Prescott, G. W. 1968. The Algae: A review. Houghton Mifflin Com-

pany, Boston, p. 376.
Snedecor, George W. and William G. Cochran. 1967. Statistical Methods. The Iowa State Univ. Press, Ames, Iowa. p. 549.

EFFECTS OF INCREASED TEMPERATURE ON POST-LARVAL AND JUVENILE ESTUARINE FISH 1 By DONALD E. HOSS, LINDA C. COSTON, and

WILLIAM F. HETTLER, JR.

National Marine Fisheries Service Mid-Atlantic Coastal Fisheries Research Center Beaufort, North Carolina

ABSTRACT

We simulated thermal increases encountered by postlarval and juvenile estuarine fishes entrained in power plant cooling systems. Three methods were used to measure the effects of thermal shock on these fishes: (1) critical thermal maximum (CTM); (2) changes in routine oxygen consumption; and (3) survival after exposure to sudden increases in temperature for various periods of time.

For menhaden, spot, and pinfish acclimated at 15° C, CTM values were 29.4, 31.0, and 31.0 respectively. Oxygen consumption of menhaden, spot, and pinfish, increased as we raised the temperature in 5° increments from the environmental temperature, indicating that additional energy expenditures are necessary to maintain the fish at the elevated tem-peratures. At temperatures of 15° C above the normal environmental temperature, all of the menhaden, spot, and pinfish died within 5 to 10 minutes. Young striped killifish acclimated at 22° C survived at 39° C for more than 30 minutes but all died at 40° C.

INTRODUCTION

Most S.E.S. (steam electric stations) in the United States use open circuit or once-through cooling systems. River, stream, lake, or estuary water is used to condense steam, and then the heated coolant is returned to the source some distance from the intake site. The amount of cooling water used by these plants is in the order of 0.1 to 3 billion gallons of water per day per plant depending on the size of the plant (Mihursky and Cronin 1967). Destruction of a high percentage of the entrained organisms in the large volume of cooling water needed may have a significant effect on aquatic population levels, especially in estuarine nursery areas.

In this investigation we are interested in the effects of a sudden increase in temperature (thermal shock) on postlarval fish that may be entrained in power plant cooling systems. At the present time information is conflicting on whether or not organisms can survive passage through cooling systems. Kerr (1953) found that small yearling salmon could survive a 16° F temperature rise in a S.E.S. condenser with no fatalities after five days and that small yearling striped bass could withstand the 16° F temperature rise with a survival rate of 94% after five days. He concluded that yearling striped bass and king salmon passing through the condenser system tested (entrainment time 3.5 to 5 min.) would have a high rate of survival. Adams (1969) found natural growth of several species of shellfish in the discharge canal of a California S.E.S. Since the net flow in this particular canal was always outward he concluded that the free-swimming stages of these bivalves had passed through the condenser system of the plant. Mihursky and Cronin (1967), however, reported up to 95% destruction of zooplankton

¹ This research was supported by U. S. Atomic Energy Contract AT (49-7)-5.

and phytoplankton upon passage through S.E.S. They also suggested that a local decrease in the sea nettle population was due to destruction upon passage through the plant.

MATERIALS AND METHODS

Fish used in these experiments were collected using a tide net described by Lewis *et al.* (1970) or by dip net in the estuary near Beaufort, N. C. The species used were menhaden (*Brevoortia tyrannus*), pinfish (*Lagodon rhomboides*), spot (*Leiostomus xanthurus*), and striped killifish (*Fundulus majalis*). Fish were maintained in the laboratory at temperatures and salinities approximating those at which they were caught for a period of 3-5 days. In experiments where salinity was an experimental factor, fish were acclimated to that salinity for at least 3 days. Light periods corresponded to those found at the time of year the fish were captured.

Three methods were used to evaluate the effects of thermal shock on fish: (1) measurement of the critical thermal maximum (CTM) (Hutchinson 1961); (2) measurement of changes in routine oxygen consumption or metabolism; and (3) measurement of percent survival after thermal shock.

