THE CRITICAL THERMAL MAXIMUM OF JUVENILE SPOT *LEIOSTOMUS XANTHURUS* LACEPEDE

By

DAVID W. BRIDGES
Department of Zoology
North Carolina State University
Raleigh, North Carolina 27607

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Summary and Conclusions

The use of coastal water as coolant during the production of electricity has created a threat to the biology of coastal waters. By 1980 the electric power industry may be using 100 trillion gallons a day (1/5 annual runoff of the contiguous U. S.) as coolant, and this use may harm organisms which get passed through the generating plants.

The temperature increase does not have to be lethal to the entrained organisms, for the large nuclear generating plants raise the temperature only about 18° F at full generating capacity. But the increase can be lethal to very young fish under certain temperature and salinity conditions. Our present problem is that we do not know the lethal temperature limits of enough of our fishes, so we can give the power industry appropriate temperature limitations.

In this study, I have measured the heat tolerance of juvenile spot (Leiostomus xanthurus), an estuarine fish. This tolerance is given in terms of their Critical Thermal Maximum (CTM) which measures the temperature above which all of the organisms will die. Because this test involves a rapid heating of the fish, it simulates the experience of an organism as it passes through the condensor of a generating plant.

In the laboratory tests, the spot of various sizes and ages were acclimated to combinations of temperatures and salinities prior to the CTM tests. It was found that the acclimation temperature was

the most important factor that affected the CTM and that larger fish had a higher CTM than did smaller ones.

When the post-larvae (smallest fish, just hatched) migrate up the estuary in the winter, they have a CTM of 85.8° F when acclimated at 50.0°. Although this is lower than their CTM later in the year, they would not be killed if they were transported through a power plant condensor. This is because the water, although warmed 18° F, has a low ambient temperature in winter and so the maximum temperature reached is still less than the CTM. During the summer, the juvenile fish had a CTM of 99.5° F when acclimated at 86°. If the cooling water were taken from the estuary or river at 83°, a common summer temperature in North Carolina, then the fish would be killed as they passed through the condensors and were warmed to 100°. Later in the fall, the cooler water now entering the plant will again allow the spot to survive entrappment.

These results imply that the most important time to worry about the mortality of juvenile spot due to heat death is in the summer and early fall. To stop this mortality in power plants, the maximum temperature encountered by these fish should not be above 91° F (a nine degree safety factor). It should also be noted that by early August, the spot in North Carolina are 2 1/2 inches long. It may be possible, therefore, to keep these fish out of the intakes with proper screening.

ABSTRACT

BRIDGES, DAVID WILDER. The Critical Thermal Maximum of Juvenile Spot, Leiostomus xanthurus, Lacepede. (Under the direction of JOHN E. HOBBIE).

The Critical Thermal Maximum (CTM) of juvenile spot was determined throughout their first growing season from the time they entered the oligohaline environment until they left it. Spot were acclimated in the laboratory to combinations of temperature and salinity levels prior to the CTM tests. When the data were analyzed by multiple regression, acclimation temperature, acclimation salinity, weight, weight squared, and length were all found to be significantly important influences on the CTM of juvenile spot. Temperature was the most important influence on CTM with an R^2 value of 0.92. Salinity was the least important with an R^2 of 0.001, and weight, weight squared and length were all intermediate influences with R^2 values of about 0.18. Response surfaces were generated from the prediction equation showing the acclimation temperature necessary to produce a fixed CTM for juvenile spot. These reveal that CTM increases with size (either length or weight) and age during the nursery period. The increase in CTM was somewhat greater between summer and fall sized spot than between spring and summer sized spot. The CTM increased as salinity increased.

The time of day at which the CTM tests were made did not effect the CTM. The increase in CTM between temperature/salinity/size combinations was consistently near 38% of the difference between two levels of acclimation temperature.

The increase in CTM with size and age may involve a reduction in cell surface concentration. The efficiency of cell metabolism may increase at lower surface concentrations, thereby providing an increase in tolerance to stress. As heat acclimation is accomplished, enzyme systems may be replaced by ones more effective at higher temperatures. The replacement of enzyme systems may be a result of physico-chemical changes in water at the specific temperature levels of 15, 30, and 45 C. The increase in CTM with salinity may be a result of the increased availability of complexing agents or other raw materials used in metabolism which permits higher temperature tolerance.

Although it is impossible to apply laboratory results directly to field situations, these data indicate that a 10 C increase in cooling water temperature of a power generating station need not be lethal during spring migration of post-larval spot providing that no other factor is reducing the heat tolerance at that time of year. However, a 10 C increase in cooling water temperature would be lethal to 100% of entrained juvenile spot from the time the water temperatures approached the mid 20's C in the early summer until they cooled below the mid 20's C in the fall.

Based on the results of this study, I would recommend to the power industry that they limit their temperature increases to 5 C below the CTM of the organisms which might be entrained in the coolant or exposed to the plume of heated water after it has been returned to the waterway.

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INTRODUCTION

A threat to the biology of coastal waters has been created by the demand for electric power. The demand is a result of expanding coastal populations which have caused a doubling in electrical power production every 10 years since 1945 (Sorge, 1969). Increased power production may be harmful to aquatic organisms because the industry uses water from rivers and estuaries as coolant to condense the steam which runs the turbines.

Temperature increases resulting from the power industry's use of water as coolant need not be lethal to all organisms, for a large nuclear generating plant usually causes a temperature increase of only about 10 C or less (Horton and Bridges, 1969). As the heat tolerance of no more than 5% of our fish species had been investigated by 1967 (Mihursky and Kennedy, 1967), the lack of this information concerning the tolerance to heat of aquatic organisms necessitates investigation in this area in order to predict the environmental consequences of the increased heated water discharges by the power industry.

I have investigated in this study the heat tolerance of an important estuarine fish, spot (Leiostomus xanthurus), by using the Critical Thermal Maximum (CTM) technique. Many thermal tolerance studies are performed with disregard for the subtle influences of both physical and biological phenomena. Therefore, the relative importance of several factors which control and influence the CTM of juvenile spot have been examined. By employing the CTM test of

heat tolerance, some of the limitations of other techniques have been avoided and, at the same time, an organism's experience of exposure to rapid thermal increases either within the condensor or in the waterway receiving the heated discharge have been more closely simulated.

Juvenile fishes may be more severely affected by heated effluents than mature fishes. This increase in susceptibility of juvenile fishes may be quite important in estuarine systems because young fish are prominent there. Alabaster (1966) found that adult rough fishes were able to avoid the heated water of a generating plant, while young of the same species were killed by it. In addition to being more successful at escaping heat, an older fish is more tolerant of heat as Brett (1956) demonstrated with salmon, Oncorhynchus. Some marine invertebrates also have been shown to attain more heat tolerance with age as exemplified by the lobster, Homerus americanus, which becomes less sensitive to environmental factors, including heat, as it progresses from one developmental stage to the next (McLeese, 1956). In contrast, marine crabs vary in their tolerance to environmental factors, such as heat, as they develop from one stage to the next (Costlow, Bookhout, and Monroe, 1960).

The size of like-aged organisms also may have some influence on their heat tolerance. Although Fry, Hart and Walker (1946) were unable to find any influence of size on the heat death of speckled trout, Salvelinus fontinalus, Prosser (1965) states that size is one of the biological influences on thermal tolerance.

