.6	86.6± 5.9
40	23	52.9±21.3	54.2±30.9	64.3± 5.6	71.8±12.6
40	26	73.1± 9.7	30.2±27.2	44.4±19.0	59.8± 8.7
40	30	57.7± 3.6	75.9± 2.3	82.0±10.9	76.5±19.9

[1] ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

* Listed observations are repeated values from the ASW/DW exposure brine

Table 10. Mean percent survival of 4 replicates of redfish embryos and larvae at 48 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 2)

Salinity ppt	Temperature °C	Mean Survival %±S.D.			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	51.8±13.3	55.1±33.3*	57.6±19.5*	58.8±12.1*
34	26	56.8± 6.3	48.0±19.6*	59.5±12.5*	40.8±25.1*
34	30	60.0±22.4	50.2±19.6*	65.6±13.4*	49.0±13.7*
36	23	17.9± 5.5	2.7± 5.5	22.8± 6.1	3.6± 3.1
36	26	30.2±21.3	8.2± 4.4	3.5± 3.1	4.2± 5.3
36	30	57.3±24.0	41.8±16.0	39.0±16.2	35.9± 6.3
38	23	23.9±17.2	6.8±10.1	20.1±12.8	7.6± 3.0
38	26	41.4±12.4	7.6± 4.9	22.1± 8.1	17.5± 6.5
38	30	45.2±22.6	25.1± 6.5	30.9± 8.3	21.9±21.1
40	23	21.3±11.6	1.9 ± 3.2	17.9± 8.1	27.1± 7.6
40	26	58.8±11.1	8.4± 8.4	20.5± 4.9	36.1±11.4
40	30	15.5± 8.9	35.9±16.6	15.4± 9.7	23.8±21.1

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 11. Mean percent survival of 4 replicates of redfish embryos and larvae at 72 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 2)

Salinity ppt	Temperature °C	Mean Survival %+S.D.			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	50.8±23.7	19.8±19.5*	49.6±15.9*	50.0±10.6*
34	26	32.8±15.8	34.7±15.8*	56.7± 7.1*	33.6±16.1*
34	30	24.7±15.4	24.4±17.4*	42.0± 3.0*	25.6±12.6*
36	23	2.9± 5.9	0.0± 0	17.5±11.0	0.0± 0
36	26	30.2±21.3	8.3± 4.4	0.0± 0	4.2± 5.3
36	30	25.1±20.3	20.9± 9.8	12.3±12.3	7.8± 8.6
38	23	5.7± 6.6	3.4±10.7	8.8± 2.4	0.0± 0
38	26	12.3±12.5	3.9± 5.8	12.6± 7.8	13.8± 2.5
38	30	13.0±11.5	8.9± 6.8	10.1± 8.7	0.0± 0
40	23	3.1± 6.2	1.9± 3.2	8.4±10.4	15.6±15.3
40	26	25.5± 7.7	2.8± 4.8	18.9± 9.4	17.8±11.6
40	30	5.6± 4.1	7.9± 2.8	10.1±13.2	7.1±11.4

[1] ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 12. Repeated measures analysis of variance on survival of redfish embryo exposed to four brine types at various combinations of temperature and salinity. (Embryo Bioassay 2)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
MEAN	387.990	1	387.990	6721.38	0.0000
SAL	8.983	3	2.694	46.68	0.0000
TEMP	1.961	2	0.980	16.98	0.0000
BRINE	2.116	3	0.705	12.22	0.0000
ST	2.121	6	0.354	6.12	0.0000
SB	1.412	9	0.157	2.72	0.0062
TB	1.112	6	0.185	3.21	0.0057
STB	1.734	18	0.096	1.67	0.0531
ERROR	7.447	129	0.058		
R(1)	69.103	1	69.103	2033.55	0.0000
R(1)S	2.843	3	0.948	27.89	0.0000
R(1)T	1.164	2	0.582	17.12	0.0000
R(1)B	0.153	3	0.051	1.50	0.2175
R(1)ST	0.588	6	0.098	2.89	0.0113
R(1)SB	0.209	9	0.023	0.69	0.7200
R(1)TB	0.594	6	0.099	2.92	0.0106
R(1)STB	0.889	18	0.049	1.45	0.1181
ERROR	4.384	129	0.034		
R(2)	0.001	1	0.001	0.10	0.7465
R(2)S	0.092	3	0.031	2.50	0.0623
R(2)T	0.940	2	0.470	38.21	0.0000
R(2)B	0.102	3	0.034	2.76	0.0451
R(2)ST	0.093	6	0.016	1.26	0.2799
R(2)SB	0.198	9	0.022	1.79	0.0764
R(2)TB	0.156	6	0.026	2.12	0.0556
R(2)STB	0.231	18	0.013	1.04	0.4201
ERROR	1.587	129	0.012		

...(Cont'd)

Table 12. (Cont'd)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
R	70.429	3	23.477	1281.33	0.0000
RS	3.189	9	0.354	19.34	0.0000
RT	2.283	6	0.380	20.76	0.0000
RB	0.466	9	0.052	2.82	0.0032
RST	0.868	18	0.048	2.63	0.0003
RSB	0.502	27	0.019	1.01	0.4473
RTB	0.825	18	0.046	2.50	0.0007
RSTB	1.519	54	0.028	1.54	0.0121
ERROR	7.091	387	0.018		

Abbreviations:

SAL = Salinity
TEMP = Temperature
BRINE = Brine Type
ST = Salinity x Temperature
SB = Salinity x Brine
TB = Temperature x Brine
STB = Salinity x Temperature x Brine
R = Time of Exposure
R(1) = Linear Component of Time
R(2) = Quadratic Component of Time

Table 13. Multiple regression analysis on survival of redfish embryos exposed to artificial sea salts/distilled water brine at various combinations of temperature and salinity (Embryo Bioassay 2).

RESPONSE MEAN: 0.806 R-SQUARE: 0.705
ROOT MSE: 0.210 COEF. OF VARIATION: 0.260

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	17.5607	0.677	132.92	0.0001
Quadratic	3	0.144	0.006	1.09	0.3548
Cross Product	3	0.584	0.022	4.42	0.0052
Total Regression	9	18.289	0.705	46.14	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	174	7.662	0.044

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	5.688	6.015	0.95	0.3457
Sal	1	-0.307	0.293	-1.05	0.2962
Temp	1	0.120	0.172	0.70	0.4877
Time	1	-0.001	0.013	-0.06	0.9552
Sal x Sal	1	0.004	0.004	1.10	0.2724
Sal x Temp	1	<-0.001	0.002	-0.15	0.8818
Temp x Temp	1	-0.002	0.003	-0.83	0.4098
Sal x Time	1	<-0.001	<0.001	-2.76	0.0064
Temp x Time	1	0.001	<0.001	2.33	0.0211
Time x Time	1	<0.001	<0.001	1.21	0.2274

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	1.406	0.351	7.98	0.0001
Temp	4	0.325	0.081	1.85	0.1222
Time	4	17.141	4.285	97.31	0.0001

df = degrees of freedom; SS = sums of squares; R-Square = multiple correlation coefficients; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 14. Multiple regression analysis on survival of redfish embryos exposed to dome salt/Brazos River water brine at various combinations of temperature and salinity (Embryo Bioassay 2).

RESPONSE MEAN: 0.672 R-SQUARE: 0.792
ROOT MSE: 0.191 COEF. OF VARIATION: 0.284

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	22.301	0.746	203.63	0.0001
Quadratic	3	1.151	0.038	10.51	0.0001
Cross Product	3	0.248	0.008	2.26	0.0816
Total Regression	9	23.699	0.792	72.13	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	170	6.206	0.036

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	25.981	5.755	4.51	0.0001
Sal	1	-0.003	0.271	-3.69	0.0003
Temp	1	-0.436	0.159	-2.74	0.0068
Time	1	-0.020	0.012	-1.57	0.1177
Sal x Sal	1	0.012	0.003	3.28	0.0012
Sal x Temp	1	0.004	0.002	1.62	0.1081
Temp x Temp	1	0.006	0.002	2.38	0.0186
Sal x Time	1	<-0.001	<0.001	-1.49	0.1385
Temp x Time	1	<0.001	<0.001	1.39	0.1651
Time x Time	1	<0.001	<0.001	4.01	0.0001

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	3.066	0.766	21.00	0.0001
Temp	4	1.949	0.487	13.35	0.0001
Time	4	18.874	4.718	129.26	0.0001

df = degrees of freedom; SS = sums of squares; R-Square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 15. Multiple regression analysis on survival of redfish embryos exposed to dome salt/distilled water brine at various combinations of temperature and salinity (Embryo Bioassay 2).

	RESPONSE MEAN: 0.791		R-SQUARE: 0.773		
	ROOT MSE: 0.187		COEF. OF VARIATION: 0.236		

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	16.657	0.701	158.73	0.0001
Quadratic	3	1.466	0.062	13.97	0.0001
Cross Product	3	9.250	0.010	2.38	0.0707
Total Regression	9	18.373	0.773	58.36	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	154	5.387	0.035

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	27.065	5.885	4.60	0.0001
Sal	1	-1.033	0.278	-3.72	0.0003
Temp	1	-0.484	0.165	-2.94	0.0038
Time	1	0.007	0.013	0.52	0.6015
Sal x Sal	1	0.013	0.004	3.67	0.003
Sal x Temp	1	0.001	0.002	0.23	0.8152
Temp x Temp	1	0.010	0.002	3.59	0.0004
Sal x Time	1	-0.001	<0.001	-2.47	0.0147
Temp x Time	1	<-0.001	<0.001	-1.20	0.2314
Time x Time	1	<0.001	<0.001	4.07	0.0001

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	2.903	0.726	20.75	0.0001
Temp	4	0.634	0.158	4.53	0.0017
Time	4	14.903	3.726	106.51	0.0001

df = degrees of freedom; SS = sums of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 16. Multiple regression analysis on survival of redfish embryos exposed to artificial sea salt/Brazos River water at various combinations of temperature and salinity (Embryo Bioassay 2).

	RESPONSE MEAN: 0.743		R-SQUARE: 0.764		
	ROOT MSE: 0.206		COEF. OF VARIATION: 0.278		

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	20.330	0.664	159.30	0.0001
Quadratic	3	2.705	0.088	21.20	0.0001
Cross Product	3	0.343	0.011	2.69	0.0475
Total Regression	9	23.378	0.764	61.06	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	170	7.232	0.042

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	40.892	5.956	6.87	0.0001
Sal	1	-1.960	0.292	-6.72	0.0001
Temp	1	-0.277	0.168	-1.65	0.1017
Time	1	0.010	0.013	0.74	0.4632
Sal x Sal	1	0.026	0.004	6.84	0.0001
Sal x Temp	1	<0.001	0.002	0.13	0.8984
Temp x Temp	1	0.005	0.003	2.02	0.0447
Sal x Time	1	-0.001	<0.001	-2.40	0.0176
Temp x Time	1	<-0.001	<0.001	-1.54	0.1248
Time x Time	1	<0.001	<0.001	3.32	0.0011

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	2.814	0.704	16.54	0.0001
Temp	4	0.410	0.102	2.41	0.0513
Time	4	20.446	5.111	120.16	0.0001

df = degrees of freedom; SS = sums of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 17. Mean percent survival of 4 replicates of redfish embryos and larvae at 12 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 3)

Salinity ppt	Temperature °C	Mean Survival %+S.D.			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	95.0± 5.0	95.0± 0*	100.0± 0.0*	97.5± 5.0*
34	26	95.0± 4.0	100.0± 0.0*	97.5± 2.9*	98.8± 2.5*
34	30	94.5± 7.9	90.5± 6.2*	95.0± 4.1*	100.0± 0.0*
36	23	98.8± 2.5	96.2± 2.5	100.0± 0.0	100.0± 0.0
36	26	98.8± 2.5	95.0± 4.1	97.5± 2.8	100.0± 0.0
36	30	95.0± 4.1	59.8± 8.3	70.0±16.3	95.0± 0.0
38	23	98.8± 2.5	98.7± 2.6	100.0± 0.0	100.0± 0.0
38	26	97.5± 5.0	98.8± 2.5	97.5± 2.9	92.5± 6.5
38	30	96.3± 4.8	95.0± 4.1	98.8± 2.5	91.3± 6.3
40	23	98.8± 2.5	100.0± 0.0	96.3± 2.5	98.8± 2.5
40	26	91.1± 5.1	96.2± 4.8	94.6± 7.9	96.3± 4.8
40	30	96.9± 6.3	97.5± 2.9	96.3± 4.8	95.0± 0.0

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 18. Mean percent survival of 4 replicates of redfish embryos and larvae at 24 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 3)

