Corvina
906

# FINAL PERFORMANCE REPORT

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## INLAND AND ANADROMOUS SPORT FISH MANAGEMENT AND RESEARCH

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Subproject VI, Salton Sea Sport Fish Research

Study No. 2, Salinity Tolerance of the Egg and Larvae

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# SALINITY TOLERANCE OF CYNOSCION XANTHULUS

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## ABSTRACT

Eggs and larvae of the sciaenid fish Orangemouth Corvina, Cynoscion xanthulus, were obtained from fish matured in the laboratory by photoperiod and temperature manipulation and induced to spawn by LH-RHa injections. The effects on gametes of parental salinity acclimation were also investigated. Successful gonadal maturation occurred in adult corvina acclimated to 35 through 50 ppt salinity. Significant growth also occurred in individuals acclimated to 35 through 50 ppt salinity. Reproductive failure of this species will occur with increased salinity due to insufficient osmotic capability in the eggs and larvae.

The Salton Sea is a saline lake located in the Imperial Valley California extending into both Riverside and Imperial Counties. The Sea was created in 1905 when the Colorado River was accidentally diverted into the Salton Basin and was allowed to flow unchecked for two years (Carpelan 1961). At that time the surface level of the sea was 65 m below sea level. The water level declined rapidly due to evaporation until 1925 when it was 83 m below sea level. Since 1925, the diversion of Colorado River water into the Imperial Valley for agriculture has raised the level of the Salton Sea to a current elevation of 75 m below sea level. It is 53 km long, 23 km wide, and approximately 14 m deep. Although the water level of the Salton Sea is currently stable, the salinity of the Sea is constantly increasing. About 5 million tons per year of salt is carried into the Sea from its tributaries, but no salt is removed because the Sea has no outlets. The Salton Sea has an ionic composition significantly different from that of ocean water (Carpelan 1961, Young, 1970) and the water temperatures range from 10°C in January to 36°C during July through September (Walker 1961; Matsui, in prep). In the 1950's, several fish species, including Sargo, Anisotremus davidsoni (Steindachner); Bairdiella, Bairdiella icistia (Jordan and Gilbert); and Orangemouth Corvina, Cynoscion xanthulus (Jordan and Gilbert) were introduced into the Salton Sea from the Gulf of California in a joint study program conducted by the California Department of Fish and Game and the University of California, Los Angeles (Walker 1961). These species have formed reproductive populations that provide one of the highest quality fisheries in the State (Black 1988). Although Brocksen and Cole (1972), Lasker et al. (1972), and May (1975a, b) found that 40 ppt salinity exceeds the upper tolerance limits of Salton Sea fish during embryonic and

larval development, recruitment is still occurring at salinity levels above 40 ppt. The development of Salton Sea solar and geothermal energy resources, as well as recently implemented water conservation and water transfer increased the urgency of determining the impact of increased salinity on the reproductive capabilities of the sport fish. The orangemouth corvina (C. xanthulus) was selected for the experiments due to the fact that the corvina, although comprising only 3% of the total sportfish catch, represented the most highly prized of the fish at the Salton Sea (Black 1988).

## MATERIALS AND METHODS

Salinity bioassays were selected as the best available technology to estimate the tolerance of organisms to total dissolved solids within the relatively short span (October 1986 through June 1989) of the study. During the course of the study 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 salinity bioassays were further designated as being:

1) Acute definitive, 96-hr tests for eggs and larvae

2) Chronic acclimation to increasing levels of total dissolved solids for fish to determine:

   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.

3) Static renewal 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 test conducted during the course of these studies required the production of Salton Sea water at various concentrations of total dissolved solids (TDS), and required an efficient reliable method of measuring TDS during the bioassays. Reverse osmosis was utilized to produce water 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/l). The water samples were analyzed both for conductivity and for TDS by summation of the components.

