Calif. Fish and Game, 58(1) : 58–66. 1972.

THE RESPONSE OF SALTON SEA FISH EGGS AND LARVAE TO SALINITY STRESS[1]

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In laboratory experiments, Salton Sea water at salinities (S) of 40 ‰ and higher adversely affected developing embryos and larvae of the croaker, *Bairdiella icistia*, and the sargo, *Anisotremus davidsoni*. Embryos developed abnormally, hatching success diminished, and mortality of larvae was greater than in normal Salton Sea water at 37.6 ‰ S.

INTRODUCTION

The Salton Sea is an inland saline lake, 58 km long and 14.5 to 24 km wide, in the desert of southeast California. Orginally formed in 1905–1907 by accidental diversion of flood water from the Colorado River, the Salton Sea has since been maintained by agricultural drainage from surrounding land. A few species of marine fish were introduced into the sea as long ago as 1929 when the Sea was about 14.5‰, but most successful introductions were made from 1948 through 1956 at higher salinities. Carpelan (1961) gives an excellent review of the physical and chemical history of the Sea. The sport fishery in the Salton Sea presently depends on three introduced species from the Gulf of California, the bairdiella, *Bairdiella icistia*, the sargo, *Anisotremus davidsoni*, and the orangemouth corvina, *Cynoscion xanthulus*. The bairdiella supports only a minor sport fishery and is primarily a forage fish for the highly prized corvina; both of these species belong to the croaker family, Sciaenidae. The sargo, of the family Pomadasyidae, is both an important game fish and a forage fish (Walker, Whitney, and Barlow, 1961). Because of a persistent increase in the salinity of the Salton Sea in recent years due to agricultural soil leaching (Federal-State Reconnaissance Report, 1969), concern has been expressed for the continued survival of these three species on a self-sustaining basis in the Salton Sea (Calhoun, 1969).

The ability to survive stresses in the environment is generally less developed in early larval stages as evidenced by the huge mortalities which occur at this time. There is ample evidence that the greatest degree of mortality occurs in fishes during the egg and larval stage of development, (Sette, 1943; Ahlstrom, 1954). Pelagic fish larvae often

[1] This work was performed as part of Dingell-Johnson Project, California F-24-R, "Salton Sea Study," supported by Federal Aid to Fish Restoration Funds. Accepted for publication September 1971.

hatch from the egg before having fully developed the organs to see, feed, or osmoregulate. Development to the feeding stage proceeds with yolk providing the energy needed. In particular the newly-hatched pelagic larvae of bairdiella and sargo lack gills, an open gut, scales, and a functional kidney, all part of the adult's final organ complement for osmoregulation. Because of these facts it seemed appropriate to study the effects of increased salinity on the eggs and larvae of Salton Sea fish. Observations are reported here which were made on the fertilization success, development, hatching and survival time of bairdiella and sargo eggs and larvae as they were affected by high salinities. The results are discussed with respect to the steadily increasing salinity of the Salton Sea.

METHODS

When this study was initiated in 1968, no method was available for maturing and inducing spawning of Salton Sea fish in the laboratory. Thus mature sargo and bairdiella had to be caught by a trawl or gill net during their spawning season (April and July 1968 respectively), then stripped and the eggs fertilized aboard a motor launch. The developing eggs were brought back to the laboratory operated by California Department of Fish and Game at Salton City, where temperature was maintained at 17 C in an air-conditioned room; there was no temperature control aboard the launch where temperatures varied between 24.5 and 27 C. In 1970, Haydock (1971) perfected a method for inducing maturation and spawning of bairdiella under laboratory conditions. This simplified the salinity study by providing viable eggs and sperm for laboratory experimentation and permitted a rigorous temperature control unobtainable during the earlier experiments. The work performed in 1968 was therefore repeated in 1970 to determine whether the earlier lack of temperature control altered the response of bairdiella eggs and larvae to salinity stress sufficiently to vitiate the earlier conclusions. The results from both years were comparable. For the sake of completeness both series of experiments are described in detail here.