Salinity PPT	Temperature °C	Mean Survival %+S.D. ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	83.8±11.5	90.0± 7.1*	71.3±11.8*	83.8± 2.5*
34	26	77.5± 8.7	89.6±11.4*	85.0± 9.1*	82.5±14.4*
34	30	81.3±15.5	86.0±11.5*	91.3± 4.8*	98.8± 2.5*
36	23	73.6±10.0	68.2± 9.7	58.8± 2.5	72.6±12.0
36	26	67.5±35.7	67.5± 6.5	57.5± 9.6	52.8±18.0
36	30	72.5± 5.0	75.3±12.5	45.0±28.3	51.3±11.1
38	23	65.8± 8.7	62.8± 7.5	52.5±15.5	57.3±12.9
38	26	51.3±11.8	36.3± 7.5	37.5± 8.7	58.8±11.1
38	30	82.5±11.9	27.5± 2.9	77.2±10.0	58.8±22.9
40	23	70.9±15.5	56.3±11.1	47.5± 9.6	45.0± 4.1
40	26	63.2± 5.4	32.0±18.9	43.2±16.2	61.0± 8.9
40	30	77.8±14.9	43.7±15.3	85.0± 3.8	81.3± 6.3

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 19. Mean percent survival of 4 replicates of redfish embryos and larvae at 48 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 3)

Salinity ppt	Temperature °C	Mean Survival %+S.D			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	60.0±21.8	51.3±15.5*	57.5± 8.7*	57.5±15.0*
34	26	41.3±33.0	70.6±16.8*	72.5±11.9*	76.3±20.6*
34	30	59.9±31.7	66.3±18.0*	81.3±12.5*	72.5±16.6*
36	23	17.7±31.7	21.3±12.4	35.0±11.5	22.4±13.2
36	26	38.8±25.3	33.8± 8.5	16.3± 9.5	8.8± 7.5
36	30	63.4±15.7	50.8±20.2	26.3±19.3	33.8±22.5
38	23	36.6±16.8	10.4± 6.2	17.5±22.5	19.6±11.8
38	26	27.5± 9.6	5.0± 5.8	12.5± 6.5	17.5±28.7
38	30	68.7±14.9	7.5± 2.9	45.0±22.0	36.3±18.0
40	23	16.4±16.9	2.5± 5.0	10.0± 9.1	16.3± 8.5
40	26	29.0±12.9	14.1± 8.5	7.6± 2.7	38.7± 7.5
40	30	59.4±16.9	13.4±15.5	60.0±14.7	48.8±21.7

[1]ASW, artificial seawater ; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine.

Table 20. Mean percent survival of 4 replicates of redfish embryos and larvae at 72 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Embryo Bioassay 3)

Salinity ppt	Temperature °C	Mean Survival %±S.D. ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	15.0±13.2	32.5±12.6*	21.3±16.5*	27.5± 6.5*
34	26	6.3± 6.3	37.2±18.3*	33.8±20.1*	43.8±26.6*
34	30	20.5± 8.6	10.9± 2.8*	33.8±13.1*	20.0±10.8*
36	23	1.3± 2.6	3.8± 4.8	3.8± 2.5	3.9± 7.9
36	26	12.5±15.0	16.3± 8.5	2.5± 5.0	0.0± 0.0
36	30	22.9± 9.1	11.4± 7.3	13.8±11.1	6.3± 2.5
38	23	11.3± 7.4	0.0± 0.0	8.8±14.4	2.6± 3.0
38	26	2.5± 2.9	0.0± 0.0	2.5± 2.9	7.5±15.0
38	30	7.5± 5.0	0.0± 0.0	9.2± 6.9	12.5± 8.7
40	23	5.0±10.0	0.0± 0.0	0.0± 0.0	2.5± 2.9
40	26	6.3± 4.8	0.0± 0.0	2.5± 5.0	3.8± 2.5
40	30	6.3± 9.5	0.0± 0.0	10.0± 4.1	3.8± 4.8

[1] ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

* Listed observations are repeated values from the ASW/DW exposure brine

Table 21. Repeated measures analysis of variance on survival of redfish embryos exposed to four brine types at various combinations of temperature and salinity. (Embryo Bioassay 3)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
MEAN	509.798	1	509.798	7861.55	0.0000
SAL	10.640	3	3.547	54.69	0.0000
TEMP	1.121	2	0.561	8.65	0.0003
BRINE	0.681	3	0.227	3.50	0.0172
ST	0.901	6	0.150	2.31	0.0366
SB	3.397	9	0.377	5.82	0.0000
TB	0.681	6	0.114	1.75	0.1135
STB	1.770	18	0.098	1.52	0.0921
ERROR	9.273	143	0.065		
R(1)	148.940	1	148.940	7132.41	0.0000
R(1)S	2.066	3	0.689	32.98	0.0000
R(1)T	0.932	2	0.466	22.31	0.0000
R(1)B	0.199	3	0.066	3.19	0.0256
R(1)ST	0.825	6	0.137	6.58	0.0000
R(1)SB	0.887	9	0.099	4.72	0.0000
R(1)TB	0.300	6	0.050	2.40	0.0309
R(1)STB	0.869	18	0.048	2.31	0.0033
ERROR	2.986	143	0.021		
R(2)	0.518	3	0.518	35.45	0.0000
R(2)S	1.597	9	0.532	36.41	0.0000
R(2)T	1.268	6	0.634	43.36	0.0000
R(2)B	0.606	18	0.202	13.81	0.0000
R(2)ST	0.153	27	0.025	1.74	0.1158
R(2)SB	0.673	18	0.075	5.11	0.0000
R(2)TB	0.243	54	0.041	2.77	0.0140
R(2)STB	0.638	429	0.036	2.42	0.0020
ERROR	2.090	18	0.015		

...(Cont'd)

Table 21. (Cont'd)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
R	149.647	3	49.882	3262.43	0.0000
RS	3.689	9	0.409	26.81	0.0000
RT	2.429	6	0.405	26.48	0.0000
RB	0.897	9	0.099	6.52	0.0000
RST	1.067	18	0.059	3.88	0.0000
RSB	1.728	27	0.064	4.19	0.0000
RTB	0.699	18	0.039	2.54	0.0005
RSTB	1.930	54	0.036	2.34	0.0000
ERROR	6.559	429	0.015		

Abbreviations:

SAL = Salinity
TEMP = Temperature
BRINE = Brine Type
ST = Salinity x Temperature
SB = Salinity x Brine
TB = Temperature x Brine
STB = Salinity x Temperature x Brine
R = Time of Exposure
R(1) = Linear Component of Time
R(2) = Quadratic Component of Time

Table 22. Multiple regression analysis on survival of redfish embryos exposed to artificial sea salts/ distilled water brine at various combinations of temperature and salinity. (Embryo Bioassay 3).

RESPONSE MEAN:	0.643	R-SQUARE:	0.333
ROOT MSE:	0.389	COEF. OF VARIATION:	0.605

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	7.532	0.187	16.62	0.0001
Quadratic	3	5.858	0.145	12.93	0.0001
Cross Product	3	0.038	0.001	0.08	0.9638
Total Regression	9	13.427	0.333	9.88	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	178	26.885	0.151

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	17.195	11.528	1.49	0.1376
Sal	1	-0.849	0.544	-1.56	0.1204
Temp	1	-0.063	0.319	-0.20	0.8434
Time	1	0.020	0.025	0.83	0.4090
Sal x Sal	1	0.011	0.007	1.54	0.1251
Sal x Temp	1	0.000	0.005	0.11	0.9155
Temp x Temp	1	0.001	0.005	0.22	0.8265
Sal x Time	1	0.000	0.001	0.25	0.7993
Temp x Time	1	0.000	0.000	0.43	0.6711
Time x Time	1	-0.000	0.000	-6.03	0.0001

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	0.757	0.189	1.25	0.2904
Temp	4	0.675	0.169	1.12	0.3499
Time	4	11.976	2.994	19.82	0.0001

df = degrees of freedom; SS = sums of squares; R-square = multiple correlation coefficient
F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 23. Multiple regression analysis on survival of redfish embryos exposed to dome salt/Brazos River water brine at various combinations of temperature and salinity. (Embryo Bioassay 3)

	RESPONSE MEAN: 0.537		R-SQUARE: 0.376		
	ROOT MSE: 0.388		COEF. OF VARIATION: 0.722		
REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	13.214	0.302	29.32	0.0001
Quadratic	3	2.158	0.049	4.79	0.0033
Cross Product	3	1.078	0.025	2.39	0.0689
Total Regression	9	16.449	0.376	12.17	0.0001
RESIDUAL	df	SS	MEAN SQUARE		
Total Error	182	27.338	0.150		
PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	18.183	11.051	1.65	0.1016
Sal	1	-0.546	0.531	-1.03	0.3049
Temp	1	-0.483	0.309	-1.56	0.1209
Time	1	0.034	0.024	1.44	0.1516
Sal x Sal	1	0.004	0.007	0.51	0.6096
Sal x Temp	1	0.009	0.004	2.00	0.0472
Temp x Temp	1	0.003	0.005	0.52	0.6007
Sal x Time	1	0.001	0.001	-1.49	0.1388
Temp x Time	1	0.000	0.000	0.99	0.3256
Time x Time	1	0.000	0.000	-3.72	0.0003
FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	7.613	1.903	12.67	0.0001
Temp	4	0.837	0.209	1.39	0.2381
Time	4	9.077	2.269	15.11	0.0001

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 24. Multiple regression analysis on survival of redfish embryos exposed to dome salt/distilled water brine at various combinations of temperature and salinity. (Embryo Bioassay 3)

	RESPONSE MEAN:	0.595	R-SQUARE:	0.401	
	ROOT MSE:	0.368	COEF. OF VARIATION:	0.618	
REGRESSION	**df**	**SS**	**R-SQUARE**	**F**	**PR>F**
Linear	3	9.974	0.243	24.61	0.0001
Quadratic	3	5.191	0.127	12.81	0.0001
Cross Product	3	1.279	0.031	3.15	0.0258
Total Regression	9	16.443	0.401	13.52	0.0001
RESIDUAL	**df**	**SS**	**MEAN SQUARE**		
Total Error	182	24.590	0.135		
PARAMETER	**df**	**ESTIMATE**	**STD. DEV.**	**T**	**PR>T**
Intercept	1	30.799	10.481	2.94	0.0037
Sal	1	-1.785	0.503	-3.55	0.0005
Temp	1	0.103	0.294	0.35	0.7266
Time	1	0.087	0.023	3.87	0.0002
Sal x Sal	1	0.025	0.007	3.81	0.0002
Sal x Temp	1	-0.003	0.004	-0.66	0.5082
Temp x Temp	1	0.001	0.005	0.27	0.7840
Sal x Time	1	-0.001	0.001	-2.59	0.0104
Temp x Time	1	-0.001	0.000	-1.53	0.1288
Time x Time	1	-0.000	0.000	-4.88	0.0001
FACTOR	**df**	**SS**	**MEAN SQUARE**	**F**	**PR>F**
Sal	4	4.275	1.069	7.91	0.0001
Temp	4	3.752	0.938	6.94	0.0001
Time	4	9.695	2.424	17.94	0.0001

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 25. Multiple regression analysis on survival of redfish embryos exposed to artifical sea salts/ Brazos River water at various combinations of temperature and salinity. (Embryo Bioassay 3)

	RESPONSE MEAN: 0.557		R-SQUARE: 0.364		
	ROOT MSE: 0.378		COEF. OF VARIATION: 0.679		
REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	5.539	0.135	12.91	0.0001
Quadratic	3	4.066	0.099	9.48	0.0001
Cross Product	3	5.285	0.129	12.32	0.0001
Total Regression	9	14.891	0.364	11.57	0.0001
RESIDUAL	df	SS	MEAN SQUARE		
Total Error	182	26.031	0.143		
PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	28.117	10.784	2.61	0.0099
Sal	1	-1.199	0.518	-2.32	0.0216
Temp	1	-0.635	0.302	-2.10	0.0370
Time	1	0.138	0.023	5.91	0.0001
Sal x Sal	1	0.012	0.007	1.83	0.0690
Sal x Temp	1	0.014	0.004	3.36	0.0009
Temp x Temp	1	0.003	0.005	0.67	0.5064
Sal x Time	1	-0.002	0.001	-4.61	0.0001
Temp x Time	1	-0.000	0.000	-2.09	0.0377
Time x Time	1	-0.000	0.000	-4.96	0.0001
FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	5.165	1.291	9.03	0.0001
Temp	4	3.901	0.975	6.82	0.0001
Time	4	11.111	2.778	19.42	0.0001

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 26. Mean percent survival of 4 replicates of redfish larvae at 24 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Larval Bioassay)