To determine the CTM, an individual fish was placed in a 500 ml distillation flask containing 300 ml of sea water at acclimation temperature. The distillation flask was then placed in a hemispherical mantle heater connected to a variable transformer adjusted to obtain a rate of heating of approximately 1° C per minute. The water in the distillation flask was used and stirred by compressed air. Flaring of the opercula was used to indicate the CTM and the temperature at this point was recorded.

Routine metabolism (Beamish and Mookherjii 1964) of postlarval fish was measured with a differential respirometer. Individual postlarval fish were transferred into 15 ml respiration flasks containing 3 or 5 ml of millipore filtered sea water at the same temperature. The flasks were then attached to the respirometer and immersed instantly into a water bath at the same temperature or at temperatures of 5° , 10° , 15° , or 20° C above acclimation. A temperature probe inserted into a respiration flask was used to measure changes in water temperature with time (Table 1). Oxygen consumption measurements were made at hourly intervals on each fish for a period of 4 hours and no fish was used in more than one experiment. Wet weights were obtained after the fourth oxygen measurement. Least squares regressions calculated

Increas	Se	Average Time (Min) 3 ml 5 ml		
above 15	° C			
5°		1.76	2.35	
10°		3.13	3.80	
15°		3.82	4.22	

 TABLE 1. Average time for water in respiration flask to reach temperature of water bath.

for logarithms of respiration as a function of logarithms of weight furnished values for the metabolism-weight coefficients in the metabolism equation: log $Q = \log a + k \log W$, where Q is the respiration rate, a and k are constants (for the species) and W is the weight of the fish.

In thermal shock experiments we transferred groups of 10 killifish from acclimation temperatures (22° C) to water 17 or 18° C higher. After exposure to elevated temperatures for 3, 5, 10, or 30 minutes the fish were returned to the acclimation temperature. Groups of control fish were treated in the same manner except temperature was held constant. The number of dead fish was counted at 0, 1, and 24 hours after exposure. Percent survival was then calculated.

RESULTS

CTM

We measured the CTM of menhaden, spot and pinfish acclimated at 15° C and 30 o/oo salinity. When first placed in the distillation flask the fish, except menhaden, were usually relatively quiet. Increasing the temperature caused: first a rapid swimming, next spasms, then flaring or spreading of the opercula and finally death. The CTM value indicates the temperature at which an animal loses its ability to escape from conditions that will kill it. In table 2 the results of the CTM experiments are summarized. The CTM value for spot is in agreement with that found by Bridges (1971) for larger fish of the same species. Our experiments indicate that a change in temperature of 14° C for menhaden and of 16° C for spot and pinfish acclimated to 15° C would be lethal.

TABLE 2. CTM values for three species of fis	n acclimated	at 15	°C
--	--------------	-------	----

Species	Number of fish	Average wet weight (mg)	Average CTM (°C)	Standar error	Increase (°C) over d acclimation Temperature
Menhaden	18	47.2	29.4	.099	14.4
Spot	32	25.2	31.1	.069	16.1
Pinfish	24	29.4	31.0	.072	16.0

Routine metabolism

Routine oxygen consumption measurements provide a useful means of determining sublethal effects of temperatures. Any stressful condition, such as increased temperature, imposed on the fish should be reflected by a change in the rate of metabolism (Steed and Copeland 1967). Using the metabolism-weight coefficients obtained in these experiments (Table 3) oxygen consumption was calculated for a 30 mg fish for each experimental temperature (Fig. 1).

TABLE 3. Metabolism-weight coefficients, experimental temperature, number of fish and weight range of fish for routine metabolism experiments.

Species T	N	w	a	k
Menhaden 15 20 25	$15 \\ 16 \\ 6$	38-62 33-65 29-58	-0.6205 0.2516 0.3839	$1.1882 \\ 0.7781 \\ 0.7688$
Spot	17 15 9	$12-26 \\ 13-34 \\ 16-28$	$\begin{array}{c} 0.2786 \\ 0.3227 \\ 0.7144 \end{array}$	0.5724 0.6819 0.5013
Pinfish	$17 \\ 15 \\ 15 \\ 15$	12-38 18-42 10-32	-0.2320 0.0278 0.0009	0.9100 0.8621 1.0024

Where: T = Experimental temperature, °C

N = Number of fish W = Weight range of fish in mg

 $a = \log_{n}$ value for intercept of calculated regression line k = slope of calculated regression line





As expected, our experiments showed that the increases in temperatures caused increases in oxygen consumption of fish surviving the sudden temperature change. At 15° C respiration was essentially the same for all three species of fish (Fig. 1). After sudden temperature increases of 5° and 10° C, however, menhaden had a higher rate of respiration in relation to either spot or pinfish. Thus, it would seem that the sudden change in temperature placed a greater stress on menhaden than on the other two species of fish.

