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*Temperature and Salinity Tolerances of
Young Gulf of California Grunion,
Leuresthes sardina
(Atheriniformes: Atherinidae)¹*

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ABSTRACT

Temperature and salinity tolerances were determined for laboratory-reared larval and juvenile Gulf of California grunion, *Leuresthes sardina*. The fish were hatched in the laboratory from artificially fertilized eggs obtained from spawning adults captured with a beach seine. Upper and lower incipient lethal temperatures were determined for prejuveniles acclimated at 22°C and at 30°C. The lowest and highest temperatures to which grunion could acclimate gradually were 8°C and 35°C, respectively, and the overall zone of tolerance (a measure of eurythermality) had an area of 436°C squared, a lower value than has been found for other temperate species. Newly hatched prolarvae were more eurythermal than the older fish. Salinity tolerance also decreased with age, from 4-67.5‰ at hatching to 5-57.5‰ at 30 days post-hatching.

Introduction. The endemic Gulf of California grunion, *Leuresthes sardina* (Jenkins and Evermann) 1888, is one of two fishes (Atherinidae: Silversides) that lay their eggs in beach sand, entirely out of water, on descending high spring tides during several months of the year. *L. sardina* and the well-known California grunion, *L. tenuis* (Ayres), which spawns on the beaches of southern California, are the only two fish known to exhibit this unique behavior (Walker 1952).

A few workers have investigated the effects of temperature on development (Hubbs 1965, Ehrlich and Farris 1971) and growth (Ehrlich and Farris 1972)

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of the California grunion, *L. tenuis*, but no one has investigated their temperature and salinity tolerances. The only publications on the Gulf of California grunion, *L. sardina*, are a popular article describing its daylight spawning habits (Rechnitzer 1952) and a brief account of its natural history and prediction of its spawning runs (Thomson 1973).

L. sardina is endemic to the upper Gulf of California, from the Colorado River delta to Guaymas, Sonora, Mexico, and it appears to be most numerous in the northern part of its range. The northern Gulf of California experiences a wide annual range of surface temperatures (Thomson et al. 1969), from less than 12°C in January to over 32°C in August. Salinities in the delta region, no longer affected by any significant freshwater inflow from the Colorado River, now range upward from 35 to 37‰ due to the dry evaporative climate. Temperatures and salinities in shallow restricted environments, such as *esteros* (hypersaline lagoons), may be more extreme, exceeding 40°C and 41‰ in the high littoral tidal channels in summer.

The eggs of *L. sardina* develop at 7 to 10 cm below the surface of the sand, just below the high spring tide level, and they are ready to hatch in about 9 days at 18°C. They will not hatch, however, until they are washed out of the sand by the succeeding high spring tides about two weeks after they are deposited. To hatch the eggs in the laboratory, they must be placed in seawater and agitated; they can be hatched at will between 9 and 32 days, although there is a considerable reduction in the hatching percentage after about 28 days.

At 22°C, the prolarval yolk sac is absorbed in 4 to 5 days, and the postlarval stage then begins. Between 30 and 40 days after hatching, the postlarvae metamorphose into juveniles, attaining the adult fin morphology. In this paper, the term "prejuvenile" will be used to refer to fish entering metamorphosis, i.e., 30 to 40 days old.

Methods and Procedure. All of the grunion used in these experiments were reared in the laboratory from artificially fertilized eggs taken from spawning adults in March and April 1972, at El Golfo de Santa Clara, Sonora, Mexico. The eggs were incubated in the laboratory in darkness at 18°C, between layers of paper towels suspended above 36‰ natural seawater, with the edges of the paper towels contacting the water. Plastic trays covered loosely with aluminum foil retarded evaporation, and the salinity was adjusted when necessary by adding distilled water.

