JOB PERFORMANCE REPORT

STATE:	California		
PROJECT TITLE:	Salton Sea Sport Fish Study	**PROJECT NUMBER:**	F-48-R-l
STUDY TITLE:	Salinity Tolerance of Salton Sea Sport Fishes	**STUDY NUMBER:**	II
JOB TITLE:	Maximum Salinity Tolerance of the Eggs, Larvae, and Adult Life Stages of Salton Sea Sport Fish	**JOB NUMBER;**	I
PERIOD COVERED:	July 1, 1987-June 30, 1988		

I. SUMMARY

Anisotremus davidsonii acclimated to 40 ppt Salton Sea water spawned with a photoperiod of 13 L/11D and 21°C. The maximum fertilization success was 86.4%. The results of the 96 hr bioassays indicated a significant effect of salinity with higher salinities showing lower survivorship. Survivorship in 35 ppt and 40 ppt was not significantly different, however, 45 ppt was distinctive as were 50 and 55 ppt. Anisotreumus davidsonii acclimated to 45 ppt Salton Sea water also spawned with the same temperature and photoperiod regime. Arrested development occurred during blastulation. The eggs remained in the developmental stage for 10 to 48 hrs after which all eggs died.

II. BACKGROUND

In the 1950's, a number of fish species were introduced into the Salton Sea, a large saline lake in Southern California (Whitney 1961). Several of these species (orangemouth corvina, Cynoscion xanthulus; bairdiella, Bairdiella icistia; and sargo, Anisotremus davidsonii) have fomed reproductive populations that provide one of the highest quality fisheries in the State (Black 1983). The Salton Sea has an ionic composition different from that of ocean water (Carpelan 1961, Young 1970) and its overall salinity, now approximately 41 ppt, was predicted to rise at a rate of 1 ppt every three years (U.S. Department of Interiror and the Resources Agency of California, 1969). A more recent study (University of California, Los Angeles, Environmental Science and Engineering 1982) has made predictions for surface elevation and salinity by the year 2000 based on different energy and water conservation scenarios. The development of Salton Sea solar and geothermal energy resources, as well as recently implemented water conservation and possible water transfer, necessitates that studies be conducted to determine the impact of increasing salinities on the survival of the sportfishery.

Brocksen and Cole (1972), Lasker (1972), and Kay (1975, 1976) found that 40 ppt salinity exceeds the upper tolerance limits of Salton Sea fish during embryonic and larval development. The Vantuna Research Group of Occidental College also found similar results when using artificial fertilization after an injection with pregnant mare serum. However, contradictory results were obtained when field collected fertilized eggs were transported to the laboratory and exposed to Salton Sea water of 38, 40, and 42 ppt salinity (Matsui 1986). These yolk sac larvae continued to thrive and metamorphosis was achieved, and the fish were successfully maintained by us in the laboratory for

a period of two months. The studies by May (1975, 1976) and Lasker (1972) utilized artificially fertilized eggs from either females ripened in the field or induced to ovulation and hydration by injection with PMS (Haydock 1971). The stripping of eggs, as well as the use of mammalian gonadotropins, to induce oogenesis not only significantly reduces fertilization success but also decreases egg viability (Hunter, pers. comm.; Leong, pers.comm.; and Prentice, pers. comm.). Because the former author's experimental conclusions were based upon an extremely small sample size (SO-100 eggs) while utillizing eggs vith potentially decreased viability, their experimental results should be investigated further. The substantiation of salinity levels at which mortality will occur to the various life stages of tested sportfish are important if methods of salinity control are to be initiated for preservation of a self-sustaining or a hatchery maintained sportfishery.

III. OBJECTIVES

Determine the lethal salinities of Salton Sea vater for each life stage of Anisotremus davidsonii.

IV. PROCEDURES

LABORATORY

Experimental Approach

Salinity bioassays were selected as the best available technology to estimate the tolerance of organisms to total dissolved solids within the relatively short span of the contract.