Percent survival also was calculated for the fish used in the routine metabolism experiments. At 30° C (15° C above acclimation temperature) all of the fish tested died within 5-10 minutes of exposure. At 25° C (10° C above the acclimation temperature) 67% of the menhaden, 50% of the spot and 17% of the pinfish died during the experiment.

Thermal Shock

The percentage survival of postlarval killifish at various temperaturesalinity-time combinations is given in table 4. Survival after 24 hours was 100% in both control groups. A t-test analysis of the percentage of fish surviving at various levels of temperature, salinity, and time of exposure (Table 4) indicated the following:

- (1) Survival was significantly greater at 10 o/oo and 39° C than at 10 o/oo and 40° C (P<.01)
- (2) Survival was significantly greater at 30 o/oo and 39° C than at 30 o/oo and 40° C (P<.01)
- (3) At 40° C survival was significantly greater at 30 o/oo than at 10 o/oo $(P \le .01)$
- (4) At 30 o/oo and 40° C survival was significantly greater at 3-5 minute exposure times than at 10-30 minute exposure times (P<.01)
- (5) At 10 o/oo and 40° C survival was significantly greater at 3-5 minute exposure times than at 10-30 minute exposure times (P<.05)</p>
- (6) At 10 o/oo and 39° C there was no significant difference between exposure times (P>.05).

Salinity o/oo	Temperature °C	Exposure time (min)	24 hour percent survival
10	39	3 5 10 30	100 100 100 77
10	40	3 5 10 30	30 30 9 0
	39	3 5 10 30	100 91 100 100
30	40	3 5 10 30	100 100 20 0

TABLE 4. Survival of striped killifish 24 hours after thermal shock.



FIGURE 2. Variations in S.E.S. discharge canal design. (A) Discharge directly into river. (B) Discharge into ocean. (C) Discharge into headwaters of a lake.

Of the three methods we used to evaluate the effects of thermal shock on postlarval estuarine fish, the percent survival method more nearly simulates the conditions entrained fish would be exposed to in an actual cooling water system than either the CTM or the routine metabolism methods. The CTM method was used by Bridges (1971) to simulate the conditions a fish would experience as it passed from environmental temperature through a condenser. The rate of temperature change a fish would experience passing from environmental temperature through a condenser, however is faster than the 1° C change per minute defined for CTM experiments. In an operating condenser fish may pass from environmental temperature to 16° C or more above environmental temperature within seconds (Auerbach *et al.*, 1971). For fish acclimated at 15° C it would take 16 minutes to reach the measured CTM of 31° C (16° C change in temperature at 1° C per minute rate of change). This is not as severe a stress as a change in temperature of 16° C i n a minute or less.

The routine metabolism method measures changes in metabolic rate caused by changes in temperature. The rate of change in temperature was more realistic in these experiments (Table 1) but the length of exposure time (3-4 hours) was longer than fish would normally experience. This method is more suited for measuring sublethal chronic effects on fish than for measuring acute lethal effects. The faster rate of temperature change (average 3° C per minute) in these experiments in part explains why the spot and pinfish died at a lower temperature (30° C) than predicted by the CTM.

In the survival experiments the change from environmental temperature to elevated temperature occurred in a matter of seconds. However, the length of exposure time can be controlled to fit the design of any plant cooling water system. Using this method we found that the lethal temperature for postlarval striped killifish acclimated at 22° C was affected by both exposure time and salinity. Striped killifish were able to withstand a 40° C temperature for a longer exposure at 30 o/oo than at 10 o/oo.

