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**Effects of Sublethal  
Entrainment Stresses  
on the Vulnerability of  
Juvenile Bluegill Sunfish  
to Predation**

Glenn F. Cada  
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ENVIRONMENTAL SCIENCES DIVISION  
Publication No. 1743

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## ABSTRACT

CADA, GLENN F., JEAN A SOLOMON, AND JAMES M. LOAR. 1981. Effects of sublethal entrainment stresses on the vulnerability of juvenile bluegill sunfish to predation. ORNL/TM-7801. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 42 pp.

This report provides a review of literature concerning the effects of sublethal stresses on predator-prey interactions in aquatic systems. In addition, the results of a preliminary laboratory study of the susceptibility of entrainment-stressed juvenile bluegill to striped bass predation are presented. Previous laboratory studies of altered predator-prey interactions have generally fallen into one of two categories: (1) "susceptibility to predation" studies, in which predation rates upon stressed and control prey in different tanks are compared, and (2) "predator preference" studies, in which stressed and control prey are mixed together and the predators are allowed to select between them.

Juvenile bluegill were exposed to thermal and physical entrainment stresses in the ORNL Power Plant Simulator and subsequently to predation by juvenile striped bass in a "susceptibility to predation" experimental design. None of the entrainment stresses tested (thermal shock, physical effects of pump and condenser passage, and combination of thermal and physical shock) was found to significantly increase predation rates as compared to controls, and no significant interactions between thermal and physical stresses were detected.

The validity of laboratory predator-prey studies and the application of indirect mortality information for setting protective standards and predicting environmental impacts are discussed.

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## 1. INTRODUCTION

The entrainment of nonscreenable larval and juvenile fishes at steam-electric power plants is a problem of continuing concern and research effort. The past decade has seen a large number of both laboratory and field studies aimed at assessing the impacts of entrainment-induced mortality on fish populations. Most of these studies have dealt with understanding and quantifying direct mortality, i.e., immediate mortality and inevitable latent mortality due to damage resulting from condenser passage. Although it was initially believed that the combined thermal, mechanical, and chemical stresses associated with power plant entrainment would be lethal to the great majority of fragile fish eggs and larvae, it has been repeatedly demonstrated that many fish can survive the entrainment process (Jinks et al., in press).

Among those fish which survive direct mortality, however, exists a potential for indirect mortality as a secondary effect of condenser or discharge plume entrainment. Indirect mortality is the premature death of entrained organisms due to sublethal effects, including increased susceptibility to predation, parasitism, disease, or other stresses, or decreased competitive and feeding abilities (Van Winkle et al. 1979). In those instances where direct mortality is not complete, it is important to estimate the magnitude of indirect mortality so that meaningful standards or impact assessments can be developed.

Although a number of published studies examined the influence of temperature shock on indirect mortality, little is known about the effects of combined mechanical and thermal entrainment stresses. The

purpose of this report is to examine one aspect of indirect mortality, the alteration of predator-prey interactions. A review of studies concerning the effects of sublethal stresses on predator-prey interactions in aquatic systems is provided, and the results of a preliminary laboratory study of the susceptibility of entrainment-stressed juvenile bluegill to striped bass predation are presented.

## 2. LITERATURE REVIEW

Laboratory studies of the effects of sublethal anthropogenic stresses on predator-prey interactions have generally fallen into one of two categories: susceptibility to predation or predator preference studies. In susceptibility to predation experiments, stressed fish are offered to predators in one tank while unstressed, control fish are offered to a different set of predators in another tank. Response parameters, such as predation rates, number of predator attacks, and number of captures/attack, are used to determine whether or not stressed fish are more susceptible to predation than unstressed fish. Predator preference studies, on the other hand, mix both stressed and unstressed fish together and offer them simultaneously to the same predators. After approximately one-half of the prey are eaten, surviving stressed and unstressed fish are counted to establish whether or not selective predation has altered the proportions of the two groups of prey relative to the initial proportions.

In this section, published studies that have utilized one and/or the other of these methodologies to examine sublethal stresses are reviewed.