The physical factors which comprise an organism's environment also influence its tolerance to lethal elements. Among these important

physical factors, Prosser (1965) includes temperature, salinity, photoperiod and oxygen.

The interactions of the biological factors of size and age with the physical factors of temperature and salinity, principally establish an organism's ability to survive. Studies of environmental tolerance, therefore, should include the influence of both biological and physical factors. These interacting factors were all considered in this study of the heat tolerance of juvenile spot, Leiostomus xanthurus, and the relative influence of each factor on the Critical Thermal Maximum (CTM).

Heat tolerance of an organism that is exposed to a generating plant's effluent can best be described by using the CTM developed by Hutchison (1961) rather than the pharmaceutical (LD 50) techniques usually employed (Finney, 1952). The CTM procedure simulates the increase in temperature to which an organism would be exposed within the condensor of a power plant and upon entering or being overtaken by a plume of heated water as it was returned to the waterway. In addition, the CTM procedure must be rapid enough to prevent temperature acclimation but not so rapid that the experimental organism goes into heat shock. In contrast, the LD 50 methods require that test organisms either suffer a heat shock from being put immediately into heated aquaria from their acclimation aquaria, or acclimate to some extent to the temperature as the aquarium water is heated slowly.

The definition of CTM as presented by Hutchison (1961, p. 92) should be followed when performing the CTM test, but some misunderstanding of it seems to exist among fishery investigators.

Hutchison states that the CTM occurs when there is a "complete loss of any ability of the animal to escape from temperature conditions that, if continued, will promptly lead to death." Some fishery investigators have stated that the CTM occurs when the organism loses equilibrium. However, Hutchison's definition precludes this idea for when one performs CTM tests, he sees that equilibrium is, in fact, lost and regained several times by the test organism. So, the point at which the CTM occurs, based upon equilibrium, becomes quite subjective. I have even found that a fish still can swim when it has completely lost its ability to remain upright. Although one may argue that a fish when swimming on its side is not able to direct its swimming, this remains to be established experimentally.

The behavioral responses shown by fish during a CTM test are quite similar to those of salamanders (Hutchison, 1961). Both types of organisms increase their activity in the flask as the temperature begins to rise. Next, fish may or may not leap out of the water several times (jumping response) and salamanders generally leap and attempt to crawl up the sides of the flask. Following the jumping response is a loss of equilibrium. Whereas, the ability to maintain their normal vertical orientation (righting response) is lost gradually by salamanders, it is lost and regained several times by fish. After equilibrium loss is reached, salamanders remain motionless for a period, but fishes retain the ability to swim while remaining on their sides. Fish then become motionless and lie at the surface or bottom of the flask beating their opercula rhythmically for a short period. This quiescent period is terminated in salamanders by a general

muscular spasm and in fish by a spasmodic opercular flutter. The temperature at which the spasms occur in both types of organisms is the CTM. It is followed immediately by a heat coma and then death within a few seconds.

METHODS

The CTM of spot was measured throughout their initial growing season on groups of juveniles acclimated to a variety of temperature and salinity combinations in the laboratory. All of the experimental animals were collected from South Creek, a tributary to the Pamlico River, in an attempt to sample from a uniform population of organisms and thus reduce any variation in results due to genetic variance between populations of fish. Temperature acclimation was carried out in 50 liter aquaria at the rate of 1 C increase per day and 1/2 C decrease per day (Fry et al., 1946). The organisms were allowed to acclimate to salinity for the length of time it took them to become acclimated to temperature. Surprisingly enough, there are few accounts of the rate of acclimation of fishes to either temperature or salinity in the literature, yet the salinity acclimation appears to be rapid. In some fishes, for example, Angelovic, White and Davis (1969), Hickman (1969), and Pots and Evans (1967) have shown that sodium transport across the gills of some fishes is so rapid that plasma osmolarity reaches equilibrium usually in less than 24 hours.

The care and handling of all experimental organisms was standardized, with particular care being given to density, food and photoperiod. Spot which weighed 1 g or more were held in acclimation squaria at a density of 1 g of fish per liter of water, while those weighing less than 1 g were held at a density of 50 fish per 50 gallon aquarium. Post-larvae were fed brine shrimp nauplii and powdered commercial trout food, whereas, the larger juveniles were fed a meal-gelatin diet developed by Peterson, Robinson and Willoughby (1967).

I held the lighting constant at 100 foot candles over the acclimation aquaria since both Prosser (1965) and Brett (1944) have stated that photoperiod could cause the thermal tolerance of fish to vary.

To control the quality of the aquarium water, the water was continually passed through a charcoal, gravel and calcium carbonate shell filter bed. In addition, the water in each aquarium was filtered through a 120 gph capacity diatomacious earth filter for at least 10 minutes daily or until the water was clear.

The actual performance of the CTM test also required standardization so as to eliminate any possible additional variation in the results. In order to eliminate any variation which might be due to poor physiological condition of the experimental organism, only the healthy appearing individuals, those devoid of hemorrhagic areas on the body or fins, were subjected to the CTM test. The CTM test began when one or two fish were transferred from the acclimation aquarium to a 2000 ml capacity distillation flask containing 1300 ml of water from the acclimation aquarium. Next, the flask was placed in a heating mantle and heated at the rate of 1 C per minute (measured with a mercury-in-glass thermometer read to 0.1 C) until the CTM occurred. During the whole experiment, air was bubbled through an air stone at a rate that maintained oxygen levels above 85% saturation, yet still allowed the fish to be observed.

To analyze the data, a multiple regression analysis (Snedecor and Cochran, 1967) was carried out on the IBM model 360 computer. This technique permitted me to develop the best prediction equation from 13 possible factors and combinations of factors which might influence CTM.

From this equation I constructed graphs, using a plotting attachment on the 360 computer, of the acclimation temperatures necessary to achieve a predetermined CTM for juvenile spot of a particular size at a particular salinity level. This analysis provides much more information than the one used by Hutchison (1961) in which he simply compared mean CTM values by a graphical method (Hubbs and Hubbs, 1953). The analysis which I used also gave comparative measures of the factors which significantly affect the CTM of juvenile spot which was not possible with Hutchison's analysis.

RESULTS

Of the 13 factors tested, only length (L), weight (W), and weight squares (W^2), temperature of acclimation (T), and salinity (S) correlated significantly at the 95% level of significance (using an F-test) with the CTM of the spot. Factors not significant were temperature squared, weight cubed, temperature x weight interaction, length squared, salinity squared, temperature x salinity interaction, weight x salinity interaction, and length x salinity interaction. Those which were significant were used to produce the prediction equation, CTM = $27.36428 + 0.38028(T) + 1.98355(W) - 0.19383(W^2) - 0.08214(L) + 0.02747(S)$. The mean CTM observed, the mean CTM predicted by the equation, and their difference (the residual) are shown in the appendix. The small difference between the sum of the squared residuals and the error sum of squares (-0.0002) indicates that most of the variation about each mean CTM is explained by the regression model.