Definition and Description

The term "bioassay" can be used to designate a variety of studies. During the course of these studies, the term "bioassay" was used to indicate 1) the suitability of the environmental condition for aquatic life and, 2) favorable and unfavorable concentrations or levels of such environmental factors as dissolved oxygen, pH, temperature, ammonia and total dissolved solids in Salton Sea water. The salininty bioassays were further designated as being:

1) Short term (acute) definitive, 96-hour tests for eggs and larvae.
2) Long term (chronic) acclimation to increasing levels of total dissolved solids for fish while determining:
   a. Adult reproductive potential with increasing total dissolved solids (35, 40, 45, 50, and 55 ppt).
   b. Fertilization and hatching success in increasing levels of total dissolved solids.
   c. 30 day grow out of successfully fertilized eggs in the increasing levels of TDS.
3) Static bioassays were conducted on adults as well as egg and larval forms. Water quality (ammonia, pH, temperature, dissolved oxygen) was monitored daily. The adult tanks also had biological, ultraviolet and sand filtration to aid in maintaining water quality.

Preparation and Characteristics of Salton Sea Test Water

The chemical composition of Salton Sea water is a dynamic product of a multitude of factors which results in significant chemical and physical variations throughout the year. The salinity bioassay tests conducted during the course of these studies required the production of Salton Sea water at various concentrations of total dissolved solids and required an efficient

reliable method of measuring TDS during the bioassays. Reverse osmosis vas utilized to produce vater equivalent in ionic composition to that of the Salton Sea. This method is basically a molecular sieve process in which salt water and fresh water are separated by a semipermeable membrane. By pressurizing the salt water, the normal osmotic pressure gradient was overcome and fresh water was forced across the membrane, leaving salt water concentrate.

The relationship between conductivity (mhos/cm) and TDS was established on samples of reverse osmosis produced water which represented the full range of concentrations of concern (35,000-55,000 mg/1). The standard water samples were analyzed both for conductivity and for TDS by sumation of the components. The resultant data were analyzed by regression analysis:

TDS =.528(conductivity) + 101. R2= .9797.

Field Collection, Transportation and Acclimation of Adult Fish

Adult fish from the Salton Sea were collected and transported to our Redondo Beach Laboratory in order to conduct the salinity bioassays. A coordinated effort by the California Department of Fish and Game, local fishing guides and laboratory personnel from the Vantuna Research Group enhanced the collection of a sufficient number of Anisotremus davidsonii for the salinity bioassays. Collection of fish from the Salton Sea during previous years of studies indicated that hook and line was the only feasible method of collection. Gillnet, trammel net and otter trawls were not used to collect the fish due to the increased levels of abrasion or opercular damage associated with these methods. Because abrasions as a result of stress from handling increased susceptibility to infection, each fish was injected with the wide spectrum antibiotic Chloramphenicol (.25 mg/lb) in the dorsal musculature. Other precautions were taken to insure the survival of the fish.

The synthetic sea salt Marine Environment was used to transport the fish back to the Redondo Beach Laboratory rather than Salton Sea water which has significantly higher levels of dissolved organic material and decreases the chances of survival while transporting already stressed fish (John Prentice, Texas Parks and Wildlife, pers. comm.). In addition, to reduce the chance of abrasion each sargo was individually placed in a plastic bag filled with the oxygenated synthetic sea salts and placed in a thermally insulated container. Since the Salton Sea varies in salinity between 38-41 ppt depending upon season and location on the sea, we mixed the synthetic sea salt in a solution of 37 ppt so that we could slowing acclimate the the fish to a lower salinity regime. Once the fish reached the Redondo Beach Lab the fish were placed in the 3000 gallon aquaria on a low "rate" of flow with Pacific Ocean water and treated with a wide spectrum antibiotic, Prefuran, for a period of one week. During this period of antibiotic treatment the need for daily water changes prohibits the use of Salton Sea water. If lesions occurred after the period of antibiotic treatment, cultures and blood samples were analyzed by Dr. Hose of the Vantuna Research Group and Dr. Britt of the Los Angeles County Department of Health Services. Sensitivity testing was performed indicating the range of antibiotics that would be suitable for treatment. After the abrasions healed, the fish were transfered to 35 ppt Salton Sea water. Often times the fish required an extended acclimation period (two months) until they were feeding and behaving normally. After the acclimation period, the adult fish can be acclimated to 40, 45, 50, and 55 ppt Salton Sea water. One experimental tank of fish was progressively staged and acclimated to each successive salinity, thus requiring up to five months to reach the highest acclimation salinity after the initial acclimation to the laboratory, After acclimating the fish to the appropriate salinity level, the photoperiod and temperature conditons simulating seasonal changes that occur at the Salton Sea were reduced into a six month period. In order to assess the reproductive condition of the adult

