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Developmental Defects in Fish Embryos from Salton Sea, California

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The Salton Sea is the largest inland body of water in California. It currently supports a sportfishery for orangemouth corvina (Cynoscion xanthulus). Other species of importance are the bairdiella (Bairdiella icistius) and sargo (Anisotremus davidsonii). The future status of the fishery is uncertain for several reasons including possible impacts from chemical contaminants entering the Sea via agricultural drains and rivers (Montgomery 1987). There are also relatively large inputs of sewage from the New River and the Alamo River. Although these rivers discharge into the south end, strong currents and winds create rapid dispersion throughout the Salton Sea. Responding to environmental concerns, the State of California Department of Fish and Game supported a study on the population dynamics of Salton Sea fishes. Ichthyoplankton samples were collected for three spawning seasons (1987-1989), and fish embryos were evaluated for normal development.

The development of fish embryos has been used for monitoring the effects of pollution in the New York Bight (Longwell and Hughes 1981) and in northern Europe (Westernhagen et al. 1988), where malformation rates of up to 50% were found in embryos collected near highly contaminated rivers and waste dumping areas. This report describes significant incidences of malformed fish embryos collected from the Salton Sea. However, because of extreme hydrographical conditions present at the Sea which might be at least partially responsible for the observed malformations, supporting information on embryonic development was obtained in this study by controlled spawning of Salton Sea fishes in the laboratory.

MATERIALS AND METHODS

Malformed fish eggs were evaluated in 572 samples taken monthly during the spring/summer spawning season in 1987, 1988 and 1989. The eggs were collected using a 1-m diameter, 333-y mesh plankton net towed for 2 min at a speed of 1 m/sec. Surface tows were performed at 10 stations around the perimeter of Salton Sea and at two stations in the center of the south end of the Sea. Oblique tows (bottom to surface) were also made at selected stations. Water temperatures and dissolved oxygen concentrations were measured using

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a Sonde water quality analyzer (HydroLab Corp., Austin, TX). Mean surface temperatures varied between 18-30°C and dissolved oxygen concentrations were always > 90% saturation. Eggs were carefully removed from the net and immediately preserved in 5% sodium borate-buffered formalin. A maximum of 1,000 embryos from each sample was classified according to species and developmental stage. Embryos at stages from epiboly to blastopore closure (stage V), tail 270° around yolk (stage VI), to tail 360° around yolk (stage VII, immediately prior to hatching) were examined for defects. Abnormalities were classified as cranial malformations (reduced brain or eye development) or axial deformities (curvature of the notochord, incomplete blastopore closure, or twinning). Embryos were identified to species whenever possible; however, field-collected abnormal embryos could be identified only as sargo (yolk sac with anterior oil globule) or sciaenid (bairdiella plus corvina, yolk sac with posterior oil globule).

Sargo and corvina were caught at the Salton Sea using hook-and-line and immediately returned to Redondo Beach Marine Laboratory, Fish were maintained in recirculated Salton Sea water at 40 ppt salinity. Water changes (1/3 volume) were performed on a biweekly basis using fresh water shipments. Water quality parameters were within APHA (1985) guidelines and fish were fed *ad libitum* with cooked shrimp and frozen smelt. Tank conditions followed a photoperiod:temperature regime which simulated environmental conditions during the normal breeding season (June- July). In fall 1988, corvina were acclimated to 10 hr light (L) and 17°C, then photoperiod and temperature were increased at a rate of 0.25-0.5 hr L and 1°C/week. Fish were maintained for three months at the breeding regime of 15.5 hr L and 26-27°C. During July, final oocyte maturation and spawning were accomplished by injection with 12.5 µg/kg of a synthetic analog of luteinizing hormone releasing hormone (LHRHa). On 22 and 23 July 1989, fertilized eggs were skimmed from the tank surface and transferred into 1-L beakers. Eggs were reared at 26-27°C for 10 hr at which time they were in developmental stages V and VI. A portion of the floating eggs were used for bioassays, the remainder preserved and examined for defects. Similar procedures were followed for spawning sargo except that the spawning and rearing temperature was 21-22°C. Sargo spawned without hormone injections during May and June 1988. Eggs were preserved at developmental stages IV and V.

