EFFECTS OF TEMPERATURE AND SALINITY ON FERTILIZATION, EMBRYONIC DEVELOPMENT, AND HATCHING IN *BAIRDIELLA ICISTIA* (PISCES: SCIAENIDAE), AND THE EFFECT OF PARENTAL SALINITY ACCLIMATION ON EMBRYONIC AND LARVAL SALINITY TOLERANCE[1]

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ABSTRACT

Eggs and larvae of the sciaenid fish bairdiella, *Bairdiella icistia*, were obtained from fish matured in the laboratory by photoperiod manipulation and induced to spawn by hormone injections. The effects of temperature and salinity on fertilization, embryonic development, hatching, and early larval survival were studied with the material thus obtained, and the effects on gametes of parental salinity acclimation were also investigated. Fertilization took place over a wide range of temperatures and salinities, but was completely blocked at salinities of 10‰ and below. A low level of spermatozoan activity may have accounted for the lack of fertilization at low salinities. Successful embryonic development occurred between temperatures of approximately 20° and 30°C, and salinities of 15 and 40‰. The production of viable larvae was estimated to be optimal at a temperature of 24.5°C and a salinity of 26.6‰. An interaction of the two factors was apparent, development at high salinities being most successful at low temperatures and development at high temperatures being most successful at low salinities. The stage of maturity of the spawning female had a great influence on the overall viability of the eggs produced, as well as on their response to temperature and salinity. Adult bairdiella matured sexually in dilute seawater with a salinity of 15‰, and the salinity tolerance of the eggs produced by these fish was unaltered.

The bairdiella, *Bairdiella icistia* (Jordan and Gilbert), is a sciaenid fish native to the Gulf of California. In 1950 the species was successfully introduced into the Salton Sea, a large saline lake in southern California (Whitney 1961). Salton Sea water has an ionic composition different from that of ocean water (Carpelan 1961; Young 1970), and its overall salinity, now approximately 38‰,[3] is rising at a rate of about 1‰ every 3 yr (U.S. Department of the Interior and the Resources Agency of California 1969). This rising salinity has caused concern that the present sport fishery in the Salton Sea (based on several fish species, including bairdiella) will fail when the upper salinity tolerances of the fishes are exceeded (Walker et al. 1961). Lasker et al. (1972) found that the survival of bairdiella eggs and early larvae was severely inhibited by Salton Sea water at a salinity of 40‰; thus, at the present rate of salinity increase, the bairdiella population may suffer a loss in recruitment within the next 10 yr.

The work reported in this paper was undertaken to provide more information on the salinity tolerance of bairdiella during early development, especially as influenced by temperature and by the acclimation of spawning parents to different salinities. Because of poor embryonic and larval survival in Salton Sea water (May 1972), these experiments were all conducted in seawater of ordinary ionic composition. The effects of Salton Sea water per se and their implications for the population of bairdiella in the Salton Sea will be discussed elsewhere (May in preparation).

Bairdiella normally spawn during April and May in the Salton Sea (Whitney 1961; Haydock 1971). However, thanks to the work of Haydock (1971), bairdiella can be induced to mature and spawn in the laboratory at any time of the year,

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[3]This value varies somewhat with season and location in the Salton Sea.

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making bairdiella eggs and larvae extremely favorable material for experimentation. In addition to providing a year-round supply of eggs, laboratory spawning techniques have permitted maintaining bairdiella at different salinities during maturation and spawning in order to test the effect of parental salinity acclimation on the salinity tolerance of the gametes, embryos, and larvae.

MATERIAL AND METHODS

Capture and Maintenance of Fish

Methods used for collecting and maintaining bairdiella were nearly identical to those described by Haydock (1971). Adult bairdiella were captured with a 60-m beach seine on the west coast of the Salton Sea, just north of the Salton Bay Yacht Club. Rectangular fiberglass tanks of 2,000-liter capacity were used to hold fish in the laboratory and were supplied with continuously flowing warm (22°C) seawater from the Southwest Fisheries Center system (Lasker and Vlymen 1969). Water was filtered through polypropylene GAF[4] snap-ring filter bags of 50-μm pore size (GAF Corp., Greenwich, Conn.). Mercury lamps provided illumination (Haydock 1971) and the photoperiod was controlled as desired by timers.

The fish were fed ad libitum twice each day with ground squid, supplemented by ground red crab, *Pleuroncodes planipes*, at a ratio of approximately 1 part of crab to 6 of squid (wet weight). The red crabs were intended as a source of carotenoids because some authors have indicated that parental carotenoid deficiency may affect the viability of offspring (Hubbs and Stavenhagen 1958).

Several outbreaks of the parasitic ciliate, *Cryptocaryon irritans* Brown, occurred (Wilkie and Gordin 1969) and were effectively controlled by adding copper sulfate at 0.2 ppm as Cu^{2+} in the morning and late afternoon, allowing the chemical to be diluted in the interim by the continuously flowing seawater. Whenever fish were handled, they were subsequently treated with Furacin antibiotic (Eaton Veterinary Laboratories, Norwich, N.Y.) at 130 ppm, which was gradually diluted in the open seawater system. This precaution effectively controlled bacterial infections and allowed repeated handling of fish without adverse consequences.

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Induced Maturation and Spawning

Fish which had ripe gonads when captured were maintained in this condition for several months by exposing them to a photoperiod of 16 h light, 8 h darkness (16L:8D) at approximately 22°C (Haydock 1971). Prolonged exposure of female fish to long days resulted in eventual resorption of the ova. After a group of fish had been spawned out or had begun gonadal resorption, they were shifted to a short photoperiod (9L:15D) and colder water (15°C). After being held on short days for a few months, fish could then be brought to maturity by increasing the photoperiod at a rate of 30 min per day until 16L:8D was reached; after about 3 mo on 16L:8D at 22°C, the fish had developed mature ovaries and were ready to spawn. Successful spawning could be induced over a period of at least two or three more months before gonadal resorption began. Photoperiod manipulation was effective in inducing ovarian maturation regardless of the time of year, and the experiments described in this paper were conducted in the summer, fall, and winter instead of during the normal spring spawning period.

Bairdiella kept in the laboratory vary considerably in their ovarian development (Haydock 1971). In the present study the maturity of female fish was assessed from ovarian biopsies taken with a glass capillary tube (Stevens 1966). At first only the maximum oocyte diameters were recorded immediately after sampling, along with qualitative notes concerning the amount of ovarian stroma in the sample. When it became apparent that this was not a sufficiently sensitive measure of the state of maturity, the samples were preserved in 3% Formalin (in 50% seawater) and all oocyte diameters of 175 μm or greater were measured with an ocular micrometer a day or so later,[5] giving an oocyte size-frequency distribution based on measurements of approximately 100 oocytes. The fish which had been biopsied in this manner were marked individually on the lower jaw with injections of the dye, National Fast Blue 8GXM (= Fast Turquoise PT) (Kelley 1967; Haydock 1971).

Mature female fish weighing 100-150 g were injected in the epaxial musculature near the dorsal fin with 100 IU of gonadotropin from pregnant mare's serum (PMS; Sigma Chemical Co., St. Louis, Mo.) in a carrier of Ringer's solution, after

[5]No measurable oocyte shrinkage occurred even after a week of preservation.

being anesthetized with MS-222 (tricaine methanesulfonate) at 150 ppm. Haydock (1971) found that salmon pituitary glands and PMS were both effective in inducing ovulation in bairdiella. PMS was used here because it had a standardized activity and was more readily available and easier to prepare than salmon pituitaries. The injected fish were checked for ovulation 30 h after injection and at hourly intervals thereafter until ovulation took place (Haydock 1971). In the vast majority of cases, ovulation occurred 30 or 31 h after the hormone injection. Spawning bairdiella of the size used in these experiments will yield 100,000 or more eggs (Haydock 1971). Male fish remained in a running ripe condition in the laboratory and did not require hormone injections. Bairdiella do not spawn spontaneously in captivity, whether injected or not, and gametes must be obtained by stripping. Haydock (1971) demonstrated that eggs must be fertilized 1 or 2 h after ovulation if maximal viability is to be retained.

Fertilization

Approximately 1,000 to 3,000 eggs were squeezed from an anesthetized, freshly ovulated female and added to a petri dish containing 75 ml of water of the desired temperature and salinity. When fertilizations under a number of conditions were to be made, eggs were added to all petri dishes before sperm was added. Sperm from a lightly anesthetized male fish was taken up in a pasteur pipette which was immediately filled and flushed with water from a petri dish containing eggs. Eggs and sperm were swirled in the dish for several seconds. This procedure was repeated for every dish, fresh sperm being obtained each time. After cleavage had begun, random samples (usually 100 to 300 eggs) were taken from each petri dish, preserved in 3% Formalin and later examined, and the number cleaving recorded. The percentage of eggs cleaving was taken as the percentage fertilized.

Spermatozoan activity was measured in various salinities by placing a drop of sperm under a cover slip, focusing on it with a compound microscope at 430× and adding seawater of the desired salinity. At frequent intervals after hydration, the activity of spermatozoa was rated on an arbitrary scale of 0 to 5, 0 being no activity and 5 being maximal activity. All such tests were conducted at approximately 25°C. More than 70 runs were made utilizing spermatozoa from nine fish, each run comprising between 4 and 15 observations, depending on the duration of activity.

Incubation

Developing eggs from the fertilization dishes were counted out by pipette under a dissecting microscope, rinsed with clean water of the test salinity to remove sperm, and transferred to incubators. The transfer of eggs was usually completed by the time the blastula stage had been reached, within 3 or 4 h after fertilization. One hundred developing eggs were placed in each incubator, and there were two replicate incubators for each experimental treatment. Each incubator (Figure 1) consisted of a 400-ml Pyrex beaker with an insert made from a truncated polypropylene beaker with its bottom covered by Nitex nylon mesh (350-μm mesh opening). Three hundred milliliters of water were added to each incubator. A slow stream of air bubbles in a centrally positioned glass tube created a flow of water such that eggs which rested on the bottom at low salinities were bathed by a continuous flow of aerated water (Figure 1).