Salinity ppt	Temperature °C	Mean Survival %±S.D. ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	95.0±10.0	95.0±10.0*	95.0±10.0*	95.0±10.0*
34	26	100.0± 0.0	100.0± 0.0*	100.0± 0.0*	100.0± 0.0*
34	30	100.0± 0.0	100.0± 0.0*	100.0± 0.0*	100.0± 0.0*
36	23	95.0±10.0	93.8±12.5	95.0±10.0	85.0±19.1
36	26	80.0±16.3	100.0± 0.0	75.0±19.1	95.0±10.0
36	30	100.0± 0.0	100.0± 0.0	100.0± 0.0	95.0±10.0
38	23	80.0±16.3	83.8±11.1	90.0±11.5	81.8±13.6
38	26	83.8±19.7	85.8±18.9	65.0±10.0	65.0±41.2
38	30	95.8± 8.5	100.0± 0.0	100.0± 0.0	95.0±10.0
40	23	80.0±28.3	78.8±16.5	80.0±16.3	70.5± 8.8
40	26	95.0±10.0	90.0±11.5	77.0±20.8	80.0±16.3
40	30	100.0± 0.0	90.0±20.0	90.0±20.0	88.8±13.1

[1] ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River water

* Listed observations are repeated values from the ASW/DW exposure brine

Table 27. Mean percent survival of 4 replicates of redfish larvae at 48 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Larval Bioassay)

Salinity ppt	Temperature °C	Mean Survival % ± S.D.			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	85.0±19.1	85.0±19.1*	85.0±19.1*	85.0±19.1*
34	26	100.0± 0.0	100.0± 0.1*	100.0± 0.0*	100.0± 0.0*
34	30	95.0±10.0	95.0±10.0*	95.0±10.0*	95.0±10.0*
36	23	95.0±10.0	93.8±12.5	85.0±10.0	85.0±19.1
36	26	80.0±16.3	100.0± 0.0	75.0±19.1	88.8±13.1
36	30	100.0± 0.0	73.8± 9.5	90.8±10.8	90.0±10.0
38	23	80.0±16.3	83.8±11.1	80.0±28.3	81.8±13.6
38	26	83.8±19.7	75.8±10.6	65.0±10.0	65.0±41.2
38	30	95.8± 8.5	95.0±10.0	100.0± 0.0	95.0±10.0
40	23	75.0±25.2	78.8±16.5	80.0±16.3	64.3±12.6
40	26	95.0±10.0	90.0±11.5	70.8±24.9	80.0±16.3
40	30	90.0±11.5	90.0±20.0	90.0±20.0	88.8±13.1

[1] ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

* Listed observations are repeated values from the ASW/DW exposure brine

Table 28. Mean percent survival of 4 replicates of redfish larvae at 72 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Larval Bioassay)

Salinity ppt	Temperature °C	Mean Survival % ± S.D			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	85.0±19.1	85.0±19.1*	85.0±19.1*	85.0±19.1*
34	26	100.0± 0.0	100.0± 0.0*	100.0± 0.0*	100.0± 0.0*
34	30	95.0±10.0	95.0±10.0*	95.0±10.0*	95.0±10.0*
36	23	90.0±11.5	93.8±12.5	70.0±11.5	85.0±19.1
36	26	80.0±16.3	100.0± 0.0	75.0±19.1	88.8±13.1
36	30	100.0± 0.0	73.8± 9.5	90.8±10.8	70.0±26.5
38	23	80.0±16.3	78.8± 2.5	70.0±25.8	81.8±13.6
38	26	71.3±21.7	70.8±12.5	57.5± 5.0	65.0±41.2
38	30	90.8±10.8	95.0±10.0	100.0± 0.0	95.0±10.0
40	23	75.0±25.2	73.8±25.0	65.0±19.1	64.3±12.6
40	26	95.0±10.0	88.8±13.1	70.0±24.5	80.0±16.3
40	30	85.0±13.2	90.0±20.0	80.0±16.3	77.5±20.6

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 29. Mean percent survival of 4 replicates of redfish larvae at 96 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Larval Bioassay)

Salinity ppt	Temperature °C	Mean Survival % ± S.D.			
		ASW/DW[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	85.0±19.1	85.0±19.1*	85.0±19.1*	85.0±19.0*
34	26	100.0± 0.0	100.0± 0.0*	100.0± 0.0*	100.0± 0.0*
34	30	95.0±10.0	95.0±10.0*	95.0±10.0*	95.0±10.0*
36	23	90.0±11.5	91.8±16.5	70.0±11.5	85.0±19.1
36	26	80.0±16.3	100.0± 0.0	75.0±19.1	88.8±13.1
36	30	100.0± 0.0	66.8± 9.4	82.5±13.6	61.7±37.5
38	23	80.0±16.3	78.8± 2.5	70.0±25.8	81.8±13.6
38	26	71.3±21.7	70.8±12.5	55.0± 5.8	65.0±41.2
38	30	90.8±10.8	83.8±11.1	82.5±13.6	90.0±11.5
40	23	75.0±25.2	73.8±25.0	61.3±25.9	64.3±12.6
40	26	95.0±10.0	86.8±16.2	70.0±24.5	80.0±16.3
40	30	85.0±13.2	80.0±28.3	75.0±19.1	72.5±22.2

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 30. Mean percent survival of 4 replicates of redfish larvae at 120 hours during exposure to four brine types at various combinations of salinity and temperature. Control condition (34 ppt S) in artificial seawater (Instant Ocean). (Larval Bioassay)

Salinity ppt	Temperature °C	Mean Survival % ± S.D.			
		ASW/DS[1]	DS/BRW[1]	DS/DW[1]	ASW/BRW[1]
34	23	75.0±25.2	75.0±25.2*	75.0±25.2*	75.0±25.2*
34	26	100.0± 0.0	100.0± 0.0*	100.0± 0.0*	100.0± 0.0*
34	30	93.8±12.5	93.8±12.5*	93.8±12.5*	93.8±12.5*
36	23	80.0±16.3	91.8±16.5	70.0±11.5	85.0±19.1
36	26	80.0±16.3	100.0± 0.0	75.0±19.1	88.8±13.1
36	30	100.0± 0.0	66.8± 9.4	81.8±13.6	61.7±37.5
38	23	80.0±16.3	77.5± 2.9	70.0±25.8	81.8±13.6
38	26	71.3±21.7	65.5± 7.1	55.0± 5.8	65.0±41.2
38	30	90.0±11.5	66.8± 9.4	81.7±13.6	61.7±37.5
40	23	75.0±21.2	75.8±21.1	61.3±25.9	57.5±15.0
40	26	93.8±12.5	74.3±21.1	66.8±23.6	80.0±16.3
40	30	85.0±13.2	80.0±28.3	75.0±19.1	72.5±22.2

[1]ASW, artificial seawater; DW, distilled water; DS, dome salt; BRW, Brazos River Water

*Listed observations are repeated values from the ASW/DW exposure brine

Table 31. Repeated measures analysis of variance on survival of redfish larvae exposed to four brine types at various combinations of temperature and salinity. (Larval Bioassay)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
MEAN	1546.790	1	1546.791	4828.32	0.0000
SAL	9.756	3	3.252	10.15	0.0000
TEMP	3.980	2	1.990	6.21	0.0026
BRINE	1.674	3	0.558	1.74	0.1611
ST	7.185	6	1.197	3.74	0.0018
SB	1.405	9	0.156	0.49	0.8813
TB	1.755	6	0.293	0.91	0.4874
STB	4.603	18	0.256	0.80	0.6998
ERROR	45.170	141	0.320		
R(1)	2.780	1	2.781	101.81	0.0000
R(1)S	0.037	3	0.012	0.45	0.7146
R(1)T	0.466	2	0.233	8.52	0.0003
R(1)B	0.191	3	0.064	2.33	0.0768
R(1)ST	0.431	6	0.072	2.63	0.0191
R(1)SB	0.337	9	0.037	1.37	0.2060
R(1)TB	0.302	6	0.050	1.85	0.0945
R(1)STB	0.423	18	0.024	0.86	0.6257
ERROR	3.851	141	0.027		
R(2)	0.098	1	0.098	8.62	0.0039
R(2)S	0.003	3	0.001	0.10	0.9607
R(2)T	0.023	2	0.012	1.03	0.3588
R(2)B	0.054	3	0.018	1.59	0.1942
R(2)ST	0.068	6	0.011	1.00	0.4309
R(2)SB	0.155	9	0.017	1.51	0.1496
R(2)TB	0.067	6	0.011	0.98	0.4396
R(2)STB	0.109	18	0.006	0.53	0.9398
ERROR	1.602	141	0.011		

...(Cont'd)

Table 31. (Cont'd)

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
R	2.879	4	0.719	58.77	0.0000
RS	0.213	12	0.018	1.45	0.1384
RT	0.606	8	0.076	6.18	0.0000
RB	0.289	12	0.024	1.96	0.0253
RST	0.654	24	0.027	2.23	0.0008
RSB	0.618	36	0.017	1.40	0.0630
RTB	0.485	24	0.020	1.65	0.0273
RSTB	0.746	72	0.010	0.85	0.8104
ERROR	6.909	564	0.012		

Abbreviations:

SAL = Salinity
TEMP = Temperature
BRINE = Brine Type
ST = Salinity x Temperature
SB = Salinity x Brine
TB = Temperature x Brine
STB = Salinity x Temperature x Brine
R = Time of Exposure
R(1) = Linear Component of Time
R(2) = Quadratic Component of Time

Table 32. Multiple regression analysis on survival of redfish larvae exposed to artificial sea salts/ distilled water at various combinations of temperature and salinity (Larval Bioassay).

	RESPONSE MEAN: 1.339 ROOT MSE: 0.264		R-SQUARE: 0.162 COEF. OF VARIATION: 0.197		
REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	2.604	0.138	12.46	0.0001
Quadratic	3	0.393	0.021	1.88	0.1319
Cross Product	3	0.052	0.003	0.25	0.8633
Total Regression	9	3.049	0.162	4.86	0.0001
RESIDUAL	df	SS	MEAN SQUARE		
Total Error	227	15.816	0.070		
PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	14.174	6.871	2.06	0.0403
Sal	1	-0.752	0.327	-2.30	0.0225
Temp	1	0.064	0.191	0.34	0.7364
Time	1	0.011	0.012	0.90	0.3680
Sal x Sal	1	0.001	0.004	2.29	0.0229
Sal x Temp	1	<0.001	0.002	0.11	0.9121
Temp x Temp	1	-0.001	0.003	-0.24	0.8091
Sal x Time	1	<-0.001	<0.001	-0.62	0.5381
Temp x Time	1	<-0.001	<0.001	-0.61	0.5445
Time x Time	1	<-0.001	<0.001	-0.54	0.5891
FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	1.186	0.296	4.26	0.0024
Temp	4	1.777	0.444	6.38	0.0001
Time	4	0.107	0.027	0.39	0.8192

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 33. Multiple regression analysis on survival of redfish larvae exposed to dome salt/Brazos River water brine at various combinations of temperature and salinity (Larval Bioassay)

RESPONSE MEAN: 1.261	R-SQUARE: 0.215
ROOT MSE: 0.261	COEF. OF VARIATION: 0.207

REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	0.368	0.025	1.80	0.1465
Quadratic	3	0.758	0.052	3.72	0.0127
Cross Product	3	2.026	0.138	9.93	0.0001
Total Regression	9	3.152	0.215	5.15	0.0001

RESIDUAL	df	SS	MEAN SQUARE
Total Error	169	11.491	0.068

PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	57.899	15.653	3.70	0.0003
Sal	1	-2.578	0.792	-3.26	0.0014
Temp	1	-0.551	0.242	-2.27	0.0242
Time	1	0.002	0.017	0.11	0.9140
Sal x Sal	1	0.026	0.010	2.48	0.0140
Sal x Temp	1	0.023	0.004	5.41	0.0001
Temp x Temp	1	-0.006	0.003	-1.64	0.1033
Sal x Time	1	<0.001	<0.001	0.39	0.6935
Temp x Time	1	<-0.001	<0.001	-0.47	0.6361
Time x Time	1	<-0.001	<0.001	-1.52	0.1312

FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	2.755	0.689	10.13	0.0001
Temp	4	2.198	0.549	8.08	0.0001
Time	4	0.220	0.055	0.81	0.5210

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 34. Multiple regression analysis on survival of redfish larvae exposed to dome salt/distilled water brine at various combinations of temperature and salinity (Larval Bioassay).