A special experiment with groups of spot acclimated to 30 C and 8 ppt revealed that the time of day of the testing had no effect (Table 1). Only the R^2 value at 0400 was high enough to suggest any correlation, but a replicate experiment gave a much reduced R^2. The prediction equation is CTM = $35.9189 + 0.0411(L) + 0.0511(W)$. Even when the days of acclimation and time of day were added to the regression of CTM on length and weight, there was no correlation between CTM and time of day (Table 2).

Nearly all of the variation due to regression is explained by the five significant factors of the model. The correlation index (R^2)

Table 1. Correlation index and error terms for diurnal measurements of the CTM of juvenile spot in July, 1968, experiments at 0400, 2000 and 2400 hours were repeated because of the observed variation between the first set of experiments; CTM = 35.9189 + 0.0411(L) + 0.511(W)

Time	0400	0400	0800	1200	1600	2000	2000	2400	2400
R^2	.6274	.3952	.3084	.2257	.1201	.0471	.0711	.2521	.2245
Mean square									
Regression	.1882	.5533	1.656	.5591	.1033	.0343	.1237	.4341	.2712
Error	.0373	.2822	1.238	.6395	.3025	.2311	.4041	.3679	.2677
d.f.	6	6	6	6	5	6	8	7	7

Table 2. Correlation indices from the regression of diurnal measurements of the CTM of juvenile spot with four combinations of length, weight, time of day the test was made, and days of acclimation

B variates	R^2	Mean square error
Length, weight	0.02768	0.46600
Time, length, weight	0.03141	0.47002
Days, length, weight	0.07908	0.44689
Time, days, length, weight	0.07990	0.45214

for the model including all of the factors is 0.9776 with a standard deviation of 0.445 and a mean square error of 0.1979. It is quite evident that acclimation temperature is the most important factor influencing CTM since its R^2 is 0.9201. It is also easily seen that salinity is not a very influential factor for the CTM of juvenile spot since its R^2 is only 0.001. Whereas temperature and salinity have the most and least influence, respectively, on the CTM of juvenile spot, weight (R^2 = 0.1788), weight squared (R^2 = 0.1550), and length (R^2 = 0.1556) are all intermediate and about the same level in their influence on the CTM.

The CTM of juvenile spot increases with age and weight over the initial growing season. Acclimation temperatures required to produce a particular CTM for spot at sizes representing the spring fish as they immigrate into the upper estuary as post-larvae, the summer fish and the fall fish prior to emmigration from the upper estuary are shown in Figures 1-3. Spring sized post-larval spot averaged 19.05 mm S.L.,

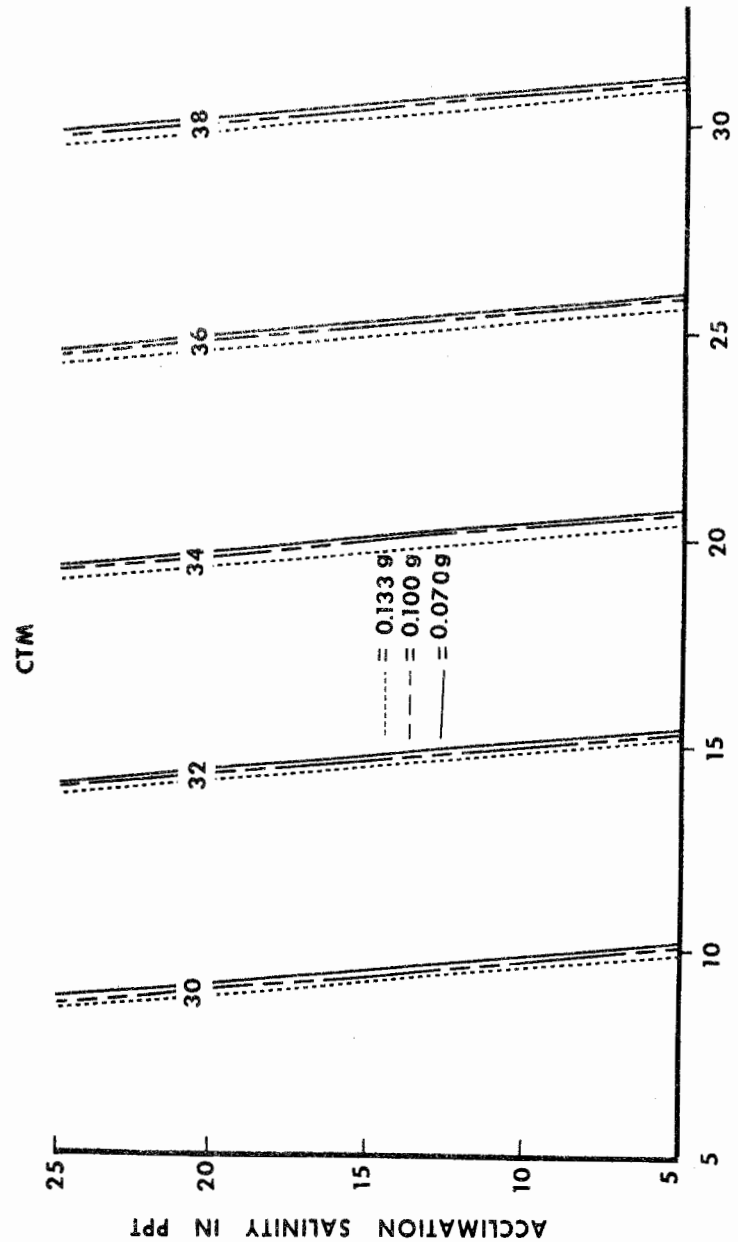

Figure 1. Acclimation temperature needed to achieve CTM's of 30.0, 32.0, 34.0, 36.0, and 38.0 for three weights of juvenile spot of 19.05 mm S.L.