Obtaining Ripe Bairdiella and Sargo

Ripe bairdiella females were taken in the Salton Sea on May 20, 29, June 10, 12, 19, and 24, 1968, by trawl between 1830 and 2130, 0.4 to 1.6 km from the western shore between Salton Bay Marina and Salton City Keys. Despite the fact that more fish were examined between 0800 and 1700 than in the evening hours, not one was ripe during daytime. Although no fish were caught after 2130 it is probable that spawning continues into the night. Whitney (1961) found that the bairdiella has a diurnal pattern of spawning, with most early cleavage eggs appearing in the plankton during early evening. One ripe sargo was caught in a gill net at 0930, April 23, 1968, and the fact that it was still alive indicated that it was in the net a short time, probably less than 3 hr. Only one running-ripe male bairdiella was caught by trawl, but sperm obtained from other mature males proved capable of fertilizing ripe eggs. The sargo fertilization was accomplished using sperm also from a mature but not running-ripe male. In the 1968 series of experiments, fertilization for both species was carried out on shipboard.

Bairdiella of the 1969 year class were maintained in large tanks at the Fishery-Oceanography Center of the National Marine Fisheries Service, La Jolla, California, for approximately 7 months prior to fertilization experiments in 1970. These fish were subjected to a photoperiod, water temperature and feeding regime conducive to accelerated growth and maturation of the gonads (Haydock, 1971). In mid-March 1970, approximately 2 months in advance of the peak spawning period for this species in the Salton Sea, viable sex products were obtained. Running-ripe females were obtained by injections of gonadotropic hormones as described by Haydock (1971). Males were running-ripe without hormone treatment. Sargo, which were captured at the Sea prior to the spawning season, were acclimatized to laboratory conditions for only 6 weeks and only one female ovulated after hormone treatment.

Artificial Fertilization

The shipboard and laboratory fertilizations were carried out in the same way. Eggs were expressed from ripe females by gentle hand pressure on the abdomen and collected on a stainless steel lab scoop. Eggs were then separated and distributed as evenly as possible in a glass Petri dish and apportioned into 10 cm diameter finger bowls, each containing 50 ml of water at an appropriate salinity.

Milt expressed from a male was drawn into a bulb-operated pipette and introduced into each bowl containing eggs. The seminal mass was repeatedly drawn into the pipette along with some of the water in the test bowl and forcefully expelled among the eggs to insure adequate mixing of the gametes. In 1970, all fertilizations were performed at 21 ± 0.5 C.

The fertilized eggs of the bairdiella and sargo are slightly buoyant and float against the surface film if undisturbed, while most unfertilized eggs remain on the bottom of the dish.

Selection of bairdiella and sargo fertilized eggs for further observation and estimates of fertilization success were made on the basis of a sink or float criterion only in test salinities (S) of 37‰ or higher. At lower salinities we observed initial cleavage stages under low-power magnification (7 to 15×), 1 to 2 hr after fertilization. In 1968, Salton Sea water evaporated to a salinity of 71.8‰ was filtered through a membrane filter (pore size 0.45 μ) and diluted with demineralized water to desired salinities. In 1970, undiluted Salton Sea water at 37.6‰ was filtered through activated charcoal to remove dissolved organic substances and a membrane filter, 0.45 μ pore size, to remove particulates. Higher salinities to 55‰ were obtained by mild heat and prolonged evaporation after filtration. Analysis for specific ions and total dissolved solids were made on Salton Sea water and 2× Salton Sea water in 1967. When compared with an analysis of sea water, these data show that some precipitation of calcium and sulfate must have occurred (Table 1).

Streptomycin sulfate assayed at 750 mg/g and penicillin-G, 1,585 units/mg, were added as dry powder (50 ppm) to some experimental vessels in 1968 and survival of eggs and larvae was compared with those without antibiotics. In 1970, only penicillin-G was used.

Usually 50 to 100 successfully fertilized eggs in a particular salinity were used for determining mortality rate. Dead eggs or larvae were removed whenever a count was made of living animals.