### 2.1 Susceptibility to Predation Studies

Goodyear (1972) utilized a predator-prey experiment to examine behavioral changes among mosquitofish following a sublethal exposure to ionizing radiation. In this study, mosquitofish were (1) exposed to largemouth bass predators but not irradiated, (2) irradiated but not exposed to predators, or (3) irradiated and exposed to predators.

Although a statistical analysis of the data was not presented, after 20 d the percent survival in the group that was both irradiated and exposed to predation was markedly lower than the survival in the other two experimental groups.

Effects of heat shock on the susceptibility of sockeye salmon fry to coho salmon predation were studied by Sylvester (1972). Prey were stressed by means of a 60-s heat shock set either at 30°C or at 10°C above the acclimation temperature, and their mean predation survival times were compared to those for unstressed control prey. These brief, sublethal thermal shocks were found to significantly decrease the mean survival times of stressed salmon fry.

Sylvester (1973) also used salmon to investigate the influence of light on the vulnerability of heat-stressed prey. In this experiment, sockeye salmon fry were given a 5-s heat shock of 23°C (shown to be sublethal in previous work), and the response variable measured was the number of survivors following a 15-min exposure to yearling coho salmon. A factorial analysis of variance of the test results indicated that while darkness increased the mean survival of all prey groups, heat-stressed fish still exhibited significantly greater susceptibility to predation than controls under both light and dark conditions.

The susceptibility of thermally shocked lake whitefish fry to yellow perch predation was examined in the laboratory by Yocum and Edsall (1974). They simulated the thermal experience of fry entrained in the condenser cooling system of a steam electric power plant by giving the whitefish heat shocks ranging from 11 to 19.5°C above acclimation temperatures for 1 min. The investigators recorded the

number of attacks, the number caught, and the number of captures per attack for both shocked and control fry. An analysis of variance of the experimental results indicated that the total number of attacks made by the perch in 30 min was lower and the total number of fry captured per attack was higher for the shocked fry than for the control fry. However, at any given acclimation temperature the total catches of shocked and control fry were not significantly different. Yocom and Edsall (1974) attributed these observations to increased vulnerability (number captured per attack) rather than increased attractiveness (number of attacks) of shocked whitefish fry to predators.

Because juvenile and adult fish may have the capability of avoiding the discharge plume of a power plant and its attendant thermal shock, recent efforts to assess temperature effects on predator-prey interactions utilized weakly swimming larval fishes as prey. Deacutis (1978) stressed Atlantic silverside and summer flounder larvae with approximately 10°C heat shocks for 15 min and introduced them to tanks containing striped killifish as predators. Nonparametric statistical tests were used to detect differences between shocked and control larvae with regard to the number of predator attacks and captures and prey escapes. Among larger silverside larvae (12.2-23.5 mm total length), the number of attacks, escapes, and escape/attack ratios were significantly greater for control than for shocked prey. These observations appear to be in agreement with those of Yocom and Edsall (1974), i.e.; shocked fish are more vulnerable rather than more attractive to predators. Deacutis (1978) noted no significant differences in susceptibility to predation among shocked and control groups of small

silverside larvae (4.0-9.8 mm total length). In contrast, heat-shocked summer flounder larvae displayed significantly greater predator avoidance abilities relative to controls, possibly due to increased sensory responsiveness or increased locomotory movements (Deacutis 1978).

The effect of different recovery times on the susceptibility of thermally shocked striped bass larvae to yearling white perch predators was studied in the laboratory (Ecological Analysts, Inc. 1979). Striped bass larvae were exposed to excess temperatures ranging from 7.9 to 10.1°C above acclimation temperatures for 10 min and allowed to recover for either 0, 30, 60, 120, 180, or 240 min before introduction to the predator tank. The mean numbers of captures per attack, which were compared by an analysis of variance and Student-Newman-Keuls multiple range tests, decreased with increasing recovery time, but were significantly greater than control values only for 0 and 30-min recovery times. The mean numbers of captures/attack for heat-shocked larvae given recovery times of 60-min or longer were not significantly greater than those of control larvae.

