

EFFECTS OF ACCLIMATION ON THE TEMPERATURE AND SALINITY TOLERANCE OF THE YOLK-SAC LARVAE OF *BAIRDIELLA ICISTIA* (PISCES: SCIAENIDAE)¹

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ABSTRACT

Eggs of the bairdiella, *Bairdiella icistia*, were fertilized and incubated in various combinations of temperature and salinity, and the salinity and upper thermal tolerances of the yolk-sac larvae were determined. The upper thermal tolerance was enhanced by acclimation to high temperatures and low salinities. Acclimation to low salinities enhanced the lower salinity tolerance of larvae at 24 h after exposure to test conditions, but an acclimation effect on the upper salinity tolerance was not apparent until 48 h after exposure. Yolk-sac bairdiella larvae are more tolerant than the embryonic stages and less tolerant than adults to extremes of temperature and salinity.

Techniques for inducing gonadal maturation and spawning under laboratory conditions are well developed for the bairdiella, *Bairdiella icistia* (Jordan and Gilbert), a sciaenid fish native to the Gulf of California and now present in the Salton Sea (Haydock 1971; May 1975). Hence bairdiella eggs and larvae are extremely favorable material for studying various facets of early development in a marine fish, and detailed information on the effects of temperature and salinity on fertilization, embryonic development, and hatching in this species has already been presented (May 1975). The present paper is concerned with the effects of acclimation on the tolerance of yolk-sac bairdiella larvae to temperature and salinity.

Acclimation has been defined as "the process of bringing the animal to a steady state by setting one or more of the conditions to which it is exposed for an appropriate time before a given test (Fry 1971:14)." In the case of yolk-sac larvae of tropical fish species which develop very rapidly, the term acclimation has a somewhat special meaning, since it necessarily refers to the conditions obtaining during embryonic development. Virtually no studies of acclimation in this context have heretofore been published. Although salinity has been shown to affect the upper thermal tolerance of

adult fish (e.g., Garside and Jordan 1968), no comparable work has been reported for fish larvae. This paper investigates the upper thermal tolerance of newly hatched bairdiella larvae and the modifying influence of acclimation, i.e., the influence of temperature and salinity during embryonic development. Since there is little likelihood that bairdiella larvae would encounter lower lethal temperatures in nature (May 1975), their lower thermal tolerance is not considered here. In addition to upper thermal tolerance, the upper and lower salinity tolerance of larval bairdiella and the effect of the acclimation salinity are also considered in this paper. This information, together with results on embryonic tolerances described earlier (May 1975) and available information concerning adult tolerances, should lead to a conclusion as to which stage in the life history of bairdiella is the most sensitive to temperature and salinity.

METHODS

General

Bairdiella eggs were obtained from fish which had been held in normal seawater (33‰) and induced to mature and spawn in the laboratory, as described previously (May 1975). Eggs were artificially fertilized at specified temperatures (within $\pm 0.2^\circ\text{C}$) and salinities ($\pm 0.5\text{‰}$) and maintained under the same conditions until hatching in specially designed incubators (May

¹Based on a portion of a dissertation submitted in partial satisfaction of the requirements for the Ph.D. degree at the University of California at San Diego, Scripps Institution of Oceanography.

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1975). These conditions (which remained constant from fertilization to hatching, plus the period of time between hatching and transfer to the test conditions) constituted the conditions of acclimation. Larvae were not fed during the experiments. Test salinities were prepared by dilution with deionized water from a stock solution of 60‰, which had been made by adding artificial sea salts to seawater (May 1975).

Upper Thermal Tolerance

Larvae were acclimated to temperatures of 21°, 24°, 27°, and 30°C, and to salinities of from 15 to 45‰ (Table 1), covering the ranges of these two factors within which successful embryonic development can take place (May 1975). Since developmental rates were more rapid at the higher temperatures (May 1975), the period of acclimation (fertilization to transfer to test vials) was shorter at the higher acclimation temperatures. The median tolerance limit (TLm)³ of yolk-sac larvae to high temperatures was determined by the method of Doudoroff (1942). Larvae were transferred directly from the acclimation conditions to a series of 25-ml capped vials maintained in the dark at a series of high temperatures in a thermal gradient block (Thomas et al. 1963) within 5 to 10 h after hatching, at which time they were 1.8 to 2.0 mm in length. The highest test temperature was 36°C, and between five and eight test temperatures, 1.5°C apart, were used depending on the acclimation temperature; the salinities in the test vials were the same as the acclimation salinities. Approximately 10 larvae were placed in each vial, and the test temperatures did not vary by more than $\pm 0.1^\circ\text{C}$. Antibiotics were added to the water in the vials (May 1975), and the survival of larvae under optimal conditions in these vials