fish and to determine the effects of the photoperiod and temperature manipulations in relation to the various salinity regimes, the fish were monitored at least once every month by means of ovarian biopsy (Stevens 1966) to determine developmental stage of the oocytes. The oocytes from tagged females were examined for yolk depositon and their diameters measured to the nearest 0.01 mm with an ocular micrometer at 40x magnification. Males were considered to have fully mature testes when milt was released upon applying light pressure to the abdomen pressure. The eggs from each parental acclimation salinity were subjected to each of the other test concentrations to determine if parental acclimation enhances the survivorship of the fertilized eggs to increasing salinity. Within the relatively short span of the contract, genetic adaptation to increasing salinities cannot be addressed. During the acute and chronic bioassays on eggs and larvae, it was necessary to document by means of photographs and measurements the developmental stages of eggs and larvae under the various temperature and salinity regimes. The measurements for the eggs included chorion diameter, yolk diameter, oil globule diameter, number of oil globules, and perivitelline space. The measurements for the larvae included notochordal length, snout to anus length, and body depth. The larvae that were alive at the end of the 96 hr bioassay were measured and preserved in 5% buffered formalin. Twenty larvae per salinity were examined histologically with observations made on: trunk muscles, cartilages, notochord, digestive tract, liver, pancreas and brain. May (1975) also documented normal and abnormal development of eggs and larvae of Eairdiella icistia but at much lower salinities. Consequently, additional documentation must occur.

Collection of Naturally Spawned *Anisotremus davidsonii* Eggs in the Field and in the Laboratory for Bioassay.

Surface ichthyoplankton samples vere collected vith a standard one meter 333 micron Nitex, conical, plankton net. All tovs vere one minute in duration at a rate of approximately 1/2 meter per second. The samples were placed in buckets with additional water while on board, then transported back to land for sorting to appropriate developmental stage prior to subjecting 100 eggs per 4 liter beaker to 35, 40, 45, 50, and 55 ppt Salton Sea. There vere four replicates of each concentration. Ahlstom's numerical designation of developmental stages (Ahlstrom, 1943) was adopted here (Table 1). Eggs selected for the bioassays were either in late gastrulation (Stage IV) or the blastopore had closed (Stage V). Since there appeared to be a degree of synchronization betveen the sexes as vell as a relatively large number of eggs shed in one reproductive act, not all of the eggs had to be handled to determine the developmental stage. In order to determine the developmental stage as well as the given density of eggs per unit volume of vater a 1 ml Hensen-Stempel pipette was used to vithdrav the eggs. Five replicate pulls of eggs were taken to determine the density. Prior to each 1 ml pull the eggs were gently poured from one beaker to another to obtain equal distribution of eggs in the water column after which the pipette vas placed in the central portion of the beaker and the sample taken. By means of the Hensen-Stempel pipette, approximately 100 eggs per beaker were added to the five salinity regimes vith four replicates each. Density of eggs vas checked intermittently during the setting up of the bioassay to guarantee equal distribution of the eggs in the beaker. If the eggs were in earlier stages of development, the eggs would remain in a temperature controlled environment until they reached Stage IV or V. Collection of the naturally spavned eggs in the laboratory vas

