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SALINITY TOLERANCE OF ANISOTREMUS DAVIDSONI

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ABSTRACT

Eggs and larvae of the haemulid fish Sargo, Anisotremus davidsoni, were obtained from fish matured in the laboratory by photoperiod and temperature manipulation. The effects on gametes of parental salinity acclimation were also investigated. Spawning occurred in adult Sargo acclimated to 35 through 45 ppt salinity. Due to Vibrio sp. infection and treatment with a wide spectrum antibiotic prior to spawning, bioassay data may be suspect. Reproductive failure of this species will occur with increased salinity above 40 ppt 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 1958). 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 sargo (A. davidsoni) was selected for the experiments due to the fact that the sargo comprised 28% of the recreational sportfish catch (Black 1988).

MATERIALS AND METHODS

Salinity bioassays were selected as the best available technology to estimate the tolerance of sargo to total dissolved solids within the relatively short span 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 standard 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 sargo from the Salton Sea were collected and transported to our Redondo Beach Laboratory in order to conduct the salinity bioassays. Collection of sargo 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 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 was 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 0.4 mm. This occurred when the photoperiod and temperature reached 13L:10D and 19-23^{o}C. Males were considered to have fully mature testes when milt was released upon applying light pressure to the abdomen.

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 contract, 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

Due to a Vibrio sp. infection in the adult Anisotremus davidsonii acclimated to the 35 ppt Salton Sea water and subsequent antibiotic treatments, spawns were not achieved by means of photoperiod and temperature manipulations until September through November. Spawns were achieved in adults acclimated to 40/45 ppt Salton Sea water from June through September. Consequently all of the bioassays on fertilized eggs from 40 ppt acclimated adults were run prior to those eggs from 35 ppt acclimated adults (Table 2). No spawns were achieved in adults acclimated to 50 or 55 ppt Salton Sea water. There were two separate processes involved in the bioassay results: egg mortality and larval mortality.

Egg Mortality

Egg mortality varied enormously across assays, ranging from a low of 3% in the June 1st experiment to a high of 31% in the November experiment (Table 3). There was, however, no particular effect of treatment salinity. Egg mortality varied from 13% to 20% across the range of treatments used with no clear pattern of increases with higher salinity (Table 4). Because the parental salinity treatments were done successively with all of the 40 ppt assays preceding all of the 35 ppt assays, and since all of the latter assays had much higher egg mortality, there was a significant effect of parental salinity on egg mortality ($p = .001$), with lower salinity being associated with higher mortality but no significant main effect of treatment salinity. There was a significant interaction of parental

acclimation and treatment salinity ($p = .0001$), however, this indicates that the pattern of egg mortality across salinity treatments is not consistent from one assay to another.

Eggs spawned by adults acclimated to 45 ppt Salton Sea water produced viable fertilized eggs which developed during a prolonged period of time. All eggs failed to reach the stage of blastopore closure. Death of the eggs occurred with either collapse or partial invagination of the chorion, suggestive of osmoregulatory failure.

Larval Mortality

The situation with larval mortality was quite different. There was no significant difference among the assays performed comparing parental acclimation of 35 and 40 ppt. Larval mortality varied from 66% to 76% (Table 5). Treatment salinity, however, had a significant effect ($p = .0001$, Table 6). There was also a significant interaction effect which was due to the fact that at high salinities in all assays, all the larvae die ($p = .0001$).

Effects Of Treatment Salinity And Parental Salinity On Larval Mortality

The effects of parental salinity acclimation shows higher larval mortality at 35 ppt than at 40 ppt (71% vs 67%). This is actually what would have been expected since adaptation to high salinities should help the larvae better to tolerate osmotic stress.

Tables 7 and 8 analyze treatment salinity effects within parental acclimation salinities, suggesting that larvae accustomed to 40 ppt survive better at low salinities.

DISCUSSION

The results of the salinity bioassays conducted upon the adult A. davidsoni were complicated by the fact that those individuals acclimated to 35 ppt Salton Sea water contracted a Vibrio sp. infection which required extensive treatment with Prefuran, a wide spectrum antibiotic. During the treatment, the environmental parameters were not altered. Subsequent to the treatment and a continuation of the ongoing photoperiod and temperature manipulations, natural spawning occurred. Due to the combination of the use of the antibiotic as well as the prolonged exposure to increasing temperatures and photoperiod, the eggs which were produced by the adults in the 35 ppt acclimation salinity may be questionable. This may explain the enormous variation in egg mortality ranging from a low of 3% in June for the 40 ppt parental acclimated eggs to a high of 31% on November 19 for the 35 ppt parental acclimated eggs (Table 3). This variation in mortality may also be seen in the eggs spawned by the 40 ppt acclimated adults when comparing the bioassays from the June spawns compared to the September spawn. Which also lends credence to the suggestion that eggs produced later in the season may be less viable thus impacting the results of these bioassays.