Susceptibility to predation studies were also used to examine the effects of chemical stresses on predator-prey interactions. Tagatz (1976) exposed both grass shrimp (prey) and pinfish (predator) to sublethal concentrations of the insecticide mirex and found that treated shrimp had significantly lower survival than untreated controls after 1, 2, and 3 d of predation. Farr (1977) showed that grass shrimp given sublethal doses of parathion were more susceptible than controls to gulf killifish predation, probably because of increased spontaneous activity (treated shrimp therefore became more visible to the predator)

and decreased physical stamina (less able to escape capture). An interesting variation to these results was observed when both prey (mosquitofish) and predators ( largemouth bass) were exposed simultaneously and continuously to sublethal concentrations of ammonia. Woltering et al. (1978) found that largemouth bass were more sensitive to ammonia than their prey and that prey consumption rates decreased as ammonia concentrations increased. In this scenario, therefore, prey appeared to benefit from sublethal levels of a pollutant as a result of decreased activity of stressed predators.

## 2.2 Predator Preference Studies

Effects of sublethal doses of various physical and chemical stressors were also examined in experiments which allowed predators to choose between simultaneously offered stressed and unstressed prey. Such experiments provide different information about the nature of pollutant effects on predator-prey relationships. While predator preference studies are superior to susceptibility to predation studies in that they allow the investigator to assess the relative selectivity of predators for or against stressed prey, they generally do not permit detailed behavioral observations of the predator-stressed prey relationship (e.g., number of attacks, number of escapes). To a great extent, the choice of experimental methodology depends upon the nature of the stress being studied and the types of experimental results that are most useful for predictive purposes.

Herting and Witt (1967) examined the preference of bowfin predators for various minnow and centrarchid prey that had been

stressed by seining, columnaris disease, parasitism, or starvation. In their experimental design, two species (only one of which was stressed) were offered to the predator, and a selection index was calculated. This index was compared to a control selection index in which neither species of the pair was stressed. The authors found that in all cases bowfin predators selected for prey fish that had been physically impaired by the stresses.

Coble (1973) used a predator preference experiment to study the selectivity of northern pike for goldfish prey. The goldfish were not stressed, but differed in such characteristics as tail form, brightness (white vs natural color), and color (orange, white, and natural color). Although northern pike are visual feeders, Coble (1973) found no significant differences between the numbers of the different types of goldfish that were eaten in the experiments.

Predator preference tests were also used to study the effects of sublethal concentrations of chemicals in aquatic systems. Hatfield and Anderson (1972) found that Atlantic salmon parr treated with 1.0 ppm Sumithion (organophosphate insecticide) suffered significantly greater predation by brook trout than did untreated controls. Mosquitofish exposed to sublethal concentrations of mercury of 0.01 ppm or greater were selected for by largemouth bass due to impairment of the normal escape behavior (Kania and O'Hara 1974). Sullivan et al. (1978) noted subtle differences in behavior, including abnormal school structure, among cadmium-treated fathead minnows. They regarded these behavioral abnormalities as responsible for the greater vulnerability of treated minnows to predation relative to simultaneously offered, untreated

fish. Farr (1978) exposed both grass shrimp and sheepshead minnows to the pesticide methyl parathion and offered them simultaneously to gulf killifish predators. As exhibited by decreased predator avoidance capabilities, grass shrimp were more sensitive than sheepshead minnows to sublethal parathion concentrations, and as a result the predators increased the proportion of grass shrimp in their diet with increasing pesticide concentrations. Thus, Farr (1978) provided an example of a mechanism by which concentrations of a chemical pollutant that do not cause direct mortality can nevertheless result in altered species composition and diversity.

Predator preference experiments were used to examine the impacts of thermal discharges on aquatic biota. Coutant (1973) noted that chinook salmon given abrupt temperature increases of 13°C for as little as 10% of the median time for equilibrium loss suffered significantly greater predation than did unshocked controls. Longer thermal shocks increased vulnerability to predation relative to controls almost exponentially. Fishes acclimated to warm discharge waters can also experience acute cold stress when winds or currents disperse the thermal plume or when the source of heat is suddenly terminated (power plant shutdown). Coutant et al. (1974, 1976) found that juvenile channel catfish and largemouth bass exposed to abrupt temperature drops of 9 and 7°C or more, respectively, were more susceptible than unshocked controls to adult largemouth bass predation. Ecological Analysts, Inc. (1979) exposed striped bass larvae to heat shocks of 7 to 10°C for 10 min before presenting them to white perch predators along with either (1) unshocked striped bass larvae or (2) unshocked