The eggs were hatched after 14 days by placing them in synthetic seawater and agitating them. The fish were fed daily on freshly hatched brine shrimp nauplii (*Artemia salina*). The 38-liter rearing tanks were maintained at 22°C, 36‰ S (using Instant Ocean synthetic sea salts), and a 12:12 light:dark cycle. Mortality was negligible with abundant food provided, and many of these fish have lived more than a year.

The upper and lower incipient lethal temperatures were determined ac-

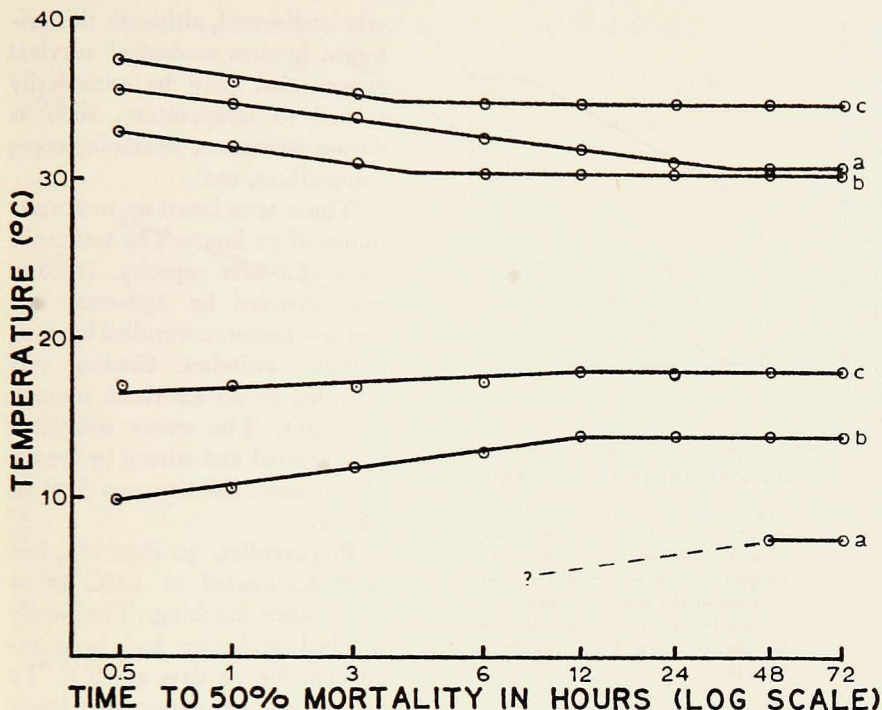


Figure 1. Time-temperature relationships for prolarvae newly hatched from eggs incubated 14 days at 18°C(a), and for 30-day-old fish acclimated since hatching at 22°C(b) or at 30°C(c); upper and lower median tolerance limits (Tlm) in relation to the duration (log time) of exposure to the test temperatures. Thirty-seven groups of ten fish (total 370 fish) were used in these temperature experiments.

according to the method of Doudoroff (1942, 1945), from resistance time experiments at constant fixed temperatures. Groups of ten fish were placed directly in 36‰ synthetic seawater at fixed temperatures close to the estimated (from preliminary experiments) lethal temperature. From the known numbers of survivors at the end of designated time intervals, the median tolerance limit (Tlm) temperatures for each time interval were interpolated between the test temperatures that were lethal to more than half and to less than half of the experimental fish (see Hoff and Westman 1966). Then, the median lethal temperatures were plotted against a logarithmic time scale, and the point of slope inflection, where the Tlm becomes constant through at least 72 hours (i.e., the slope = 0), was taken as the incipient lethal temperature. This point marks the boundary between the zone of resistance, where survival is severely limited by exposure time, and the zone of tolerance, where the fish should be able to survive indefinitely (see Brett 1956). Theoretically, 50‰ of a fish population can survive indefinitely at the incipient lethal temperature if the fish are prop-