One or two days before each experiment, seawater with a salinity of approximately 60‰ was made by adding artificial sea salts ("Instant Ocean"; Aquarium Systems, Inc., Wickliffe, Ohio) to HA Millipore-filtered seawater. This solution was filtered through paper (Whatman No. 1) to eliminate a residual cloudiness and then diluted with deionized water to the desired test salinities. Batches of seawater were aerated for several

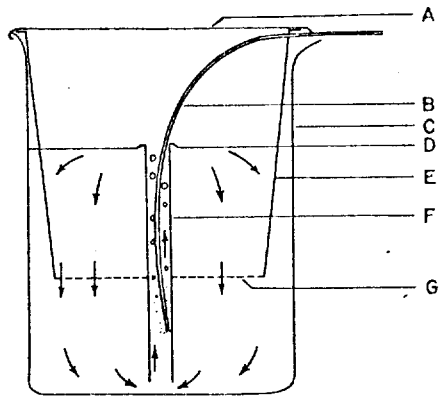


FIGURE 1.—Egg incubator. A) Parafilm cover; B) polyethylene air tube; C) 400-ml Pyrex beaker; D) water line; E) 250-ml polypropylene beaker, cut off at bottom; F) glass chimney; G) Nitex mesh. Arrows indicate direction of water flow.

hours before each experiment to stabilize oxygen tension and pH. Potassium penicillin G (50 IU/ml) and streptomycin sulfate (0.05 mg/ml) were added to the water just before it was placed in the incubators. Salinities were calculated by multiplying chlorinity values (Schales and Schales 1941) by 1.80655 (Johnston 1964) and remained within ±0.5‰ of the original salinity during an experiment. Temperatures were maintained within ±0.2°C of the desired value by immersing petri dishes and incubators in water baths equipped with cooling coils and thermostatically controlled heaters. The incubators were illuminated continuously from fluorescent room lamps which gave an intensity of from 320 to 480 lx at the water surface. Dissolved oxygen concentration in the incubators decreased with increasing temperature and salinity, and measured concentrations were within 2 or 3% of the saturation values given by Kinne and Kinne (1962). The highest oxygen content (at 18°C and 15‰) was 6.24 ml/liter, and the lowest (at 30°C and 55‰) was 4.05 ml/liter. The pH in the incubators increased with increasing salinity and decreasing temperature, varying between 8.08 and 8.27.

The percentage hatching and the condition of the larvae at hatching were recorded for each incubator. Supplementary containers (20-ml petri dishes) with 30 fertilized eggs each, were provided at each treatment to allow examination of eggs during development without disturbing the eggs in the incubators. Hatched larvae were not fed; some were kept in 400-ml beakers (without the polypropylene inserts used prior to hatching) and the pattern of mortality of the starved larvae recorded, and some were used in experiments on the temperature and salinity tolerance of yolk-sac larvae (May 1972).

During an early experiment, histological preparations were made of newly hatched larvae from different salinities at 25°C. Larvae were fixed in Bouin's solution, dehydrated in ethanol-normal butyl alcohol, embedded in paraffin, and sectioned transversely at 8 μm. Sections were stained with Mayer's hemalum and eosin.

Experimental Series

Two series of experiments on fertilization success, embryonic development, and hatching success were conducted, each series involving observations at 25 different combinations of temperature and salinity. Each series included two separate hormone-induced spawnings of fish held under identical conditions. The two spawnings in each series constituted a composite factorial array of treatments (a 3 × 5 plus a 2 × 5 factorial); this design, similar to those employed by Alderdice and his colleagues (Alderdice and Forrester 1967, 1971a, b; Alderdice and Velsen 1971), allowed coverage of a large factor space without utilizing all possible combinations of treatments. The ranges of temperature and salinity employed covered the viable ranges for bairdiella eggs, as determined in preliminary experiments. Table 1 indicates the temperatures and salinities in which eggs were fertilized and incubated in the two spawnings of each series.

The fish utilized for Series A were captured toward the end of the spawning season in the Salton Sea on 7 June 1971 and maintained on a 16L:8D photoperiod in 22°C water until the first hormone-induced spawning of the series on 23 August 1971 and the second on 1 September 1971. Ovarian biopsies indicated that the eggs were ready for spawning at this time, but only maximum oocyte diameters were measured and no oocyte size-frequency distributions were obtained. The tests were repeated in a second series of experiments, Series B. A group of fish captured in the Salton Sea on 20 May 1970 was shifted gradually

TABLE 1.—Dates and temperature-salinity conditions for experiments in Series A and B, 1971. There were two spawnings, performed at different dates, in each series; each spawning utilized eggs and sperm from different fish.

Temperature (°C)	Salinity (‰)	Series A		Series B	
		23 Aug.	1 Sept.	25 Nov.	3 Dec.
18	10	x		x	
	20	x		x	
	30	x		x	
	40	x		x	
	50	x		x	
21	15		x		x
	25		x		x
	35		x		x
	45		x		x
	55		x		x
24	10	x		x	
	20	x		x	
	30	x		x	
	40	x		x	
	50	x		x	
27	15		x		x
	25		x		x
	35		x		x
	45		x		x
	55		x		x
30	10	x		x	
	20	x		x	
	30	x		x	
	40	x		x	
	50	x		x	

from a short photoperiod to a 16L:8D photoperiod between 25 June and 10 July 1971. Half of these fish were transferred gradually to 15‰ and allowed to mature in that salinity as described below, while the other half were kept in seawater (approximately 33‰) and used to supply eggs for the Series B experiments. Prior to these spawnings, ovarian biopsies were taken and oocyte size-frequency distributions determined to assure that the fish were fully mature.

Acclimation of Spawning Fish to Low Salinity

These fish came from the same collection as those used to supply eggs in the Series B experiments and were brought to maturity simultaneously with them. The salinity was lowered to 15‰ over a period of 8 days by mixing seawater with an increasing proportion of fresh water. The day length was then increased from 9 to 16 h in 30-min increments, and the temperature was raised from 16° to 22°C over the same period (Figure 2). The tap water had been dechlorinated by passage through a commercial charcoal filter, and the mixed tap water and seawater flowed through the fish tank at 1,000 liters per hour (the same flow rate was maintained in the tank receiving straight seawater). Salinity was monitored daily in the seawater and low-salinity tanks. Variations were relatively slight during the period of gonadal maturation, monthly means ranging from 32.7 to 33.3‰ in the seawater tank and from 15.2 to 15.7‰ in the low-salinity tank.

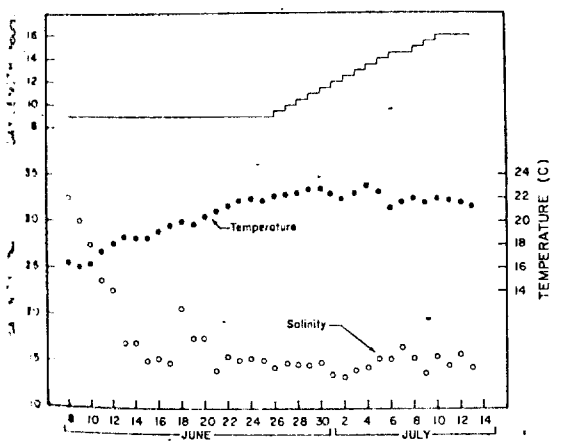


FIGURE 2.—Day length, temperature, and salinity during transition period, when fish were transferred to low-salinity water, ambient temperatures, and long days.

Female fish living at 15‰ were injected with PMS on 25 October, 8 November, and 16 November 1971. Eggs were fertilized (with sperm from males also acclimated to 15‰) and incubated as described above, at salinities of 10, 15, 20, 30, 40, 45, and 50‰. The temperature was 24.0°±0.2°C in all experiments with eggs from fish acclimated to low salinity. Hatched larvae were kept in 400-ml beakers at their original salinity to determine the percentage surviving to yolk exhaustion. The activity of spermatozoa from fish acclimated to 15‰ was assessed at various salinities as described above.

RESULTS

Spermatozoan Activity

Bairdiella spermatozoa measured 40 μm in total length, the head being about 2.5 μm long. In distilled water and dechlorinated tap water, spermatozoa showed at most only slight movement, usually in the form of very slow undulations which lasted at least 10 min. After approximately 1 min, the heads of many of these spermatozoa seemed to acquire bright rings, which an oil-immersion lens revealed to be the tail curled around the head, still undulating slowly.

Bairdiella spermatozoa became activated immediately upon contact with seawater (Haydock 1971), and the intensity of activity varied with salinity and time after initial contact with water. Spermatozoa were most active at the higher salinities but remained active longest at the lower salinities. At 10 and 15‰, a small smount of activity remained even as long as 10 min after hydration, but at 10‰ spermatozoa seldom showed activity above level 3 and at 15‰ they only rarely and briefly attained level 5 (Figure 3). At 25‰ all activity ceased by 4 min after hydration, and at 35‰ no activity was usually seen after 3 min. At 45 and 55‰, activity had completely stopped by 1.5 min after hydration. On rare occasions, at salinities between 15 and 55‰, slow undulations of some spermatozoa were observed after other movements had ceased. No difference was noted between spermatozoan activity in seawater and in Salton Sea water, nor between the activity of spermatozoa from fish acclimated to a salinity of 15‰ and from those kept at 33‰. Spermatozoan activity in dilute suspensions of

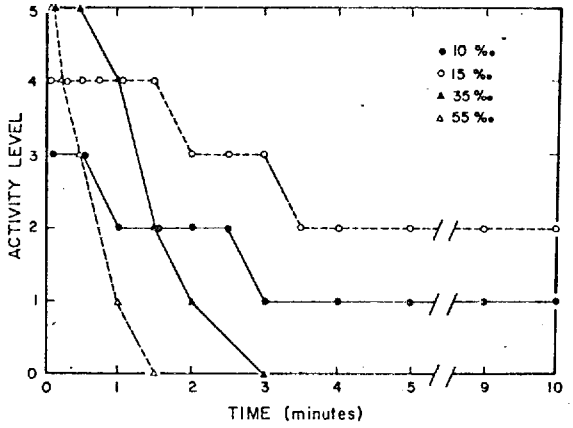


FIGURE 3.—Spermatozoan activity in four salinities as a function of time after hydration. The activity levels are described in the text.