Using a Wild dissecting microscope at 16X or 32X, embryos were examined for developmental malformations. Defects at these stages were classified as axial malformations (failure of the blastopore to close [stages VI-VII only], twisting of the notochord, and partial twinning) and abnormal cranial development or differentiation (failure to complete neurulation, brain deformities and eye defects). About 10% of the malformed field-collected embryos were prepared for histopathological examination. Eggs were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin. For each embryo, six organ systems were evaluated: nervous (eyes, brain and nerve tube), soft tissue (muscle and connective tissue), integument, digestive, skeletal (primarily

notochord), and yolk sac epithelium. Developmental defects, abnormal differentiation and degenerative cellular changes were recorded.

RESULTS AND DISCUSSION

Incidences of abnormal embryos collected from the Salton Sea are shown in Table 1. Monthly values varied from 0.0% to a maximum of 62.4% for sargo in July 1987. For sargo, highest incidences of deformities were generally observed near the termination of the 1987 and 1988 breeding seasons; this pattern was not seen in 1989. Although ambient water temperatures are elevated in summer, no deformed sargo embryos were present in the last months of the 1987 (August) and 1988 (July) seasons when temperatures were greatest. During 1987, a high incidence of abnormal sargo (15.2%) was found in February. The overall incidences of deformed sciaenid embryos were lower than those of sargo; although in some months, they were greater. Unlike sargo, incidences of abnormal sciaenids did not appear to follow any temporal pattern. These values represent an overall average from the 10 sampling sites; incidences at particular stations were not considered indicative of local influences because of rapid water turnover throughout the Sea. It was necessary to sample a range of sites to obtain embryos at the desired developmental stages since each species has different breeding areas.

Histopathological examination revealed that the most prevalent type of malformation was retarded development of the nervous system, particularly the brain and eyes (82%). This condition was also observed in most of the embryos with axial malformations. Usually, brain differentiation appeared to be arrested at the time of brain specialization or just after optic vesicle formation. Eyes were small and lacked lenses, consisting solely of optic vesicles. In other embryos, eyes and brain were formed but were less differentiated. All these embryos had degenerative changes in the brain, nerve tube or eyes. A spectrum of changes was observed, from pycnosis of individual neurons to isolated necrotic foci to severe disorganization and degeneration of all neural tissue. In most of these embryos, degeneration of the integument was also present, Disorganization of muscle, notochord, gut, or yolk sac epithelium was observed in some affected embryos and in those with axial deformities. In the 6% with retarded differentiation, development appeared arrested at the embryonic shield stage prior to neurulation. Embryos consisted of undifferentiated tissue which, although highly mitotic, contained many degenerating cells.

Because of the possibility that these malformations could have been produced by the elevated temperatures or other unusual conditions in the Salton Sea, corvina and sargo were returned to the laboratory and spawned under controlled conditions. Three corvina spawned following acclimation to a controlledtemperature:photoperiod regime (simulating environmental conditions present during the breeding season) and hormone induction. Of the 1,810 eggs examined, 5.7% (stage V) and 10.9% (stage VI) were abnormal. Axial malformations

Table 1. Incidences of deformed sargo and sciaenid (bairdiella, corvina plus unidentifiable sciaenid) embryos at the Salton Sea, California from February 1987 through June 1989

Month	Deformed Sargo Embryos % (n/total)	Deformed Sciaenid Embryos % (n/total)	% Sciaenid Embryos Examined		
			Bairdiella	Corvina	Unidentifiable Sciaenid
Feb 87	15.2% (49/322)	0.0% (0/0)	0	0	0
Mar 87	0.6% (205134587)	3.7% (10/276)	0	0	100.0
Apr a7	0.7% (100/14093)	0.8% (444/57690)	98.5	0	1.5
May a7	3.3% (781/23729)	1.0% (1433/138308)	65.7	2.6	31.7
Jun 87	9.6% (10/104)	0.2% (27/13529)	al.7	9.4	8.9
Jul a7	62.4% (206/330)	0.5% (4018429)	79.7	15.6	4.7
Aug a7	0.0% (0/47)	0.1% (30/22297)	83.7	14.0	2.3
Mar 88	1.4% (290/21052)	0.0% (0/80)	0	0	100.0
Apr 88	0.0% (0/3659)	0.6% (205/32611)	12.3	0	87.7
May 88	3.5% (103/2910)	7.4% (1105/14836)	79.4	1.9	18.7
Jun 88	24.5% (187/764)	8.9% (1900/21277)	86.5	4.3	9.2
Jul 88	0.0% (0/41)	0.8% (59/6983)	76.4	21.6	2.0
Mar a9	4.4% (746/17046)	0.0% (0/74)	29.7	0	70.3
Apr a9	1.9% (1181/62447)	8.0% (1722/21486)	53.3	0	46.7
May a9	0.9% (93/10933)	4.2% (3720/87996)	83.8	0.1	16.1
Jun a9	2.8% (163/5725)	0.6% (486/87209)	32.6	3.2	64.2