The resultant data were analyzed by regression analysis:

$$TDS = 0.528 \text{ X (conductivity)} + 101 \quad (R^2 = 0.9797)$$

## Field Collection, Transportation, and Acclimation of Adult Fish

Adult corvina from the Salton Sea were collected and transported to the Southern California Edison Research and Development Laboratory located in Redondo Beach, California in order to conduct the salinity bioassays. Collection of fish from the Salton Sea during previous years of studies at Occidental College 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 to the lab 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 fish was transported in a thermally insulated container. Since the Salton Sea varied in salinity between 38-42 ppt depending upon season and location on the Sea, the synthetic sea salts were mixed in a solution of 39 ppt so that the fish could be slowly acclimated to a lower salinity regime. Once the fish reached the lab, they were placed in 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 prohibited the use of Salton Sea water. If lesions occurred after the period of antibiotic treatment, cultures and blood samples were analyzed. Sensitivity testing was performed indicating the range of antibiotics that would be suitable for treatment. After abrasions healed, the fish were transferred 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 were acclimated to 35, 40, 45, 50, 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 condition 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 (Steven 1966) to determine developmental stage of the oocytes. The oocytes from tagged females were examined for yolk deposition and their diameters measured to the nearest 0.01 mm with ocular micrometer at 40X magnification. The oocytes were considered fully mature when the mean diameter of the largest oocytes was greater than 0.5 mm and were injected with LH-RHa. This occurred when the photoperiod and temperature reached 15.5L:8.5 D and 25-28$^{o}$C. Males were considered to have fully mature testes when milt was released upon applying light pressure to the abdomen.

LH-RHa (des-gly$^{10}$-[D-Ala$^{6}$]-luteinizing hormone-releasing hormone (1-9) ethylamide, Sigma Chemical Company, St. Louis, Missouri) was dissolved in saline solution and injected intramuscularly (Prentice and Thomas 1987). The fish were then allowed to spawn naturally. 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 study, genetic adaptation to increasing salinities could not be addressed. The measurement 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.

Collection of laboratory spawned eggs was done by means of a flow-through egg collection basket. Eggs were not removed from the basket prior to developmental Stages IV (late gastrulation) or Stage V (when the blastopore closes). Ahlstrom's numerical designation of developmental Stages (Ahlstrom 1943) was adopted here (Table 1). If the eggs were in an earlier stage of development, the eggs would remain in a temperature controlled environment similar to parental salinity and temperature until they reached Stage IV or V. Approximately 100 eggs per beaker were added to the five salinity regimes with four replicates each by means of a 1 ml Hensen Stempel pipette. Density of eggs was checked intermittently during the setting up of the bioassay to guarantee equal distribution of the eggs in the beaker.

## RESULTS

Spawns were achieved with a photoperiod and temperature manipulation of 15.5L, 25°C. Corvina in 35 and 40 ppt Salton Sea water spawned 20 to 30 hours after an injection with LH-RHa (Table 2). Although corvina acclimated to 45 and 50 ppt Salton Sea water were also injected with LH-RHa after successful gonadal maturation, no spawns occurred.

### Egg Mortality

Approximately 7% of the eggs died in these bioassays. This percentage was identical for animals with a parental acclimation salinity of 35 and 40 ppt ($p = 0.875$). There was, therefore, no main effect of acclimation salinity on egg mortality. Figure 1, however, shows that there was a significant interaction between acclimation salinity and the treatment salinity factor: treatment salinity affected mortality only for eggs that were acclimated to the lower salinity regime ($p < .001$). The mortality differences among eggs that were acclimated to 40 ppt were not significant ($p = 0.361$). Egg mortality was higher at an acclimation level of 40 ppt than 35 ppt only for low treatment salinities (35 ppt and 40 ppt. $p < .01$). Egg mortality was the same at the two acclimation levels for treatment salinity of 45 ppt ($p = 0.744$). At 50 ppt treatment salinity, the effect was reversed: eggs acclimated to 35 ppt had higher mortality than those acclimated at 40 ppt ($p = .007$).

Apparently acclimation of the parent fish to 40 ppt does improve the ability of their eggs to handle high salinities, but it also stresses the eggs leading to higher mortality under non-elevated salinities. Comparing eggs spawned by adults at 35 ppt and

developing at that same salinity with those spawned and developing in 40 ppt, the higher salinity regime would increase egg mortality approximately five times (1.57% versus 8.61%). Salinity stress is cumulative in its effects on embryonic mortality.

## Larval Mortality

There was no main effect of parental salinity ($p = 0.415$) on larval mortality. Mortality was 11.16% for samples that had been acclimated to 35 ppt and tested at 35 ppt and 8.52% for those acclimated and tested at 40 ppt. There was a massive effect of treatment salinity on larval mortality, ($p < .001$) with LD-50 occurring at 45 ppt. Unlike the egg mortality, the effect of treatment salinity on the larvae is virtually identical at the two acclimation levels. If we accept these survival rates for eggs and larvae, we would anticipate that 87.27% of eggs spawned at 35 ppt would survive. This figure drops to 82.87% at 40 ppt. It is reasonable to infer from these data that the optimal salinity regime for Corvina lies below 40 ppt, but that successful reproduction can occur at these tested salinities.