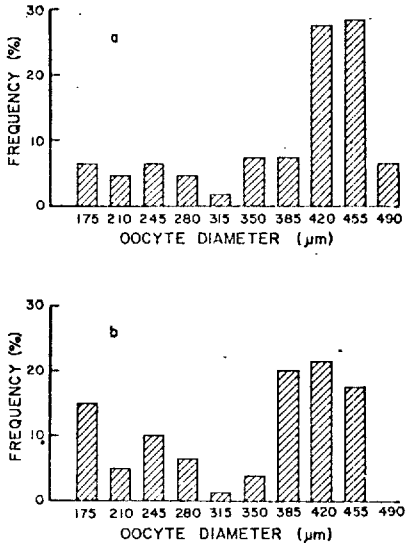


FIGURE 4.—Oocyte size-frequency distributions, based on ovarian biopsies, from fish used in Series B experiments. a) fish spawned on 25 November 1971, b) fish spawned on 3 December 1971.

sperm was the same as when hydration was carried out underneath a cover slip, indicating that the high concentration of spermatozoa in the latter case did not seriously affect the level or duration of their activity.

Maturity of Spawning Fish

Examination of many fish during this project showed that 500 μm was approximately the maximum diameter attained by oocytes in bairdiella before gonadal hydration. During hydration, which occurs in the laboratory only after an injection of gonadotropic hormone, the accession of water swells the eggs to 700 μm or more, the size at spawning. Ovarian biopsies showed that the two female fish used to supply eggs in the Series A experiments had oocytes as large as 500 μm before injection. The oocyte size-frequency distributions for the fish used in Series B (Figure 4) also showed maximum diameters of about 500 μm, and there were modes at 420 to 455 μm for the first fish and 385 to 455 μm for the second in Series B.

The much poorer fertilization and hatching success in Series A (see below) indicates that maximum oocyte diameter is not necessarily a good index of readiness for spawning. By this method it is impossible to tell whether there is a mode at the large end of the size-frequency distribution, as is characteristic of fish which are ready to spawn. The fish used to supply eggs in Series A were probably captured after the peak of spawning in the Salton Sea and their gonads at that time were probably either partly spent or beginning to be resorbed (see Haydock 1971). It was hoped that subsequent exposure to long days would induce ovarian recrudescence, but instead this treatment over a period of 2.5 mo apparently maintained the gonads at a suboptimal state of maturity or allowed them to regress even further (see Haydock 1971). A postspawning refractory period (Harrington 1959; Sehgal and Sundararaj 1970) may exist in bairdiella, but it cannot be very pronounced, since not only were eggs obtained from these fish after hormone injections in August and September, but at least 60% of the eggs could be fertilized under optimum conditions (see below). The fish used in Series B had completely regressed gonads when they were first exposed to long days in July 1971. By November 1971 or earlier they had developed ovaries capable of producing a large proportion of viable eggs, showing as much as 90% fertilization.

Fertilization

Although fertilization did take place at a salinity of 15‰, it was completely blocked at 10‰ (Table 2). In order to examine this phenomenon further, unfertilized eggs were placed in 10‰ water for various periods of time and then trans-

TABLE 2.—Percentage fertilization at various combinations of temperature and salinity in Series A and B.

Temperature (°C)	Salinity (‰)	Percentage fertilization	
		Series A	Series B
18	10	0	0
	20	14.5	28.9
	30	48.5	81.6
	40	24.4	87.5
	50	14.8	41.6
21	15	13.4	18.4
	25	43.5	69.9
	35	35.8	49.4
	45	38.8	59.9
	55	1.9	52.5
24	10	0	0
	20	63.1	62.3
	30	43.2	87.8
	40	7.4	81.3
	50	5.7	50.8
27	15	33.7	30.3
	25	52.6	77.2
	35	60.1	76.7
	45	16.8	82.0
	55	0	67.4
30	10	0	0
	20	48.7	74.6
	30	15.3	89.8
	40	2.7	68.7
	50	0	23.1

ferred to 20‰ and immediately exposed to sperm. The results (Table 3) showed that 10‰ water did not render the eggs infertile: even after 20 min at 10‰, a large proportion of the eggs could be fertilized at 20‰ and develop to hatching, although there was no fertilization in controls kept at 10‰. It was also found that eggs fertilized at 20‰ could be transferred to 10‰ and develop to hatching (Table 4). Thus the actual process of fertilization was somehow blocked at 10‰.

In Series A, fertilization was much more sensitive to high salinities and there seemed to be a greater temperature-salinity interaction than in Series B, with fertilization being more successful at high salinities when the temperature was low (Table 2). In Series B, at salinities above 10‰,

TABLE 3.—Effect of exposure to 10‰ water for various periods of time on fertilizability of bairdiella eggs at 20‰. At each time interval, between 200 and 400 eggs were transferred from 10 to 20‰, exposed to sperm, and later examined for fertilization. Thirty fertilized eggs from each group were followed until hatching.

Time at 10‰	Fertilized at 20‰ (%)	Hatching at 20‰ (%)
45 s	92.5	60.0
2 min	90.2	66.7
5 min	75.4	74.2
10 min	48.4	43.3
20 min	44.8	75.9

TABLE 4.—Survival and hatching of fertilized eggs transferred from 20‰ to 10‰ at various stages. Eggs were incubated in 20-ml petri dishes. The stages are described in Table 6.

Stage at transfer	Number of eggs transferred	Survival to stage VI (%)	Hatching (%)
IIe	30	96.7	63.3
IV	31	93.6	41.9
V	30	100	66.7
VII	29	—	62.1

fertilization was in nearly all cases over 50%, the few exceptions being at low temperature/low salinity and high temperature/high salinity combinations. A maximum of 89.8% fertilization was observed at 30°C-30‰ in Series B. The thermal limits for fertilization in Series B were evidently beyond the range tested (18°-30°C).

Normal Development

It will be helpful to outline the normal pattern of development of bairdiella eggs before discussing alterations in this pattern induced by various combinations of temperature and salinity. Newly spawned bairdiella eggs are approximately 725 μm in diameter and contain an oil globule with a diameter of about 18 μm. Occasionally there are two or three smaller oil globules instead of a single one. Like most pelagic eggs, bairdiella eggs float with the animal pole downward. The development of *Bairdiella icistia* eggs (Table 5, Figure 5) follows the pattern typical for small pelagic fish eggs and is not greatly different from that of *B. chrysura* as described by Kuntz (1915). Ahlstrom's numerical designation of developmental stages (Ahlstrom 1943) has been adopted here (Table 5), although some slight modifications of his scheme were necessary, and some of the stages have been broken down into substages. The times required to reach certain stages are listed (Table 5) for eggs at 33‰ at 25°C, based on observations made during a preliminary experiment in 1970. The newly hatched larvae are approximately 1.7 mm in length (snout to tip of notochord) and in ordinary seawater float upside down near the surface of the water.

Incubation Time

The time between fertilization and hatching varied with temperature and with salinity, and the patterns of hatching determined from the supplementary containers in Series B are shown in

TABLE 5.—Normal development of bairdiella eggs. Designation of stages in general follows Ahlstrom (1943), and times required to reach various stages are given for eggs in 33‰ water at 25°C.

Ahlstrom stage	Sub-stage	Approximate time after fertilization	Description
I	a	—	Unfertilized egg
	b	2 min	blastodisc
II	a	40 min	2 blastomeres
	b	50 min	4 blastomeres
	c	60 min	8 blastomeres
	d	2 h	Morula
	e	3 h	Blastula, periblast very apparent
III	a	6 h	Early gastrula, germ ring encircles as much as 1/3 of yolk, embryonic shield rudimentary
	b	7 h	Mid gastrula, embryonic shield expands, germ ring encircles as much as 2/3 of yolk
IV		8 h	Late gastrula, primitive streak forms
V		9 h	Blastopore closes, optic vesicles and Kupffer's vesicle form
VI	a	10 h	Somites begin to form; scattered melanophores appear, most dorsally behind optic vesicles, a few extending posteriad along notochord
	b	12 h	Lens and otic vesicles form, tip of tail reaches oil droplet
VII		15 h	Tail has moved beyond oil droplet and lifted off yolk; finfold apparent
VIII		17 h	Tail well beyond oil droplet; embryo twitches occasionally; heartbeat regular
—		20 h	Hatching

Figure 6. Series A showed similar patterns, but due to poorer survival the data are less complete and are not shown. In Figure 6 the cumulative percentage hatched has been plotted on a probability scale against time on an arithmetic scale; a straight line in this type of plot indicates a normal distribution (Sokal and Rohlf 1969), which is to be expected if differences in hatching time are due simply to random individual variation. At 30°C hatching was normally distributed for all salinities, but this was not true at the lower temperatures. At 27°C there was a plateau at 25‰, indicating that the hatching of certain eggs was delayed. At 24°C, hatching was distributed approximately in a normal fashion at 20, 40, and 50‰, but at 30‰ there was an inflection, the rate of hatching being slower after 23 h than before. At 21°C, hatching was distributed normally for 15, 35, and 45‰, but at 25‰ hatching took place in two phases separated by a 3-h period during which no hatching took place.