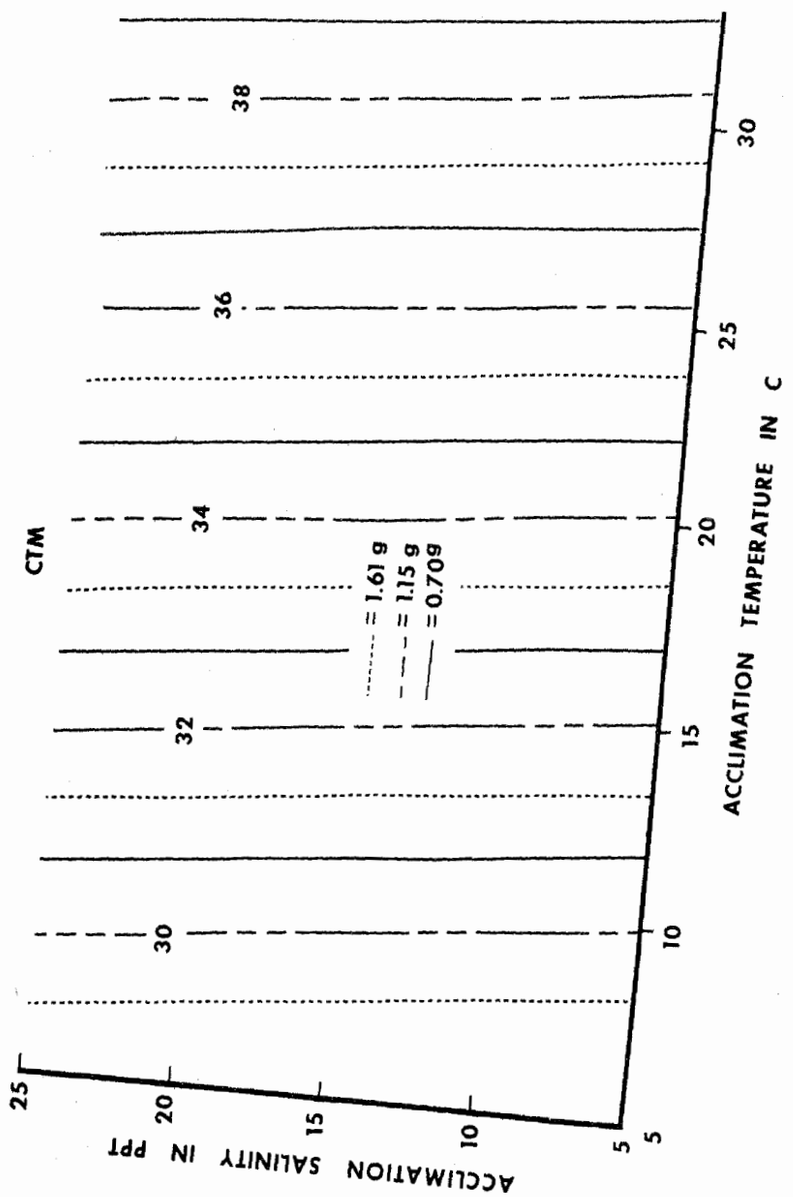

Figure 2. Acclimation temperature needed to achieve CTM's of 30.0, 32.0, 34.0, 36.0, 38.0 for three weights of juvenile spot of 40.33 mm S.L.

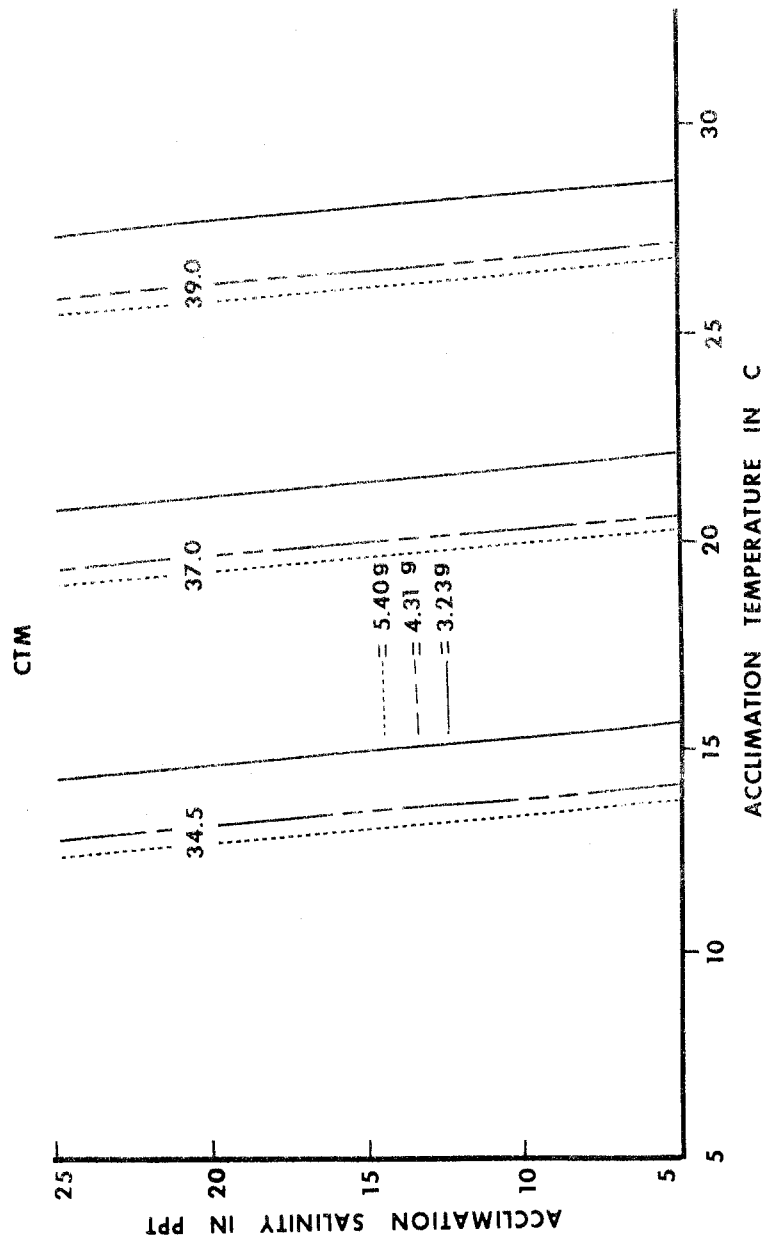

Figure 3. Acclimation temperature needed to achieve CTM's of 34.5, 37.0 and 39.5 for three weights of juvenile spot of 60.37 mm S.L.

summer sized spot averaged 40.33 mm and fall sized spot averaged 60.37 mm. In comparing the CTM of spring and summer sized spot, it is evident that the increase in CTM with age has not occurred in the smallest summer sized spot (0.70 g) for they require a higher acclimation temperature to achieve a set CTM than the post-larvae do at all three sizes (0.07, 0.10, and 0.133 g). But that is the only group which does not follow the general trend of increased CTM with age. When comparing the effect of weight on the CTM of spot at the three age periods one can see in Figure 1 that heavier fish need a lower acclimation temperature to achieve a given CTM than do lighter fish, and this effect is even more marked for the summer fish two to four months later (Figure 2). By the time the fish are ready to leave the estuary in the fall, a slightly higher maximum CTM has been attained (Figure 3). Also one can see that the CTM changes more between the medium and lightest fish than between the medium and heaviest.

Salinity increases the CTM of all ages of juvenile spot. This is indicated in Figures 1-3 since the required acclimation temperature decreases at all three sizes at all three ages; however, salinity becomes less significant at higher acclimation temperatures. Although the CTM generally increases with salinity, there is some variation and the increase is not always a significant one (Table 3).

The variation in pattern is not great, however, since only one experiment revealed a CTM value from a group of spot acclimated to 5 ppt which was significantly higher than that from a group acclimated to 15 ppt. Furthermore, only one experiment revealed a CTM value from a group acclimated to 15 ppt which was significantly higher than that

Table 3. Least significant difference at the 95% level of confidence (*) between 5, 15 and 15, 25 ppt salinity for juvenile spot acclimated to several temperature/salinity combinations in the laboratory throughout their initial growing season

