MASTER

EFFECT OF THERMAL SHOCK ON DEVELOPMENTAL
STAGES OF ESTUARINE FISH

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Abstract

Present dogma would have it that the larval and juvenile stages of aquatic species are most sensitive to environmental perturbations. However, there is some evidence that this might not be consistent for all species. The present study has examined the response of the larval and juvenile stages of the estuarine fish Fundulus heteroclitus to acute thermal shock.

The Scope

Present dogma would have it that the larval and juvenile stages of aquatic species are most sensitive to environmental perturbations. However, there is some evidence that this might not be consistent for all species. The present study has examined the response of the larval and juvenile stages of the estuarine fish Fundulus heteroclitus to acute thermal shock.

Related work is being conducted with oceanic fishes by Dr. Donald Hoss and his colleagues at the National Marine Fisheries Service Laboratory at Beaufort, NC and Drs. Schubel and Koo, at SUNY Stony Brook and the University of Maryland.

Known age larval and juvenile stages of Fundulus heteroclitus, the mummichog, were exposed to temperatures of 40, 41.5, 42 and 42.5°C. Groups of 20 fish, acclimated at 21°C, were placed in an exposure cup and transferred to an exposure bath at the desired temperature. Preliminary exposures were conducted at each experimental temperature to determine the approximate time interval over which mortality occurs. For the final exposure, groups of 10 fish were placed in two replicate cups and put in the exposure bath. The cups were removed at the appropriate time for respective exposure temperatures and transferred to a recovery bath. Fish handled in the same manner as the experimentals, but not exposed to the temperature increment, served as a control. Fish were left in the recovery bath for 24 hours and total mortality was then determined. Results were plotted on probit paper and the LT₅₀ determined for each plot.

Results

The results of this year's work were intriguing. As shown in Table 1, the LT_{50} for each exposure temperature was related to the age of the larvae tested. There was increased sensitivity to thermal stress with increasing age. There seems to be an initial high thermal tolerance for the prehatch embryos (results of 1975 work), and 0-day-old fish; and then increasing sensitivity to thermal shock which stabilized 2-3 days after larval hatching. There was very little apparent change in sensitivity thereafter for the ages tested. The most intriguing aspect of the data is that when fish were hatched at 22°C and exposed to 40°C (an increment of 18°C of thermal shock), 24 hours of exposure gave no lethal effect nor was there latent mortality. Similar results were obtained at 40.5°C .

Tests at 41.5°C gave very distinctive results which are presently difficult to interpret. There was higher thermal tolerance for 1-day-old larvae (exposed for 65 minutes) than observed for larvae 3-7 days old, in which a trend of increased sensitivity is obvious (Table 1). There was no latent mortality for these older age groups, and the test temperature was less than the upper incipient lethal temperature. It would appear that 3-7-day-old larvae exhibited some capability for higher tolerance to the extent that they might possess a mechanism for rapid acclimation to a temperature of 41.5°C for a brief time interval. However, after day 8, total mortality in the 41.5°C group with typical LT_{50} of 10-12 minutes were routinely measured. Thus, after day 8 the temperature of exposure was above the incipient lethal temperature.

At an exposure temperature of 42°C the anticipated pattern of higher tolerance of 0-day-old larvae with decreased tolerance with increased age was observed. The differences were not as dramatic nor did the temporary tolerance distribution observed at 41.5°C show at 42°C. Tests at 42.5°C gave the predicted response of higher tolerance for 0-day-old larvae and decreased tolerance with increased age. An apparent stabilization in sensitivity at 42.5°C was observed after day 2. There was no significant difference between 9 and 11.5 minutes, for example.

Another observation in this year's data is the nonlinear mortality response to the acute thermal exposure by the different age groups of fish (Fig. 1). The decreasing tolerance with age as well as the nonlinear response can be seen in Figure 1. In addition, there is some indication that the nonlinearity of the early stages becomes more linear in older larvae. The significance of this is that the response appears to be linear through the 50% mortality level, which is the point at which so many standards of water quality have been based. But, it is certainly not linear at the lower percentage mortalities or lower thermal doses, so a prediction of other than a 50% mortality level could not be extrapolated on a linear basis. Preliminary analysis suggested that a quadratic equation will describe the line.

Experiments with sublethal exposures gave no latent mortalities. Differences in size groups of the same age were tested by rearing groups at 30°C and 15°C for 3 weeks immediately post-hatch and then acclimating at 20°C. There was no apparent difference in response, in spite of the significant difference in size. I would like to repeat this in the future.

Because of the apparent differences in response to acute thermal shock of the larvae at relatively small increments of temperature, specifically the difference between 41.5 and 42°C, we attempted to determine the individual time to death. We found that this was not possible with larvae, using conventional methods. Therefore, we devised a system that may make it feasible to quantify this observation. The drawing (Fig. 2 a, b) indicates the system which was used. The individual larvae was placed in the cup, and water of the appropriate temperature was circulated through the cup. Quantitative observations could be made of physiological responses such as cessation of opercular movement, blood flow and heartbeat. However, after the cardiac arrest and transfer of the apparently dead larvae to a recovery tank, some of the larvae recovered. Further observations would be required to determine the individual larval response to thermal shock, increment of exposure, and time of exposure. The system does hold promise and could be a very useful tool.

Certain difficulties in conducting this year's work prevented the accumulation of more data. We were not successful in getting adequate numbers of the sheepshead minnow, Cyprinodon variegatus, to do the comparative study that we had suggested. In addition, several pump failures led to the loss of spawns of incubating Fundulus eggs and reduced the numbers of available larvae. It took much longer to do the individual exposures than we had originally presumed, and an early onset of the refractory period reduced the availability of spawning fish. We also have some indication that there may be a variation in daily sensitivity to thermal shock, that is, a diurnal difference possibly due to some circadian rhythm. Thus, we deliberately limited ourselves to conducting

the exposure at the same time of day so the results were comparable on a day-to-day basis.

We also observed indications that physical handling, or other such perturbations occurring at the one- to two-cell stage of embryonic development was deleterious to the hatching success of the fish. This could have some importance relative to entrainment of eggs of species and should perhaps be followed up. This is consistent with our previous results that the early cell division stages are very sensitive.

The results of the previous year's work, in cooperation with Doug Middaugh of the E.P.A. Bears Bluff Lab, is in a manuscript form ready for submission to a refereed journal.

Table 1

Age of Larvae	Temperature of Exposure			
	40°	41.5°	42°	42.5°
0	no effect	>65 min	11.6 11.0	28
1				10
2			6	9
3	=28 ²		5.5	11.5
4	=35		5.9	11.5
5	=30			8.5
6	=20-25		6	8.5
7	=35			10.5
8		10	8	8
9		10		11.5
10		11.7 10	5.7 6.0	

¹LT₅₀ from probit analysis in minutes. LT₅₀ = lethal time for 50% mortality under defined experimental conditions, i.e. 1-day-old larvae exposed at 42°C.

² indicates 30-40% mortality occurred at 28 minutes.

Figure 1.

Fig. 2A

Fig. 2 B