The time required for 50% of the larvae to hatch, estimated by graphical interpolation, decreased from 35.2 h at 21°C-25‰ to 16.0 h at 27°C-25‰. The estimated time at 50% hatching was slightly later at 30°C than at 27°C, although hatching began 2 h earlier in the former (see Figure 6). No clear-cut effect of salinity on median hatching times is discernible, but Figure 6 shows that hatching was completed more rapidly at the higher salinities (35‰ and above). The duration of hatching (the time between the appearance of the first and last hatched larvae) tended to be greater at the lower salinities and temperatures.

Embryonic Mortality

In certain treatments some surviving embryos failed to hatch but continued to develop within the chorion. Alderdice and Forrester (1971b) introduced the apt term, "postmature unhatched eggs" to describe such cases. Almost without exception, the postmature unhatched embryos were deformed in some way, usually bent and abnormally small. Often in such eggs part of the chorion was eventually digested away (Figure 7f), presumably by hatching enzymes, but the weak embryo was incapable of breaking completely free. Postmature unhatched eggs were most common at the low salinities, and the greatest proportion occurred at 30°C-20‰ (Table 6).

Eggs in Series A showed much higher mortality than those in Series B, especially at the higher temperatures and salinities (Table 7). The following description of embryonic mortality refers primarily to the eggs in Series B, which are considered more representative of normal, healthy eggs. The higher mortality in Series A usually showed up very early in embryonic development (prior to stage V); otherwise the two series showed similar trends.

No eggs hatched at 18°C and nearly all died during stage IIe (blastula). After the second cleavage at 18°C the blastomeres assumed a cloverleaf appearance which was not seen at higher temperatures (Figure 7; cf. Figure 5). Subsequent cleavages at 18°C were rather irregular, and during the blastula stage much of the cytoplasm gathered into isolated clumps, and the periblast became unusually large (Figure 7). Nearly all eggs stopped developing at this stage.

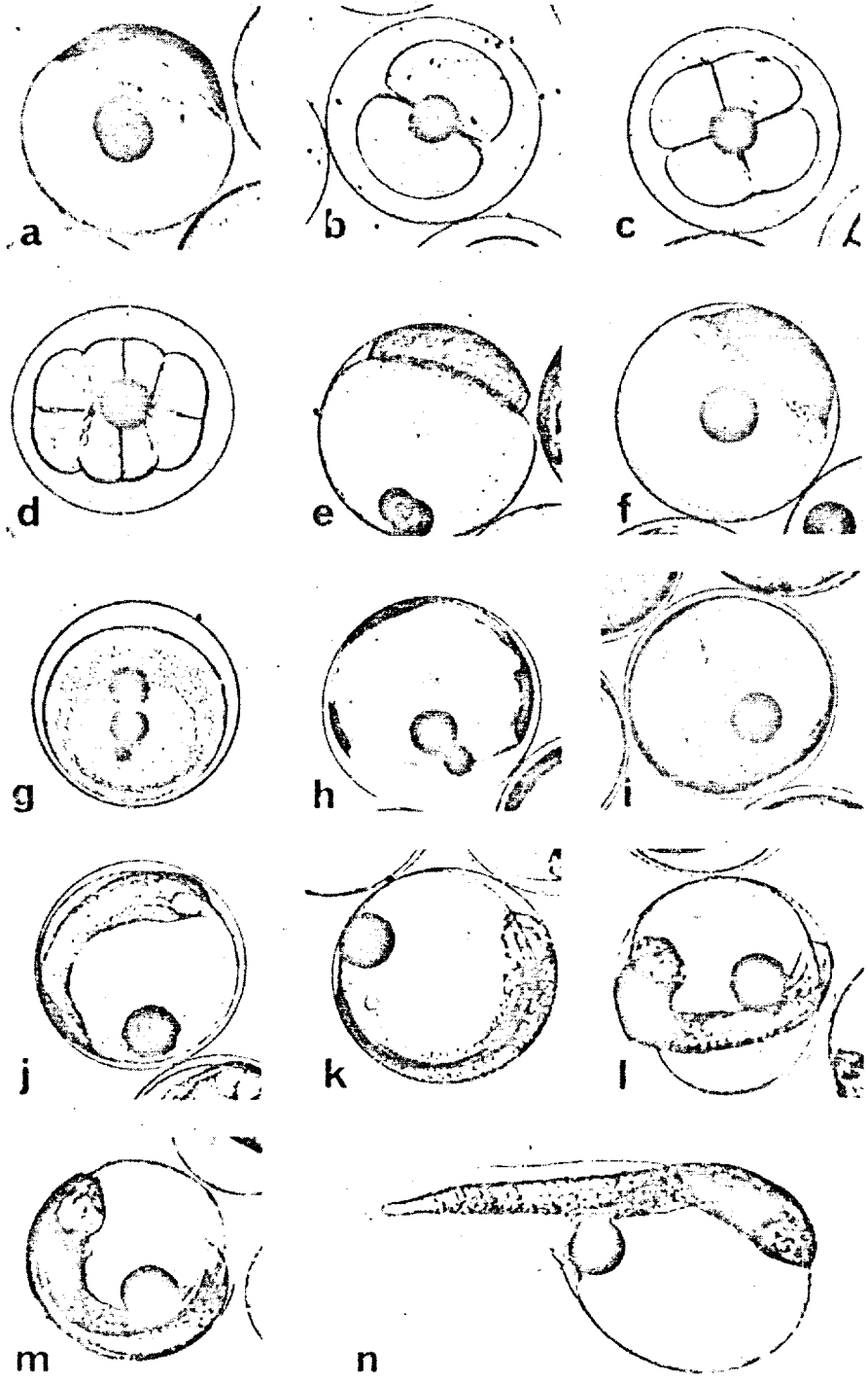


FIGURE 5.—Normal developmental stages of *Bairdiella icistia* at 25°C-33‰. a) stage Ib, 4 min after fertilization; b) stage IIa, 40 min; c) stage IIb, 50 min; d) stage IIc, 60 min; e) stage IId, 2 h; f) stage IIe, 3 h; g) stage IIIa, 6 h; h) stage IIIb, 7 h; i) stage IV, 8 h; j) stage V, 9 h; k) stage VI, 12 h; l) stage VII, 15 h; m) stage VIII, 17 h; n) newly hatched larva.

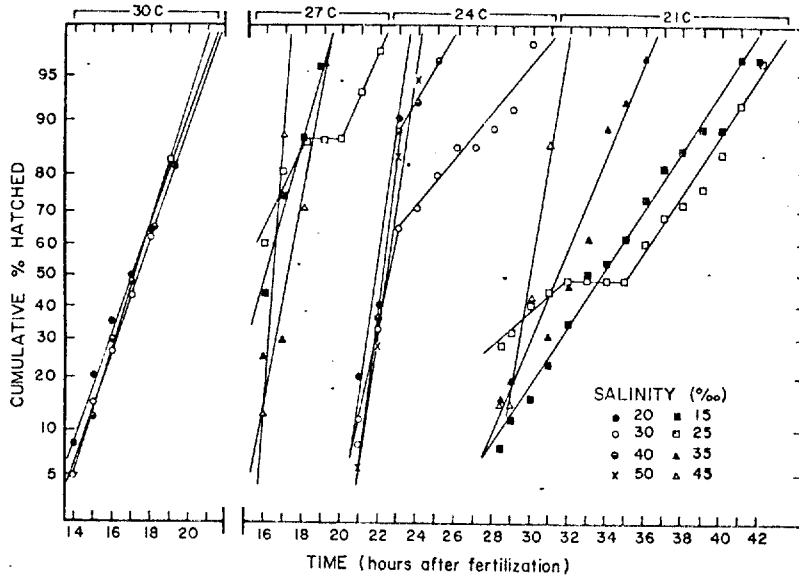


FIGURE 6.—Cumulative percentage of larvae hatching, as a function of time after fertilization, for the Series B experiments. Percentage hatching is plotted on a probability scale; lines were fitted by eye.

TABLE 6.—Percentage of postmature unhatched eggs at various combinations of temperature and salinity in Series B. There are two replicates at each treatment combination. Series A showed similar trends.

Salinity (‰)	Temperature (°C)			
	21	24	27	30
15	17.9		16.4	
	23.5		11.1	
20		19.2		43.4
		13.9		31.8
25	2.1		4.0	
	2.0		3.2	
30		7.8		6.7
		4.4		23.0
35	4.0		7.2	
	7.5		5.2	
40		0		3.1
		0		2.1
45	0		5.5	
	4.0		9.9	
50		0		0
		0.9		0
55	0		0	
	0		0	

TABLE 7.—Percentage total and viable hatch of fertilized eggs in various combinations of temperature and salinity in Series A and B. In each series there were two replicate groups of eggs (a and b) at each treatment combination.