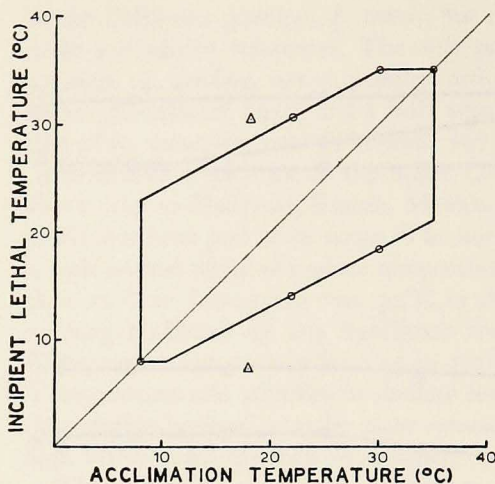


Figure 2. The thermal tolerance zone for fish 30 days old at start of tests. The two triangles represent upper and lower incipient lethal temperatures for prolarvae incubated at 18°C. Area of the polygon, a measure of eurythermality, is 436°C squared. These data are derived from Fig. 1 (points of slope inflection).

erly acclimated, although this criterion ignores ecological survival factors that may be secondarily related to temperature, such as disease resistance, metabolic scope, competition, etc.

These tests lasted up to a maximum of 72 hours. The test tanks were of 8-liter capacity. Heating was provided by 250-watt immersion heaters controlled by thermostatic switches. Cooling was provided by an Eberbach immersion unit. The water was kept well aerated and stirred by means of airstones. Salinity was kept at 36‰.

Prejuveniles, 30 days old, had been acclimated at 22°C or at 30°C since hatching. The newly hatched prolarvae had been incubated for 14 days at 18°C. To determine the upper and lower

extremes to which the grunion could be acclimated, groups of ten initially 30-day-old fish were slowly acclimated upward or downward by 1°C each 72 hours until a temperature was reached at which no fish could survive for 72 hours. This method, which is similar to a very slow CTM (critical thermal maximum; see Fry 1971), is here assumed to give an outside estimate of the ultimate upper and lower incipient lethal temperatures.

Altogether, the data for fish that were 30 days old and older were used to generate a polygon that approximately describes the zone of temperature tolerance for juvenile grunion; the upper and lower incipient lethal temperatures for the 18°C-acclimated prolarvae were added to the figure for comparison. A total of 37 groups of ten fish (i.e., 370 fish) were used in the temperature experiments.

Salinity tolerance data were determined in an analogous manner, with all fish acclimated at 36‰ and 22°C. The test salinities were prepared from Instant Ocean synthetic sea salts and distilled water. Three age groups were tested: newly hatched prolarvae, 10-day-old postlarvae, and 30-day-old prejuveniles. Fifty-two groups of ten fish (altogether 520 fish) were used in the salinity tests, which were conducted at 23°C.

Results. Fig. 1 shows the resistance times at various temperatures for prejuveniles acclimated at 22°C and at 30°C, and for prolarvae incubated at 18°C.