Date	Sal.	Temperature	N	$\bar{x}$ CTM	LSD 5:15	LSD 15:25	Ranked salinity	Ranked $\bar{x}$ CTM
4/16/69	5	29.95	20	38.00	0.284		15	38.26
	15		20	38.26		0.284	25	38.01
	25		20	38.01			5	38.00
4/2/69	5	20.04	20	33.45	0.480		15	33.73
	15		20	33.73		0.480	25	33.59
	25		20	33.59			5	33.45
4/15/69	5	10.08	17	29.72	0.470		25	30.59
	15		15	30.10		0.500*	15	30.10
	25		17	30.59			5	29.73
6/10/68	5	28.08	18	36.94	0.255*		25	37.75
	15		19	37.45		0.247*	15	37.45
	25		16	37.75			5	36.94
5/2/68	5	24.31	9	34.42	0.452*		25	35.48
	15		7	35.10		0.452	15	35.10
	25		9	35.48			5	34.42
4/29/68	5	24.53	8	34.89	0.439*		15	35.61
	15		6	35.61		0.474	25	35.32
	25		8	35.32			5	34.89
4/25/68	5	24.13	5	35.50	0.499*		25	35.96
	15		4	34.98		0.473*	5	35.50
	25		6	35.96			15	34.98
4/22/68	5	17.48	8	32.51	0.438*		25	33.09
	15		10	32.98		0.464	15	32.98
	25		10	33.09			5	32.51
4/30/68	5	17.10	11	32.45	0.531		25	33.29
	15		11	32.93		0.543	15	32.93
	25		12	33.29			5	32.45
5/21/68	5	7.58	4	28.03	1.036		25	30.17
	15		9	28.67		0.934*	15	28.67
	25		3	30.17			5	28.03

from a group acclimated to 25 ppt. So the indication that increased salinity results in increased CTM's seems valid, for there are only minor variations in the trend. Although the analysis of these data do not show that temperature and salinity interact significantly, some interaction is evident in Figures 1-3, and the interaction may have been statistically detectable had experiments been performed using more extreme acclimation salinity levels.

The predicted CTM increases more sharply between summer (40 mm) and fall (60 mm) fish than between post-larvae (20 mm) and summer fish (Figure 4). At 40 and 60 mm the difference in CTM is greatest between 5 and 15 ppt. The predicted CTM of the largest, and therefore oldest, spot is consistently higher at all salinities and temperatures than that of the two smaller, and therefore younger, spot; however, as shown in Figure 5, the affect of salinity on CTM may decrease somewhat at higher salinities.

The five factors found to significantly affect the CTM of juvenile spot can act to increase the CTM to the maximum which is genetically possible, and this change is expressed as a percent from the ratio of the increase in CTM to the increase in acclimation temperature. For example, if the CTM increased 5 C between groups acclimated to 10 and 20 C, the ratio would be 5:10 and the change would be 50%. When I computed the percent change for juvenile spot, I found it to be quite constant at near 38% with some minor variations among the younger fish (Table 4). There is some variation in CTM changes between salinity levels for the post-larvae and the summer sized fish, but the variation became minor for the fall sized fish.

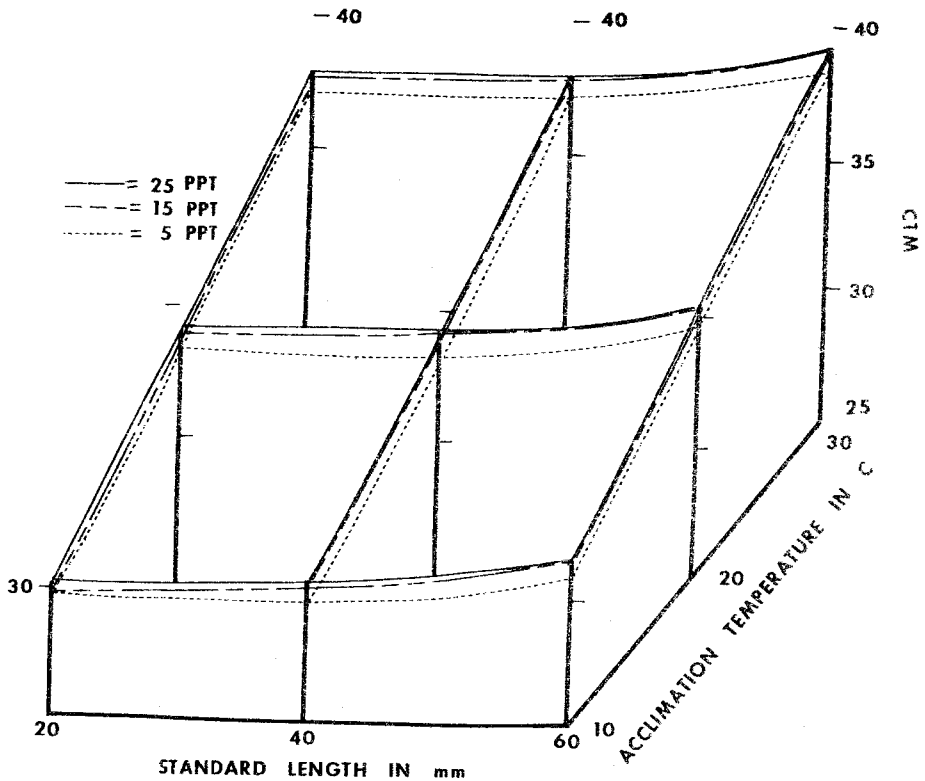

Figure 4. Critical Thermal Maximum of juvenile spot at three levels of salinity at each of three levels of thermal acclimation and standard length

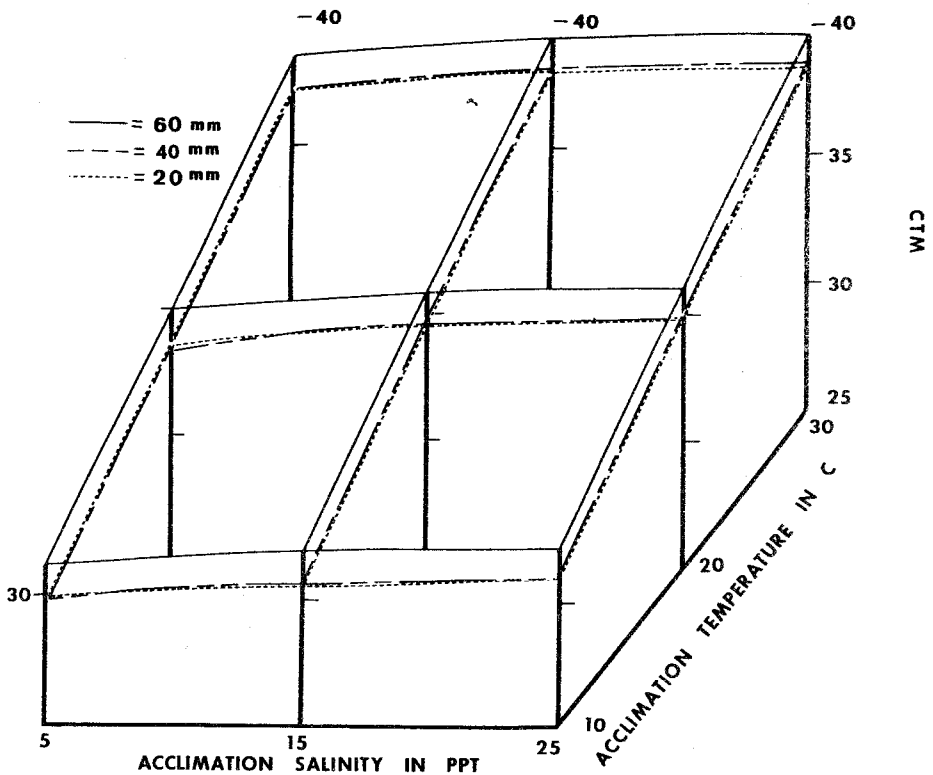

Figure 5. Critical Thermal Maximum of juvenile spot at three lengths at each combination of three levels acclimation temperature and acclimation salinity