Temperature (°C)	Salinity (‰)	Percentage total hatch				Percentage viable hatch			
		Series A		Series B		Series A		Series B	
		a	b	a	b	a	b	a	b
18	10	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0
21	15	66.7	82.5	88.4	76.5	2.0	7.5	77.9	68.4
	25	25.0	40.4	94.7	92.9	20.0	29.3	77.9	59.7
	35	27.6	37.0	72.7	68.8	16.3	35.0	50.6	38.8
	45	1.9	7.1	72.8	47.5	0	2.0	18.1	11.9
	55	0	0	0	0	0	0	0	0
24	10	0	0	0	0	0	0	0	0
	20	62.6	39.8	96.0	96.0	33.3	23.5	70.0	80.2
	30	34.3	53.5	75.6	89.0	28.3	44.6	63.0	70.2
	40	24.2	51.4	66.3	80.0	10.1	38.3	34.6	53.8
	50	8.0	9.9	66.3	41.2	1.3	2.8	0	0
27	15	67.7	66.0	76.2	89.9	7.1	4.1	68.2	81.1
	25	22.6	34.1	79.8	86.4	22.6	30.6	70.8	81.0
	35	40.9	41.5	78.4	79.2	30.7	29.8	57.5	54.7
	45	11.9	4.5	42.9	31.9	0	1.5	0	0
	55	0	0	1.0	2.0	0	0	0	0
30	10	0	0	0	0	0	0	0	0
	20	12.0	14.4	85.9	73.8	7.0	3.1	38.2	28.6
	30	1.0	1.0	62.2	60.0	0	0	15.2	14.6
	40	0	0.9	4.1	6.2	0	0	0	0
	50	0	0	0	0	0	0	0	0

At 30°C, all eggs died at or before gastrulation at 50‰; at 40‰, a small proportion of the eggs survived the high early mortality but most of these failed to hatch, only 4-6% hatching successfully in Series B (Table 7). At 20 and 30‰ at 30°C, most embryonic mortality occurred after the embryos had developed pigmentation, although at 30‰ abnormal development was apparent in many eggs during cleavage and gastrulation

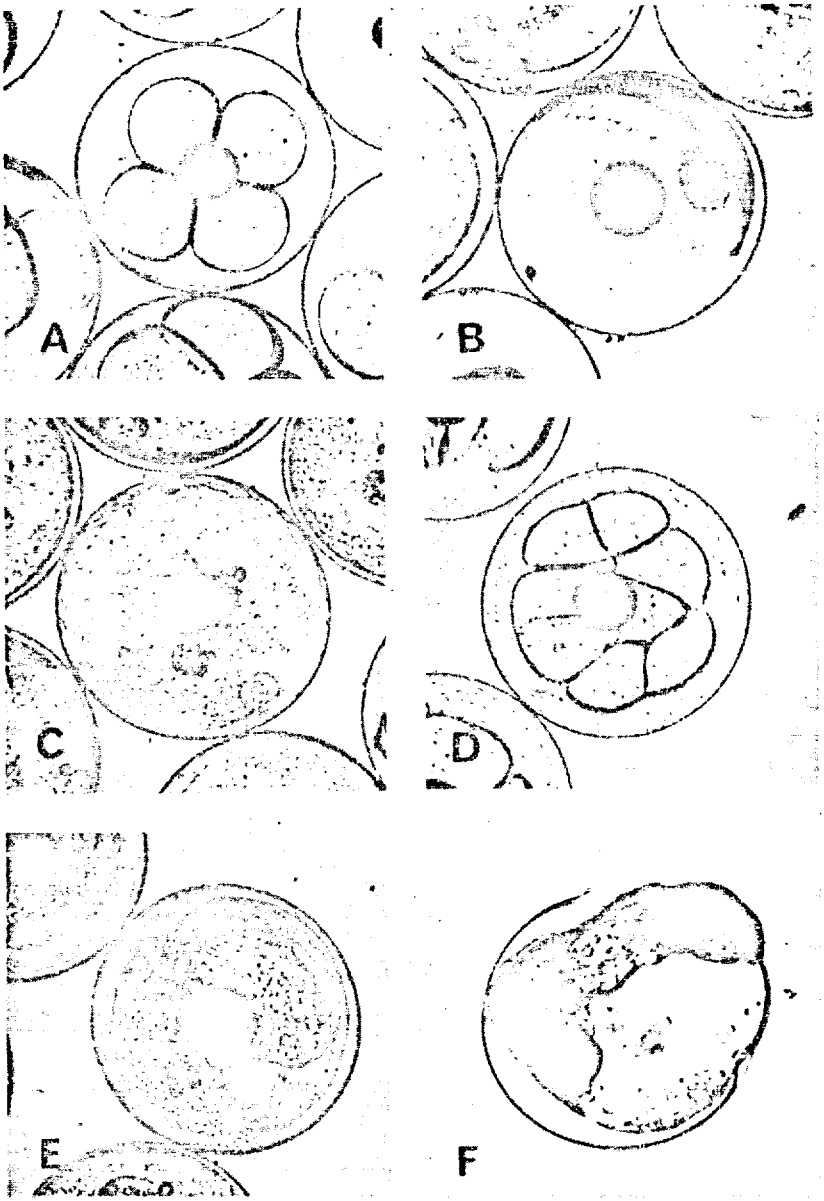


FIGURE 7.—Developmental abnormalities. A) stage IIb, at 18°C-30‰, showing unusual cloverleaf appearance of blastomeres; B) stage IIe, 18°C-30‰, showing enlarged periblast and clumped cytoplasm; C) stage IIe, 18°C-30‰, showing clumped cytoplasm; D) stage IIc, 30°C-30‰, showing irregular cleavage pattern; E) stage IIIa, 30°C-30‰, showing abnormal germ ring and clumping of cytoplasm; F) deformed embryo unable to free itself completely from the chorion, 24°C-20‰.

(Figure 7), and some eggs showed clumping of the cytoplasm similar to that observed at 18°C. Larvae hatching at 30°C were inactive. By following the development of individual eggs in the supplementary containers, it was noted that, no matter what the temperature or salinity, irregularly cleaving eggs usually died before completing gastrulation, and none ever hatched. At 21°, 24°, and 27°C, hatching was generally poorer at the higher salinities (Table 7). At 55‰ virtually no hatching

took place. A maximum of 96% hatching of fertilized eggs was observed at 24°C-20‰.

Deformed Larvae

Immediately after hatching, larvae often had curved bodies reflecting the curvature necessitated by confinement within the chorion (Figure 8), but such larvae soon straightened out. Some larvae, however, had sharply bent or kinked notochords at hatching, a deformity which was irreversible and which prevented normal swimming. These deformities were most common at high salinities (40‰ and above) and at 30°C. In Series A, salinities of 15 and 20‰ produced a high proportion of larvae with a strange deformation, in which the tail was recurved and fused to the trunk (Figure 8). Up to 78% of the larvae hatching at 27°C-15‰ showed this irreversible deformity in Series A, but the figure was only about 15% at 21°C-15‰ and less than 10% at 24°C-20‰; with one or two minor exceptions, other treatments in Series A did not produce this particular distortion, and it was not observed in any treatment in Series B. A greater proportion of late-hatching larvae in a given treatment displayed deformities than early-hatching larvae.

Larvae hatching at 15 and 20‰ showed pronounced edema (Figure 9). Histological sections showed that the size of the subdermal space was inversely related to salinity (Figure 10), an osmotic phenomenon which Battle (1929) also observed in larvae of *Enchelyopus cimbrius*. The yolk sac of newly hatched bairdiella larvae was larger and contained more water at lower salinities (May 1972).

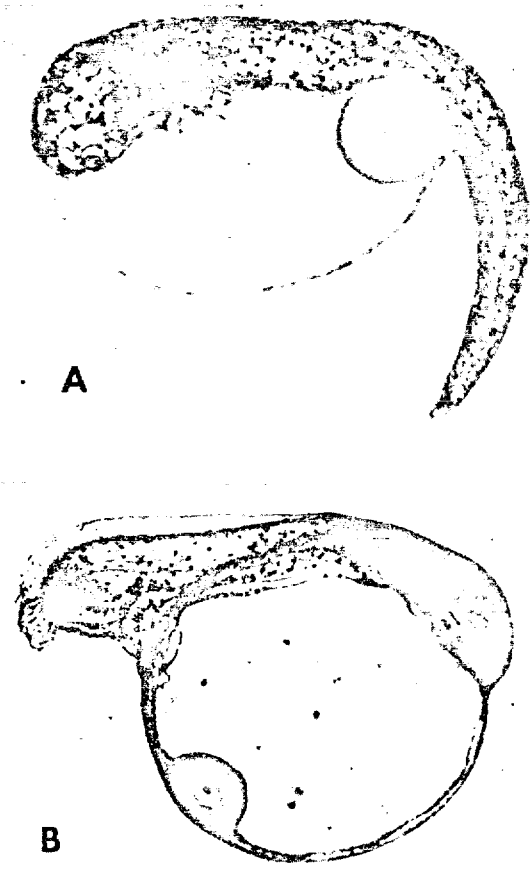


FIGURE 8.—Newly hatched larvae. A) ventral view of a normal larva, showing curvature often seen just after hatching, 24°C-30‰; B) lateral view of a larva with a recurved tail, 24°C-20‰, Series A.

Survival of Starved Larvae

Besides showing deformities, at high temperatures and salinities many larvae died before exhausting their yolk supplies. At 45 and 50‰ all larvae in Series A were dead within 1 day after hatching, and the same was true of the few hatched larvae at 40‰ at 30°C (Figure 11). The time of major mortality and the maximum survival time of starved larvae were inversely proportional to temperature and salinity. Because some of the larvae from Series B were used in tests of temperature and salinity tolerance (May 1972), a complete set of survival curves is not available for them. However, estimates of the percentage of larvae surviving to yolk absorption were obtained from the remaining larvae and from larvae in the least stressful conditions in the tolerance experiments, and these estimates indicated better larval survival in Series B than in Series A. For example, the Series B curves for 27°C (Figure 12) did not show the high mortality before yolk exhaustion at 25 and 35‰ seen in Series A, and a similar difference between the two series occurred at 21°C. At 24°C-40‰, an estimated 70% of the larvae were alive at yolk exhaustion in Series B, compared with only about 20% in Series A. At the highest temperatures and salinities, however, Series B showed heavy early mortality similar to Series A.