TABLE 1—Analysis of the Ionic Content of Normal Sea Water, Salton Sea Water, and 2X Salton Sea Water

	Sea water* (parts per thousand)	Straight Salton Sea water† (parts per thousand)	2X Salton Sea water† (parts per thousand)
Cations			
Ca	0.41	0.96	0.92
Mg	1.27	0.86	1.68
Na	10.6	11.5	22.5
K	0.38	0.22	0.37
Anions			
CO_3	0.07	0.02	0.05
HCO_3	0.14	0.15	0.27
SO_4	2.65	7.0	12.0
Cl	19.98	17.8	35.5
Total dissolved solids	34.5	38.2	69.6

* Sverdrup, Johnson and Fleming (1946).
† Analyses by E. S. Babcock and Sons, Riverside, California.

RESULTS

Fertilization and Hatching Success

Successful fertilization of more than half of extruded eggs occurred at all salinities tested but there was a tendency toward lower success in the higher salinities. Usually, at least 50% of the eggs expressed from a sargo or bairdiella female could be fertilized regardless of salinity up to 55‰. In 1968, no eggs of bairdiella developed in 50 or 55‰ beyond blastodisc formation. Hormone-induced ovulation produced fertilizable eggs which occasionally survived to an advanced embryonic stage at 50‰ but always produced embryos which exhibited deformed tails and did not hatch. Fertilization success for sargo eggs was almost 100% at all salinities from 35 to 55‰.

Survival

Embryos of sargo and bairdiella in 45 and 50‰ Salton Sea water developed more slowly than those in lower salinities and hatching success diminished with increasing salinity. For example, in an experiment with bairdiella eggs in water containing antibiotics, hatching success was 84% in 35‰, 15% in 40‰, 7% in 45‰, and 0% in 50 and 55‰, although some eggs developed to late embryonic stages in the last two salinities.

In the experiment described in Table 2 which was performed in 1968 at 17 C, high salinities reduced the survival of bairdiella eggs and larvae. All eggs in the elevated salinities were treated with 50 ppm each of streptomycin sulfate and penicillin-G; however, there were no antibiotics in the 35‰ S Salton Sea water. Forty percent of the eggs survived to the yolk-sac larval stage even in the untreated 35‰ Salton Sea water 35 hr after fertilization and 78% survived in 33.5‰ La Jolla sea water, while in each higher salinity complete mortality occurred before 35 hr had elapsed, despite the addition of antibiotics. This effect was clear even in the 40‰ S experiment.

TABLE 2—Percent Survival of *Bairdiella icistia* Eggs and Larvae at Different Salinities. (Fifty eggs were fertilized in each salinity (n = 50 fertilized eggs = 100%) and observed over 35 hours at 17 C. Antibiotics, 50 ppm each of Streptomycin sulfate and penicillin-G, were added at the beginning of the experiment except in Salton Sea water of 35 ‰ S. Ripe bairdiella were caught by trawling. Stages of development are described in Mansueti and Hardy, 1967.)

Time from fertilization (hr)	Stage of development	Salinity (parts per thousand)					
		La Jolla	Salton Sea				
		33.5	35	40	45	50	55
0	Fertilized eggs	100	100	100	100	100	100
9	Early embryo	88	94	58	37	0	0
14	Early embryo	88	64	44	10	--	--
21	Yolk sac larvae	82	58	30	0	--	--
35	Yolk sac larvae	78	40	0	--	--	--

Antibiotics have a beneficial effect on survival. Salton Sea water at 35‰ was treated with antibiotics and the resultant mortality of bairdiella eggs and larvae measured over time. All the larvae were dead by 46 hr after fertilization when no antibiotics were used. In treated water 62% were alive after 46 hr, and 36% were still living and developing after 60 hr (Table 3).

TABLE 3—Percent Survival of 50 (= 100%) *Bairdiella icistia* Eggs in Antibiotic-Treated and Untreated Salton Sea Water at 17C and 35 ‰ S

Time from fertilization (hr)	Stage of development	Without antibiotics	With antibiotics
0	Fertilized eggs	100	100
13	Early embryo	86	84
22	Late embryo	78	84
35	Yolk sac larvae	16	72
46	Yolk sac larvae	0	62
60	Post yolk sac larvae	--	36

Three experiments with bairdiella eggs and larvae were conducted in 1970. Eggs were obtained by hormone-induced ovulation and temperature was controlled at a constant 21° ± 0.5 C. In every instance there was a slight to strong increase in mortality rate with increased salinity (Table 4). In two experiments the lower salinities also resulted in similar increases in mortality rates (Table 4). When Penicillin was used (Table 5), the increased mortality rate appeared during the larval stage at 40‰ and in the embryonic stages at 45‰ and higher salinities.