Table 4. Percent increase in CTM of juvenile spot due to differing laboratory conditions throughout their initial growing season (groups are partitioned by salinity acclimation (S), temperature (T), weight over all three salinities (W), and length over all three salinities (L); $I\%_{10}$ = percent increase from 10 C increase in acclimation temperature; $I\%_{20}$ percent increase from a 20 C increase in acclimation temperature; 10-20, 20-30, and 10-30 = the temperature ranges over which the percent increases were computed; percent increase = CTM increase: difference in acclimation temperature X 100)

S	T	W	L	CTM	$I\%_{10}$ 10-20	$I\%_{10}$ 20-30	$I\%_{20}$ 10-30
5	10	0.1	19.05	29.94			
	20			33.61	36.7	38.1	37.40
	30			37.42			
15	10			30.40			
	20			34.15	37.5	38.0	37.75
	30			37.95			
25	10			30.55			
	20			34.29	37.4	38.0	37.70
	30			38.09			
5	10	1.15	40.33	29.89			
	20			33.40	35.1	41.0	38.05
	30			37.50			
15	10			30.43			
	20			34.23	38.0	38.0	38.00
	30			38.03			
25	10			30.57			
	20			34.37	38.0	38.0	38.00
	30			38.17			
5	10	4.31	60.33	31.17			
	20			34.97	38.0	38.1	38.05
	30			38.78			
15	10			31.71			
	20			35.51	38.0	38.0	38.00
	30			39.31			
25	10			31.84			
	20			35.65	38.1	38.0	38.05
	30			39.45			

Table 4. Percent increase in CTM of juvenile spot due to differing laboratory conditions throughout their initial growing season (groups are partitioned by salinity acclimation (S), temperature (T), weight over all three salinities (W), and length over all three salinities (L); $I\%_{10}$ = percent increase from 10 C increase in acclimation temperature; $I\%_{20}$ percent increase from a 20 C increase in acclimation temperature; 10-20, 20-30, and 10-30 = the temperature ranges over which the percent increases were computed; percent increase = CTM increase: difference in acclimation temperature X 100)

S	T	W	L	CTM	$I\%_{10}$ 10-20	$I\%_{10}$ 20-30	$I\%_{20}$ 10-30
5	10	0.1	19.05	29.94			
	20			33.61	36.7	38.1	37.40
	30			37.42			
15	10			30.40			
	20			34.15	37.5	38.0	37.75
	30			37.95			
25	10			30.55			
	20			34.29	37.4	38.0	37.70
	30			38.09			
5	10	1.15	40.33	29.89			
	20			33.40	35.1	41.0	38.05
	30			37.50			
15	10			30.43			
	20			34.23	38.0	38.0	38.00
	30			38.03			
25	10			30.57			
	20			34.37	38.0	38.0	38.00
	30			38.17			
5	10	4.31	60.33	31.17			
	20			34.97	38.0	38.1	38.05
	30			38.78			
15	10			31.71			
	20			35.51	38.0	38.0	38.00
	30			39.31			
25	10			31.84			
	20			35.65	38.1	38.0	38.05
	30			39.45			

DISCUSSION

Thermal acclimation alters the CTM of juvenile spot more than salinity, size or age. Acclimation temperature acts as a controlling factor, while age, size and salinity only modify heat tolerance. Of course, all of these factors can only influence the CTM of spot to the extent determined genetically. The mechanisms by which the factors influence the CTM are not known, but several theories do exist.

Investigators have postulated that enzymes, lipids and hormones, as well as membrane phenomena may all be involved in temperature acclimation in fishes. Since different enzymes have different temperature sensitivities and different biochemical systems have different temperature constants, one should be able to predict a priori that different poikilotherms will have different temperature sensitivities according to the temperature at which their metabolism functions most efficiently. One enzymatic change found to occur in relation to temperature in fresh-water fishes is an inactivation of the cholinesterase activity in response to temperature increases (Kusakina, 1963; Baslow and Nigrelli, 1964). Although Holliday (1969), in discussing the temperature sensitivity of teleost eggs, specifies that only enzymes change, he does indicate that temperature acclimation is accomplished through more extensive biochemical modifications. This probably involves many more elements of the protoplasm than just enzymes. One such element may well be the lipids, as it is known that their state and quantity changes as the temperature rises (Prosser, 1965; de Sylva, 1969). Hormonal changes have also occurred in fish with rising temperatures as demonstrated by the thyroid stimulating

hormone (TSH) increase with increasing temperature in freshwater cyprinids (Fortune, 1958); however, the amount of increase was shown to be independent of acclimation temperature. A theory of temperature acclimation which may include changes in all of the elements just mentioned postulates that critical anomalies in biophysical processes occur in the membrane phenomena at temperature near 15, 30, and 45 C (Drost-Hansen, 1969). The implication here is that an organism may be genetically able to acclimate to changing temperatures by replacing metabolic pathways; and if so, the pathways may change at the temperatures, 15, 30 and 45 C, at which the critical anomolies are said to occur.

Temperature and size both alter the metabolism of fish, and metabolism may be intimately involved with temperature acclimation of fishes. According to Bartholomew (1968) metabolic rate, as measured by oxygen uptake, nearly doubles for every 10 C increase in temperature. However, temperature is not the only parameter which influences metabolism since size also changes metabolic rate. Davison (1955) states that it is an accepted general phenomenon that in the animal kingdom small animals exhibit a higher standard metabolic rate than related animals of greater body weight. Because increases in both metabolic rate and susceptibility to cold death are inversely related to increasing size among young salmon, Brett (1956) has suggested that temperature acclimation of marine fish may be governed by metabolic rate.

The variation of thermal tolerance with organism size may be the result of a change in cell surface concentration as the organism grows.

I have shown that CTM increases with size at all three ages measured which agrees with the statement made by Brett (1969) that embryonic stages of fishes are more sensitive to temperature than adults. In contrast, however, Fry et al. (1946) were unable to find any relationship between size and the time of death in thermal tolerance experiments involving speckled trout, Salvelinus fontinalis. Increases in CTM occurring with the growth of juvenile spot may be the result of changes in the cell surface to volume ratio of the skeletal musculature. The surface to volume ratio decreases as skeletal muscles increase in size, and Davison (1955) found that changes in cell surface concentration and changes in metabolic rate coincided in the ontogeny of anurans. So, perhaps the thermal tolerance increases with growth when the decreasing cell surface to volume ratio reduces the amount of energy required by active transport mechanisms for organism maintenance, and therefore, makes more energy available for thermal acclimation.

The heat tolerance of juvenile spot increases with age during the first growing season in the upper estuary. This increase in temperature tolerance with age seems to occur in most fishes which have been investigated (Brett, 1969). Since spot probably spawn in coastal water (Dawson, 1958) rather than in the estuaries, their eggs and larvae may be less tolerant of thermal changes than more advanced stages, as is the case with marine fish in general (de Sylva, 1969). As the newly spawned spot age and gain tolerance to environmental fluctuations, they begin immigrating toward the upper estuary which will serve as a nursery their first growing season. This increase in tolerance is necessary for survival in the nursery, for fluctuating environmental conditions are quite common there.

