# Upper Thermal Tolerance of Early Life Stages of South Carolina and Florida Largemouth Bass[1]

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*Abstract:* Upper thermal tolerance limits (TL50's) for early life stages of Par Pond and Florida stocks of largemouth bass (*Micropterus salmoides* intergrade and *M. s. floridanus*) were determined. Comparisons were made between 3 stages (non-feeding and feeding pro-larvae, and post-larvae), 2 initial temperatures (18° and 23° C), and instant vs. gradual (5° C/day) exposures to test temperatures between 18° and 38° C. Upper thermal tolerance limits did not differ between largemouth bass stocks, life stages, or initial temperatures. Ninety-six hour TL50 was 32.8° C for instant exposures and 34.1° C for gradual exposures (5° C/day) to high temperatures. Percent survival for 96-hour exposures to high temperatures was significantly higher for gradual exposures than instant exposures for all stocks, initial temperatures, and life stages. Calculated Maximum Weekly Average Temperature (MWAT) to protect early life stages of largemouth bass is 28.1° C.

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Temperature is one of the most important physical parameters affecting success of poikilothermic vertebrates. Rates and efficiencies of metabolic functions such as reproduction, growth, digestion, and respiration are partially controlled by water temperature. Thermal requirements for survival and growth differ between life stages and among species of fishes (NAS/NAE 1972, Brungs and Jones 1977, McCormick 1978). Numerous studies have been conducted to determine thermal tolerance limits and optimum temperatures for survival and growth of many fish species (e.g. Coutant 1971, Talmage and Coutant 1979, Cravens 1982, Cravens et al. 1983). Emphasis has been placed on studying adult fish. However, larval fish

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are of particular importance in assessing thermal effects, since they have limited ability to escape adverse temperatures. Larval fish often have distinct thermal requirements for survival and growth and are generally more sensitive to temperature stresses than adults (Hokanson et al. 1973, Brungs and Jones 1977). Few studies have been reported regarding thermal tolerance of larval fish.

Upper incipient lethal temperatures at various initial temperatures for northern and Florida largemouth bass juveniles have been reported (Hart 1952, Cincotta et al. 1984). McCormick and Wenger (1981) determined the 24-hour upper lethal threshold (TL50) for 2 sources of northern largemouth bass yolksac fry. Ninety-six hour upper TL50's have not been reported for largemouth bass larval stages. Furthermore, no one has investigated the effects of gradual versus instant exposures to elevated temperatures on larval largemouth bass.

Largemouth bass is a top predatory species is most southern reservoirs and is an important resident fish in Savannah River Plant (SRP) aquatic ecosystems. Cooling water from reactors at SRP is presently discharged into onsite creeks and swamps. These swamps and creeks are important spawning and nursery areas for many resident fish species (Paller et al. 1983). The rate and magnitude of temperature fluctuations due to reactor operations during spawning season have the potential to affect survival and growth of early life stages of fish spawning in these habitats. Knowledge of maximum temperatures and rates of temperatures change which allow survival and growth of larval fishes is necessary to ensure that proposed thermal mitigation alternatives will be adequate to protect these stages.

Objectives of this study were to: 1) determine acceptable maximum temperatures for survival of early life stages of Par Pond and Florida stocks of largemouth bass in order to decide which strain to stock in a newly constructed cooling reservoir at SRP; 2) determine the effect of an instant vs. 5° C/day exposure to high temperature extremes on percent survival; 3) determine effects of different initial (acclimation) temperatures on percent survival when exposed to high temperature extremes; 4) determine differences in thermal tolerance of different developmental stages of largemouth bass larvae.

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## Materials and Methods

### Test System

Studies were conducted at Par Pond Laboratory at SRP, Aiken, South Carolina. Test system water was supplied from Par Pond on a once through basis and was filtered by an in-line sand filter. A portion of incoming water was chilled to 8° C. Hours of light and darkness in the laboratory were maintained equivalent to actual seasonal photoperiod at SRP during the study period.

Four trough and headbox combinations and 1 additional water bath comprised the test system. Temperatures were maintained at 18°, 23°, 28°, 33° and 38° C throughout all studies. Test temperatures were obtained by mixing ambient and chilled water entering headboxes to slightly below desired test temperatures. Exact test temperature was achieved by placing stainless steel immersion heaters controlled by mercury thermoregulators and electronic relay boxes in each of the headboxes.

Test (incubation) chambers were constructed of 600-ml polypropylene beakers. Bottoms of the beakers were removed and replaced with 0.64 mesh/mm stainless steel screens. Each incubation chamber was supplied with water at the test temperature. Flow rate through each incubation chamber was maintained at 25-100 ml/min.

Water temperatures were measured by thermocouples linked to a microcomputer system which continually monitored temperatures and recorded hourly temperature means and ranges for each test temperature.