## Larval Morphology

Notochordal length, snout to anus length and body depth decreased with increasing treatment salinities however, the effect is significant only for notochordal length ($p < .001$).

## Adult Acclimation

The adult corvina were able to maintain their body weight as they were acclimated to increasing levels of total dissolved solids of Salton Sea water. Significant growth occurred in individuals acclimated to 35, 40, 45 and 50 ppt Salton Sea water without an incremental increase in feed. In 35 ppt, Salton Sea water the initial mean weight was 3.99 kg resulting in an end mean weight of 5.55 kg. In 40 ppt, the initial mean weight was 2.4 and an end mean weight of 5.20 kg. In 45 ppt, initial mean weight was 3.35 kg with an end mean weight of 5.14 kg and in 50 ppt the initial mean weight was 3.40 kg resulting in an end mean weight of 4.78 kg (Table 3). The food consisted of shrimp, juvenile Tilapia, anchovies, silver smelt and Columbia River smelt at 3-4% wet weight per day.

## Ovarian Biopsies

Ovarian biopsies were performed to assess gonadal development in response to photoperiod and temperature manipulation. Oocyte maturation occurred in the adult corvina acclimated to 35 through 55 ppt water with oocytes reaching 0.5 mm diameter when the photoperiod and temperature reached 15.5L:8.5D and 25-28°C during the acclimation (Figs. 2-6). When biopsied eggs reached the 0.5 mm diameter, an injection of LH-RHa was administered to stimulate spawning. Spawns only occurred in 35 and 40 ppt Salton Sea water. Corvina injected with LH-RHa in 45 and 50 ppt Salton Sea water did not demonstrate increased oocyte development.

## DISCUSSION

Simmons (1957) found sciaenids in the upper Laguna Madre of Texas to be fairly resistant to hypersalinity. Spotted seatrout, Cynoscion nebulosus, occurred at levels up to 75 ppt. Silver perch, Bairdiella chrysura, were found in 45 ppt, and croakers, Micropegan undulatus, were numerous to 70 ppt. Hanson (1970) tested the salinity tolerance of Cynoscion xanthulus young of the year with 96 hour bioassays and found that most corvina survived to 57.5 ppt but all died at 62.5 ppt. These tests were conducted without subjecting the fish to incremental acclimation to higher salinities.

Similarly, Brockson and Cole (1972) determined that the optimal range of salinity was between 33-37 ppt for the juvenile stages of all three species of Salton Sea sport fish and that growth, food consumption, food assimilation, and respiration were adversely affected at the extreme salinities of 29 and 45 ppt, however, whether acclimation was truly achieved during the two-week acclimation and testing period is questionable (Hochachka and Somero 1973).

The knowledge of the effects of increasing salinity and the impact upon the reproductive success of the Salton Sea sport fish is necessary for the management of the Salton Sea water conservation efforts. It has been demonstrated by the relations shown in Table 3 that significant growth occurred in individuals acclimated to 35, 40, 45, and 50 ppt Salton Sea water. Oocyte maturation occurred in adult corvina acclimated to 35 through 50 ppt and males in these same salinities were running ripe, drumming, and displaying reproductive behavior. Reproductive success was achieved with injections of LH-RHa administered to fish in 35 and 40 ppt yielding natural spawns however no spawning occurred in individuals in 45 or 50 ppt.

Although the adults can survive and grow at salinity levels significantly higher than previously believed, reproductive failure of this species will occur with increased salinities due to insufficient osmotic capabilities in the eggs and larvae. Adult acclimation to increasing salinities has afforded an advantage to the eggs, however, not to larvae. It can be stated that either chronic high salinity ("acclimation") or acute high salinity will significantly increase egg mortality. As the salinity of the Salton Sea increases, the population of corvina will begin to decline due to senescence, fishing pressures and osmotic stress. Since the adults can continue to grow with increasing levels of total dissolved solids in Salton Sea water a population of non-reproductive adults can be maintained for recreational purposes by the development of a hatchery. Optimal stocking size will need to be determined.