was comparable to that in larger containers. The number of larvae surviving at each test temperature was recorded at 0.5, 1, 3, 6, 12, 24, 48, and 72 h after transfer, and for each time the TLm—the temperature at which just 50% of the larvae survived the given time interval—was estimated by graphical interpolation as described by Doudoroff (1942). At each observation, larvae which showed no movement were removed from the vials by pipette and examined under a dissection microscope. If the heart was not beating and the larva was opaque, the larva was considered dead and was discarded; live larvae were returned to the vials. In one instance (see Results) moribund larvae were found and counted as dead.

Salinity Tolerance

Larvae were acclimated to salinities of 15, 20, 25, 30, 35, and 40‰, and their upper and lower salinity TLm's were determined by transferring larvae directly to a series of 25-ml vials containing test salinities ranging from 0 (deionized water) to 58‰. Between five and seven larvae were transferred to each vial within 8 h after hatching. Because of limited material, only the upper TLm was determined for larvae from an incubation salinity of 40‰, and only four larvae from this salinity were available for each vial. The temperature during fertilization, incubation, and testing was maintained at $24 \pm 0.2^\circ\text{C}$ by thermostatically controlled water baths, and vials were kept under continuous room light of low intensity (May 1975). The number of larvae surviving at each salinity was recorded 24, 48, and 72 h after transfer, and the upper and lower TLm's were estimated graphically for each time interval as in the case of thermal tolerance. Larvae were considered dead on the basis of the same criteria used in the study of thermal tolerance.

³The term "median tolerance limit" and the symbol TLm are recommended in "Standard Methods" (American Public Health Association 1971).

TABLE 1.—Acclimation conditions for larvae used in determination of heat tolerances.

Acclimation salinity (‰)	Acclimation temperature (°C)			
	21	24	27	30
15	×		×	
20		×		×
25	×		×	
30		×		×
35	×		×	
40		×		
45	×			

RESULTS

Upper Thermal Tolerance

The upper TLm dropped with increasing time intervals (Figures 1-4), and there was a leveling off of the time-temperature curves in the lower salinities as time increased. Most of the time-temperature curves have been separated by eye-fit lines into two major segments, the horizontal segment defining the "incipient lethal temperature"

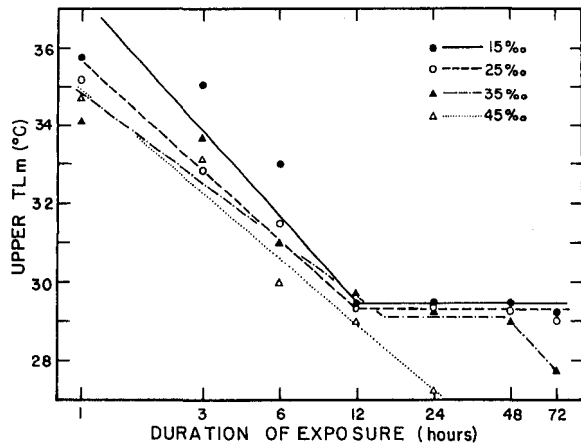


FIGURE 1.—Heat tolerance of larval bairdiella acclimated to 21°C in various salinities. The upper median tolerance limits (TLM) are plotted for various durations of exposure. The time scale is logarithmic, and lines were fitted by eye.