### Test Organisms

Par Pond largemouth bass were collected from the nonthermal intake arm of Par Pond on 27 and 30 of March 1985. Par Pond is a 1,220-ha reservoir on SRP receiving heated discharge water from nuclear-reactor cooling processes. Water temperatures at collection times were 22.2° and 23.0° C. Florida largemouth bass were collected from Florida Game and Fresh Water Fish Commission's Richloam Hatchery on 1 March 1985. Hatchery brood stock was collected from Lake Parker and Lake Hollingsworth in Polk County, south central Florida. Water temperatures at time of collection ranged from 22.5° – 23.3° C.

Electrophoretic studies show that largemouth bass from the Savannah River Basin (Par Pond stock) are intergrades between *M. s. salmoides* and *M. s. floridanus;* Florida largemouth bass collected in the same geographic areas as Florida largemouth bass in this study are 100% *M. s. floridanus* (Phillip et al. 1983).

### Procedures

Embryos were tempered over approximately three hours to initial temperatures of 18° and 23° C. Largemouth bass were hatched and reared at 18° or 23° C until utilized for the study. Three distinct developmental stages, early pro-larvae, late pro-larvae, and post-larvae, were studied. Early pro-larvae were 24 hours post-hatch and not able to feed or swim. Late pro-larvae had begun to feed and were capable of limited swimming, but had not absorbed the yolksac. Post-larvae were those fish which had completely absorbed the yolk sac and were capable of sustained swimming. Size and age of these developmental stages varied according to water temperature. Duplicate groups of 20 larvae were exposed to 18°, 23°, 28°, 33° and 38° C water. Larvae were exposed to test temperatures by 1 of 2 methods; temperature shock or gradual temperature change. Larvae which were shocked (instant temperature change) were removed from their initial temperatures and placed directly into water at 1 of the 5 test temperatures. Observations of mortality were made at 1, 2, 4, 8, 12 and 24 hours after transfer, and at 24-hour intervals thereafter, until study termination 96-hours later.

Larvae which were gradually exposed to test temperatures were moved from the initial temperature (18° or 23° C) to the next higher temperature at a rate of 5° C per 24-hours. This was accomplished by transferring a 20-liter aquarium and its incubation chambers from the initial or intermediate temperature to the next trough which was 5° C warmer. Larvae were tempered to the intermediate temperature over several hours by trickling water slowly into aquaria containing incubation chambers. Dead fish were counted and removed after 24-hours at each intermediate temperature, immediately before larvae were transferred to the next temperature. This transfer procedure was continued at 24-hour intervals until the test temperature was obtained. Mortality at test temperatures was recorded at 24-hour intervals for 96-hours.

Larvae were fed 24-hour post-hatch brine shrimp nauplii twice per day. Each incubation chamber was fed the same quantity regardless of test temperature or number of survivors. Studies were terminated by counting and preserving surviving fish in 95% ethanol.

### Statistical Methods

Data was analyzed using SAS (SAS Institute Inc. 1982). Overall effects of test temperatures on 96-hour percent survival (for each stock, life stage, and initial

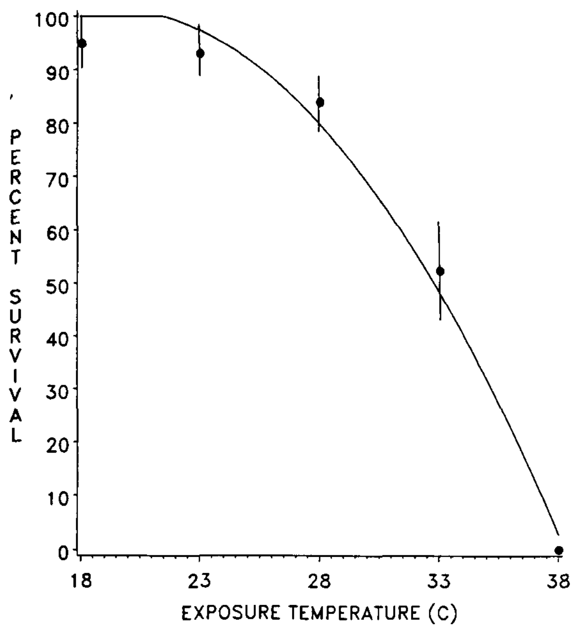


**Figure 1.** Mean percent survival of largemouth bass fry, exposed instantly for 96-hours to temperatures above their acclimation temperature. (Vertical lines represent ± 2 SE.) Percentage survival = (10.602 * temperature) − (0.277 * temperature$^2$), $F_{regression}$ = 1142.73, df = 2,179, $P \leq 0.001$.)

temperature) were examined with multiple regression to determine the model (linear vs. culvilinear) that resulted in lowest unexplained variances. Then the effects of stock, initial temperatures, life stage, and type of exposure (sudden vs. gradual exposure) on 96-hour percent survival were analyzed using ANCOVA with test temperature as covariate. Significance testing was performed at the 0.05 level.