## ACKNOWLEDGMENTS

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Table I
Numerical Designation of Developmental Stages (Ahlstom, 1943)

| Ahlstom 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 | Mid 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 few 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 |

**Table 2.** Summary of 1989 Spawning Data For Orangmouth Corvina after injection of LHRHa

| Trial No. Date | Tank (Salinity ppt) | Tag No. | Weight lbs/kg | LHRHa mg * | Sex | Maturity Eggs or Milt | Photoperiod Hours Light | Temperature C | Time Injected | Spawn | Spawn Time (Date) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 July 21 | LMS2 (40) | YW0007 | 13.00/5.90 | 0.74 | F | 0.3mm - 0.5mm | 15.5 | 25 | 1300 | Yes | 1730 (7/22) |
| | LMS2 (40) | YW0008 | 10.25/4.60 | 0.57 | F | 0.3mm - 0.4mm | 15.5 | 25 | 1310 | | |
| | LMS2 (40) | YW0004 | 10.50/4.80 | 0.60 | F | 0.4 | 15.5 | 25 | 1320 | | |
| | LMS2 (40) | YW0010 | 7.50/4.10 | 0.42 | M | Running Ripe. Drumming when handled | 15.5 | 25 | 1335 | | |
| 2 July 26 | LMS2 (40) | YW0002 | 8.00/3.60 | 0.45 | F | 0.4mm - 0.5mm | 15.5 | 25 | 1320 | Yes | 1700 (7/27) |
| | LMS2 (40) | YW0015 | 14.50/6.60 | 0.82 | ? | No eggs tubed | 15.5 | 25 | 1330 | | |
| 3 July 30 | LMS3 (35) | WT0015 | 9.10/3.80 | 0.47 | F | 0.40mm - 0.50 | 15.5 | 27 | 1320 | Yes | 2030 (7/31) 2330 (8/1) |
| | LMS3 (35) | WT0200 | 10.00/4.50 | 0.56 | M | Not running ripe Milt tubed | 15.5 | 27 | 1335 | | |

* Dosage of LHRHa administered was 0.125 mg/kg BW

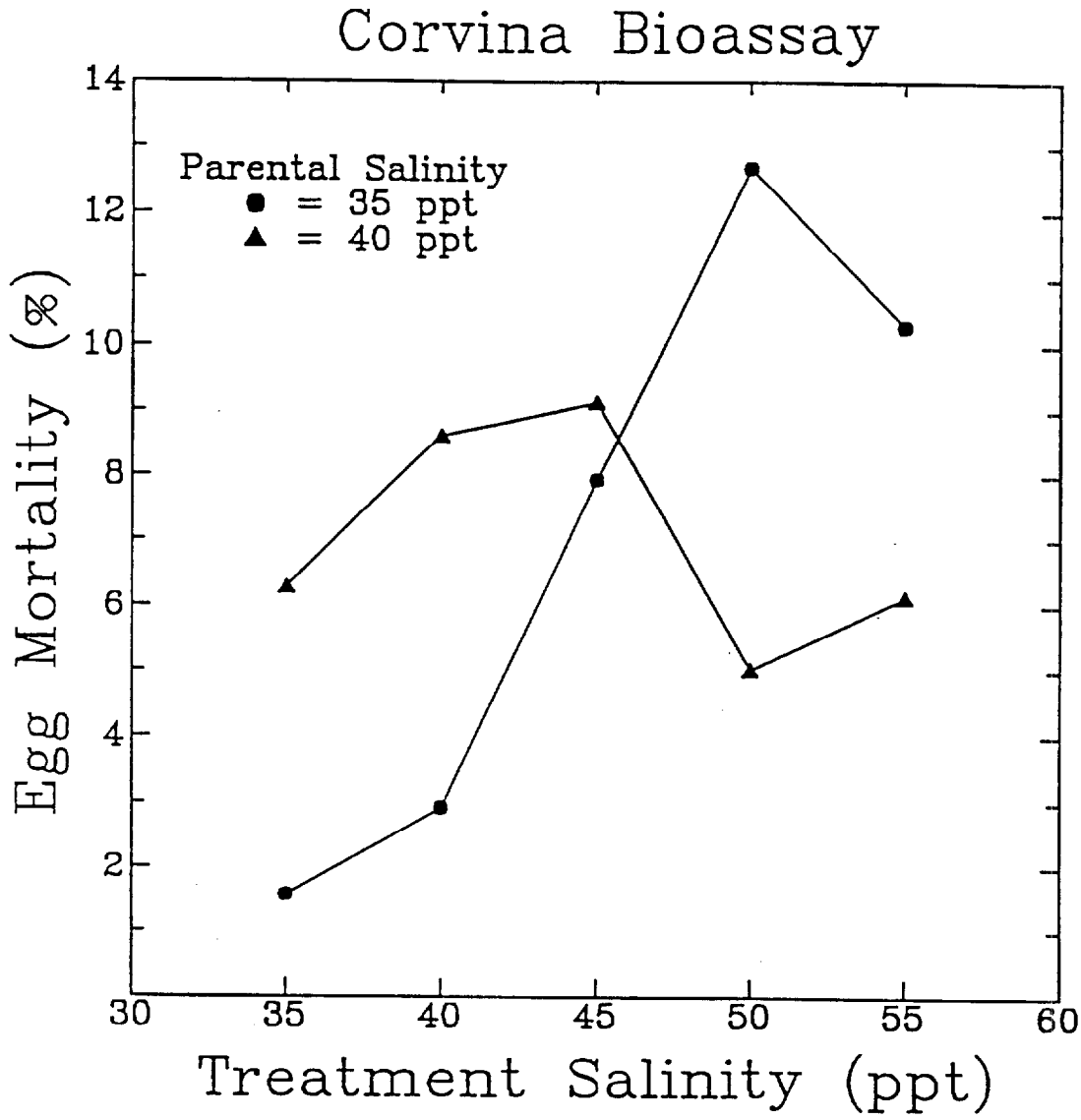
Table 3. Acclimation of Adult Corvina to Increasing Salinity and Growth