Photoperiod may have influenced the effect of age on CTM. Although I maintained a photoperiod of 100% (constant light) over all the experimental organisms, the time that they were exposed to it may not have been enough to cancel the effect of the photoperiod to which they were exposed naturally prior to being captured. However, during most of the summer and fall, water temperatures at the time of capture were approaching 30 C, so that acclimation to 20 and 10 C required 20 and 40 days, respectively, at the rate of 0.5 C decrease per day. This acclimation time may have been sufficient to remove the effect of any former photoperiod (Hoar and Robertson, 1959). Since the increase in CTM between summer and fall spot is greater than that between spring and summer spot, the increase in CTM as spot grow older seems more the result of age rather than photoperiod.

Salinity proved to be the least important factor among those which affected the CTM of juvenile spot; however, it did generally increase the CTM. This increase is consistent with the findings of Strawn and Dunn (1967) who found that the presence of any dissolved solids (total salinity) increased the temperature of heat death of several salt- and freshwater-marsh fishes. As the temperature became more tolerable, the salinity level which gave optimum survival dropped for freshwater fishes but increased for saltwater fishes. In this study, the salinity changes from 5 to 25 ppt increased the CTM of juvenile spot at all temperatures, all sizes and all ages tested. The increase was minor, sometimes not even statistically significant, but it was consistent. Conceivably, as spot approach maturity, they may be affected differently by changes in the salinity. This is perhaps reflected by the migration

toward the ocean's more stable conditions and away from the upper estuary's unstable conditions as spot age. Other organisms which show the same increase in heat tolerance with increasing salinity are the lobster, Homarus americanus, (McLeese, 1956); and the trout of the genus, Salmo sp. (Holsband, 1953). Holsband (1953) even found that the lethal temperature for heat was raised for trout (Salmo) by the addition of calcium and magnesium ions to the water.

One might speculate that the increase in ionic content in the water may enhance the ability of the organism to withstand stresses by supplying additional, needed complexing agents. These agents may be used to facilitate transport across membranes and thereby speed up the replacement of one enzyme system with another which is more efficient and effective under the stressful conditions. This speculation gains some support from the conclusion of Prosser, Mackay, and Kato (1970) that ionic concentration and temperature effects also are intimately involved in membrane transport systems in fishes. They further state that in general high salt concentrations of marine fishes at low temperatures and low salt concentrations in freshwater fishes at low temperatures is a possible means of reducing the osmotic work necessary for survival and that these ion shifts may be membrane limited. Drost-Hansen's (1969) hypothesis would be supported if an investigator found significant reactions to salinity at temperatures around 15 and 30 C, the temperatures at which Drost-Hansen (1969) has said that the membranes replace their transport mechanisms. Since the maintenance of proper osmotic balance is accomplished, at least in part, by transport mechanisms, an irregularity in transport at

15 or 30 C, the temperatures proposed to be points at which the transport mechanisms are replaced, would create heat tolerance anomalies and thereby support Drost-Hansen's theory. The data obtained in my study show a general increase in CTM with salinity, but no anomalies occur at 15 or 30 C. Crost-Hansen's theory offers an intriguing area for more investigation.

In summation, several statements seem appropriate. Acclimation temperature is the major controlling factor of CTM; however, its control is not absolute. Other factors, including age, size, and acclimation salinity also influence CTM, and although their influence is much less than that of temperature, they are still statistically significant influences. These data establish that size definitely influences CTM as contrasted with the lack of evidence of this influence in speckled trout investigated by Fry et al. (1946). Age within one growing season seems to be an influence on CTM also; however, the natural photoperiod to which the experimental fish were exposed before capture may have shaded the influence of age. Salinity, over the range of 5-25 ppt, was a minor influence on the CTM of juvenile spot, although extreme salinities approaching freshwater and full strength seawater concentrations (0 and 35 ppt) may have given more emphasis to salinity's affect on heat tolerance. This is the impression one receives from most investigators involved with the study of marine or estuarine organisms, for they consistently state that the investigation of the effects of temperature may be influenced by salinity, and the two parameters should always be investigated together (Brett, 1969; Kinne, 1964; Prosser and Brown, 1965).

ECOLOGICAL SIGNIFICANCE

It is not possible to say that the reactions of an organism in a natural situation are the same as the reactions of the same organism in an unnatural situation, such as a laboratory experiment. There are, however, certain inferences, observations and insights provided by laboratory studies which one can apply to natural situations. In this study, I have manipulated some parameters and tried to control others which might influence the CTM of juvenile spot. This may not be totally realistic in that an organism in the natural setting has all of the influencing parameters impinging upon it to varying degrees of each at all times. Accepting the limitations of a laboratory experiment and admitting that there is a danger in extrapolating the results into the natural habitat of an organism, I shall try to point out some of the implications of this study for predicting the effect of the power industry on estuaries.

Although the heat added to the cooling water in the condensors of a power plant is potentially lethal to entrained organisms, the results from this study show that death due to overheating can be avoided with appropriate precautions. When spot migrate up the estuary as post-larvae in the winter, they are more susceptible to heat damage than they will be as they grow older. However, entrained post-larvae would not be killed by the thermal increase in the condensors of a generating plant at this time, because the ambient temperature of the coolant before entering the power plant would be low enough so that the heat added in the condensors would not approach lethal levels. This is evidenced by a CTM of 29.9 C of post-larvae acclimated to 10 C. Even though this

shows that post-larvae would not be killed by the thermal increase in the condensors, these experiments provide no information relating to other affects of a trip through the condensors or to any effects on the metabolism of the fish as a result of the temperature change. These data show that if a temperature of near 30 C is reached in the condensors, all of the post-larval spot passing through the condensors when ambient temperatures are near 10 C will be killed by the heat increase alone. During the summer in North Carolina when the ambient water temperatures range throughout the 20's and low 30's C, the CTM for juvenile spot, when acclimated to 20 C, is 33.4 C and, when acclimated to 30 C, is 37.5 C. The implication is that spot entrained in the cooling water during the summer stand a good chance of being killed just from the increase in temperature experienced in the cooling condensors. In the fall when ambient temperatures may range from 30 down to 10 C, the CTM of juvenile spot ranges from 39.5 at 30 C acclimation temperature to 31.2 at 10 C acclimation temperature. These data imply that as a spot grows to near 60 mm S.L. in late summer and early fall when water temperatures are near 30 C, it can stand nearly a 10 C increase in temperature--but not quite. A 10 C increase in temperature will kill 100% of those exposed to it at that time of year. However, later in the fall when ambient temperatures drop, spot can withstand the 10 C temperature increase created by the generating plant.