TABLE 1

Ahlstrom's Numerical Designation of Developncntal Stages

Ablstrom Stage	Sub-Stage	Description
I	a	Unfertilized egg
	b	blastodisc
II	a	2 blastomeres
	b	4 blastomeres
	c	8 blastomeres
	d	morula
	e	Blastula, periblast very apparent
III	a	Early gastrula, germ ring encircles as much as 1/3 yolk, embryonic shield rudimentary
	b	Hid gastrula, embryonic shield expands, germ ring encircles as much as 2/3 of yolk
IV		Late gastrula, primitive streak forms
V		Blastopore closes, optic vesicle and Kupffer's vesicle form
VI	a	Somites begin to form; scattered melanophores appear, most dorsally behind optic vesicles, a fev extending posterior along notochord
	b	Lens and otic vesicles form. Tip of tail reaches oil droplet
VII		Tail has moved beyond oil droplet and lifted off yolk; finfold apparent
		Hatching

by means of a flow-through egg collection basket. Eggs were not removed from the basket prior to Stages IV or V. The bioassays were conducted at the parental acclimation temperature. A continuous monitoring temperature probe (TempMentor) was used.

V. FINDINGS

SALINITY BIOASSAY

Sublethal Stress Assessment

The demonstration of survival, growth and reproduction require that stress factors be ascertained with biological endpoints other than death. These determinations of an organism's well-being are made through the study of sublethal effects. "Sublethal effects may be defined as those responses to environmental change, histological, morphological, physiological, or ethological, that may be induced in one stage of development but be expressed in a later stage of organization or development in terms of reduced survival potential" (Rosenthal and Alderdice, 1976).

There are certain critical life stages and activities that should be examined to assess sublethal stress. These life stages should include gametogenesis, spawning ability, fertilization, development, hatching, feeding success, and growth rate. Sprague (1976) in reviewing the literature on sublethal stress concluded that studies related to reproductive cycle or effects on young stages were the most important in defining stress.

1. During the course of our salinity bioassay assessments, adult Anisotremus acclimated to 35, 40, and 45 ppt Salton Sea water demonstrated gametogenesis. These same adults demonstrated varying degrees of reproductive success. As previously mentioned ovarian biopsies of tagged

fish were necessary to determine reproductive state. The handling during these times and the stress induced led to Vibrio sp. infections. The Vibrio sp. was successfully treated with injections of Chloramphenicol (.25 mg/lb) in the dorsal musculature when lesions were readily apparent and Prefuran baths given to fish after netting or handling. The Vibrio infections were a recurrent problem which led to frequent dosages of Chloramphenicol injections for the fish acclimated to 35 ppt Salton Sea water. This may have affected both gametogenesis and fertilization success in these fish. Thirteen spawns naturally occurred in 35 ppt. The first four spawns were relatively low in number (maximum 12,000 eggs) with < 1% fertilization success. This was true of all of the initial spawns by fish not only acclimated to 35 ppt but also 40 and 45 ppt. Although the males were running ripe for a period of weeks prior to the initial spawns, a degree of synchronization was not achieved generally until a number of spawning sequences had occurred. As the number of spawning sequences increased the number of eggs increased. Although the number of eggs spawned increased with time, the fertilization success in 35 ppt remained fairly low (maximum fertilization success 26%). The fertilized eggs were measured and were found to be equivalent in dimension (chorion diameter, oil globule diameter, oil globule number, yolk diameter, and perivitelline space) as those collected in the field. A bioassay was not conducted on these eggs due to the fact that the fertilization success was fairly low and it was felt that since this represented the fourth spawning sequence, subsequent spawns may yield greater numbers of fertilized eggs. Contrary to this, subsequent spawns yielded greater number of eggs but most were unfertilized. Unfortunately by this time, a Vibrio infection was rampant in the tank resulting in Chloramphenicol injections given with increased frequency. Some individuals in the tank remained uninfected and continued to spawn with increased number of eggs being produced, however, the

fertilization success plummeted to 1% of 97,460 eggs. The eggs that were fertilized underwent abnormal cell division and died in late stage two or early stage three. Another factor that may have contributed to the developmental abnormalities observed in eggs spawned in 35 ppt Salton Sea water is the decrease in levels of calcium ions. Standard Salton Sea water has significantly higher levels of calcium ions than ocean water. Seasonal variations in calcium concentration range from 920 mg/l to 1030 mg/l. Diluting standard Salton Sea water to achieve 35 ppt salinity dropped the calcium ion concentration to 760 mg/l. Although these levels are higher than those found in ocean water, the decreased concentrations may affect sperm mobility and fertilization success (Yamomota 1976) and the functioning of microtubules. Recently collected Anisotremus are now being acclimated to 35 ppt Salton Sea water so that we may attempt a bioassay in the near future.