striped bass larvae and unshocked Gammarus. In 4 of 14 tests, shocked larvae were significantly more susceptible to predation than simultaneously offered unshocked larvae ( $\alpha = 0.05$ ). The presence of an alternative prey (Gammarus) had no effect on preferential predation but did reduce the overall level of predation on both shocked and control striped bass larvae (Ecological Analysts, Inc. 1979).

### 3. MATERIALS AND METHODS

The ORNL Power Plant Simulator (PPS) is an experimental device that reproduces many features of the internal hydraulics of open-cycle condenser cooling systems. The PPS circulates temperature-controlled water through a closed loop and has as key components, a pump, a condenser bundle, and vertically adjustable piping (Fig. 1). Thus, entrainment stresses associated with pump passage, condenser tube passage, vacuum conditions, and thermal effects can be examined separately or in concert in a controlled, experimental fashion. A detailed description of the PPS is provided by Cada et al. (in press).

Susceptibility to predation experiments were conducted using juvenile bluegill sunfish (Lepomis macrochirus) as prey and juvenile striped bass (Morone saxatilis) as predators. Striped bass ranged from 170 to 215 mm in total length (mean length of 188 mm) while the bluegill were approximately 20 to 30 mm in total length.

All bluegill were obtained from the Cohutta National Fish Hatchery, Cohutta, Georgia, and were held in a 525-L rectangular stock tank prior to testing. The stock tanks were maintained at 20°C by the addition of filtered well water at a rate of 4 to 5 L/min. Illumination was provided by cool-white fluorescent lights controlled by an outside photoswitch to reproduce the seasonal photoperiod. The bluegill were fed twice a day on a mixture of canned salmon and ground trout chow.

Striped bass predators were held in six 1.2-m diam, cylindrical fiberglass tanks that also served as test tanks for the predation

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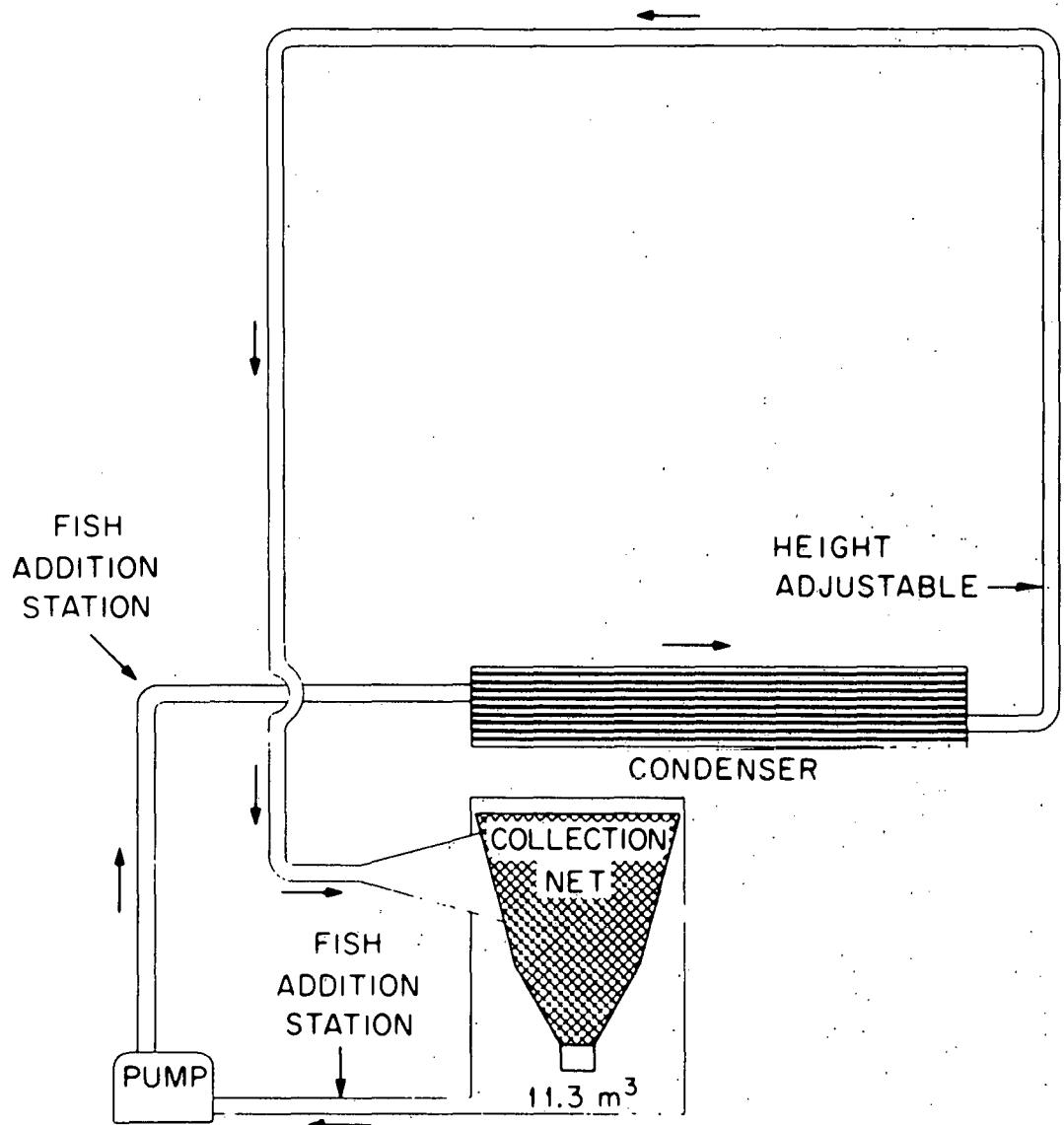