Viable Hatch

The percentage hatching of viable larvae (Table

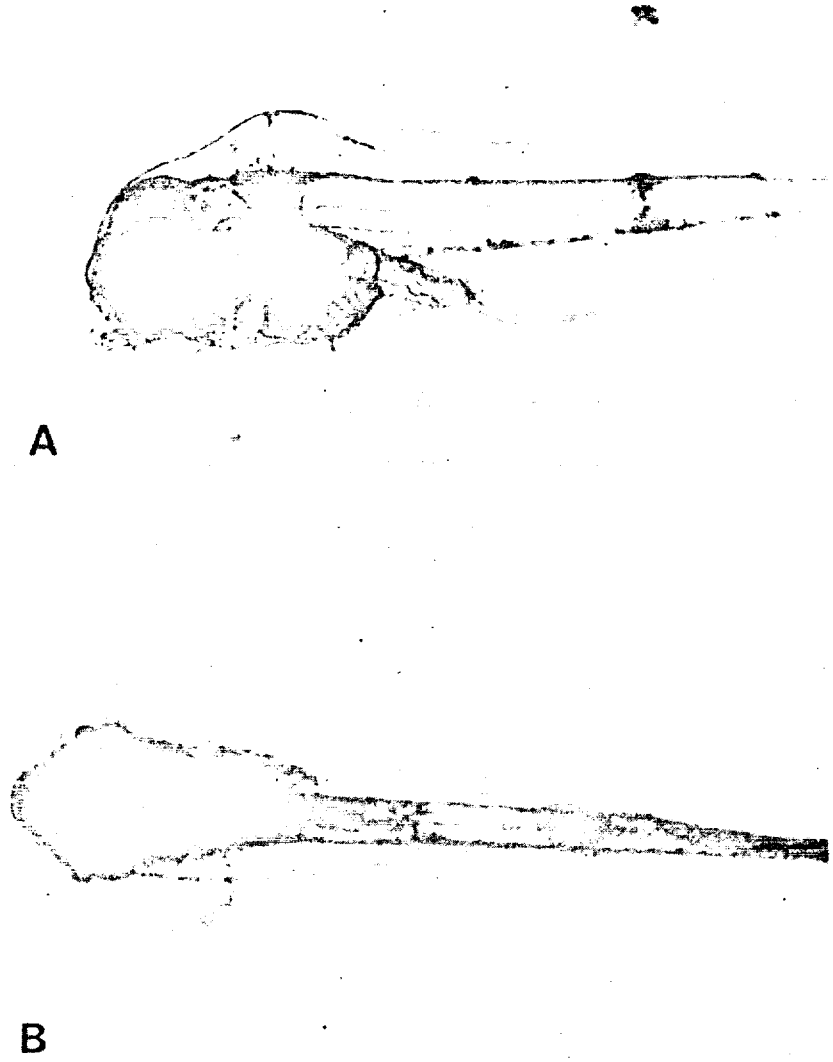
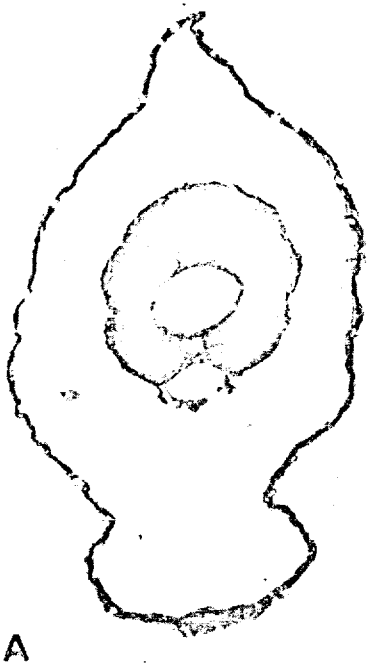


FIGURE 9.—Two-day-old larva, 24°C-20‰, with enlarged subdermal space. A) side view, B) dorsal view.

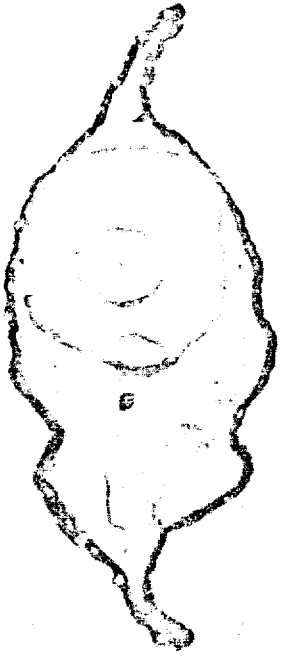
7), calculated from the preceding information, may be considered the ultimate criterion of successful development in these experiments. Viable larvae are defined here as morphologically normal larvae capable of surviving to yolk absorption, since all other larvae would not survive in nature. Series A showed a much lower viable hatch than Series B at very high and very low temperatures and salinities. Even for the best eggs, it is clear that salinities above 40‰ are detrimental to early survival, and that 30°C is extremely stressful. Survival at higher salinities was considerably better at low temperatures. The various observations on embryonic and larval survival in Series B are summarized (Figure 13) in the manner of Alderdice and Forrester (1967).

Response Surfaces

It has become customary to describe a biological



A



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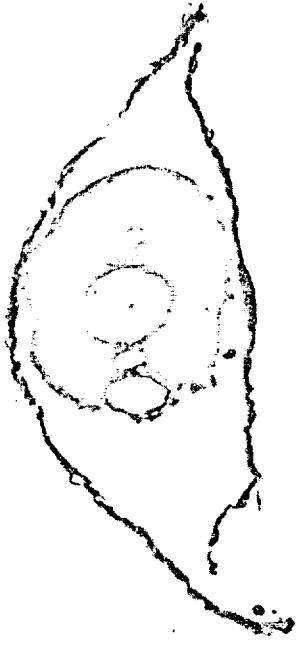

C

D

FIGURE 10.—Transverse sections of newly hatched larvae incubated in various salinities at 25°C. Serial sections were made of each larva, and the sections illustrated were located two sections posterior to the anus. A) 20‰, B) 33‰, C) 45‰, D) 50‰.

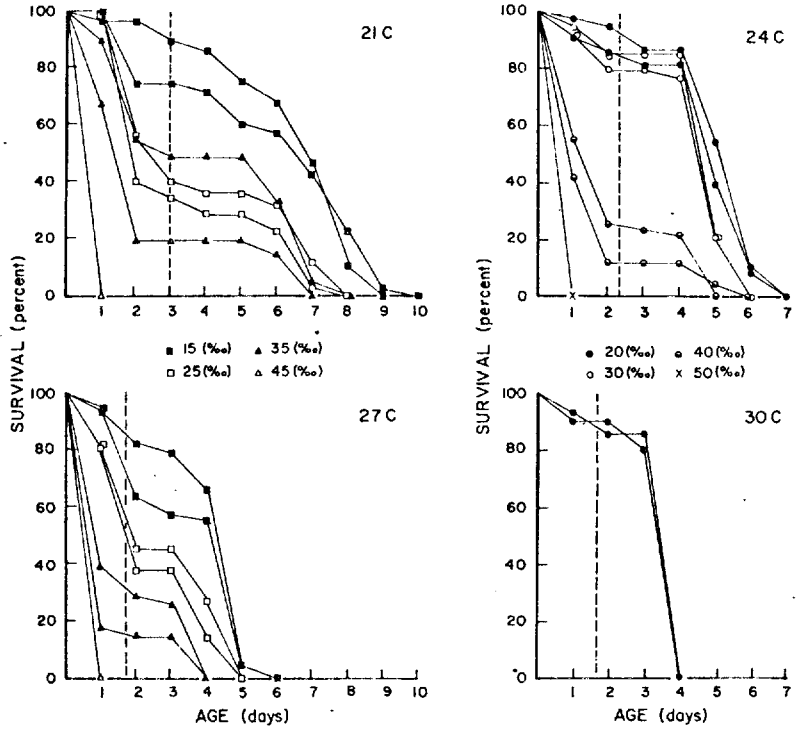

FIGURE 11.—Survival curves for unfed larvae at various temperatures and salinities in Series A. There were two replicate groups of larvae at each treatment, and the vertical dashed lines indicate the time of complete yolk absorption at each temperature.

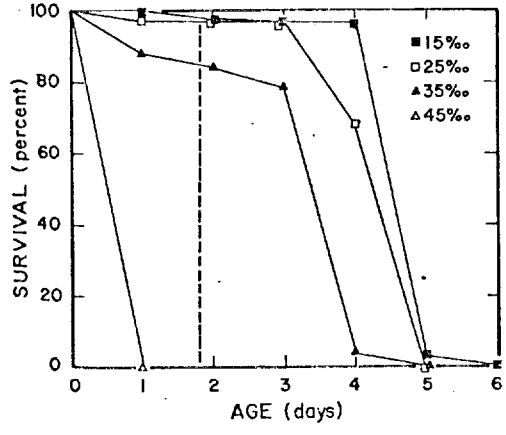

FIGURE 12.—Survival curves for unfed larvae in various salinities at 27°C, Series B. Vertical dashed line indicates the time of complete yolk absorption.

response to temperature and salinity by fitting a second order polynomial to the data and presenting response surfaces calculated from this equation (e.g., Costlow et al. 1960; Alderdice and Forrester 1967; Haefner 1969). This procedure was applied by computer to the results for fertilization, total hatch, and viable hatch, and the resulting equations are given in Table 8. Analysis of variance (ANOVA) showed that, although regression accounted for most of the variance in these data, deviations from regression were highly significant for all equations. This probably reflects the difficulty of fitting a second order polynomial to data of this sort, especially when abrupt thresholds are present, as between 10 and 15‰ and 18° and 21°C. A higher order polynomial, or a nonlinear model (Lindsey et al. 1970), would no doubt yield a better fit. Nonetheless, the second

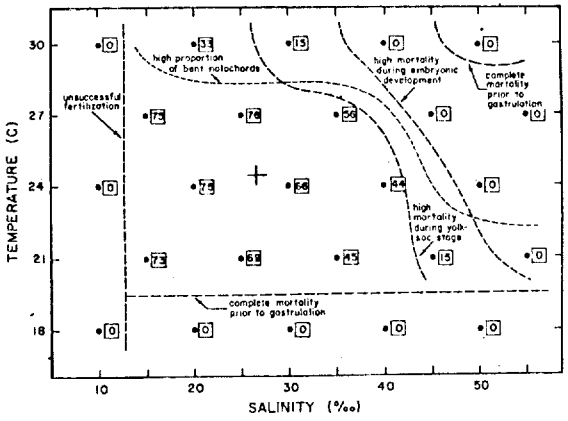

FIGURE 13.—Summary of the effects of temperature and salinity on early development of bairdiella. Closed circles identify treatment combinations utilized in the experiments, and the numbers in squares beside them give the mean values for viable hatch in Series B. The cross marks the estimated position of maximum viable hatch.

significance of interaction by existing statistical techniques.