2. Adult Anisotremus acclimated to 40 ppt Salton Sea water with a photoperiod of 13 L/11 D and 21°C temperature regime spawned nine times during a period of two months with a maximum fertilization success of 86.4%. The embryos hatched in 48 hrs with yolk sac absorption occurring in 96 hrs at 21°C. Laboratory and field bioassays utilizing naturally fertilized eggs from 40 ppt parental acclimation salinity and placing 100 eggs per beaker with four replicates per salinity yield similar results (Fig. 1). The results of the laboratory bioassays for eggs from 40 ppt parental acclimation indicated that in 35 ppt 92% of the eggs hatched, however, 83% had completed yolk sac absorption and were alive at the end of 96 hrs. In 40 ppt 94% of the eggs hatched with 72% completing yolk sac absorption and were alive at the end of the 96 hr bioassay. In 45 ppt 97% of the eggs hatched, 93% died prior to yolk sac absorption and only 4% were alive at the end of the 96 hrs. In 50 ppt 94% of the eggs hatched, all died prior to the end of the 96 hrs. In 55 ppt the results were very

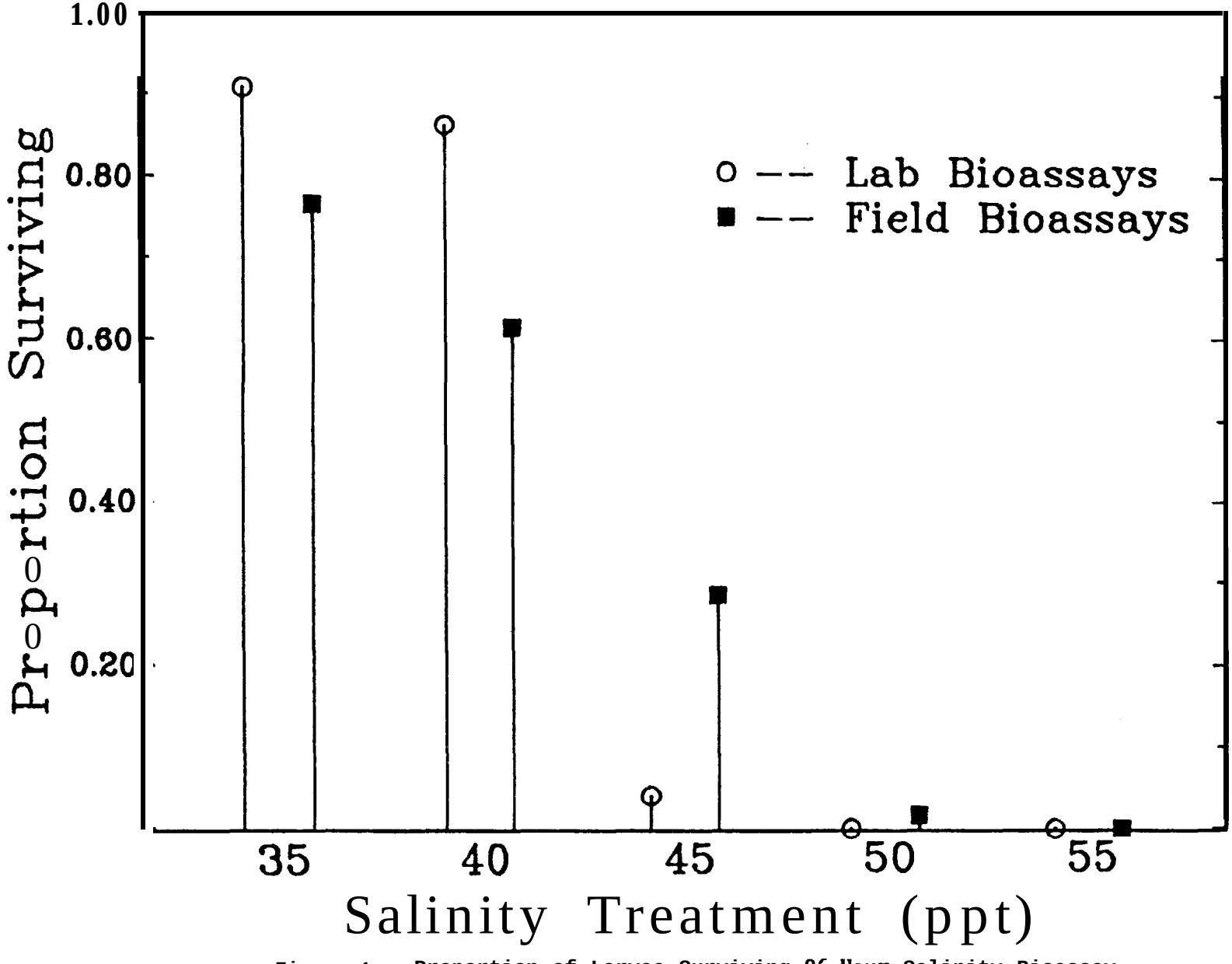

Figure 1. Proportion of Larvae Surviving 96 Hour Salinity Bioassay

similar, vith 95% of the eggs hatching, however, all died prior to the end of the 96 hr close dovn of the bioassay. In all of the salinity regimes, approximately 2-5% of the eggs died prior to hatching.

As stated previously the results of the field bioassays were similar to the findings for the laboratory bioassays. In 35 ppt, 76% completed yolk sac absorption and vere alive at the end of the 96 hr bioassay. In 40 ppt 61% survived the 96 hrs. In 45 ppt, 24% of the larvae survived through the end of the 96 hr bioassay. This represents a significant increase