Fig. 1. Schematic diagram of the ORNL Power Plant Simulator.

studies. The tanks contained no structures other than the inlet hose and center outflow standpipe. Each tank contained  $0.5 \text{ m}^3$  of water (set at a constant  $20^\circ\text{C}$ ) and two striped bass. All striped bass had been maintained in laboratory tanks on a diet of trout chow for several months prior to testing. Two weeks prior to testing, striped bass were switched to a diet of minnows to accustom them to living prey.

The entrainment stresses utilized in this experiment included (1) thermal ( $10^\circ\text{C } \Delta T$ ), (2) net collection stresses (ambient water temperature), (3) combined pump passage, condenser passage, and net collection stresses (ambient water temperatures), and (4) combined heat shock ( $10^\circ\text{C } \Delta T$ ), pump passage, condenser passage, and net collection stresses. Pumping rate for all tests was  $4.16 \text{ m}^3/\text{min}$ , or 50% of capacity. Bluegill sunfish were exposed to these stress regimes, and their susceptibility to striped bass predation, expressed as predation rates (number consumed in 30 min), was compared to that of unstressed controls.

Predation rates among four experimental groups of bluegill were compared. These groups were:

- (1) Control Group: For each replicate, twenty-five bluegill were dipnetted out of the stock tank into a transfer aquarium. The contents of the transfer aquarium were then poured into a predation tank containing two adult striped bass. This control group was used to determine the susceptibility to predation of bluegill not exposed to entrainment stresses.

(2) Thermal Shock Group: Twenty-five bluegill per replicate were dipnetted out of the stock tank into a transfer aquarium. The contents of the transfer aquarium were poured into a screened bottle and exposed to the water in the PPS reservoir for a period of time equivalent to the time necessary to pass an organism through the PPS and retrieve it from the collection net (350-400 s). The fish were then poured into a predation tank. This group was used to detect any change in susceptibility to predation as a result of a brief 10°C temperature shock [entrainment stress (1) above] (if any) and exposure to water circulating within the closed system of the PPS.