	RESPONSE MEAN: 1.157		R-SQUARE: 0.250		
	ROOT MSE: 0.276		COEF. OF VARIATION: 0.239		
REGRESSION	**df**	**SS**	**R-SQUARE**	**F**	**PR>F**
Linear	3	2.683	0.155	11.73	0.0001
Quadratic	3	1.647	0.095	7.20	0.0002
Cross Product	3	0.005	<0.001	0.02	0.9906
Total Regression	9	4.336	0.251	6.32	0.0001
RESIDUAL	**df**	**SS**	**MEAN SQUARE**		
Total Error	170	12.970	0.076		
PARAMETER	**df**	**ESTIMATE**	**STD. DEV.**	**T**	**PR>T**
Intercept	1	6.133	16.572	0.37	0.7118
Sal	1	0.287	0.838	0.34	0.7325
Temp	1	-0.800	0.256	-3.12	0.0021
Time	1	0.002	0.018	0.14	0.8876
Sal x Sal	1	-0.004	0.011	-0.40	0.6924
Sal x Temp	1	0.001	0.004	0.14	0.8890
Temp x Temp	1	0.015	0.004	4.19	0.0001
Sal x Time	1	<0.001	<0.001	0.22	0.8235
Temp x Time	1	<-0.001	<0.001	-0.04	0.9711
Time x Time	1	<-0.001	<0.001	-1.96	0.0516
FACTOR	**df**	**SS**	**MEAN SQUARE**	**F**	**PR>F**
Sal	4	0.221	0.055	0.72	0.5769
Temp	4	3.417	0.854	11.20	0.0001
Time	4	0.704	0.176	2.31	0.0601

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 35. Multiple regression analysis on survival of redfish larvae exposed to artificial sea salts/Brazos River water brine at various combinations of temperature and salinity (Larval Bioassay)

	RESPONSE MEAN: 1.201		R-SQUARE: 0.123		
	ROOT MSE: 0.319		COEF. OF VARIATION: 0.266		
REGRESSION	df	SS	R-SQUARE	F	PR>F
Linear	3	1.705	0.088	5.58	0.0013
Quadratic	3	0.098	0.005	0.32	0.8125
Cross Product	3	0.577	0.030	1.89	0.1314
Total Regression	9	2.380	0.123	2.60	0.0080
RESIDUAL	df	SS	MEAN SQUARE		
Total Error	167	16.997	0.102		
PARAMETER	df	ESTIMATE	STD. DEV.	T	PR>T
Intercept	1	2.548	19.165	0.13	0.8944
Sal	1	0.234	0.970	0.24	0.8094
Temp	1	-0.392	0.230	-.132	0.1886
Time	1	0.002	0.021	0.08	0.9325
Sal x Sal	1	-0.008	0.013	-0.62	0.5376
Sal x Temp	1	0.011	0.005	2.22	0.0279
Temp x Temp	1	<-0.001	0.004	-0.06	0.9540
Sal x Time	1	<0.001	0.001	0.40	0.6908
Temp x Time	1	<-0.001	<0.001	-0.83	0.4059
Time x Time	1	<-0.001	<0.001	-0.83	0.4086
FACTOR	df	SS	MEAN SQUARE	F	PR>F
Sal	4	1.613	0.403	3.96	0.0042
Temp	4	1.172	0.293	2.88	0.0244
Time	4	0.207	0.052	0.51	0.7292

df = degrees of freedom; SS = sum of squares; R-square = multiple correlation coefficient; F = factor MS/Error MS; PR > F = probability of a larger F-value; T = t statistic; PR > T = probability of a greater T value; Sal = salinity; Temp = temperature; Time = time of exposure

Table 36. Probit analysis of results of two juvenile bioassays using dome salt/Brazos River water brine. Dilution water was 34 ppt artificial seawater salts/distilled water.

Time	Probability	Concentration (% Brine)	95% Fiducial Limits Lower	95% Fiducial Limits Upper
Experiment 1				
96 h	LC_{10}	2.68	1.26	3.65
$\chi^2 = 6.25$ ($P<0.10$)	LC_{50}	6.63	5.16	11.37
	LC_{90}	10.59	7.80	20.35
7 day	LC_{10}	1.56	-*	-
$\chi^2 = 10.73$ ($P<0.10$)	LC_{50}	6.84	-	-
	LC_{90}	12.12	-	-
14 day	LC_{10}	1.14	-	-
$\chi^2 = 10.77$ ($P<0.10$)	LC_{50}	6.50	-	-
	LC_{90}	11.86	-	-
Experiment 2				
24 h	LC_{10}	2.93	2.26	3.31
$\chi^2 = 6.26$ ($P>0.10$)	LC_{50}	3.97	3.66	4.29
	LC_{90}	5.01	4.63	5.69
48 h	LC_{10}	2.89	2.23	3.27
$\chi^2 = 8.09$ ($P>0.10$)	LC_{50}	3.92	3.62	4.24
	LC_{90}	4.96	4.59	5.62
96 h	LC_{10}	1.99	-3.65	3.08
$\chi^2 = 24.46$ ($P>0.10$)	LC_{50}	3.65	2.29	5.98
	LC_{90}	5.30	4.09	13.02

*Data at these time intervals were not ammenable to computation of confidence intervals by probit analysis.

Table 37. Salinities of exposure media of two acute bioassays using dome salt/Brazos River water brine.

	Experiment #1	Experiment #2
Brine %	285.0 ppt	297.0 ppt
0.5	35.3 ppt	-
1	36.5 ppt	37.0 ppt
2	39.0 ppt	39.8 ppt
3	41.5 ppt	42.1 ppt
4	44.0 ppt	44.6 ppt
5	46.6 ppt	47.0 ppt
7.5	-	53.1 ppt
10	-	59.6 ppt

APPENDIX FIGURES

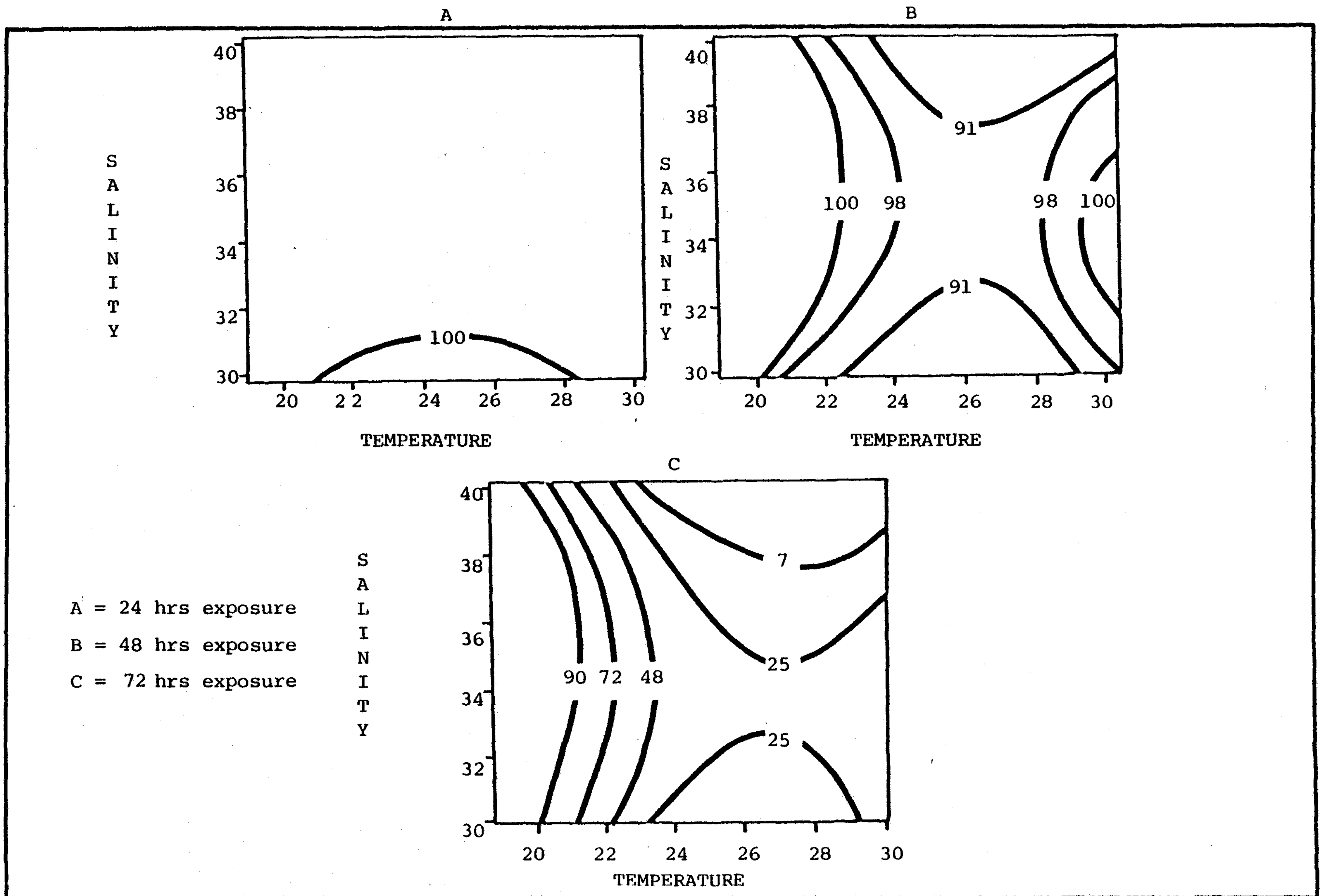


Fig. 1. Response surface diagrams predicting mean percent survival of embryos exposed to artificial sea salts/distilled water in Embryo Bioassay 1.

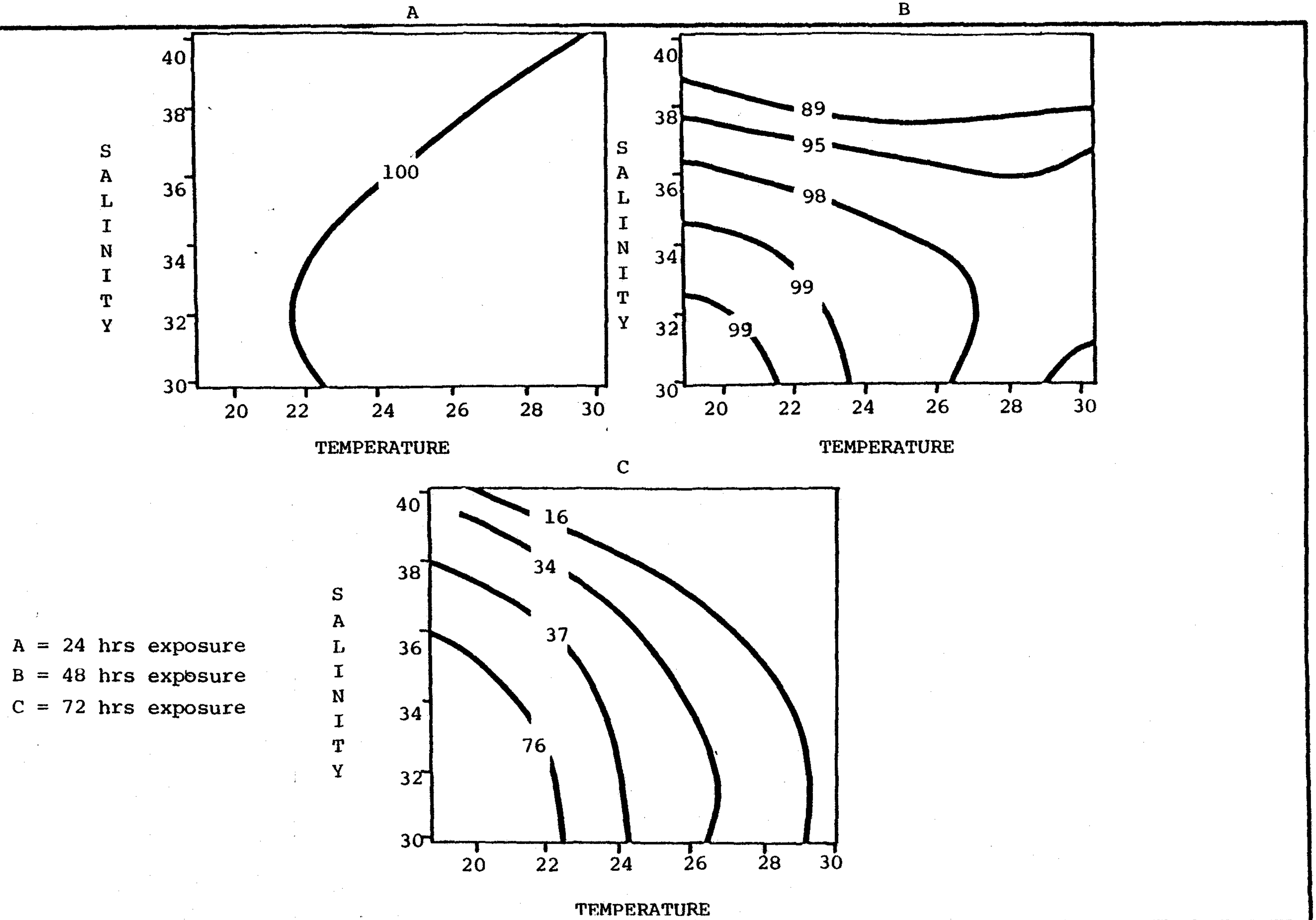
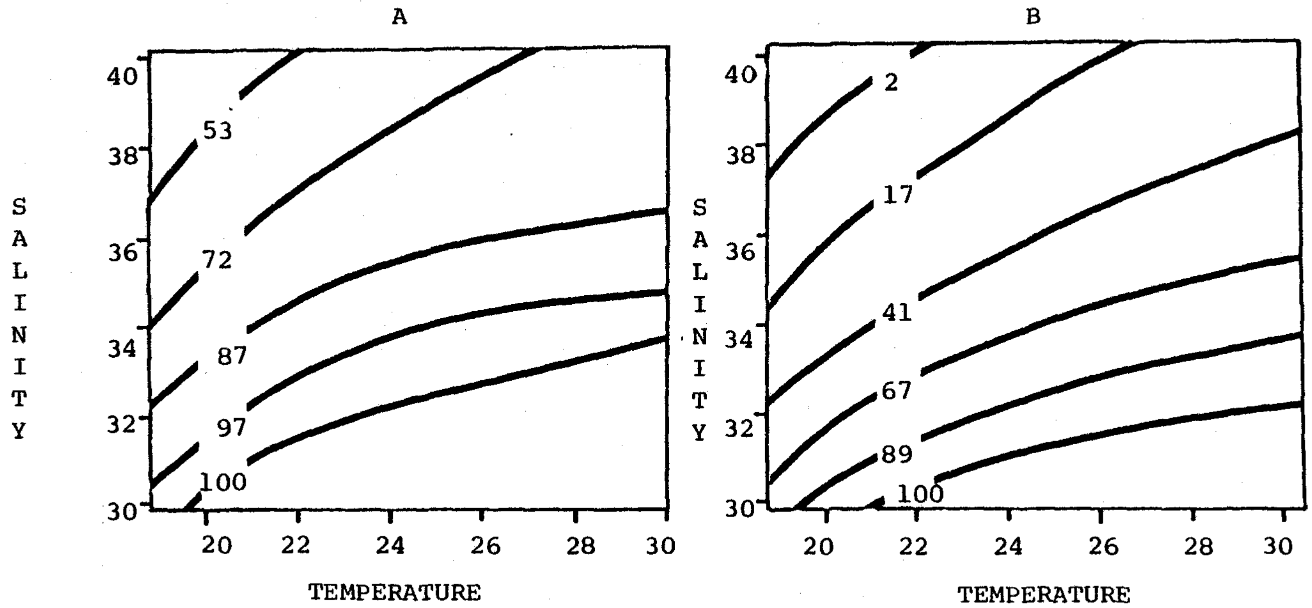