(Brett 1956), but in several curves there is a suggestion of an early plateau during the first few hours of exposure to the test conditions (Figures 1, 3, 4). At any given time the TLM was usually higher in the lower salinities. Since the highest test temperature used in the experiments was only 36°C, at acclimation temperatures above 21°C the 50% mortality point was usually not reached until 3 or more hours after exposure to the test conditions. Survival was very poor among larvae from eggs maintained at 30°C (a temperature highly stressful to eggs—May 1975) in 30‰, and at 24 h, survival in this group was below 50% at all test temperatures. For purposes of comparison, the

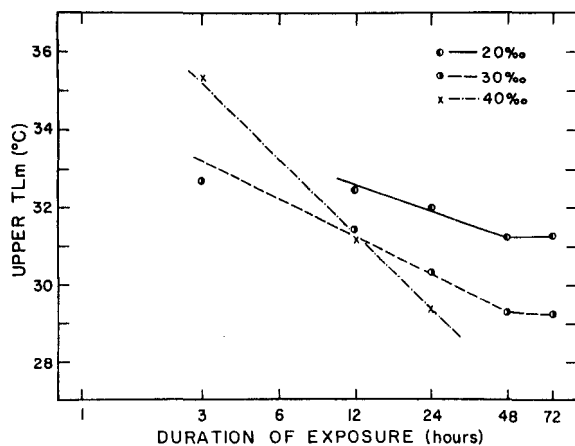


FIGURE 2.—Heat tolerance of larval bairdiella acclimated to 24°C in various salinities.

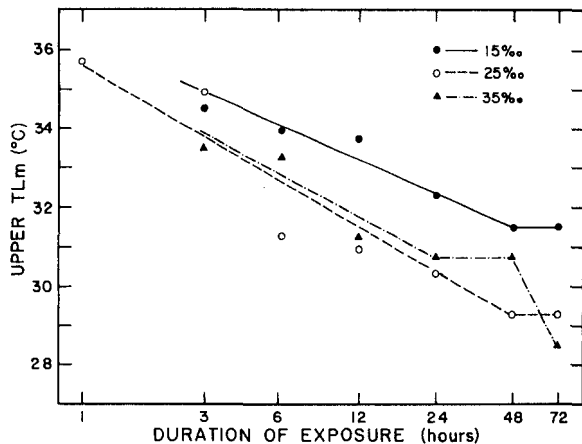


FIGURE 3.—Heat tolerance of larval bairdiella acclimated to 27°C in various salinities.

24-h upper TLM has been plotted against salinity for various acclimation temperatures (Figure 5); in this graph the increase in TLM at lower salinities is clear, as is the general increase in TLM effected by higher acclimation temperatures. In a salinity of 15‰, all larvae alive at 12 and 24 h in the 21°C acclimation group were moribund in test temperatures of 30°C and higher, i.e., they were contorted and totally immobile and unresponsive to touch, although their hearts were beating and they were not opaque. These larvae have been considered dead for the purpose of data presentation; if considered alive, they would raise the calculated 12-h upper TLM from 29.5° to 32.1°C (Figures 1, 5). At the salinity of normal seawater, the 24-h upper TLM of larval bairdiella lies

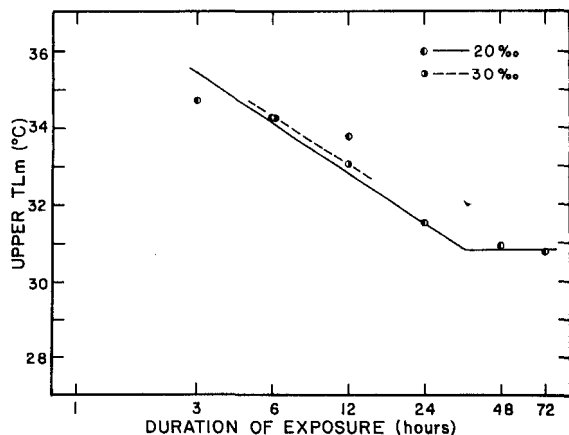


FIGURE 4.—Heat tolerance of larval bairdiella acclimated to 30°C in two salinities.

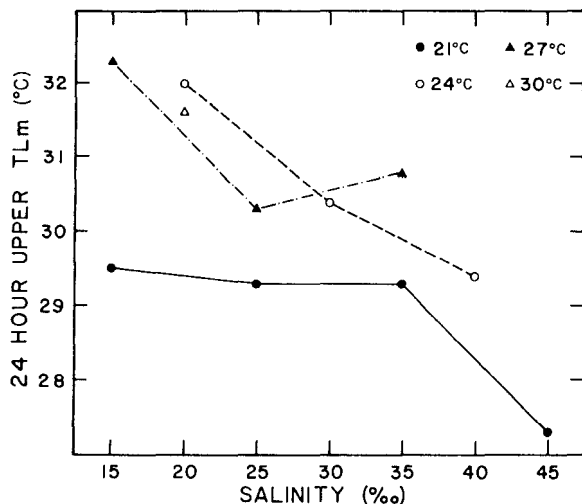


FIGURE 5.—Twenty-four hour upper median thermal tolerance limits (TLM) at various salinities for larvae acclimated to 21°, 24°, 27°, and 30°C.

between 29° and 31°C, depending on the acclimation temperature. Larvae could resist higher temperatures for shorter periods of time.