## Results

Multiple regression analysis which regressed 96-hour percent survival on test temperature and test temperature squared produced the best fit for sudden and 5° C/ day exposures to test temperatures above initial temperatures. Effects of brood stock (Florida and Par Pond), life stage (early pro-larvae, late pro-larvae and post-larvae) and initial temperature (18° or 23° C) on 96-hour percent survival were not significant ($P > 0.05$). A single regression equation was generated from pooled data in order to predict 96-hour percent survival to instant exposure to increasing water temperatures (Fig. 1). Predicted 96-hour TL50 for instant exposure was 32.8° C. A single regression equation was also generated from pooled data in order to predict percent survival to a 5° C/day increase in water temperature (Fig. 2). Predicted 96-hour TL50 was 34.1° C.

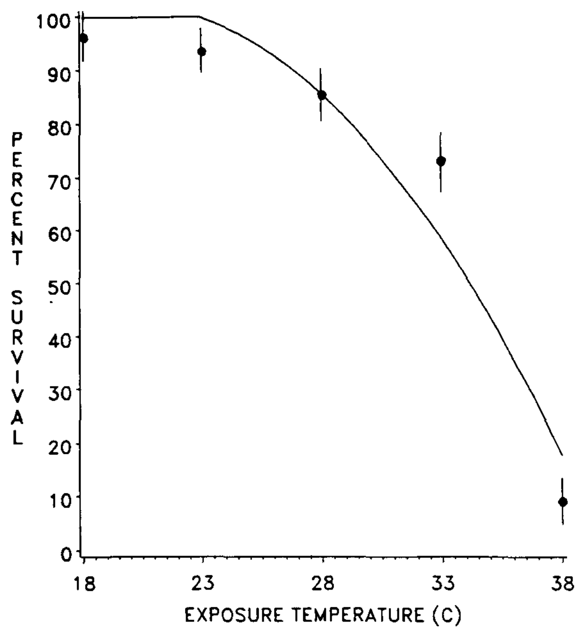


**Figure 2.** Mean percent survival of largemouth bass fry exposed at a rate of 5° C/day for 96-hours to temperatures above their acclimation temperature. (Vertical lines represent ± 2 SE.) Percent survival = (10.273 * temperature) − (0.258 * temperature$^2$), $F_{regression}$ = 1065.04, df = 2,141, $P \leq 0.0001$.)

Survival rates of largemouth bass larvae shocked to test temperatures and survival rates of those exposed to test temperatures at a rate of 5° C/day were significantly different (F type = 15.07, df = 2,323, $P \leq 0.0001$). Five degree C/day exposure to temperatures above initial temperature increased percent survival above that for an instant exposure.

## Discussion

### Differences Between Stocks

Survival was not affected by source of brood stock. Clugston (1964) found no evidence of differences in early growth of *M. s. salmoides* and *M. s. floridanus* grown in Florida ponds. McCormick and Wenger (1981) found no significant differences in thermal tolerance and growth for larval and juvenile largemouth bass from Tennessee and Minnesota.

### Differences Between Life Stages

Survival during instant or gradual exposures to high temperatures was not influenced by life stage. McCormick et al. (1977) found the upper TL50 for newly hatched and swim-up fry white sucker larvae approximately the same. Brett (1970) states that embryonic stages of fish are most stenothermal. Northern pike embryos are most sensitive to thermal shock within the first five hours after fertilization, with the thermal tolerance range increasing with development (Hassler 1982). Irvin (1974) found progressive development of high temperature tolerance with development stages of Dover sole. Largemouth bass larvae in this study did not exhibit this increasing ability to withstand higher temperatures as they developed. Since optimal temperature for survival and growth of largemouth bass early life stages is nearly equal to the maximal survival temperature, they may not respond the same as northern pike or Dover sole which spawn at temperatures lower than their optimum temperature for survival and growth.

### Influence of Initial Temperature

Initial temperature did not increase the upper lethal temperature of larvae. McCormick and Wenger (1981) also reported this. Generally, higher initial temperatures will increase the upper lethal limit (Brett 1956). All fish have an ultimate incipient lethal temperature, which cannot be increased by increasing initial temperature (Brungs and Jones 1977). Largemouth bass larvae did not show a significantly higher upper TL50 with increasing initial temperature. Lack of physiological development to enable acclimation or the possibility that early life stages may not have been adjusted to the initial temperature due to the length of time (<15 days) at that temperature may explain this effect.

### Comparison with EPA Temperature Criteria

Brungs and Jones (1977) reported 32.2° C and 21° C as maximum weekly average temperatures (MWAT) for adult largemouth bass growth and spawning, respectively. No criteria have been developed for MWAT of early life stages of largemouth bass. EPA calculates MWAT as: optimum temperature + (TL50 − optimum temperature/3). Optimum survival temperature for 21-day post-hatch largemouth bass was 25.8° C and upper incipient lethal temperature was 32.8° C (Storms 1985). Based on this data MWAT for largemouth bass fry is 28.1° C; an intermediate value compared to MWAT for spawning and adult growth. EPA maximum temperature values for short term exposures are 34° C for adults and 27° C for spawning compared to the value observed in this study of 32.8° C for largemouth bass early life stages.

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