(3) Collection Stress Group: Twenty-five bluegill per replicate were dipnetted out of the stock tank into a transfer aquarium. The contents of the transfer aquarium were poured into the collection net with the pump operating. After 60 s, the pump was turned off and the net was slowly raised out of the water and the contents washed off the sides by means of a gentle spray of PPS water applied to the outside of the net. Fish were retrieved from a collection bucket at the bottom of the net and placed back in the transfer aquarium. The contents of the transfer aquarium were poured into a predation tank. This experimental group was used to detect changes in susceptibility to predation as a result of collection stresses [entrainment stress (2) above] and thermal shock (if any).

(4) Pump-Condenser Passage Group: For each replicate, twenty-five fish from the stock tank were transferred to a fish addition station located upstream of the pump. The fish were carried by circulating water through the pump, the condenser tube bundle, and the elevated return piping before being discharged into the collection net (Fig. 1). The bluegill were retrieved in the same manner as the Collection Stress Group and offered to striped bass predators in laboratory tanks. This experimental group was used to assess the effects of combined stresses of pump passage, condenser passage, negative pressures, collection, and thermal shock (if any) on susceptibility of bluegill to predation [entrainment stresses (3) and (4) above].

The three treatment groups and three replicates of the control group were randomly assigned each day to six predation tanks, each containing two striped bass. Bluegill prey were poured into the striped bass holding tanks and were left undisturbed for 30 min, a time period during which approximately one-half of the prey had been eaten in preliminary trials. At the end of the predation period, surviving bluegill were dipnetted out of the tanks, counted, and poured back into the tanks to standardize the food ration for each tank on each day. The striped bass in each tank were offered a single group of prey (25 bluegill) each day and received no other food during the 16-d study.

Statistical analyses were carried out to determine whether any of the stresses imposed on the bluegill resulted in an increased rate of predation. The number of fish surviving the predation period (out of

the original 25) was log-transformed and analyzed by standard analysis of variance techniques. Differences in predation rates between controls and different entrainment stresses were examined, blocked on date, and, when the analysis of variance was significant for a particular main effect, the means were sorted with Duncan's Multiple Range Test.

## 4. RESULTS

Table 1 shows the number of replicates of the control and treatment groups assigned to each of the predation tanks (numbered tanks 1-6). It was observed during the course of the experiment that striped bass in tank 6 appeared to be more voracious than those in the other tanks, i.e., nearly all bluegill were consumed during the 30-min period regardless of the experimental stress. This possibility was examined statistically using a one-way analysis of variance on log-transformed data. The results showed that two tanks, tanks 2 and 6, had consistently lower survival than the remaining four. Consideration of the stresses involved revealed that the bluegill placed in tank 6 had been exposed to a stress other than "control" in only one out of six instances (Table 1). The bluegill in tank 2, however, were all exposed to additional stresses beyond minimal handling. It was concluded that survival rates in tank 6 were indeed abnormally low, and as a result tank 6 was eliminated from further consideration.

The results of the analyses of variance of the five remaining tanks are provided in Table 2. The type of stress imposed upon bluegill was found to be a significant source of variation ( $\alpha = 0.05$ ), whereas the date was not a significant factor. Collection stresses resulted in a mean survival rate that was significantly lower than those of the other experimental groups at the ( $\alpha = 0.05$ ) level (Table 3). The differences between mean survival rates among control, thermal shock, and pump-condenser passage groups were not statistically significant ( $\alpha = 0.05$ ).

Table 1. Number of replicates for each tank-experimental stress combination. Each replicate involved 25 bluegills.

Tank number	Experimental stress			
	Control	Thermal shock	Collection stress	Pump-condenser passage
1	2	2	1	1
2	0	2	2	2
3	3	0	2	1
4	3	1	0	2
5	5	1	0	0
6	5	0	1	0

Table 2. Analysis of variance of bluegill survival rates following exposure to various entrainment stresses and then to striped bass predation<sup>a</sup>.

Source of Variation	df	SS	MS	F	Pr > F <sup>b</sup>
Stress	3	5.648	1.883	3.78	0.03
Date	5	0.679	0.136	0.27	0.92
Error	21	10.454	0.498		
Total	29	16.781	0.579		

<sup>a</sup>Model:  $\ln(\text{no. of survivors}) = \mu_{ij} + \text{stress}_i + \text{date}_j$ ;  
 where  $i = 1, 2, 3, 4$ .  $\text{stress}_1 = \text{control}$ ,  $\text{stress}_2 = \text{thermal shock}$ ,  
 $\text{stress}_3 = \text{collection}$ ,  $\text{stress}_4 = \text{pump-condenser passage}$  and  
 $\text{collection}$ .