including incomplete blastopore closure were present in 63% of the deformed embryos, with the remainder exhibiting retarded cranial development. Incidences of abnormalities in laboratory-spawned *sargo were* similar for stage V embryos, 9.9% (58/587) and 15.8% (64/405) for the two groups spawned on 7 May 1988. The malformation rate. in a third spawn (2 June 1988) of embryos evaluated at an earlier stage of development (stage IV) was higher, 24.9% (49/197). Types of defects were identical to those observed in corvina. The similarity of malformations in field-collected and laboratory-spawned embryos *suggests* that ambient contamination rather than temperature or other water quality characteristics contributed to the observed developmental toxicity.

Malformations of the head and body axis have been previously reported following exposure to a variety of environmental contaminants - insecticides (Weis and Weis 1976; van Leeuwen et al. 1986), chlorinated organics such as DDT (Smith and Cole 1973) and PCBs (Mauck et al. 1978), metals (Weis and Weis 1977; Somasundaram et al. 1984) and cyanide (Rosenthal and Alderdice 1976). These contaminants have all been detected in tributaries draining into the Salton Sea, many at levels exceeding U.S. Environmental Protection Agency biological criteria for chronic and acute exposure (Kennedy/Jenks 1985; U.S. Environmental Protection Agency 1986). The types of malformations observed in Salton Sea embryos are not specific for a particular contaminant, however, and appear to represent the manifestation of environmental stress at a life stage during which the range of potential responses is limited (Rosenthal and Alderdice 1976; Weis and Weis 1987; Westernhagen 1988). It is likely that the malformation rates reported here underestimate the effects of highly toxic point sources such as the New and Alamo Rivers because exposed embryos may be rapidly transported to less contaminated areas, producing a lower overall' incidence of developmental defects. Future laboratory studies should focus on defining the individual contributions of toxic point sources to the observed developmental abnormalities.

In laboratory-spawned Salton Sea fish, malformation rates in late-stage corvina embryos ranged between 4 and 16%; however, 25% of early stage sargo embryos were abnormal. It has been estimated that corresponding aberration rates of the earlier stages (prior to organogenesis) are four to ten times higher even though defects in undifferentiated embryos are more difficult to recognize (Westernhagen 1988). Malformations are less prevalent in late stages because embryonic death frequently results from aberrant development during the period between cleavage and gastrulation. It is thus possible that the reproductive success of Salton Sea fishes is being impaired by ambient contamination through direct uptake by the developing embryo or via exposure prior to spawning by contaminants transferred into maturing oocytes. The morphological anomalies we describe in Salton Sea embryos are severe and would certainly cause embryonic death before or soon after hatching (Westernhagen 1988). It is currently difficult to recognize more subtle defects in the embryo which lead to malformations presently found in adult Salton Sea fishes, such as

minor vertebral defects and deformed fins, gill covers, and jaws. Such malformations were first observed in the initial investigation of resident fishes almost 40 years ago (Walker 1961) and are also prominent in other fish species inhabiting polluted areas of the Rhine River (Sloof 1982) and the Baltic Sea (Bengtsson et al. 1988).

Westernhagen et al. (1988), who found malformation rates in late stage embryos similar to those reported here, concluded that the resulting embryonic mortality would not, by itself, produce observable reductions in fish recruitment. However, population reductions would become significant if secondary stresses were present. Although sportfish do successfully reproduce at the Salton Sea, developmental toxicity is already evident and embryonic mortality might be increased to a critical level by anticipated salinity increases or other environmental stresses.

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