in the survival of field-collected eggs from that seen in laboratory. In 50 ppt only tvo larvae survived the 96 hrs. In 55 ppt, all larvae died prior to yolk sac absorption and prior to the end of the 96 hrs. An analysis of variance conducted on the laboratory and field bioassay data indicated a significant main effect of salinity with higher salinities shoving lower survivorship ($F(4, 10) = 246$), $P < .0001$). 35 ppt and 40 ppt were not significantly different, however, 45 ppt was distinctive in both lab and field experiments as vere 50 and 55 ppt. An analysis of variance performed on the lab and field data also indicated a significant lover average survival in the lab ($F(1.10) = 29.2$, $p < .0003$). This may be indicative of nutritional differences in the lab and field. There was no literature on gut analyses for Anisotremus, however, when transporting nevly caught fish to the lab from the Salton Sea, the sargo often regurgitated barnacles as vell as fish. Arthropods are often fed to fish to provide carotenoids which are important for oogenesis. In the lab we fed the sargo frozen anchovy, smelt and shrimp. Although the eggs produced in the lab were equivalent in dimension to those collected in the field, they may not have been comparable.

The three morphological measurements taken on 20 larvae per replicate per salinity were notochordal length, snout to anus length, and body depth. Neither body depth nor snout to anus length varied significantly

in 35, 40, or 45 ppt, however, notochordal length did show significant differences. Optimal growth was achieved in 35 and 40 ppt in both the lab and field (Fig. 2) and a significant reduction in growth was demonstrated by those larvae in 45 ppt ($F(2,6) = 8.23$, $p < .02$).