TABLE 4—Percent Survival of *Bairdiella icistia* Eggs and Larvae at 21C in Different Salinities. (Eggs were produced by hormone-induced ovulation. No antibiotics were used in these experiments. n = ~ 100 fertilized eggs = 100%.)

Time from fertilization (hr)	Stage of development	Salinity (parts per thousand)							
		30	33.5	35	37.6	40	45	50	55
	Experiment 1								
0	Fertilized eggs	100.0		100.0		100.0	100.0	100.0	100.0
13	Tail-bud embryo	36.5		72.3		35.9	38.0	19.4	0.0
21	Tail-bud embryo	35.6		66.3		33.0	35.0	7.8	--
30	Tail-free	29.8		56.4		23.3	22.0	4.9	--
36	Yolk sac larvae	24.0		51.5		18.4	16.0	0.0	--
42	Yolk sac larvae	18.3		50.5		17.5	12.0	--	--
47	Yolk sac larvae	18.3		50.5		17.5	12.0	--	--
	Experiment 2								
0	Fertilized eggs		100.0	100.0	100.0	100.0	100.0	100.0	100.0
12	Tail-bud embryo		49.0	47.6	72.5	25.0	43.0	14.3	0.0
23	Tail-free		49.0	43.8	60.4	21.0	30.0	8.2	--
30	Yolk sac larvae		48.0	37.1	52.7	21.0	28.0	7.1	--
37	Yolk sac larvae		47.0	35.2	49.5	18.0	25.0	4.1	--
45	Yolk sac larvae		47.0	33.3	49.5	18.0	24.0	4.1	--
60	Post yolk sac larvae		14.0	0.0	17.6	0.0	0.0	0.0	--
	Experiment 3								
0	Fertilized eggs			100.0		100.0	100.0	100.0	100.0
12	Tail-bud embryo			65.9		66.3	67.0	44.5	19.2
20	Tail-bud embryo			52.3		60.4	59.0	31.8	10.1
31	Tail-free			44.3		53.6	54.0	30.0	6.1
36	Yolk sac larvae			42.0		53.5	51.0	28.2	3.0
42	Yolk sac larvae			42.0		52.5	47.0	16.4	3.0
46	Yolk sac larvae			42.0		52.5	47.0	16.4	3.0

TABLE 5—Percent Survival of *Bairdiella icistia* Eggs and Larvae in Different Salinities. (Eggs were produced by hormone-induced ovulation. Penicillin-G, 50 ppm was used in each salinity. n = ~ 100 fertilized eggs = 100%.)

Time from fertilization (hr)	Stage of development	Salton Sea (S) (parts per thousand)				
		35	40	45	50	55
0	Fertilized eggs	100	100	100	100	100
13	Tail-bud embryo	92	94	81	58	11
20	Tail-bud embryo	92	91	78	55	4
30	Tail-free	89	90	73	48	3
36	Yolk sac larvae	88	89	72	48	2
56	Yolk sac larvae	17	1	1	0	0

Table 6 has similar data obtained with sargo embryos and larvae in 1968 and 1970. In the experiment performed in 1968 (Experiment 1), 50 surviving, normal-appearing embryos which had been fertilized in their appropriate salinities were observed over a 96-hr period. Mortality was virtually absent in 35‰ Salton Sea water but the rate escalated with increased salinity. In the late larval stage, only 8% (4 larvae) were alive at 96 hr and 50‰ whereas 90% (45 larvae) were alive in the control (35‰) Salton Sea water.

TABLE 6—Percent Survival of Sargo, *Anisotremus davidsoni* Eggs and Larvae in Different Salinities. (Experiment 1 was started with 50 fertilized eggs (n = 50 = 100%) which survived 10 hr in each appropriate salinity. Eggs were obtained from spawning females caught in the Salton Sea in Experiments 1 (1968) and 2 (1970). In Experiment 3, eggs were obtained from hormone-induced ovulation. A variable number of fertilized eggs was used in each salinity in the latter two experiments ranging from 31 to 103 in Experiment 2 and 28 to 31 in Experiment 3.)