Salinity Tolerance

The 24 h upper TLM for salinity was not greatly affected by the acclimation salinity and ranged from 43 to 48.5‰, but the 24-h lower TLM was appreciably higher among larvae incubated at higher salinities (Figure 6). The lower TLM's (24 h)

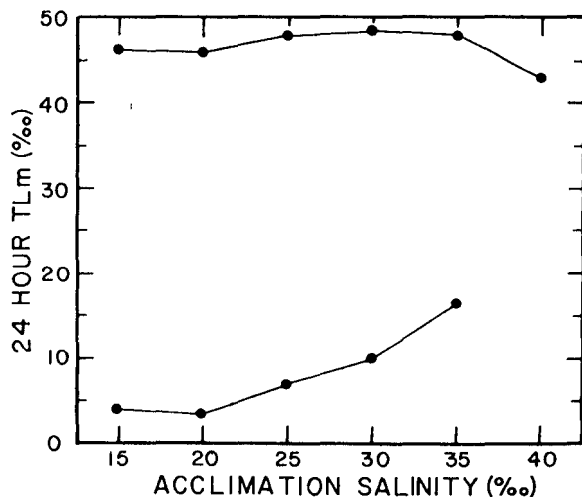


FIGURE 6.—Upper and lower median tolerance limits (TLM) of salinity for a 24-h exposure. Larvae were acclimated to various salinities at 24°C.

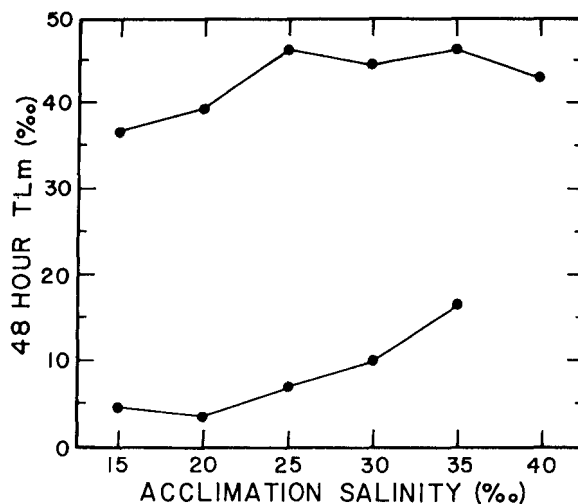


FIGURE 7.—Upper and lower median tolerance limits (TLM) of salinity for a 48-h exposure.

for larvae acclimated to 15 and 20‰ were 4.2 and 3.5‰, respectively, whereas those for larvae acclimated to 30 and 35‰ were 10 and 16.5‰, respectively. The major difference between the 24-h TLM's and those for 48 and 72 h is the progressive lowering of the upper TLM for larvae acclimated to low salinities (Figures 7, 8). The upper TLM for larvae from 15‰ shifted from 46.2‰ at 24 h, to 36.6‰ at 48 h, and to 30.0‰ at 72 h; between 24 and 72 h, the upper TLM remained the same (43‰) for larvae acclimated to 40‰, and decreased only from 48 to 46.2‰ for

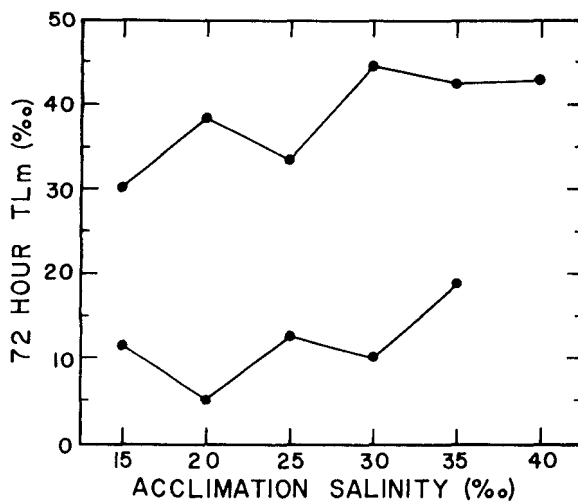


FIGURE 8.—Upper and lower median tolerance limits (TLM) of salinity for a 72-h exposure.

those acclimated to 35‰. There was little change in the lower TLM's between 24 and 48 h, but a slight rise occurred between 48 and 72 h in all but the 30‰ acclimation group.