Histological examination of the laboratory raised bioassay larvae was conducted by Dr. Stephen Goldberg. Histological investigation has proven to be a useful method for determining the relative health of larval fishes raised under both laboratory and natural conditions (Umeda and Ochial 1975; Ehrlich et al. 1976; Groman 1982; O'Connell 1976, 1980, 1981; Theilacker 1978, 1986). The notochord, cartilage, trunk musculature, digestive tract, liver and pancreas were studied in the sargo. The liver and pancreas were only occasionally present due to the relatively early stage of larval development. The other structures were found to have developed normally in all salinity regimes.

3. The Anisotremus acclimated to 45 ppt had four natural spawns with a photoperiod of 13L/11D at 21°C. There was a relatively low fertilization success (13.8%). The fertilized eggs were measured and were comparable in dimension to field collected eggs. The developing eggs of sargo acclimated to 40 ppt salinity required an average time of 8-10 hr to complete gastrulation at 21°C. The eggs fertilized by sargo acclimated to 45 ppt required 48 hrs to achieve gastrulation then soon after all eggs died. Although the fish acclimated to 45 ppt salinity had been treated with antibiotics initially, no antibiotics had been administered for a period of two months prior to the natural spawns, consequently, it is doubtful that the arrested development was a result of the use of antibiotics. The temperature regime was held constant (21°C) *to* avoid developmental problems due to fluctuating temperature. Also, great care was taken *to* maintain other water quality parameters (dissolved oxygen, pH, ammonia) at optimal conditions. A general mineral analysis was