Acclimation of Spawning Fish to Low Salinity

On 20 October 1971 it was discovered that only 4 of the 26 fish acclimated to 15‰ seawater were females, whereas 15 of the 26 fish at 33‰ were females. The random assignment of fish to the two tanks had somehow resulted in a great disparity in their sex ratios. Two of the four female fish from 15‰ biopsied on 20 October 1971 had well-developed ovaries, showing that gonadal maturation can take place in a salinity of 15‰. The two well-developed females, as well as one of the poorly developed ones, were spawned with hormone injections; the oocyte size-frequency distributions from biopsies of the three fish shortly before injection are shown in Figure 14.

TABLE 8.—Multiple regression equations for percentage fertilization, total hatch, and viable hatch, as functions of temperature and salinity. Y = arcsin (percentage)$^{1/2}$, X = temperature (C), X_2 = salinity (‰).

		Fertilization	
Series A:	Y	=	$-3.89030 + 0.25964X_1 + 0.10770X_2 - 0.00476X_1^2 - 0.00125X_2^2 - 0.00128X_1X_2$
Series B:	Y	=	$-2.76156 + 0.14033X_1 + 0.12125X_2 - 0.00240X_1^2 - 0.00149X_2^2 - 0.00055X_1X_2$
		Total hatch	
Series A:	Y	=	$-8.55800 + 0.72293X_1 + 0.04177X_2 - 0.01482X_1^2 - 0.00073X_2^2 - 0.00014X_1X_2$
Series B:	Y	=	$-13.40397 + 1.06566X_1 + 0.10817X_2 - 0.02115X_1^2 - 0.00157X_2^2 - 0.00070X_1X_2$
		Viable hatch	
Series A:	Y	=	$-3.91134 + 0.31755X_1 + 0.02932X_2 - 0.00645X_1^2 - 0.00046X_2^2 - 0.00017X_1X_2$
Series B:	Y	=	$-9.99277 + 0.81039X_1 + 0.07829X_2 - 0.01620X_1^2 - 0.00117X_2^2 - 0.00066X_1X_2$

order equations are useful in that they allow computation of optimal conditions (Box 1956). The resulting values (Table 9) show a thermal optimum at about 24°C for total and viable hatch in both series, and optima of 23° and 25°C for fertilization in Series A and Series B, respectively. The calculated salinity optimum for fertilization was considerably higher in Series B than in Series A (36 vs. 31‰), but in both series the optimal salinities for hatching were below those for fertilization, ranging from 26 to 29‰. The optimal responses estimated at these points from the equations (Table 9) are below the maximal values actually recorded (cf. Tables 2 and 7), another indication of the lack of fit of the second order polynomial. The calculated positions of the optima, however, are the best available estimates of the true optima. These experiments were designed primarily to cover wide ranges of the two factors under consideration, and the arrangement of treatments unfortunately does not allow testing of the

TABLE 9.—Optimum temperatures and salinities for fertilization, total hatch, and viable hatch, estimated from the regression equations (Table 8). Also listed are the optimum percentage fertilization, total hatch, and viable hatch, calculated from the regression equations at the estimated temperature and salinity optima.

Item	Temperature (°C)	Salinity (‰)	Percentage
Fertilization:			
Series A	23.1	31.3	50.3
Series B	25.1	36.1	85.8
Total hatch:			
Series A	24.3	26.3	47.6
Series B	24.7	28.9	94.3
Viable hatch:			
Series A	24.3	27.4	11.2
Series B	24.5	26.6	67.3

The freezing point depression of blood serum from fish acclimated to 15‰, determined by the melting point method of Gross (1954), was 0.64 ± 0.066°C (mean ± SD, n = 12 fish), and that of fish from 33‰ was 0.63 ± 0.076°C (n = 12 fish). The two groups did not differ significantly, nor was

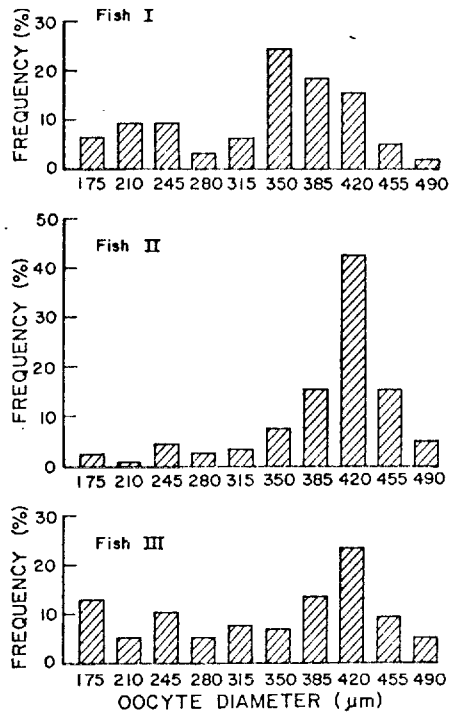

FIGURE 14.—Oocyte size-frequency distributions, based on ovarian biopsies, from fish acclimated to 15‰.

TABLE 10.—Fertilization success for eggs obtained from fish acclimated to 15‰.

Salinity (‰)	Percentage fertilization		
	Fish I	Fish II	Fish III
10	0	0	0
15	19.7	73.8	42.2
20	24.2	89.8	53.4
30	2.5	31.1	88.5
40	0.9	18.7	79.2
45	0	21.2	85.3
50	0	20.4	63.3

TABLE 11.—Percentage total and viable hatch of fertilized eggs at various salinities for eggs from fish acclimated to 15‰. For each fish, there were two replicate groups of eggs at each salinity.

Salinity (‰)	Fish I		Fish II		Fish III	
	a	b	a	b	a	b
			Total hatch			
15	23.4	22.0	87.1	79.8	97.0	92.6
20	30.5	44.2	86.7	86.3	97.0	95.7
30	10.9	2.0	46.9	66.0	97.9	94.9
40	—	—	80.0	80.9	84.8	68.8
45	—	—	71.2	71.4	65.7	76.3
50	—	—	35.9	31.0	22.8	45.8
			Viable hatch			
15	17.0	20.0	66.3	50.5	79.2	72.6
20	27.1	26.9	62.7	70.3	82.7	76.5
30	8.7	0	37.5	49.2	84.3	79.9
40	—	—	43.0	56.1	55.3	44.7
45	—	—	18.1	12.6	19.4	22.9
50	—	—	0	0	0	0

there a significant difference between sexes within each group (Mann-Whitney *U* test; Siegel 1956).

The fish with poorly developed ovaries (Fish I) became listless and swam in a disoriented manner after the hormone injection; 5 days after spawning, it still spent most of its time resting on its side on the bottom of the tank. At this point the fish was sacrificed and dissected, revealing some large, hydrated eggs with coalesced yolk, 665-735 μm in diameter, along with many unhydrated eggs still in their follicles, measuring 350 μm in diameter. Eggs obtained from the hormone-induced spawning of this fish showed low fertility, significant numbers being fertilized only at 15 and 20‰, with a maximum of 24.2% fertilized at 20‰ (Table 10). The hatching success of fertilized eggs was also poor, with a maximum total hatch of 44.2% at 20‰ (Table 11). A few embryos and larvae produced by this fish displayed various degrees of cyclopia, a deformity rarely seen in other batches of eggs.

As expected, the two ripe fish produced much better eggs, with maximum fertilization percentages of almost 90% (Table 10). Eggs from Fish II had a lower optimum salinity than those from Fish III and were more sensitive to high salinities (Table 10). No fertilization took place at 10‰, as was the case with eggs from fish living at 33‰.

Hatching at 15, 20, and 30‰ was better in eggs from Fish III than in those from Fish II, despite the better fertilization success of the latter at 15 and 20‰ (Table 11). Hatching at 40, 45, and 50‰ was comparable in the two batches of eggs. Eggs from Fish II hatched more successfully at 15 and 20‰ than at 30‰, whereas those from Fish III hatched equally well at 15, 20, and 30‰. The incidence of postmature unhatched eggs was similar to that in eggs from Series A and Series B, with most appearing at 15 and 20‰, few at 30‰, and very few or none above 30‰.

The hatching success at various salinities (20, 30, 40, and 50‰) of the best batch of eggs from fish living at 15‰ (i.e., from Fish III) was compared with that of the best batch of eggs from fish at 33‰ (Series B) at the same temperature (24°C) and salinities, by ANOVA (an arcsin-square root transformation was applied to the percentages).

Neither total nor viable hatching success differed significantly between the two groups. Therefore, acclimation of spawning fish to a low salinity did not affect the salinity tolerance of the eggs in any detectable way. Effects of acclimation salinity on egg size and buoyancy will be discussed elsewhere (May in preparation).

DISCUSSION

Fertilization and early development in *Bairdiella icistia* are stenothermal and stenohaline processes. The approximate limits for successful development, from fertilization to yolk exhaustion, are 20° to 28°C and 15 to 40‰, although a certain interaction of the two factors is apparent, development being more successful at the higher salinities when the temperature is relatively low, and at the higher temperatures when the salinity is relatively low. The limits within which successful reproduction can take place are defined by the most sensitive stages and events in development. The lower limit of salinity for bairdiella reproduction is defined by fertilization, since eggs cannot be fertilized at 10‰ or below, even though eggs fertilized at a higher salinity will develop at 10‰. However, the lowest salinity at which eggs remain buoyant may in some cases determine the lower salinity threshold for successful reproduction (May 1972). The upper salinity limit, and both the upper and the lower limits of temperature, are defined by the abilities of the embryos to develop. Fertilization is successful at 18°C but development is not; likewise, fertilization does take place at 30°C, and at salinities of 45‰ and above, but the hatching of viable larvae is greatly curtailed.