| Tank | Sex | Fish Tag No. | Start Weight (kg) | End Weight (kg) | Paired t-test t | p | |
|---|---|---|---|---|---|---|---|
| C1 | Females | BL-0029 | 2.27 | 4.93 | -6.554 | 0.0316 | |
| | | OR-0022 | 2.24 | 4.28 | | | |
| 35 ppt | | OR-0030 | 6.35 | 7.91 | | | |
| 10 months | Males | BL-0030 | 4.00 | 6.41 | -5.085 | 0.0009 | |
| | | OR-0011 | 3.74 | 4.88 | | | |
| | | OR-0015 | 4.25 | 4.90 | | | |
| | | OR-0018 | 5.67 | 6.69 | | | |
| | | OR-0021 | 5.33 | 5.90 | | | |
| | | OR-0026 | 3.54 | 4.99 | | | |
| | | OR-0027 | 2.72 | 5.38 | | | |
| | | OR-0029 | 3.40 | 4.88 | | | |
| | | OR-0031 | 6.12 | 6.26 | | | |
| | | OR-0032 | 2.27 | 4.74 | | | 1.391 |
| | Combined | | 3.99 | 5.55 | -6.668 | 0.0001 | |
| LMS2 | Females | YW-0008 | 2.38 | 5.78 | -6.274 | 0.0335 | |
| | | YW-0015 | 4.76 | 6.69 | | | |
| 40 ppt | | YW-0017 | 1.59 | 4.68 | | | |
| 31 months | Males | YW-0001 | 1.81 | 4.59 | -11.223 | 0.0012 | |
| | | YW-0010 | 1.59 | 4.99 | | | |
| | | YW-0011 | 1.70 | 4.82 | | | |
| | | YW-0014 | 3.52 | 5.44 | | | |
| | | YW-0016 | 1.93 | 4.68 | | | |
| | Combined | | 2.41 | 5.21 | -13.406 | 0.0001 | 2.162 |
| B1 | Females | BL-0015 | 2.61 | 4.63 | -10.974 | 0.0004 | |
| | | BL-0018 | 2.04 | 3.69 | | | |
| 45 ppt | | BL-0021 | 4.99 | 6.14 | | | |
| | | BL-0026 | 4.20 | 5.82 | | | |
| 7 months | | BL-0027 | 2.95 | 5.23 | | | |
| | | BL-0033 | 1.47 | 3.47 | | | |
| | Males | BL-0014 | 6.69 | 5.90 | -0.302 | 0.7773 | |
| | | BL-0023 | 3.97 | 6.33 | | | |
| | | BL-0031 | 5.73 | 5.09 | | | |
| | Combined | | 3.85 | 5.14 | -3.243 | 0.0117 | 1.356 |

Table 3. Continued.