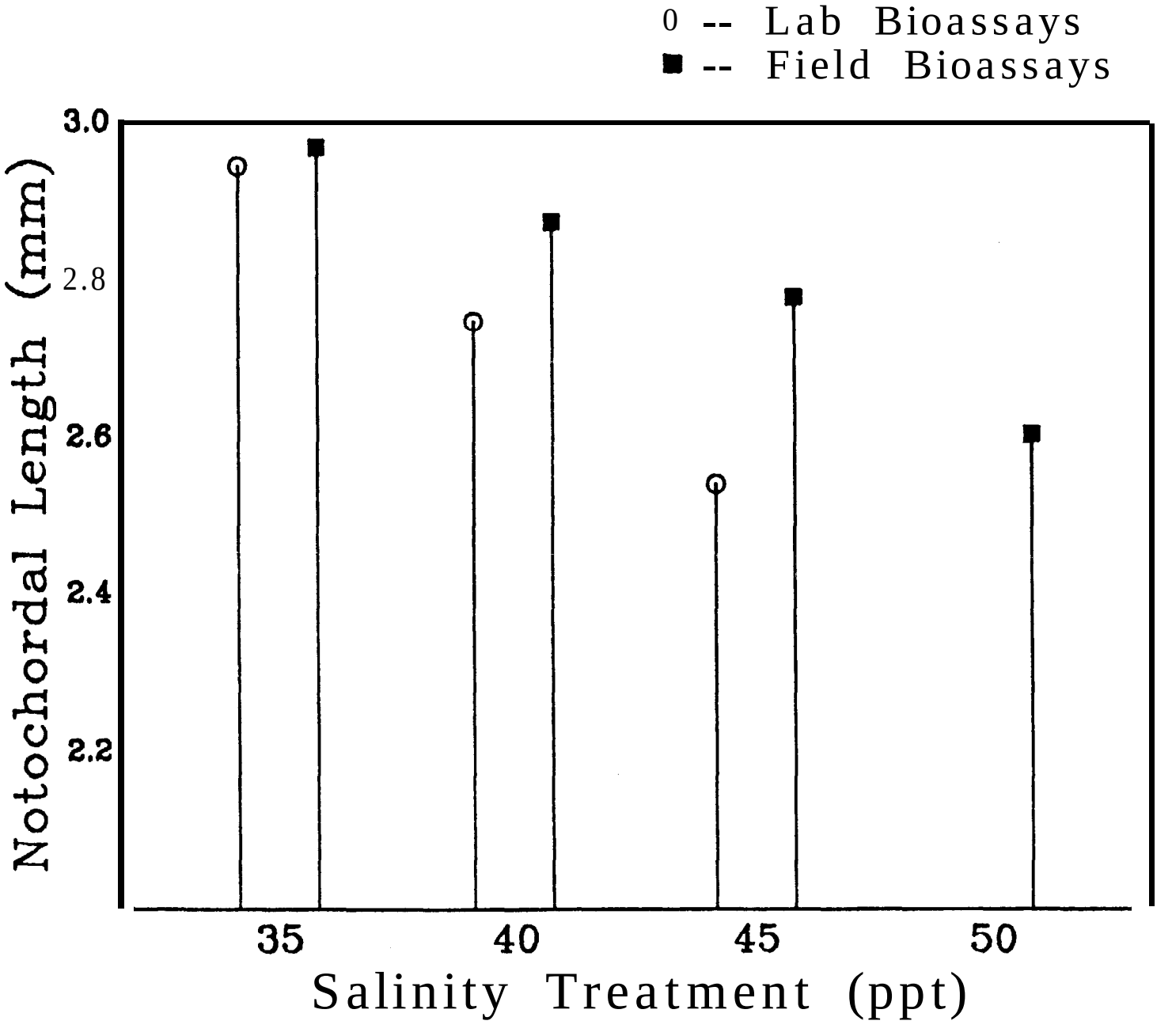

Figure 2. Notochordal Length of Larvae Surviving the 96 Hour Salinity Bioassay

performed to determine the relative proportions of the major anions and cations. Calcium, a very important ion involved in sperm mobility and microtubule mobility, did not vary significantly from levels in 40 ppt. It is believed that the arrested development and ultimate death of the eggs from adult sargo acclimated to 45 ppt was due to an inabilitiy of the eggs to withstand the increase in salinity.

Adult Anisotreumus have been acclimated in 50 ppt Salton Sea water. Their general feeding levels, behavior patterns, and level of activity do not differ from those fish acclimated to 45 ppt. No spawns have occurred.

A Question of the Applicability of the Bioassay Results:

The protocol for this bioassay was based on allowing the fertilized eggs to rest undisturbed for 8-10 hrs at 21°C prior to collection for the bioassay. This time guideline was determined during previous experiments when eggs were collected during Stage II (blastula) and placed in the five designated concentrations of Salton Sea water. These previous experiments yielded dubious results with the majority of eggs dying in all salinities. This was not testing the effects of salinity but rather the effects of handling. The previous investigators (Lasker 1971, May 1975) also utilized eggs either in the blastula or morula stages of development with variable results. However, when utilizing eggs in late gastrulation (Stage V) and subjecting these eggs to the five designated salinity regimes, we were no longer looking at the effects of handling but the effects of salinity. The bioassay data do indicate that parental acclimation salinities of 35, 40, and 45 ppt are suitable for gametogenesis and that fertilization occurs at these salinities. The bioassays also indicate that the larvae can survive 40 ppt salinity, however, 45 ppt is a critical salinity which relatively few larvae can survive. Contrary to this the eggs spawned by parents acclimated to 45 ppt were never able to complete

gastrulation. This may point to the limited applicability of the bioassay data utilizing eggs after late gastrulation but using eggs prior to late gastrulatin would only cause dubious results due to the effects of handling and not the effects of salinity.

VI. RECOMMENDATIONS

1. Salinity bioassays from 35 ppt parental acclimation salinity at 21°C need to be readdressed.
2. Salinity bioassays on all parental acclimation salinities with increased temperature regimes need to be conducted to determine the combined effects of temperature and salinity.
3. Salinity bioassays conducted with a parental acclimation salinity of 42.5 ppt to determine if hatching success and larval development can occur.
4. These salinity bioassays will be conducted concurrently while additional Cynoscion xanthulus are being collected and acclimated to appropriate parental acclimation salinities for lethal salinity determinations. The bioassays must be conducted on the orangemouth corvina and the data on sargo should not be extrapolated to other species since previous investigators (Brocksen and Cole 1972) have demonstrated that there are species specific responses to increasing salinity and temperature regimes.
5. Additional histological examinations need to be conducted on larval stage from the salinity bioassay 30 day grow-outs to determine the developmental stage when organisms are morphologically capable of osmoregulation.

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