Fertilization in bairdiella is more limited by salinity than temperature over the ranges studied. The complete block to fertilization which occurs at 10‰ may be related to an inability of spermatozoa to function properly at this salinity. Although the egg itself seems to be unharmed by water of 10‰, at this salinity spermatozoa never attain the high intensity of activity that they do at higher salinities. At 15‰, where spermatozoan activity is more intense than at 10‰ but less intense than at higher salinities, fertilization occurs but is poorer than at higher salinities. Fairly high salinities seem to aid fertilization: the calculated optimum salinities for fertilization were higher than the optima for hatching in both Series A and Series B. It is possible that low calcium levels at low salinities inhibit the activity of spermatozoa (Yanagimachi and Kanoh 1953). In general, the greater the intensity of spermatozoan activity, the shorter is the overall duration of activity (Figure 3). Thus a shortlived but extremely high level of spermatozoan activity may be necessary for fertilization in bairdiella, perhaps because penetration of the micropyle requires a considerable expenditure of energy on the part of the spermatozoa. This implies that the actual process of fertilization takes place during the first few seconds after hydration of the sperm, when spermatozoan activity is maximal. Haydock (1971) reports that bairdiella spermatozoa are no longer able to fertilize eggs 30 s after sperm hydration. In such a situation, experimental technique could have a marked influence on the success of artificial fertilization, since a delay of a few seconds between hydration of the sperm and contact of the sperm with eggs could significantly reduce the percentage of eggs which become fertilized. A technical problem of this sort may explain the puzzling differences in fertilization success between eggs from Fishes II and III, acclimated to 15‰ (Table 10).

Several previous investigations of salinity effects on spermatozoan activity in other fishes provide interesting contrasts with the present results. Ellis and Jones (1939) found that spermatozoa of Atlantic salmon, *Salmo salar*, a fish which spawns in fresh water, were active for over 180 min in seawater diluted to 15 and 20% and that the duration of activity dropped off sharply above and below these salinities. Working with the longjaw mudsucker, *Gillichthys mirabilis*, Weisel (1948) observed that spermatozoa showed only feeble activity in seawater diluted to 17-24%, but activity was intense in 25% seawater and above; the duration of spermatozoan activity was maximal (over 50 h!) in 25% seawater and decreased at higher salinities, as it did in the case of bairdiella. Yamamoto (1951) found that spermatozoa of the flounder, *Limanda schrenki*, were active in normal seawater and in seawater diluted to 50%, but showed no activity (and no fertilizing capability) in 25% seawater. Hines and Yashouv (1971), on the contrary, found that mullet, *Mugil capito*, spermatozoa exhibited a gradual increase in duration of activity with increasing salinity up to the salinity of normal seawater, rather than a threshold. Dushkina (1973) reported that spermatozoa of Pacific herring, *Clupea harengus pallasi*, were most active at higher salinities (17-23‰), but remained active longest at the

lowest salinities (0.3-0.5‰), as was true for bairdiella; however, in herring the duration of spermatozoan activity was much longer (4-8 days at 6-7°C) and some fertilization occurred even in fresh water. Spermatozoan activity and its response to salinity appear to be extremely variable among fish species, which is hardly surprising in view of the diversity of habitats and modes of reproduction of fishes.

The large proportion of postmature unhatched eggs at low salinities (Table 6) reflects a high incidence of malformations under these conditions, the embryos being physically unable to break from the chorion. Edema seen among larvae in low salinities suggests that deformities and the inability to hatch may be related to osmotic problems. Battle (1929) noted a similar difficulty in hatching among embryos of fourbeard rockling, *Enchelyopus cimbrius*, in low salinities and attributed it to abnormally developed musculature, which prevented movements required to free the embryo from the egg case. An inability to complete hatching at low salinities has been reported for other species as well (Ford 1929; McMynn and Hoar 1953; Alderdice and Forrester 1967; Dushkina 1973). The generalization that gastrulation and hatching are the two developmental stages most sensitive to physical disturbance (e.g., Holliday 1969) seems valid in the case of bairdiella.

The finding that unfed bairdiella survive longest in low salinities and low temperatures is not unique. Nakai (1962) and Hempel and Blaxter (1963) likewise found that starving larvae of *Sardinops melanosticta* and *Clupea harengus* survived longer at lower salinities, and more rapid mortality among unfed larvae at higher temperatures has been observed on a number of occasions (e.g., Qasim 1959; Bishai 1960; Hempel and Blaxter 1963; Alderdice and Velsen 1971; Hamai et al. 1971). High temperatures increase metabolic rate, accelerate yolk absorption (May 1972), and no doubt hasten death from starvation. The effect of high salinities on larval physiology is less certain: a salinity effect on embryonic or larval oxygen consumption has not been demonstrated except after abrupt transfer (Holliday 1969), and salinity has only a small effect on the rate of yolk absorption in bairdiella (May 1972). High salinities may increase larval mortality by causing osmotic or ionic changes in the interior milieu, although the larvae of some species have proved capable of osmoregulating over rather wide ranges of salinities (Holliday 1969). Lower levels of activity have been observed among larvae of some species in low salinities (Hempel and Blaxter 1963; Holliday 1965), and may reduce their metabolic demand and thus extend their survival time (Holliday 1965).

The salinity tolerance of bairdiella eggs is not significantly affected by acclimation of the parent fish to low salinity (15‰). This might suggest that the enhanced survival at low salinities which Solemdal (1967) observed in eggs from the Finnish population of flounder, *Pleuronectes flesus*, has a genetic basis. If acclimation of spawning fish to low salinities does not cause an increase in embryonic tolerance to low salinities, one might expect that high-salinity acclimation would be similarly ineffectual in aiding embryonic survival at high salinities. This supposition should be verified experimentally; but, if valid, it implies that salinity responses determined on eggs from fish living in ordinary seawater should be accurate predictors of reactions to different salinities in nature, except where genetic adaptation has occurred. This could be a significant advantage in cases where it is important to estimate the effects of rising salinities in specified habitats, such as the Salton Sea or the Gulf of California, where high salinities may in the future pose a threat to existing stocks of fish.

Because of the unusual chemical nature of the Salton Sea, it is impossible to estimate the salinity tolerance of bairdiella eggs in Salton Sea water from the present data concerning their responses in ordinary seawater. There is evidence that the ionic composition of Salton Sea water has a deleterious effect on the survival of eggs and larvae (Lasker et al. 1972; May 1972), so that the upper salinity limits defined in the present study are probably higher than those which hold for bairdiella in the Salton Sea.

The spawning season of bairdiella occurs during a period of rapidly rising temperatures. In the Salton Sea this species spawns mainly in April and May, with a peak of spawning probably in mid-May (Whitney 1961; Haydock 1971). Maximum surface temperatures in the Salton Sea are plotted in Figure 15, where the spawning time of bairdiella is also shown. It is clear that some bairdiella may spawn in water of 30°C or higher, although most spawning is probably finished before temperatures reach this level. Whitney (1961) reports finding bairdiella eggs in 1955 as late as 1 August, which means they could have been exposed to the undoubtedly lethal temperature of

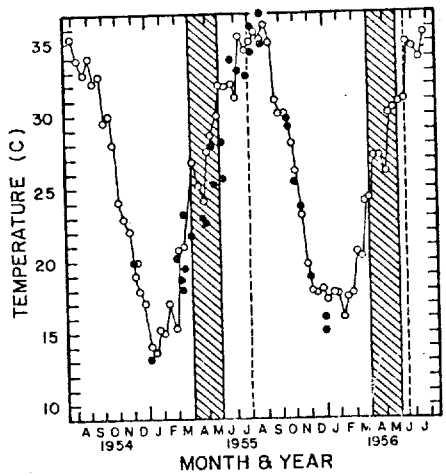

FIGURE 15.—Maximum surface temperatures in the Salton Sea. Open circles: measurements made at Sandy Beach, Salton Sea, from August 1954 to July 1956 (after Carpelan 1961). Closed circles: measurements made at various stations on the Salton Sea during 1967 (after Young 1970). The shaded areas indicate the major spawning period of *Bairdiella icistia*, and the vertical dotted lines indicate the latest records of bairdiella eggs in the plankton in 1955 and 1956, according to Whitney (1961).

35°C. Such late spawning by bairdiella seems unlikely, however, and Whitney may have collected eggs of the orangemouth corvina, *Cynoscion xanthulus*, which spawns during the summer and probably produces similar eggs, rather than bairdiella. In any event, it seems possible that late spawning bairdiella in the Salton Sea could release their eggs in water with a temperature high enough to reduce embryonic and larval survival severely. In view of the temperature-salinity interaction which occurs in the case of both embryonic and larval tolerance, bairdiella which spawn at relatively low temperatures early in the season will probably have a selective advantage as the salinity of the Salton Sea rises.

In the absence of detailed information on the distribution of bairdiella and the physical conditions obtaining in its native habitat, the Gulf of California, it is difficult to apply the present findings to the ecology of this species in that area. However, the utilization of Colorado River water for irrigation has caused an increase in the river's salinity (Wolman 1971); if this, and the accompanying reduced flow of fresh water into the upper Gulf of California, results in a significant rise in salinity in areas where bairdiella spawn, the combined action of salinity stress and heat in this arid region could adversely affect early survival in the local bairdiella population. The warm brine effluent from a proposed desalination plant in this area (Thomson et al. 1969) could aggravate the situation considerably if dispersal of the effluent is not adequate.

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