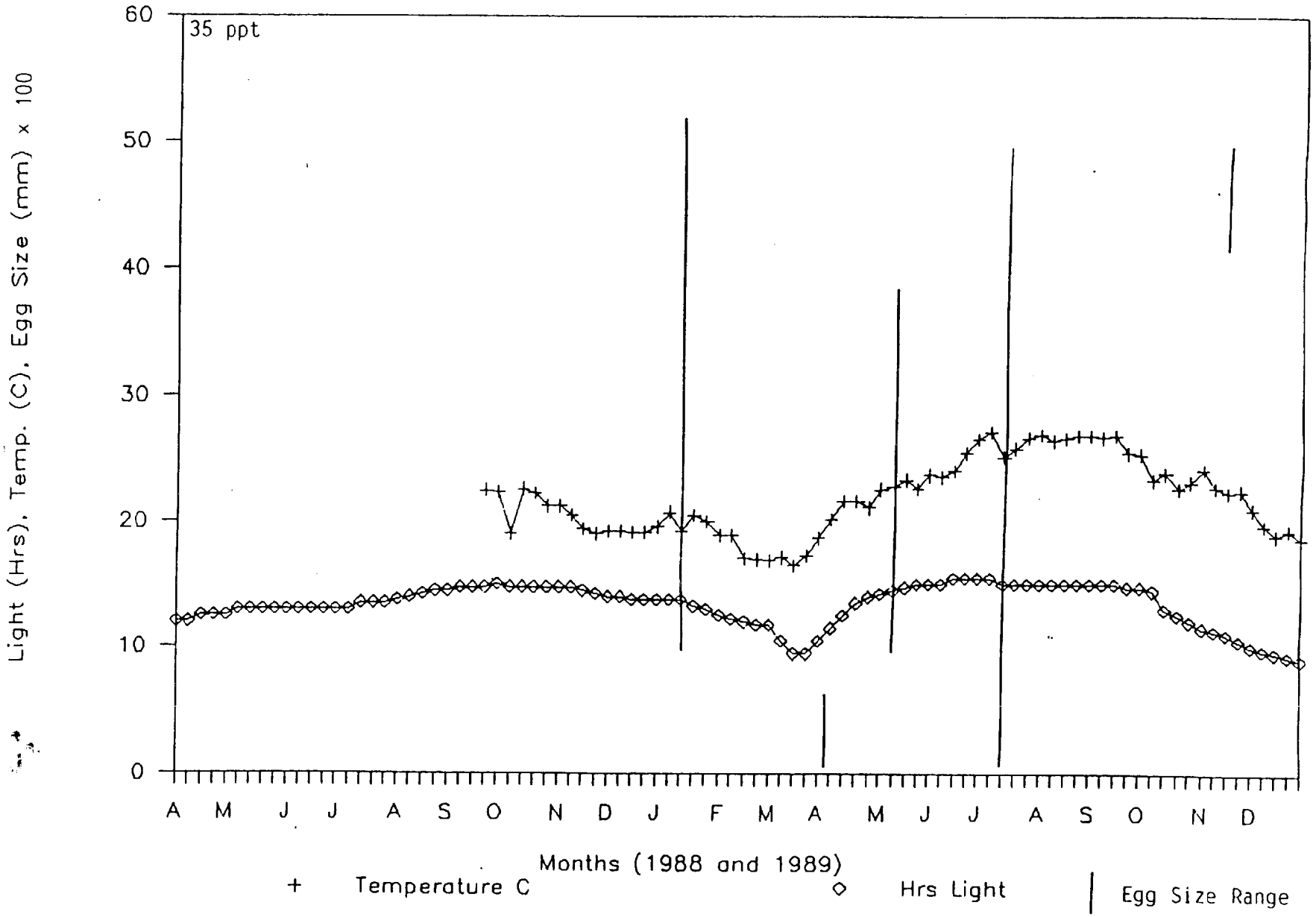
| Tank | Sex | Fish Tag No. | Start Weight (kg) | End Weight (kg) | Paired t-test t | p | |
|---|---|---|---|---|---|---|---|
| B2 | Females | GY-0036 | 2.15 | 4.42 | na | | |
| 50 ppt | Males | GY-0016 | 5.78 | 5.90 | -3.634 | 0.0153 | |
| | | GY-0017 | 5.10 | 6.01 | | | |
| 14 months | | GY-0018 | 4.05 | 6.69 | | | |
| | | GY-0031 | 3.69 | 5.61 | | | |
| | | GY-0034 | 2.83 | 3.66 | | | |
| | | GY-0043 | 3.66 | 5.95 | | | |
| | Combined | | 3.89 | 5.46 | -4.390 | 0.0051 | 1.484 |
| LMS3 | Females | WT-0015 | 3.69 | 3.83 | na | | |
| 55 ppt | Males | GY-0020 | 3.60 | 5.07 | -2.047 | 0.1095 | |
| | | WT-0016 | 3.18 | 5.98 | | | |
| 15 months | | WT-0019 | 4.65 | 5.25 | | | |
| | | WT-0020 | 3.97 | 5.44 | | | |
| | | WT-0050 | 4.99 | 4.39 | | | |
| | Combined | | 4.81 | 4.99 | -2.009 | 0.0995 | 1.245 |