Fig. 2. Response surface diagrams predicting mean percent survival of embryos exposed to dome salt/Brazos River water in Embryo Bioassay 1.



A = 48 hrs exposure

B = 72 hrs exposure

Fig. 3. Response surface diagrams predicting mean percent survival of embryos exposed to artificial sea salts/distilled water in Embryo Bioassay 2.

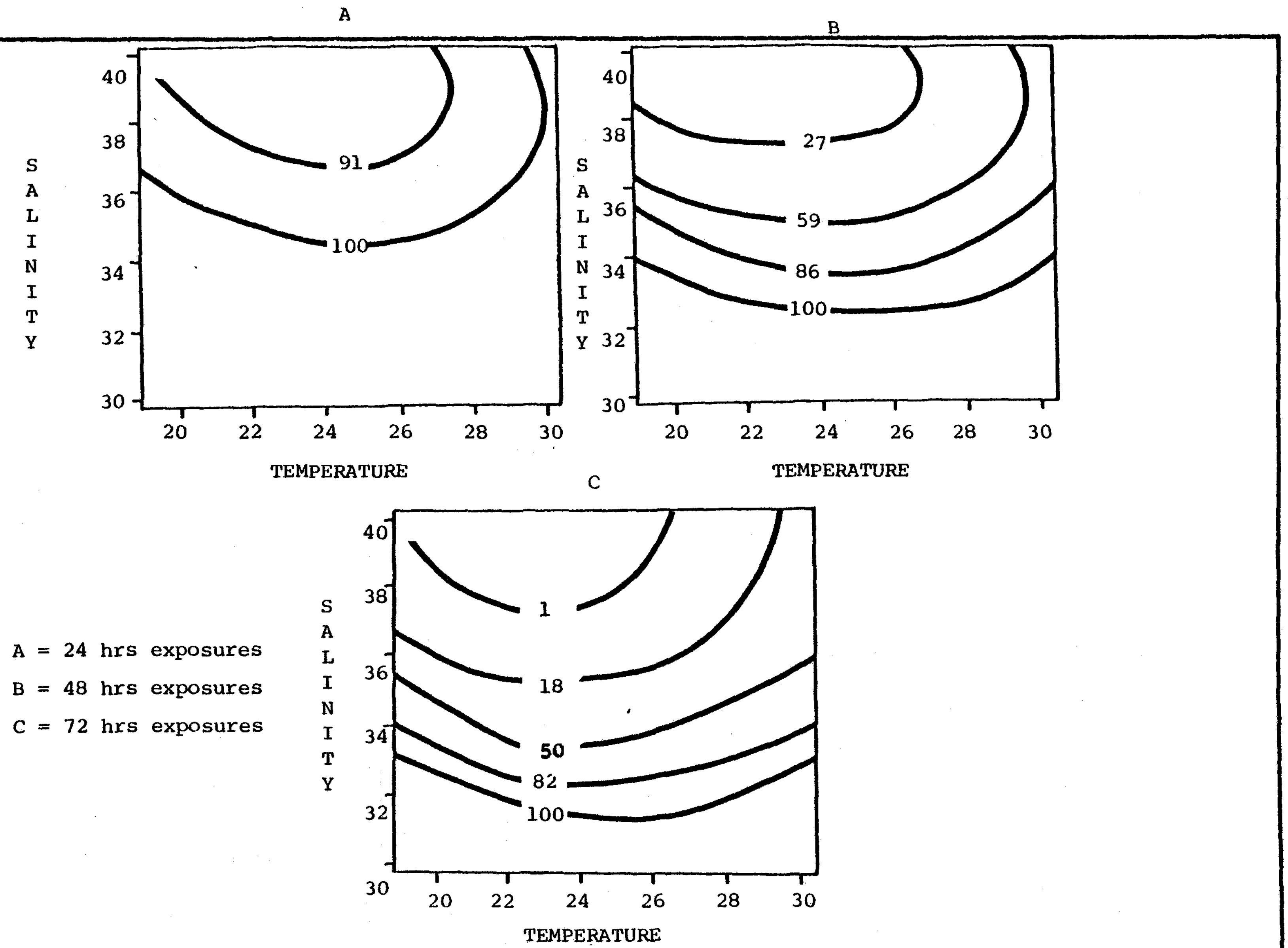


Fig. 4. Response surface diagrams predicting mean percent survival of embryos exposed to dome salt/Brazos River water in Embryo Bioassay 2.

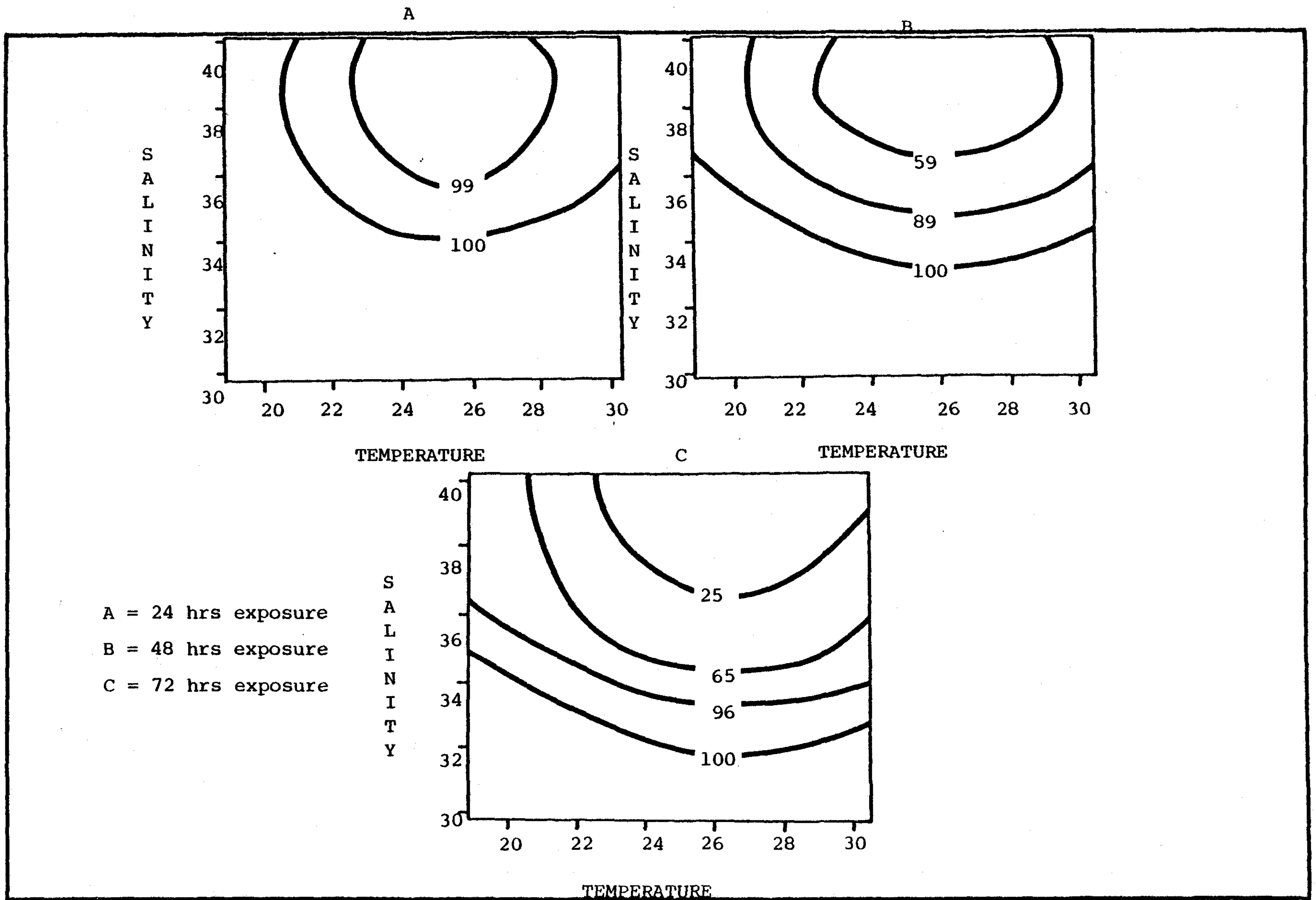


Fig. 5. Response surface diagrams predicting mean percent survival of embryos exposed to dome salt/distilled water in Embryo Bioassay 2.