The results of this study may help the power generating industry to design and operate generating facilities without causing massive heat death of entrained organisms, and these results may help control

agencies to establish more meaningful water quality criteria. This study shows that if a generating plant operates at peak production during the summer and early fall, thereby causing a heat increase of 10 C in the coolant, the entrained juvenile spot will all be killed. Since the greatest demand for electricity occurs at this time, when a 10 C temperature increase is the most dangerous to entrained organisms, an alteration in operation of the generating plant would be necessitated to avoid reaching lethal temperatures during capacity generating operation. In addition to changing generating operations to protect aquatic organisms, the controlling agency can be more realistic by using the results of studies such as this one to set appropriate standards. When establishing temperature criteria for industry, one should consider that the temperature tolerances as determined in the laboratory may be the maximum temperatures at which the organism can survive, and if this is so, they will be too high for the survival of that animal in the natural environment (de Sylva, 1969). However, de Sylva's opinion is not universally accepted among environmental scientists, for the rigors to which the experimental animal is exposed while being removed from a natural situation and being maintained in the laboratory may actually produce additional stresses on the organism. These added stresses may lower the organism's tolerance in the laboratory below the level it would be in nature. Following his theory of maximum tolerance expressed in the laboratory, de Sylva (1969), however, advocates that maximum allowable temperature increases should be kept to 1 to 2 C below those found to be lethal to 50% of that species in the laboratory. Brett (1969) states that any temperature which approaches the stage of exterminating

10% of a population is reaching the catastrophic level, for when any portion of a population of organisms is dying from an identifiable cause, the rest of that population which is subjected to that causative factor is surviving under some level of stress created by the factor. I, therefore, suggest that a safety factor of 5 C below the CTM of an organism be maintained when establishing temperature increase criteria until such time as that temperature level shall be proven to not cause death to the organism in question.

The results of this study imply that under the appropriate conditions the heated plume from a power plant may be lethal to small fishes. Death could occur during the summer and fall when the differential between the ambient water temperature and the CTM is at its lowest point, so that it would be impossible for juvenile spot to withstand the 10 C rise in temperature produced by a power plant. This lethal condition could develop when a shift in the wind direction causes the heated plume to crowd the shoreline or to change the direction of flow and overtake juvenile organisms.

The fact that the heated plume can be lethal and that physical phenomena, such as wind, may influence the extent to which the lethality is expressed makes it important for the power industry to select plant sights with extreme care. The power industry would be well advised, when planning a power installation, to build in safeguards to organisms against the worst possible sets of circumstances. If the power industry would do this, they could include any necessary installations into the initial construction and prevent costly alterations after the plant

was operable, and most important, they would do minimum damage to the ecosystem of which they had become a part.

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A P P E N D I X

Appendix Table 1. Acclimation temperature, acclimation salinity, mean weight, mean length, date of tests, number in each test group, mean observed CTM, mean predicted CTM and residual (observed minus predicted) of juvenile spot (tests were made throughout their initial growing season; see test for prediction equation)

Temperature	Salinity	Weight	Length	Date	Number	CTM		
						Observed	Predicted	Residual
10.08	5.00	0.08	18.26	4/15/69	17	29.72	29.89	-0.27
10.08	15.00	0.08	17.59	4/15/69	15	30.06	30.32	-0.26
10.08	25.00	0.07	17.77	4/15/69	17	30.59	30.56	0.03
29.95	5.00	0.11	19.61	4/16/69	20	38.00	37.50	0.50
29.95	15.00	0.13	20.81	4/16/69	20	38.26	37.71	0.55
29.95	25.00	0.13	20.25	4/16/69	20	38.01	38.04	-0.03
17.48	5.00	0.22	25.20	4/22/68	10	32.51	32.51	0.00
17.48	15.00	0.23	24.56	4/22/68	10	32.98	32.85	0.13
17.48	25.00	0.16	25.12	4/22/68	8	33.09	32.95	0.14
24.13	5.00	0.26	26.33	4/25/68	4	35.50	35.02	0.48
24.13	15.00	0.30	27.12	4/25/68	6	34.98	35.30	-0.32
24.13	25.00	0.34	27.56	4/25/68	5	35.96	35.61	0.35
24.53	5.00	0.34	29.84	4/29/68	8	34.89	35.03	-0.14

Appendix Table 1 (continued)

Temperature	Salinity	Weight	Length	Date	Number	CTM		
						Observed	Predicted	Residual
24.53	15.00	0.52	32.76	4/29/68	8	35.61	35.39	0.22
24.53	25.00	0.39	32.90	4/29/68	6	35.32	35.42	-0.10
17.10	5.00	0.57	30.58	4/30/68	12	32.45	32.56	-0.11
17.10	15.00	0.63	32.90	4/30/68	11	32.93	32.75	0.18
17.10	25.00	0.59	31.70	4/30/68	11	33.29	33.05	0.24
24.31	5.00	0.56	32.09	5/2/68	9	34.42	35.16	-0.74
24.31	15.00	0.66	32.86	5/2/68	7	35.10	35.55	-0.45
24.31	25.00	0.55	31.24	5/2/68	9	35.48	35.76	-0.28
17.84	25.00	0.33	27.35	5/6/68	2	33.00	33.22	-0.22
7.58	5.00	0.70	35.90	5/21/68	3	28.03	28.73	-0.70
7.58	15.00	0.72	35.10	5/21/68	9	38.66	29.10	-0.44
7.58	25.00	0.94	38.48	5/21/68	4	30.17	29.47	0.70
28.11	5.00	1.61	45.38	6/10/68	16	36.94	37.15	-0.21
28.13	15.00	1.38	44.27	6/10/68	19	37.45	37.21	0.24
28.08	25.00	1.23	42.86	6/10/68	18	37.75	37.35	0.40

Appendix Table 1 (continued)

Temperature	Salinity	Weight	Length	Date	Number	CTM		
						Observed	Predicted	Residual
29.64	5.00	3.36	56.05	7/31/68	14	38.44	38.65	-0.21
29.64	15.00	3.66	57.50	7/31/68	14	39.01	38.99	0.02
29.95	5.00	3.91	52.30	8/1/68	14	38.75	38.39	-0.64
30.76	5.00	4.11	58.04	8/8/68	9	39.50	39.31	0.19
30.76	25.00	4.16	59.18	8/8/68	9	39.41	39.78	-0.37
19.23	5.00	3.76	58.10	8/20/68	9	35.47	34.77	0.70
19.45	25.00	3.22	57.17	8/20/68	9	35.61	35.39	0.22
19.53	5.00	3.40	55.70	8/20/68	1	35.40	34.86	0.54
19.42	25.00	3.99	62.79	8/20/68	7	35.87	35.11	0.76
23.49	5.00	3.83	60.60	9/23/68	7	35.89	36.21	-0.32
23.87	25.00	3.58	58.78	9/23/68	5	36.56	36.92	-0.36
23.79	5.00	4.45	60.50	9/30/68	5	36.45	36.57	-0.12
23.67	25.00	4.23	62.82	10/3/68	6	35.63	36.81	-1.18
24.02	5.00	5.15	65.27	10/14/68	10	36.58	36.35	0.23
16.93	5.00	4.05	59.42	10/14/68	6	34.13	33.91	0.22

Appendix Table 1 (continued)

Temperature	Salinity	Weight	Length	Date	Number	CTM		
						Observed	Predicted	Residual
23.79	5.00	5.40	62.77	10/16/68	7	36.55	36.45	0.10
16.94	5.00	3.67	58.33	10/24/68	6	33.90	33.82	0.08
16.95	5.00	4.50	66.00	11/5/68	1	34.00	33.53	0.47
16.97	25.00	4.33	67.57	11/5/68	20	33.43	33.91	-0.48