Figure 1. Egg Mortality By Treatment Salinity



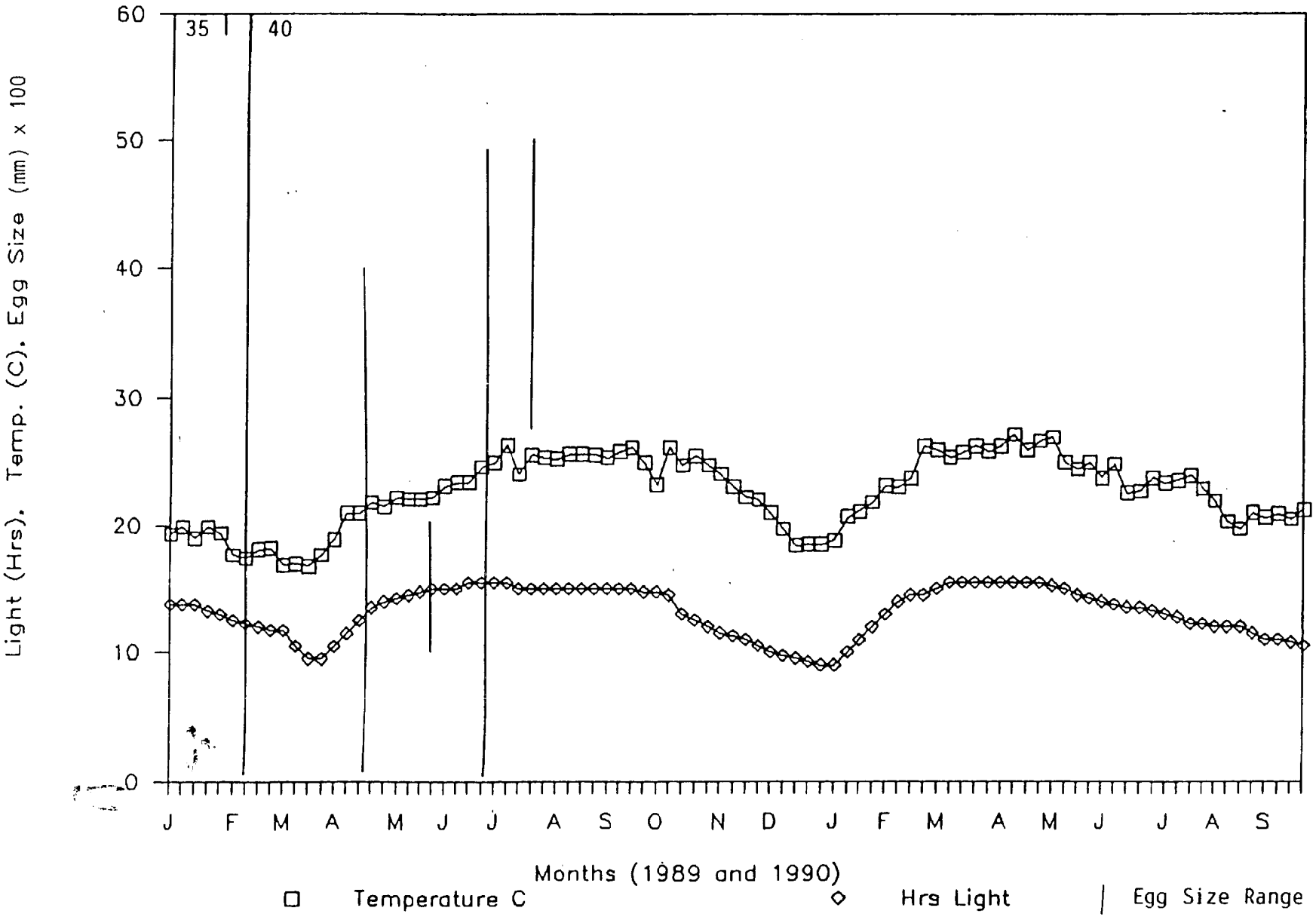
# FIG. 2. CORVINA EGG SIZE WITH PHOTOPERIOD & TEMPERATURE

LMS3: Salinity 35 ppt



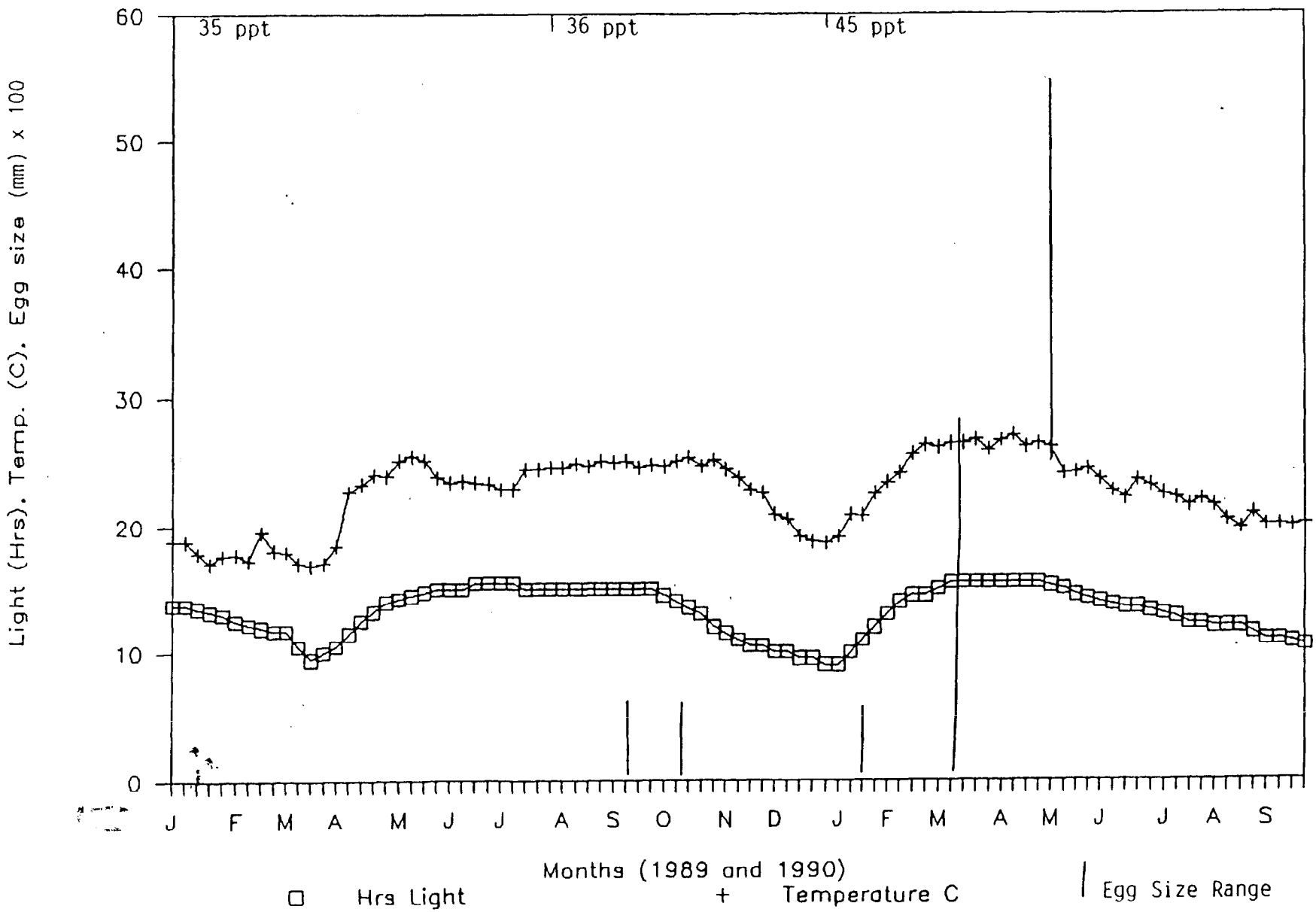
# FIG. 3. CORVINA EGG SIZE WITH PHOTOPERIOD & TEMPERATURE

LMS2: Salinity 35 through 40 ppt



# FIG. 4. CORVINA EGG SIZE WITH PHOTOPERIOD & TEMPERATURE

B1: Salinity 35 through 45 ppt



# FIG. 5. CORVINA EGG SIZE WITH PHOTOPERIOD & TEMPERATURE

B2: Salinity 35 through 50 ppt