DISCUSSION

Fry et al. (1946) define the "zone of tolerance" as the range of any environmental factor within which an animal can live indefinitely, and the "zone of resistance" as the range within which the animal can live for only a finite period of time, depending on the level of the factor. The zone of tolerance is bounded by the upper and lower "incipient lethal levels." In work on the upper thermal tolerance of fishes, the incipient lethal level is defined by an abrupt flattening of the time-temperature curve at a temperature below which less than 50% of the exposed individuals succumb (Brett 1956). Some of the curves generated in the present study (Figures 1-4) suggest that the incipient lethal level has been reached, but curves from the higher salinities lack a horizontal segment. This points up a difficulty in working with early larvae: at 24°C the larval yolk supply is 95% consumed by about 40 h after hatching (May 1974), and this occurs even sooner at higher temperatures. The 48- and 72-h TLM's therefore apply to starving larvae. Unlike adult fish, larvae which hatch from pelagic eggs are extremely sensitive to food deprivation (e.g., Lasker et al. 1970) and begin dying of starvation soon after yolk absorption if food is not provided for them, and unfed bairdiella larvae die sooner at high temperatures and salinities (May 1975). Therefore, prolonging these tests would not have helped in defining the upper incipient lethal temperature for larvae in the higher salinities—the TLM would simply continue to fall. Even at the lower salinities, the TLM would decline after a sufficient period of time; the curves for a salinity of 35‰ (Figures 1, 3) show how a flat segment is reached, only to be followed by another drop in TLM. A further difficulty in estimating tolerance limits for warmwater larvae is that these larvae develop morphologically at an extremely rapid rate and are very different organisms 1 or 2 days after hatching than they are at hatching. Newly hatched bairdiella are poorly developed and rather inactive (May 1975), whereas by 45 h after hatching (at 24°C) they have acquired functional eyes and an open mouth and are quite active. In this situation, consideration of the TLM at a more or less arbitrary time after exposure to

the test conditions, such as 24 h, is at least a useful approach for comparative purposes.

Larval bairdiella are more sensitive to high temperatures when the salinity is also high, as are bairdiella gametes and developing embryos (May 1975). This adds further weight to the suggestion (May 1975) that in nature, eggs spawned late in the season at high temperatures will have a reduced chance of contributing recruits to the population when natural salinities rise as they are doing in the Salton Sea. The survival of bairdiella larvae in the Salton Sea would be significantly reduced at temperatures above 31°C, and temperature data from the Salton Sea (May 1975) indicate that some larvae could be exposed to thermal stress of this level or greater. The highest TLM is reached in 15‰, the lowest salinity in which larvae were tested and the nearest to being isosmotic with larval body fluids. Older fishes of various species are also most tolerant of high temperatures in isosmotic or nearly isosmotic salinities (Arai et al. 1963; Strawn and Dunn 1967; Garside and Jordan 1968; Simmons 1971). The added burden of osmotic work seems to reduce the ability of both larval and adult fish to tolerate extremely high temperatures.

It is clear that acclimation can alter the tolerance of early bairdiella larvae to both temperature and salinity, even though the rapid developmental rate of bairdiella eggs restricts the period of acclimation to between 20 and 40 h (the time between fertilization and transfer to test conditions, which is a function of incubation temperature). Incubation of bairdiella eggs at higher temperatures produces larvae with a higher upper thermal TLM. However, increasing the acclimation temperature from 27° to 30°C does not increase the upper TLM, even though the TLM's are generally above 30°C. Hence the lethal levels determined for an acclimation temperature of 27°C may represent "ultimate" incipient lethal temperatures (Fry et al. 1946), but here again one must consider the unique problems of working with early larvae. If the effect of thermal acclimation on the tolerance of yolk-sac larvae is to be studied, acclimation must take place during embryonic development, but the embryos are more sensitive to temperature than are the larvae to which they give rise (cf. May 1975). A temperature of 30°C is extremely stressful for developing eggs, and the larvae produced at this temperature survive poorly, a trait magnified at higher salinities.