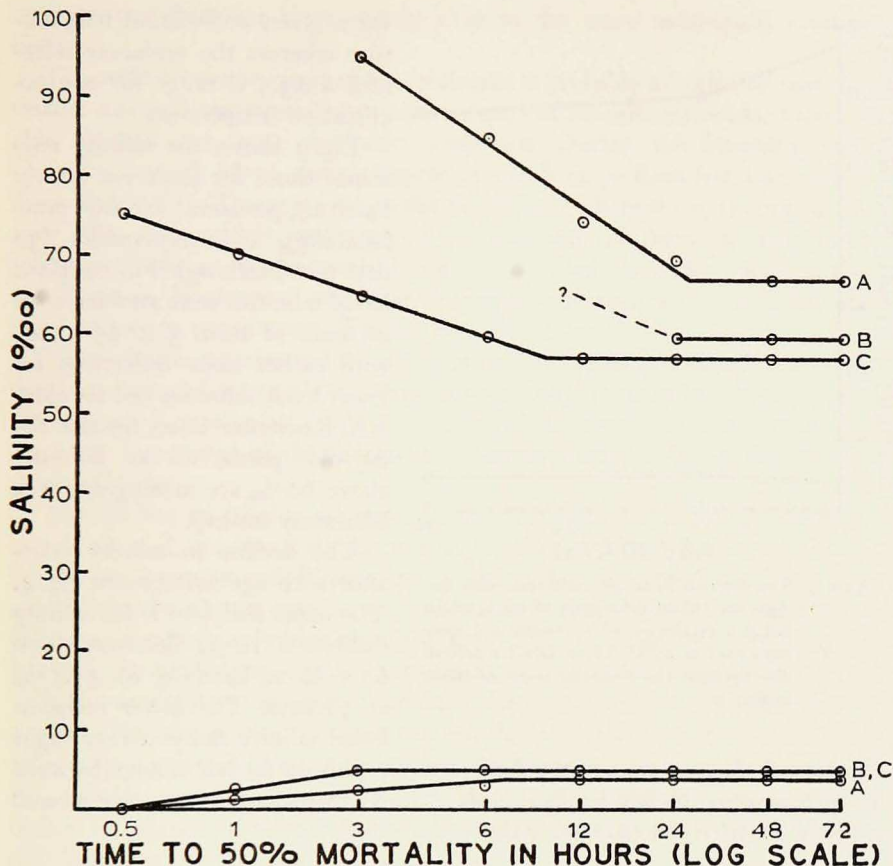


Figure 3. Time-salinity relationships for newly hatched prolarvae (A), 10-day-old postlarvae (B), and 30-day-old prejuveniles (C), all acclimated at 36‰; upper and lower median tolerance limits in relation to the duration (log time) of exposure to the test salinities. Fifty-two groups of 10 fish (total 520 fish) were used in these salinity experiments.

The incipient lethal temperatures were attained after intervals of from 4 to 48 hours, with earlier slope inflections for upper lethal temperatures and for the older fish. Resistance times were not available for the prolarvae below 7°C due to inability of the cooling unit to lower the test temperatures beyond this point.

The zone of tolerance for the older fish, a measure of the eurythermality of the species, comprises an area of 436°C squared (Fig. 2). The lower and upper limits of acclimation were 8°C and 35°C, respectively. The triangles representing the lower (7.5°C) and upper (31°C) lethal temperatures for 18°C-acclimated prolarvae fall outside the tolerance zone polygon, indicating that the newly hatched fish are more eurythermal than the older fish. While the older fish could acclimate within a 27°C range, they could tolerate only a 17°C range

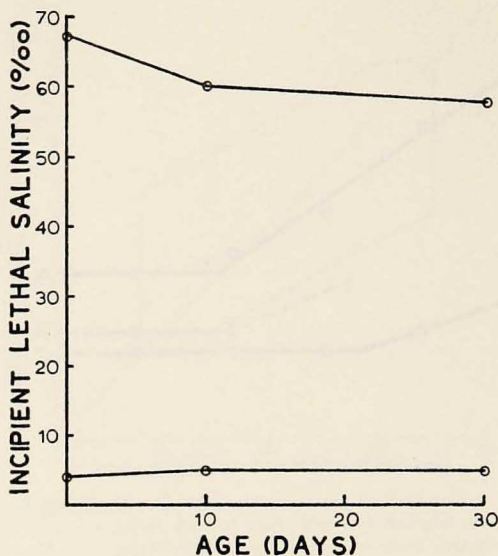


Figure 4. The decrease in salinity tolerance with age. Ages are indicated for start of tests, which lasted a maximum of 72 hours, and were conducted at 23°C. These data are derived directly from the points of slope inflection in Fig. 3.

decrease, from 4 to 5‰ in the first 10 days, with no further change between 10 and 30 days. Newly hatched prolarvae are, then, more euryhaline as well as more eurythermal than the older fish.