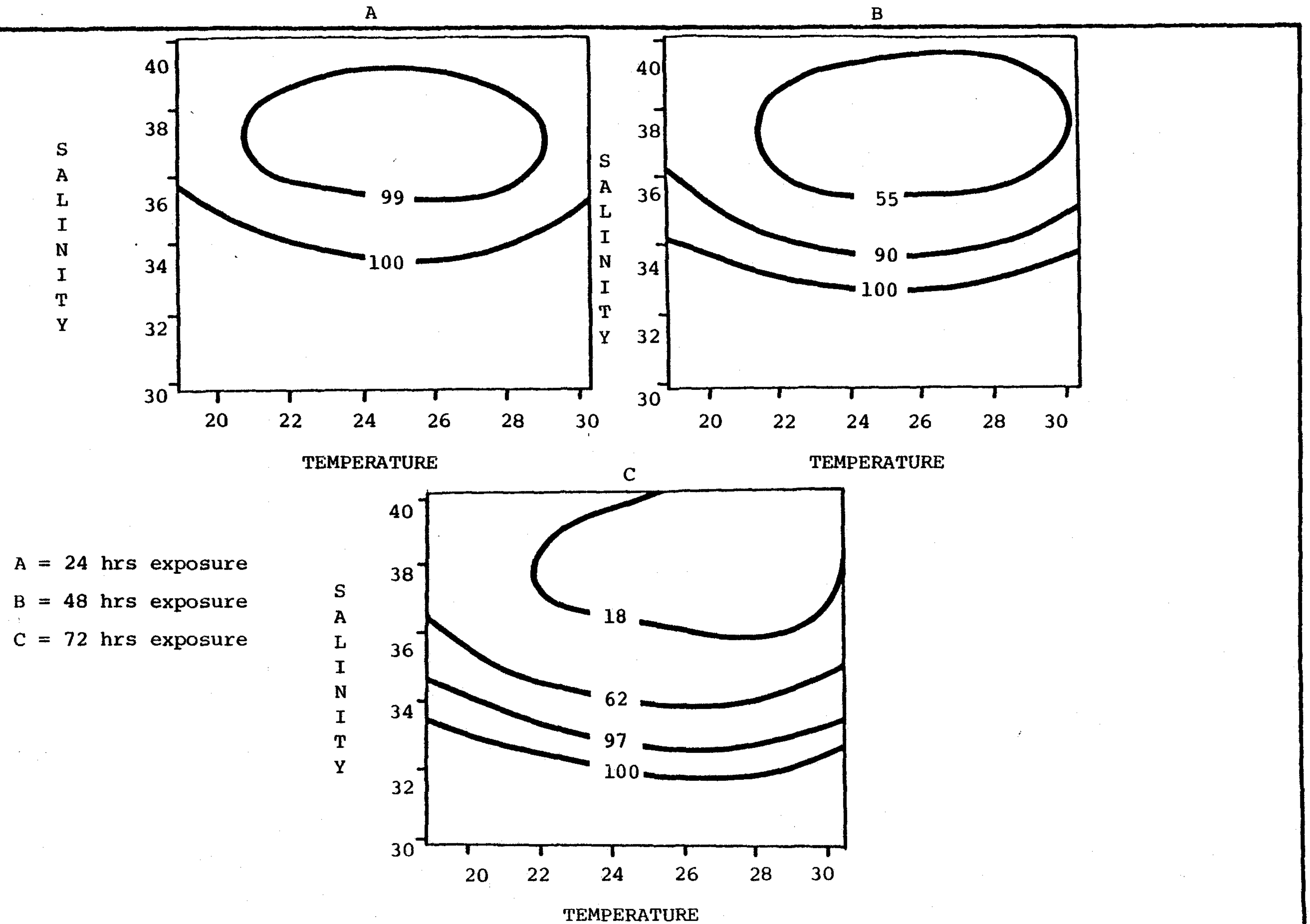
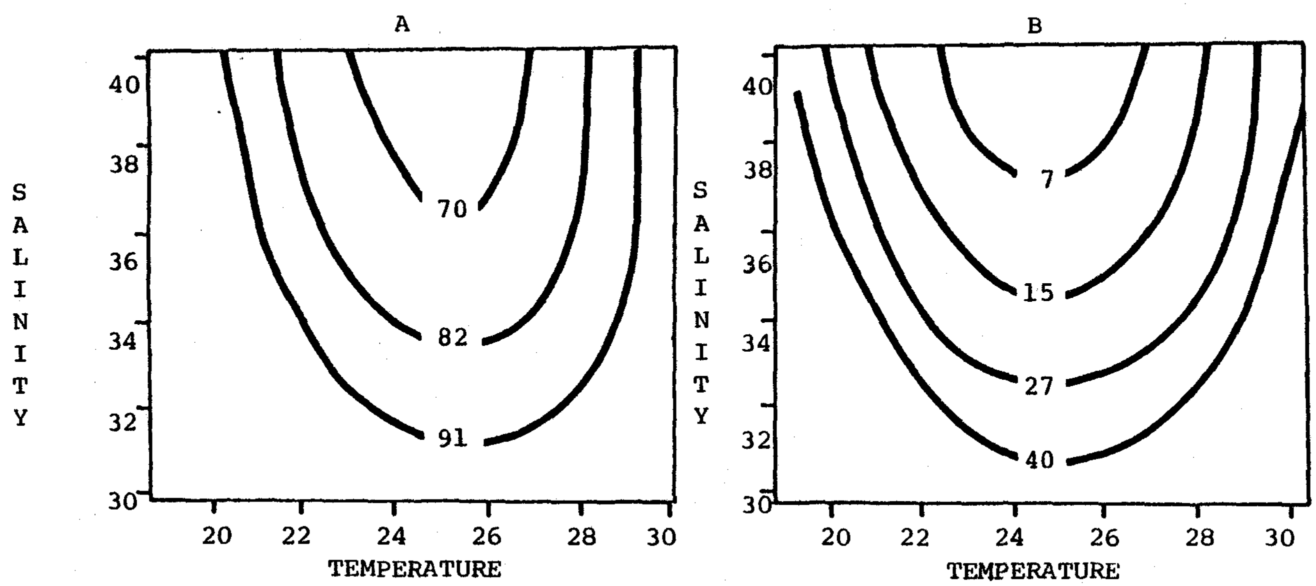


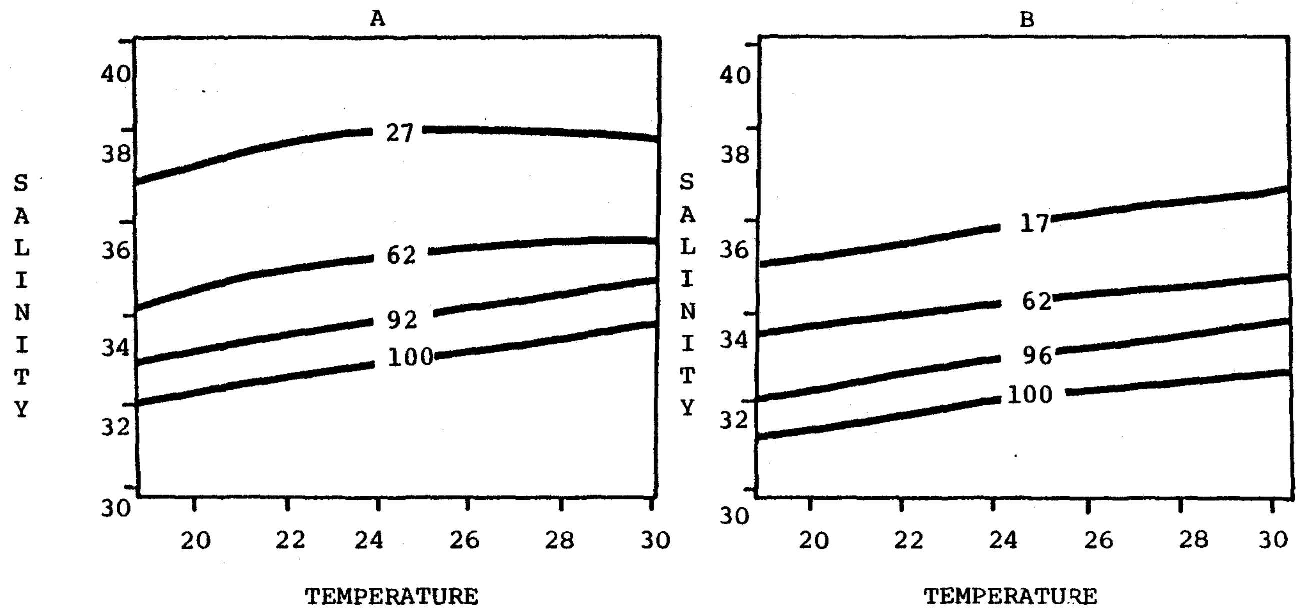
Fig. 6. Response surface diagrams predicting mean percent survival of embryos exposed to artificial sea salts/Brazos River water in Embryo Bioassay 2.



A = 48 hrs exposure

B = 72 hrs exposure

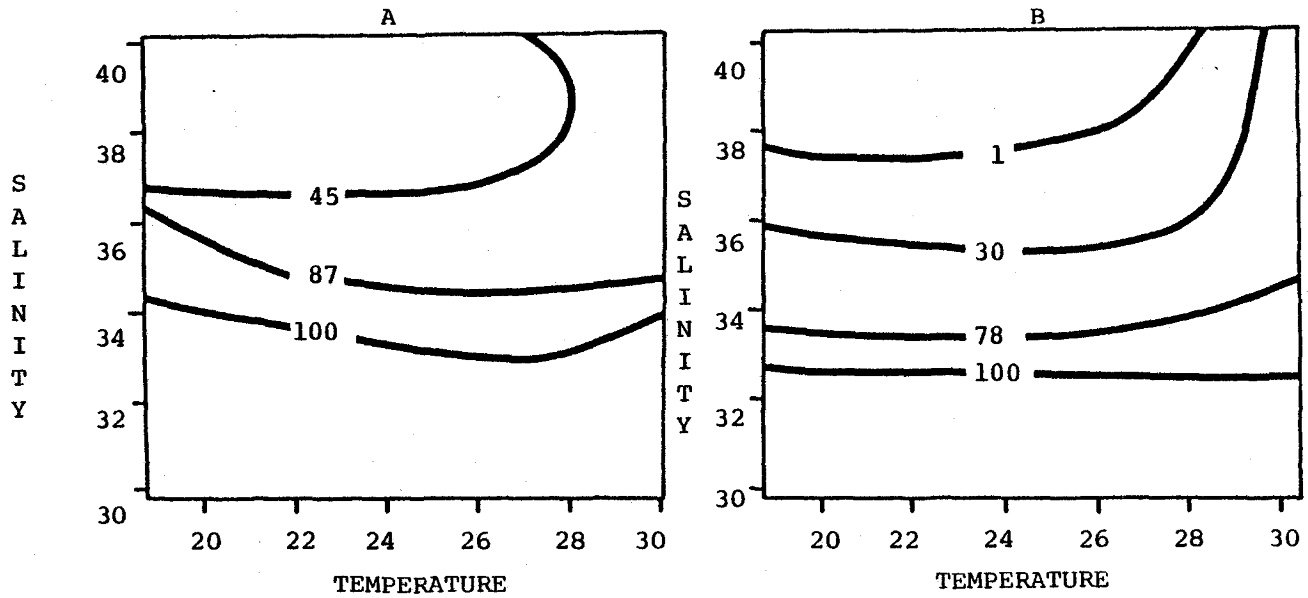
Fig. 7. Response surface diagrams predicting mean percent survival of embryos exposed to artificial sea salts/distilled water in Embryo Bioassay 3.



A = 48 hrs exposure

B = 72 hrs exposure

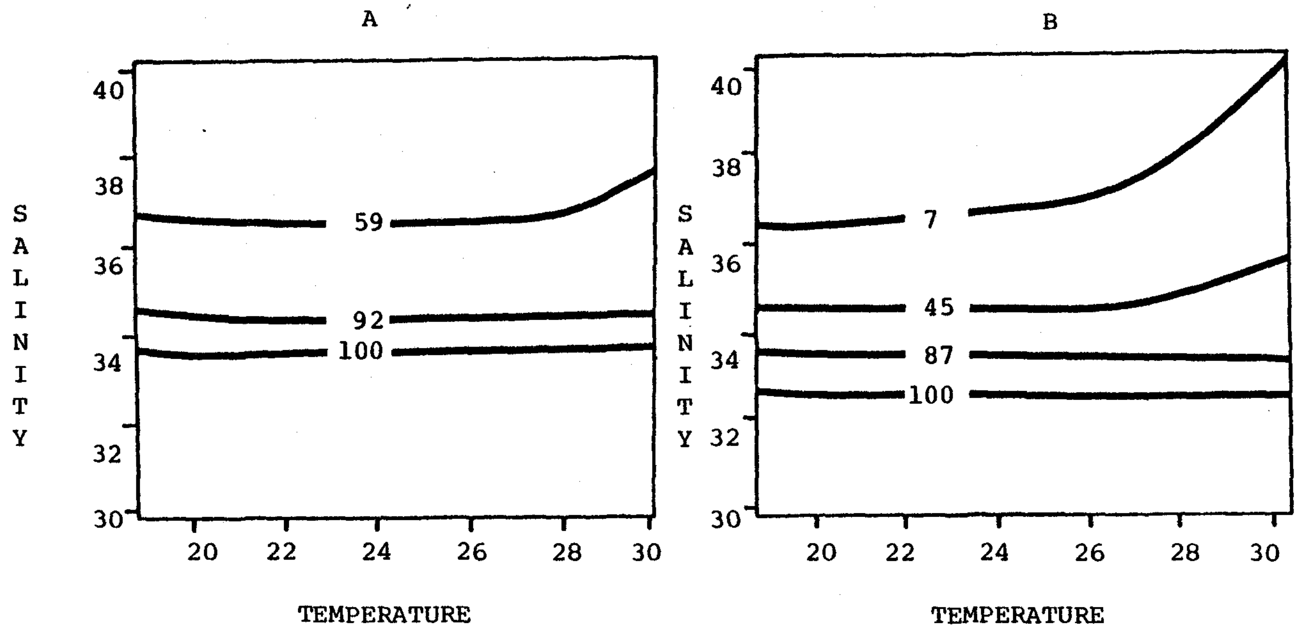
Fig. 8. Response surface diagrams predicting mean percent survival of embryos exposed to dome salt/ Brazos River water in Embryo Bioassay 3.



A = 48 hrs exposure

B = 72 hrs exposure

Fig. 9. Response surface diagrams predicting mean percent survival of embryos exposed to dome salt/ distilled water in Embryo Bioassay 3.



A = 48 hrs exposure

B = 72 hrs exposure

Fig. 10. Response surface diagrams predicting mean percent survival of embryos exposed to artificial sea salts/Brazos River water in Embryo Bioassay 3.