<sup>b</sup>Probability of observing a larger value of F, under the null hypothesis that the group means for that effect are equal.

Table 3. Results of Duncan's Multiple Range Test used to determine significant differences between mean survival rates of bluegills exposed to various entrainment stresses and striped bass predators. Mean values connected by the same line are not significantly different at the  $\alpha = 0.05$  level.

Stress	Number of observations	Geometric mean survival rate <sup>a</sup>
Control	13	18.58
Thermal shock	6	14.26
Pump-condenser passage	6	12.91
Collection	5	4.63

<sup>a</sup>Survival rate = number of stressed fish (out of original 25) which survived the 30-min predation period.

Table 4 presents the results of an analysis of variance used to detect interactive effects between thermal shock and physical stress (pump-condenser passage). Neither the main effects of physical stress and thermal shock nor the interaction between physical stress and thermal shock were significant sources of mortality at the  $\alpha = 0.05$  level.

Table 4. Analysis of variance of interaction between thermal and physical stresses among bluegills exposed to both entrainment stresses and striped bass predation<sup>a</sup>.

Source of variation	df	SS	MS	F	Pr > F <sup>b</sup>
Physical stress (P)	1	2.231	2.231	2.20	0.18
Thermal shock (T)	1	0.359	0.359	0.35	0.57
P X T	1	0.012	0.012	0.01	0.92
Error	7	7.083	1.012		
Total	10	9.684	0.968		

<sup>a</sup>Model:  $\ln(\text{no. of survivors}) = \mu_{ij} + \text{physical stress}_i + \text{thermal shock}_j + (\text{physical} \times \text{thermal})_{ij}$ ; where  $i = 3, 4$ ; stress<sub>3</sub> = collection, stress<sub>4</sub> = pump-condenser passage and collection.  $j = 1, 2$ ; thermal<sub>1</sub> =  $0^\circ\text{C} \Delta T$ , thermal<sub>2</sub> =  $10^\circ\text{C} \Delta T$ .

<sup>b</sup>Probability of observing a larger value of F, under the null hypothesis that the group means for that effect are equal.

## 5. DISCUSSION

Previous studies using the Power Plant Simulator showed significant short-term direct mortalities among bluegill exposed to the same thermal and physical stresses used in the present experiment. For example, the mean short-term conditional mortalities for bluegill passed through the PPS under these conditions ( $10^{\circ}\text{C } \Delta T$ ; 50% pumping rate) ranged from 0.03 for the effects of thermal shock to 0.22 for the effects of pump passage (Cada et al., in press). It was hypothesized that those fish which initially survived the experimental stresses might nevertheless be stunned or have their behavior altered in such a way as to increase their susceptibility to predation. Although collection stresses in the PPS reduced the ability of bluegills to avoid predation, our experiments failed to demonstrate statistically significant differences between the predation survival rates of entrainment-stressed fish and those of unstressed controls.

The most obvious conclusion, that is, that the combined physical and thermal stresses of entrainment are not severe enough to have deleterious effects on prey behavior, seems unlikely in view of previous studies. Thermal shocks alone were found to produce significant differences in predator-prey relationships (Section 2.1), although in some cases these differences were reflected in such parameters as total number of attacks or number of captures per attack rather than in the total number of captures (Yocom and Edsall 1974, Deacutis 1978). Because the design of our experiment involved disturbing the predators as little as possible, we were precluded from making any observations

other than the total number of captures at the end of the 30-min predation period. Consequently, if significant differences did exist, for example, in number of captures per attack, they would not have been observed in our study.