Discussion and Conclusions. The 35°C ultimate upper incipient lethal temperature for young *L. sardina* is the maximum tolerated by most marine fishes (de Sylva 1969). The 436°C squared tolerance zone is smaller than values reported for other temperate species. For example, the value for a temperate marine atherinid, *Menidia menidia*, has been reported as 715°C squared (Hoff and Westman 1966). The three relatively eurythermal marine species tested by Hoff and Westman had an average tolerance value of 675°C squared, which is less than 75% of the average for the 23 freshwater species listed by Brett (1956).

Brett (1970) has observed that, in general, the range of tolerance for marine fish varies from about 20°C in tropical latitudes to 27°C in temperate latitudes, narrowing to 8°C near the poles. In this context, the 27°C tolerance range (8°C to 35°C) for *L. sardina* fits nicely into the temperate latitudes. This seems reasonable in light of its affinity to *L. tenuis* of the temperate San Diego fauna and also because of its range in the northern Gulf of California, where

for a given acclimation temperature whereas the prolarvae tolerated a 23.5°C range for one acclimation temperature.

Fig. 3 shows the salinity resistance times for prolarvae (newly hatched), postlarvae (10 days post-hatching), and prejuveniles (30 days post-hatching). The incipient lethal salinities were attained after intervals of from 3 to 25 hours, with earlier slope inflections for lower lethal salinities and for older fish. Resistance times for the 10-day-old postlarvae at salinities above 60‰ are missing due to a laboratory mishap.

The decline in salinity tolerance with age is shown in Fig. 4. The upper incipient lethal salinity exhibits a 10‰ decrease, from 67.5‰ at hatching to 57.5‰ at 30 days. The lower incipient lethal salinity shows a very slight

temperatures fluctuate more widely than in the more subtropical southern Gulf.

The wide tolerance range of the prolarvae is probably adaptive in that their smaller size and correspondingly lesser powers of locomotion render them less able to seek out more favorable conditions. During the spawning season (January through May), water temperatures may range from less than 12°C to more than 25°C. Temperatures in the beach sand where the eggs develop may vary even more widely, and perhaps this accounts for the wide temperature tolerance displayed by the newly hatched fish. There is some indication that, for *L. tenuis*, biochemical reactions during development are unusually stable with respect to temperature (Ehrlich and Farris 1971).

This relative eurythermality of the prolarvae stands in marked contrast to frequent statements regarding the great sensitivity of early larval stages (e.g., Brett 1970, Lewis 1965, Norris 1963, de Sylva 1969). There is a great need for more knowledge of the thermal requirements for the earlier life stages of marine species.

The decline in salinity tolerance that occurs between hatching and metamorphosis in *L. sardina* is similar to that for *Clupea palasii* (Kurata 1959) and for *Clupea harengus* and *Pleuronectes platessa* (Holliday 1965; also 1971: fig. 4-167). In these three species, as in *L. sardina*, there is a greater decrease in tolerance for high salinities than for low salinities. The tolerated range at hatching for all four species lies between 0-5‰ and 60-70‰.

Such euryhalinity is now recognized for the prolarvae of some marine teleosts (Holliday 1971). Possibly this euryhalinity is due to short-term tissue tolerance to internal osmotic changes in the relatively undifferentiated cells of the early larva, and secondarily, to the ability of the undifferentiated epidermal tissues to regulate the body fluids and thereby reverse the internal changes (Holliday 1971).

In summary, the larvae and juveniles of *L. sardina* appear to be well suited to tolerate the temperature and salinity regimes presently existing in the Gulf of California, in spite of the damming of the Colorado River. Of course, the acute tolerances measured here may not reflect more subtle chronic or ecological effects on growth, reproduction, or survival. Furthermore, hot brine effluents from a possible future nuclear power/desalting plant near the Colorado delta (see Thomson et al. 1969) may cause salinities and temperatures to approach or exceed viable limits for the grunion within at least a part of its presently most favorable range.

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