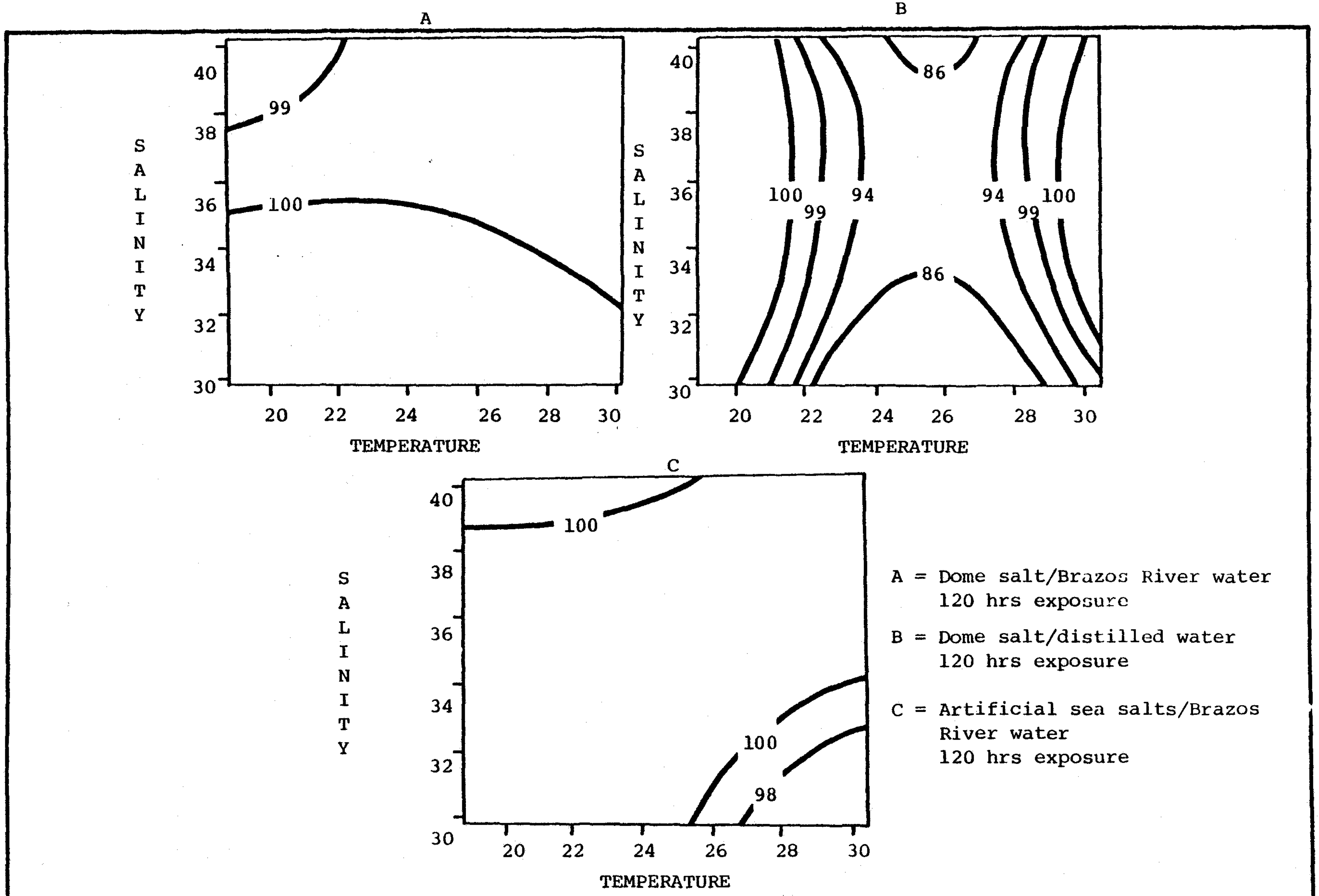


Fig. 11. Response surface diagrams predicting mean percent survival of larval exposed to the brines for 120 hrs in the larval bioassay.

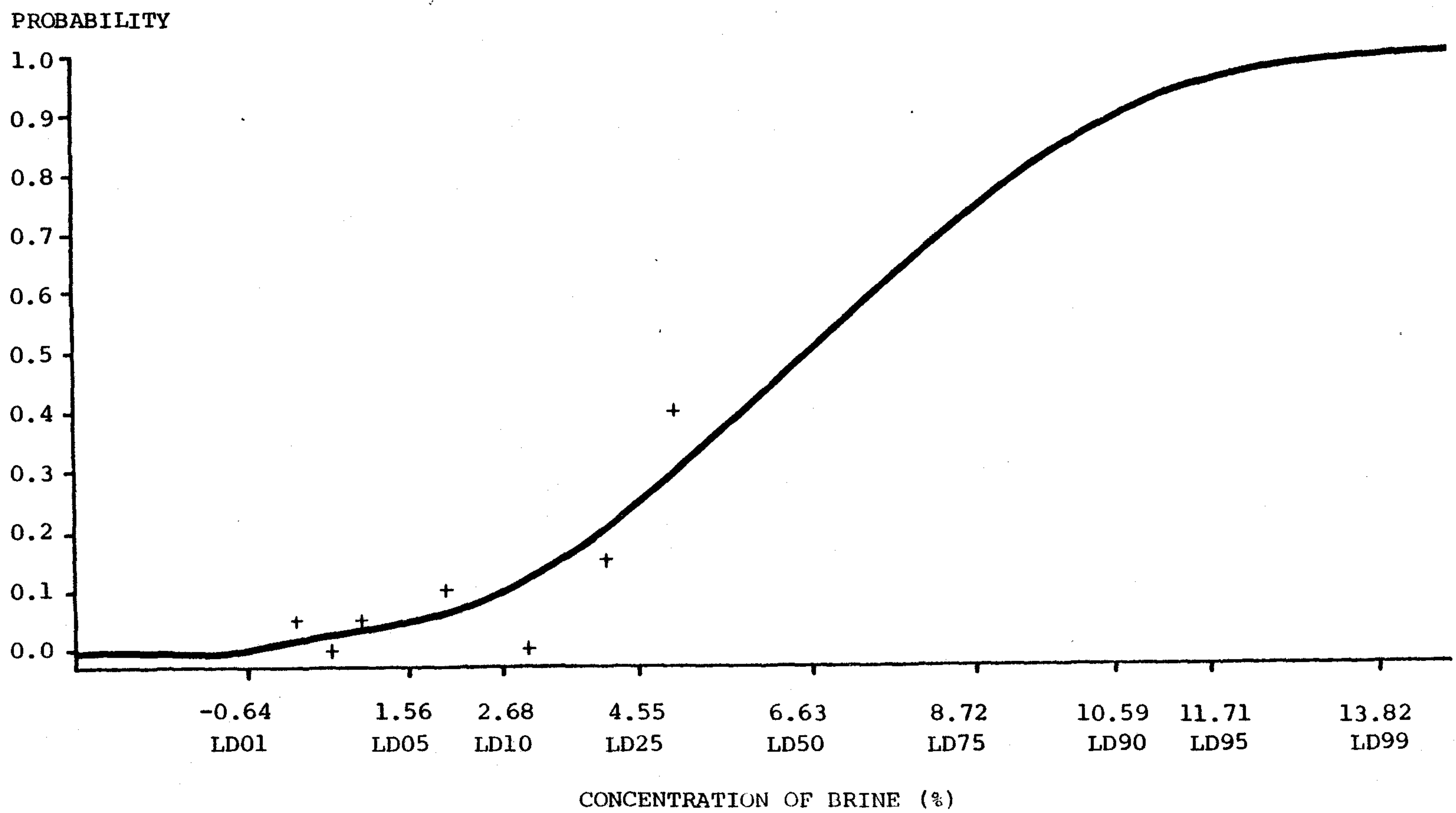


Fig. 12. Probit analysis on survival of juvenile redfish exposed to acute concentrations of dome salt/Brazos River water for 96 h (Juvenile Bioassay 1).

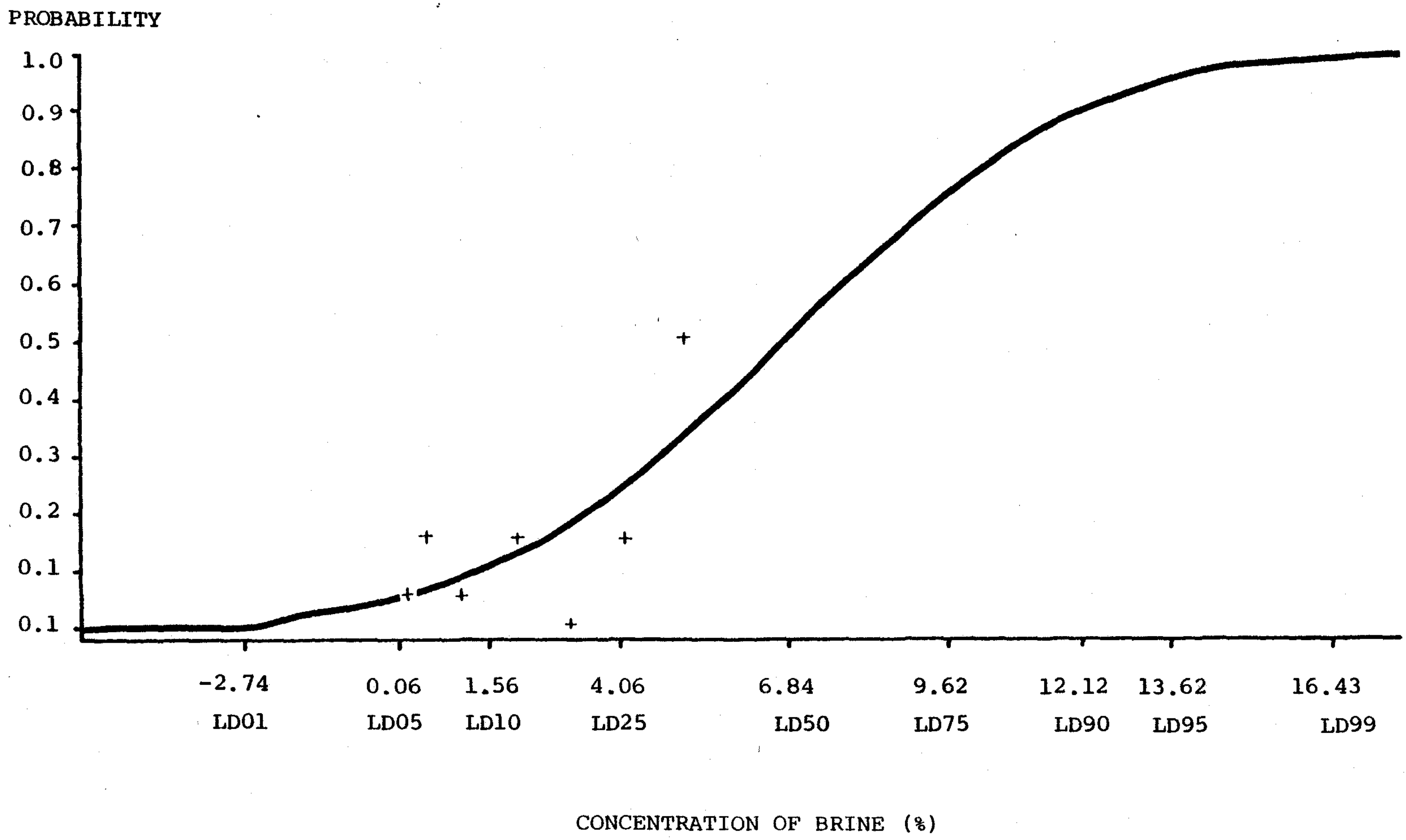


Fig. 13. Probit analysis on survival of juvenile redfish exposed to acute concentrations of dome salt/Brazos River water brine for 7 days (Juvenile Bioassay 1).