A problem that was encountered concerned the large variability in predation rates among the six different tanks, despite the fact that experimental conditions (e.g., water temperature and volume, predator size, predator ration, and lighting) were similar. It was this variability that led to the exclusion of tank 6 from the analysis and may have been the reason why the trends in mean survival rates between the different treatments (Table 3) were not statistically significant. Previous investigators noted great differences between capture rates among similar groups of predators, and they chose to report such parameters as captures per attack instead of total captures (Yocom and Edsall 1974, Ecological Analysts, Inc. 1979). The problem of inter-tank variability could also be reduced to some extent by a "predator preference" experimental design, where both stressed fish and unstressed controls are added to the same tank simultaneously.

The larger question of the applicability of laboratory predator-prey studies to real world power plant impacts must also be considered. Because indirect mortality is virtually impossible to study in the field, information must be obtained in laboratory studies and extrapolated to field situations. Thus, the researcher is faced with the problem of recreating natural conditions, such as realistic predator and prey densities, as well as providing appropriate

alternative prey, turbidity levels, and refuges (Ecological Analysts, Inc. 1979). Such factors as prey switching and aggregation of predators in the discharge area (Van Winkle et al. 1979, Coutant et al. 1979) may be highly variable but important contributors to the magnitude of indirect mortality that might occur in a particular situation. It will never be possible to duplicate in the laboratory all of the factors which may influence predation in nature. However, when used as a standardized bioassay procedure (Sprague 1971), the unequivocal demonstration of altered predator-prey relationships in simple laboratory systems can be a crucial step in setting meaningful, comprehensive standards for permissible levels of stress or pollutant discharge limits.

Van Winkle et al. (1979) concluded that information on sublethal effects and indirect mortality can be used in two ways. In the case where the biological response variable is mortality (as opposed to a response such as reduced feeding ability or growth), a conditional mortality rate can be calculated and used in estimating population-level impacts through simulation modeling. Although it is possible to calculate conditional mortality rates resulting from increased predation upon entrainment-stressed fish, to a great extent these rates will vary depending upon the experimental design (e.g., number of predators per tank, size of the tank) and therefore would have little meaning when modeling the responses of stressed populations in actual ecosystems. The conditional mortality concept might be used to compare the magnitudes of component sources of multiple stresses, much as has been done in previous direct mortality studies with the PPS. For

example, under identical laboratory conditions, physical stresses may be found to have greater effect in increasing susceptibility to predation than thermal stresses. Such observations could be used to modify or strengthen the conclusions already drawn from direct mortality studies about the relative importance of the various sources of entrainment stress.

A second application of indirect mortality information is in the setting of standards for pollutant discharges (Van Winkle et al. 1979). Standards based solely on direct mortality data may not afford adequate protection to affected populations because they are not sensitive to the suite of sublethal stresses which may ultimately result in "ecological death" (Coutant et al. 1979). The PPS could be used in this context to establish threshold limits for single- or multiple-entrainment stresses, i.e., to determine the levels of stresses at which vulnerability of entrained fish to predation is significantly different from that of controls.

## 6. SUMMARY AND RECOMMENDATIONS

One form of indirect mortality which may later affect fishes surviving power plant entrainment is increased susceptibility to predation. The significance of this factor was examined in experiments that exposed juvenile bluegill to various combinations of entrainment stresses in the Power Plant Simulator (PPS) and subsequently to predation by juvenile striped bass. Although the collection procedure was found to be detrimental to the survival of bluegill prey, none of the entrainment stresses tested (thermal shock, physical effects of pump and condenser passage, and combination of thermal and physical shock) was found to significantly lower the predation survival rates, as compared to control fish. Moreover, no significant interactions between thermal and physical stresses were detected in these experiments.

It is certainly possible that entrainment stresses of the magnitude used in this study, which are sufficiently great to cause immediate direct mortality in some individuals, may not alter the behavior of bluegill in ways that would increase their susceptibility to predation. However, all treatments showed a trend, albeit not statistically significant, toward higher predation rates than the controls. It is possible that true differences in susceptibility to predation may have been masked by the large variability between experimental replicates. The problem of comparing survival rates of stressed and control fishes exposed to different sets of predators can be reduced by utilizing "predator preference" experiments in which the

treated and control fish are mixed together and introduced to the same predator tank simultaneously. Future indirect mortality studies with the PPS will concentrate on single-tank, predator-preference experiments and the requisite development of a suitable tag for separating mixtures of treated and control larval fish.

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