Time from fertilization (hr)	Stage of development	Salinity (parts per thousand)				
		35	40	45	50	55
	Experiment 1					
10	Tail-bud embryo	100.0	100.0	100.0	100.0	100.0
25	Tail-free	90.0	92.0	80.0	66.0	24.0
52	Yolk sac larvae	90.0	90.0	68.0	52.0	10.0
73	Post yolk sac larvae	90.0	78.0	62.0	0.0	0.0
96	Post yolk sac larvae	90.0	8.0	0.0	0.0	0.0
	Experiment 2					
0	Fertilized eggs	100.0	100.0	100.0	100.0	100.0
16	Tail-bud embryo	54.8	63.8	72.7	69.2	74.8
27	Tail-bud embryo	12.9	14.9	36.4	43.6	57.3
34	Tail-free	12.9	12.8	36.4	41.0	55.3
40	Yolk sac larvae	12.9	10.6	36.4	41.0	50.5
51	Yolk sac larvae	12.9	10.6	36.4	41.0	50.5
	Experiment 3					
0	Fertilized eggs	100.0	100.0	100.0	100.0	100.0
14	Tail-bud embryo	71.0	81.8	19.4	21.4	0.0
19	Tail-bud embryo	64.5	77.3	19.4	0.0	--
32	Tail-free	58.1	40.9	9.7	--	--
38	Yolk sac larvae	48.4	40.9	9.7	--	--
44	Yolk sac larvae	48.4	4.5	0.0	--	--
65	Post yolk sac larvae	9.7	0.0	--	--	--
84	Post yolk sac larvae	0.0	--	--	--	--

Experiments 2 and 3 (Table 6), performed with sargo eggs obtained by hormone-induced spawning in 1970, similarly showed a clear detrimental effect of salinity on survival rate at 40‰ and higher.

Although antibiotics prevent the drastic mortality rates of embryos and larvae at salinities of 40‰ and higher, bairdiella and sargo embryos which survived under these conditions exhibited developmental abnormalities. Bent tails and the inability to swim straight after hatching were the most common signs of abnormal development.

DISCUSSION

Salton Sea water of 40‰ salinity apparently exceeds the upper tolerance limits of bairdiella and sargo to salinity during embryonic and larval development. In most experiments 40‰ was clearly detrimental to life at these early stages as reflected in increased mortality rates. When mortality was not accelerated in a few experiments, embryos at 40‰ S and higher were abnormally developed and produced larvae which did not survive beyond the yolk-sac stage. At 40‰ S fertilization success was usually lower, abnormal embryos developed from fertilized eggs, the mortality rate of eggs and larvae at salinity was accelerated over controls at normally experienced salinities, and any larva hatched out at 40‰ salinity or higher was also abnormal; these facts suggest that bairdiella and sargo will not reproductively survive a rapid change in salinity over their normal habitat, now about 37‰ S in the Salton Sea. However, it is still possible that slow acclimation and genetic adaptation of the adults to rising salinity will favor production of gametes that will survive and live normally. The experiments reported here can be logically extended to include (i) acclimation of adults to high salinities and artificial spawning to ascertain gamete viability, (ii) mortality rate of eggs and larvae from acclimated fish, and (iii) the effect of temperature on salinity tolerance of eggs and larvae. The fact that fertilized eggs sink in La Jolla sea water at 33.5‰ and float in Salton Sea water at 37.6‰, suggest that adaptation has occurred toward higher salinities. However, no information is available on the parent stock of bairdiella from the Gulf of California. Simmons (1957) found *Bairdiella chrysura* in the Laguna Madre of Texas only in salinities of 45‰ and less. Other sciaenids live in the Laguna Madre, Texas, at salinities up to 75‰ but probably do not spawn in water above 45‰.

ACKNOWLEDGEMENTS

We wish to thank Alex Calhoun (California Department of Fish and Game, Sacramento, California) for suggesting this problem to us and also Hiram Li, Jack Hanson, David Crear, and Irwin Haydock for material assistance at the Salton Sea.

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