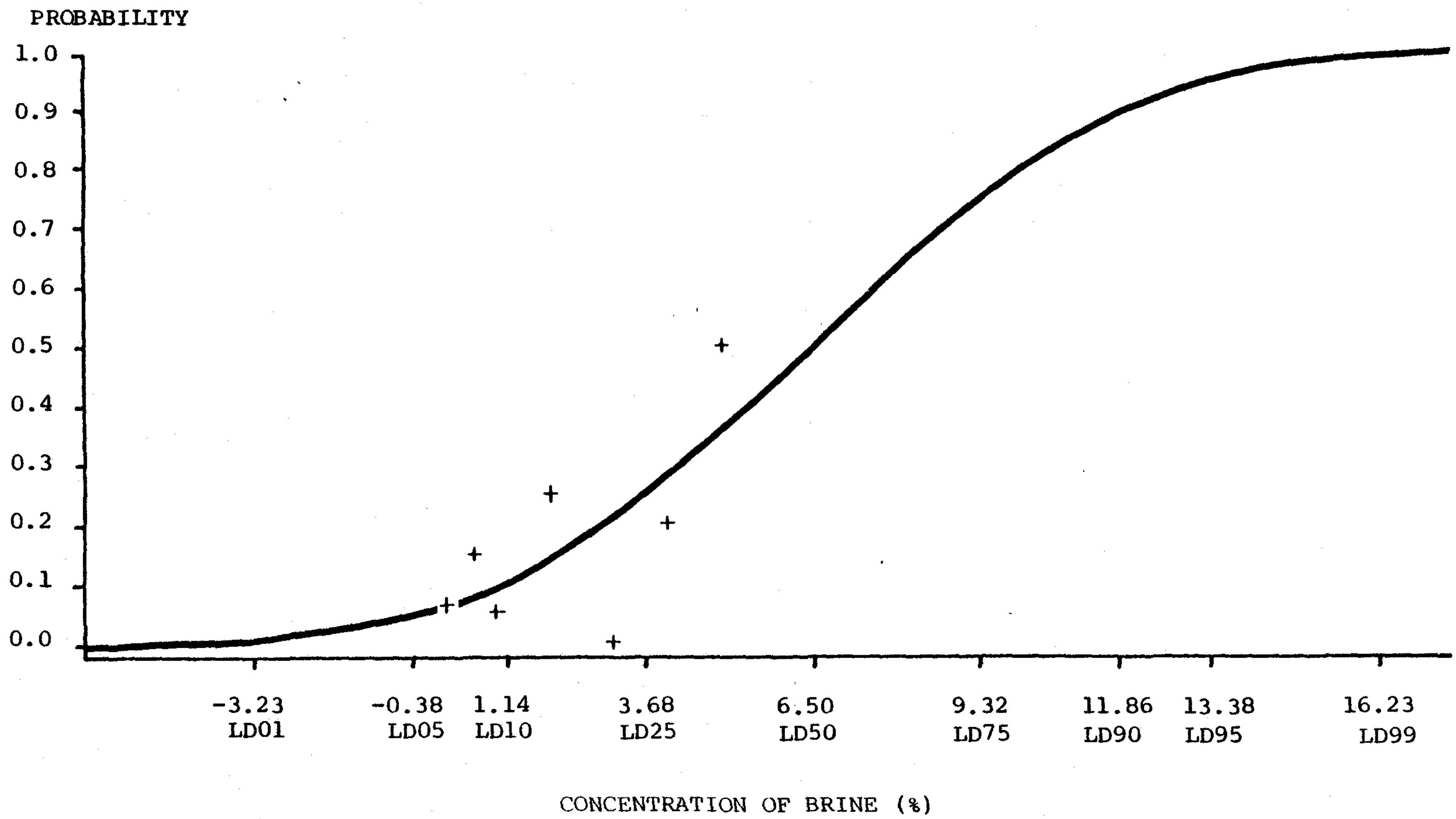


Fig. 14. Probit analysis on survival of juvenile redfish exposed to acute concentrations of dome salt/Brazos River water brine for 14 days (Juvenile Bioassay 1).

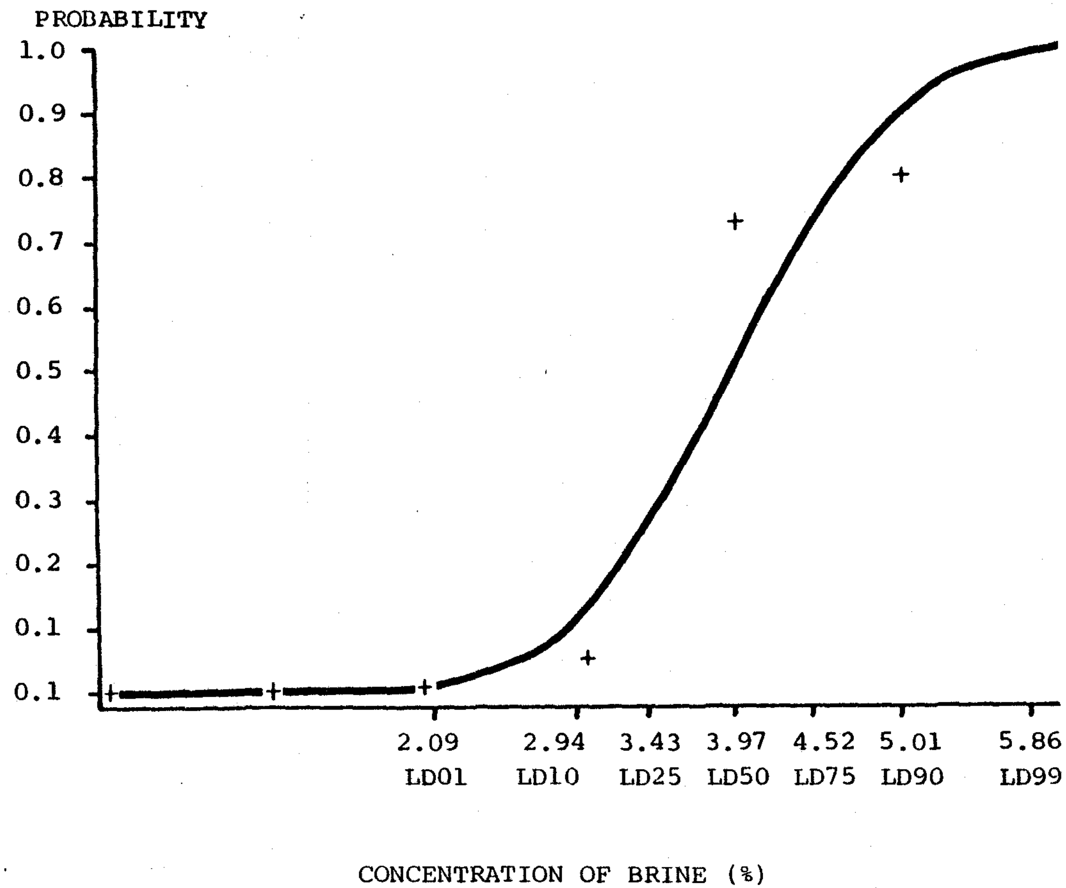


Fig. 15. Probit analysis on survival of juvenile redfish exposed to acute concentrations of dome salt/Brazos River water brine for 24 h (Juvenile Bioassay 2).

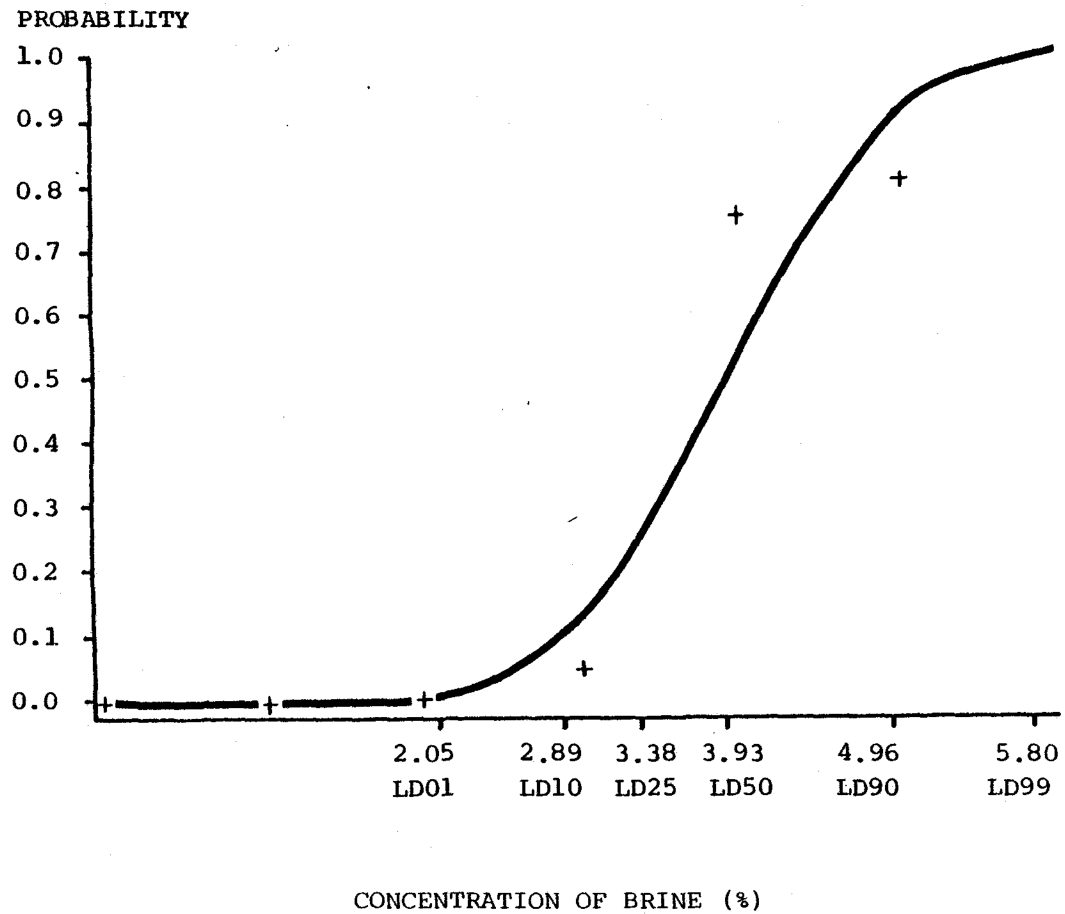


Fig. 16. Probit analysis on survival of juvenile redfish exposed to acute concentrations of dome salt/Brazos River water brine for 48 h (Juvenile Bioassay 2).

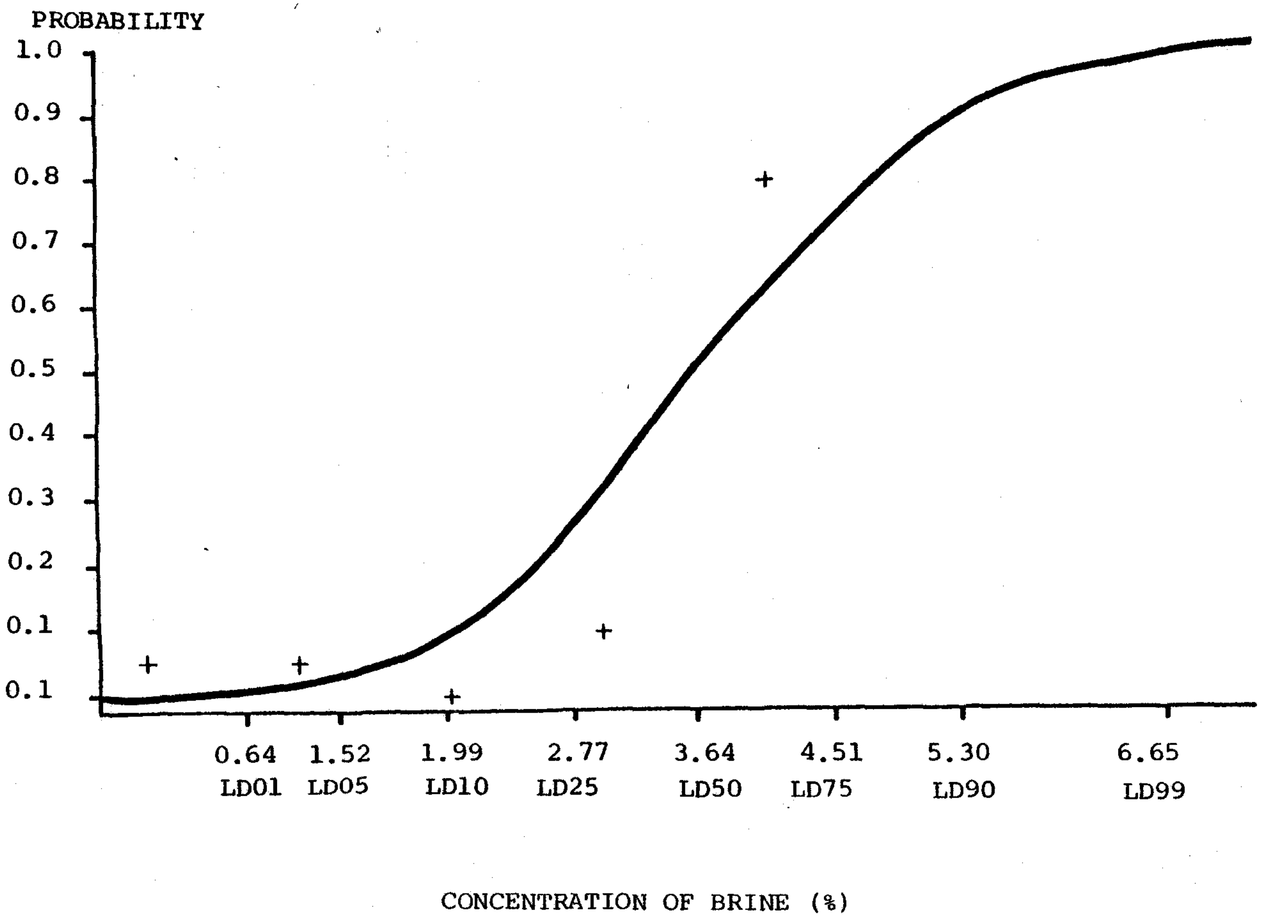


Fig. 17. Probit analysis on survival of juvenile redfish exposed to acute concentrations of dome salt/Brazos River water brine for 96 h (Juvenile Bioassay 2).