Survival of estuarine fish entrained in power plant cooling water systems will depend in part on the length of time the fish are exposed to the elevated temperatures. Length of exposure time in turn depends on the design of the cooling system. In figure 2 three variations in cooling water canal design are shown. It is apparent from the diagrams that the length of time the fish is exposed to heated water will depend to a large extent on the length of the discharge canal. Adams (1969) gives a range of from 2 to 10 minutes for passage time from intake headwork to the end of the discharge canal in tidal power stations in California. These stations have short discharge canals similar to figure 2-A. In the longer discharge may exceed 30 minutes. Since very little cooling takes place in the discharge canal $(1-2^{\circ} C)$ the organisms are exposed to the elevated temperatures the entire time they are entrained.

Although these results are preliminary they do indicate that experiments can be designed that will be helpful to the power generating industry in designing plant cooling systems to minimize harmful effects to entrained organisms.

We thank W. E. Schaaf for statistical assistance and C. W. Lewis for preparation of the figures.

LITERATURE CITED

Adams, J. R. 1969. Ecological investigations around some thermal power stations in California tidal waters. Chesapeake Sci. 10: 145-154.

Auerbach, S. I., D. J. Nelson, S. V. Kaye, D. E. Reichler, and C. C. Coutant. 1971. Ecological considerations in reactor power plant siting, p. 803-820. In Environmental aspects of nuclear power stations. International Atomic Energy Agency, Vienna. Beamish, F. W. H., and P. S. Mookherjii. 1964. Respiration of fishes

Beamish, F. W. H., and P. S. Mookherjii. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, *Carassius* auratus L. Can. J. Zool. 42:161-175.

Bridges, D. W. 1971. The critical thermal maximum of juvenile spot, Leiostomus xanthurus, Lacepede. Water Resour. Res. Inst. Univ. N. C. Rep. No. 43, 39 p.

Hutchinson, V. H. 1961. Critical thermal maxima in salamanders. Physiol. Zool. 34: 92-125.

- Kerr, J. E. 1953. Studies on fish preservation at the Contra Costa steam plant of the Pacific Gas and Electric Company. Calif. Dept. Fish Game, Fish. Bull. 92. 66 p.
- Fish Game, Fish. Bull. 92. 66 p.
 Lewis, R. M., W. F. Hettler, Jr., E. P. H. Wilkens, and G. N. Johnson. 1970. A channel net for catching larval fishes. Chesapeake Sci. 11: 191-198.
- Mihursky, J. A., and L. E. Cronin. 1967. Progress and problems in thermal pollution in Maryland. Contrib. Natur. Resour. Inst., Univ. Md. No. 344. 11 p.
- Steed, D. L., and B. J. Copeland. 1967. Metabolic responses of some estuarine organisms to an industrial effluent. Contrib. Inst. Mar. Sci. Univ. Tex. 12: 143-159.

TOXICITY OF VARIOUS OFF-SHORE CRUDE OILS AND DISPERSANTS TO MARINE AND ESTUARINE SHRIMP

By EARL R. MILLS

Texas A & M Marine Laboratory Galveston, Texas

and

DUDLEY D. CULLEY, JR.

Assistant Professor School of Forestry and Wildlife Management Louisiana State University Baton Rouge, Louisiana

ABSTRACT

The acute effects of four crude oils and two oil spill removers on four species of marine shrimp (*Penaeus setiferus*, *P. aztecus*, *Palaemonetes vulgaris*, and *P. pugio*) were determined. Results of 48-hour bioassays showed that distinctive differences in toxicity existed between crude oils from different areas with all shrimp tested. The oil spill removers were much more toxic than the crude oils. Addition of the oil spill removers to all crude oils at recommended application ratios increased the toxicity of both the crude oils and the oil spill removers, indicating a synergistic effect. The *Palaemonetes* species appeared more tolerant to all toxicants.

Evidence indicates that the most serious effects of oil pollution would be noted in the shallower areas where high concentrations of toxic compounds may build up.

INTRODUCTION

Recent studies of oil pollution have mainly been concerned with the ecological effects of spilled petroleum products on the marine ecosystem (Diaz-Piferrer, 1962; Hawkes, 1961; McCauley, 1966; North, 1961; O'Sullivan and Richardson, 1967; Rutzler and Sterrer, 1970). Due to lack of quantitative field data, it is impossible to predict the biological