The response of these larvae to elevated temperatures is therefore not a true reflection of thermal "acclimation," as the term is generally used, but is more a reflection of thermal stress during sensitive periods of morphogenesis. In an analogous way, salinity stress on embryos during acclimation at 40‰ probably accounts for the observation that the larvae have a reduced upper TLM for salinity when compared with larvae acclimated to 30 and 35‰.

Thermal acclimation has also been shown to affect the thermal tolerance of larval herring (Blaxter 1960), menhaden (Lewis 1965), and salmonids (Bishai 1960; Iwai 1962), although only Blaxter's study utilized larvae which hatched from eggs maintained at the acclimation temperature. The mechanisms involved in thermal acclimation during early development have never been investigated, but the present results for bairdiella suggest that they must be activated quite rapidly, within a day or two at most. A similarly rapid rate of acclimation to warm temperatures has been found in older fish (Brett 1970; Allen and Strawn 1971), so that a similar mechanism may be operating in both cases. Factors involved in setting thermal tolerance limits in fishes are little understood (Fry 1967), but thermal inactivation of enzymes has been suggested as a possible mechanism (Hochachka and Somero 1971).

Holliday and Blaxter (1960) found that the salinity prior to hatching had a limited effect on the salinity tolerance of larval herring. This effect was more pronounced in the present experiments with larval bairdiella, but there was a delay in the appearance of the acclimation response to high salinities. The upper TLM (salinity) was similar for all acclimation salinities 24 h after initial exposure to the test conditions, but at 48 h the larvae acclimated to high salinities had a higher TLM than those from low salinities (a very slight indication of the same phenomenon can be discerned in the results of Holliday and Blaxter 1960). This observation is difficult to explain, especially in view of the rudimentary state of our knowledge of larval osmoregulatory mechanisms; perhaps it is related to the opening of the mouth between 35 and 45 h after hatching (May 1974), which could expose the internal larval tissues more directly to the ambient salinity. Incubation at low salinities enables larvae to tolerate much lower salinities than larvae incubated in more saline water. Again, it is difficult to speculate on how this effect might be mediated.

Early larvae of *Bairdiella icistia* are more tolerant than the embryonic stages and less tolerant than adults to extremes of temperature and salinity. Very few bairdiella eggs develop normally at 30°C (May 1975), and 15 to 40‰ is the approximate salinity range for normal fertilization and embryonic development. In contrast to the eggs, 50% of the newly hatched larvae are capable of withstanding temperatures between 30° and 33°C for 24 h or longer, except at the lowest acclimation temperature and highest salinity; and with proper acclimation, larvae can tolerate salinities ranging from about 4 to 48‰ for 24 h, or 5 to 45‰ for 72 h. Juvenile and adult bairdiella must tolerate temperatures ranging from 10° to 34° or 35°C in the Salton Sea (Carpelan 1961). These fish have been found in freshwater (R. G. Hulquist, California Department of Fish and Game, pers. commun.) and can tolerate Salton Sea water with a salinity of 52.5‰ for 96 h after direct transfer from ordinary Salton Sea water (approximately 38‰), and 58‰ for over a week after gradual acclimation (Hanson 1970). The early larvae of some other species have also been shown to be more tolerant of temperature and salinity than their eggs. McCauley (1963) reports that prolarvae of the sea lamprey, *Petromyzon marinus*, are considerably more tolerant of high temperatures than are the eggs, and data presented by Holliday (1965) show that newly hatched herring, *Clupea harengus*; plaice, *Pleuronectes platessa*; and Atlantic cod, *Gadus morhua*, larvae are more tolerant of both high and low salinities than are their respective eggs. However, in the case of the herring and plaice, further larval development and metamorphosis are accompanied by a decrease in salinity tolerance (Holliday 1965), a pattern quite different from that found in bairdiella.

ACKNOWLEDGMENTS

I thank Reuben Lasker for his advice and material aid during this work. The University of California Institute of Marine Resources and the Southwest Fisheries Center, National Marine Fisheries Service, NOAA provided financial support.

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