The larval bioassays however were not impacted by the sequence in which the bioassays were performed. Treatment salinities above 40 ppt resulted in significant larval mortality regardless of the parental acclimation salinity.

Although previous investigators had suggested that adaptation and acclimation to higher salinities might afford a greater degree of osmoregulatory capability, the spawning and ultimate reproductive failure that occurred in the sargo acclimated to 45 ppt Salton Sea water is indicative of the limits to salinity acclimation.

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Table 1. Ahlstrom's numerical designation of developmental stages.

Ahlstrom 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
IV	a	Somites begin to form; scattered melanophores appear, 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 1988 Sargo spawns used in bioassays.

Month	Tank System Salinity	Date Spawned	Date Found	Egg Stage	Egg Count	Fertilization Percent	Temperature C
JUNE							
	C3(40ppt)	6/1	6/1	V	7,080	86.4%	21.7
	C3(40ppt)	6/2	6/2	IV	15,413	31.6%	21.1
JULY							
	C3(40ppt)	7/10	7/11	IV,V	17,850	9.0%	22.5
SEPTEMBER							
	B2(35PPT)	9/28	9/28	II	7,583	46.0%	22.4
	B2(35PPT)	9/30	9/30	II	4,600	63.0%	22.7
OCTOBER							
	LMS1(35PPT)	10/29	10/30	IV,V	107,432	1.0%	21.0
NOVEMBER							
	LMS1(35PPT)	11/18	11/18	II	50,326	5.7%	17.7
	LMS1(35PPT)	11/28	11/28	I	36,958	39.1%	17.8

Sargo were spawning in the lab from April through December.

Table 3. *Anisotremus* egg mortality by parental acclimation.

Parental salinity		Larvae	Dead eggs	Total
01 June 88	40 ppt	1534 96.72%	52 3.28%	1586
02 June 88	40 ppt	1728 92.80%	134 7.20%	1862
01 Sept 88	40 ppt	435 71.43%	174 28.57%	609
29 Sept 88	35 ppt	805 88.66%	103 11.34%	908
01 Oct 88	35 ppt	635 76.14%	199 23.86%	834
19 Nov 88	35 ppt	772 69.36%	341 30.64%	1113
28 Nov 88	35 ppt	828 69.99%	355 30.01%	1183
Total		6737 83.22%	1358 16.78%	8095 100.00

Table 4. *Anisotremus* egg mortality by treatment salinity.

Treatment salinity	Larvae	Dead eggs	Total
35	1362 83.25%	274 16.75%	1636
40	1319 83.80%	255 16.20%	1574
45	1361 86.74%	208 13.26%	1569
50	1375 82.68%	288 17.32%	1663
55	1320 79.85%	333 20.15%	1653
Total	6737 83.22%	1358 16.78%	8095 100.00

Table 5. *Anisotremus* larval mortality by parental salinity.

Parental salinity	Live Larvae	Dead Larvae	Total
01 June 88 40 ppt	512 33.38%	1022 66.62%	1534
02 June 88 40 ppt	594 34.38%	1134 65.63	1728
01 Sept 88 40 ppt	113 25.98%	322 74.02%	435
29 Sept 88 35 ppt	252 31.30%	553 68.70%	805
01 Oct 88 35 ppt	219 34.49%	416 65.51%	635
19 Nov 88 35 ppt	204 26.42%	568 73.58%	772
28 Nov 88 35 ppt	198 23.91%	630 76.09%	828
Total	2092 31.05%	4645 68.95%	6737 100.00

Table 6. *Anisotremus* larval mortality by treatment salinity.

Treatment salinity	Live Larvae	Dead larvae	Total
35	1050 77.09%	312 22.91%	1362
40	913 69.22%	406 30.78%	1319
45	127 9.33%	1234 90.67%	1361
50	2 0.15%	1373 99.85%	1375
55	0 0.00%	1320 100.00%	1320
Total	2092 31.05%	4465 68.95%	6737 100.00

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40	913 69.22%	406 30.78%	1319
45	127 9.33%	1234 90.67%	1361
50	2 0.15%	1373 99.85%	1375
55	0 0.00%	1320 100.00%	1320
Total	2092 31.05%	4465 68.95%	6737 100.00

Table 7. Anisotremus larval mortality by treatment within parental salinity (35ppt)

Treatment salinity	Live Larvae	Dead Larvae	Total
35 ppt	416 64.70%	227 35.30%	643
40 ppt	365 63.81%	207 36.19%	572
45 ppt	91 14.61%	532 85.39%	623
50 ppt	1 0.16%	631 99.84%	632
55 ppt	0 0.00%	570 100.00%	570
Total	873 28.72%	2167 71.28%	3040 100.00

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