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Thermal Effects on Eggs, Larvae and Juveniles of Bluegill Sunfish

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THERMAL EFFECTS ON EGGS, LARVAE AND JUVENILES OF BLUEGILL SUNFISH

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Project 18050 GAB

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ABSTRACT

Bioassay experiments were conducted to determine thermal tolerance of early life history stages of bluegill sunfish. Bluegill eggs hatched at temperatures from 18 to 36C during two incubation tests. Maximal hatch occurred at 22.2 and 23.9C. Lower TL_{50} temperature for hatch of normal fry was 21.9C and upper TL_{50} temperature was 33.8C.

Juvenile bluegills acclimated to 12.1C had a lower 96-hour TL_{50} of 3.2C and an upper 96-hour TL_{50} of 27.5C. Juveniles acclimated to 32.9C had a lower 96-hour TL_{50} of 15.3C and an upper 96-hour TL_{50} of 37.3C. TL_{50} increased with increasing temperature of acclimation. For juveniles acclimated to a given temperature, upper TL_{50} decreased with longer exposure.

A preliminary test determined ranges of thermal tolerance for sac-fry and swim-up fry. In another preliminary test, juvenile bluegills were acclimated to 12.1, 19.0, 26.0 or 32.9C, and reared at a series of test temperatures for three to six weeks to define optimal temperature ranges for growth and survival.

Additional research determined conditions for the culture of *Lepomis macrochirus*, including spawning induction, hatching, and growth of larvae and juveniles.

This report was submitted in fulfillment of Project 18050-GAB, Contract 14-12-913, under sponsorship of the Office of Research and Monitoring, Environmental Protection Agency.

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SECTION I

CONCLUSIONS

1. Based on two experiments, bluegill eggs that are spawned at 26C have a lower mean TL_{50} of 21.9C and an upper mean TL_{50} of 33.8C.

2. Juvenile bluegills have a greater thermal tolerance range than eggs. The upper 96 hr. TL_{50}'s were 27.5C and 37.3C for juveniles acclimated at 12.1C and 32.9C respectively, and the lower 96 hr. TL_{50}'s were 3.2C and 15.3C for juveniles at the same acclimation temperatures. TL_{50} temperatures for juvenile bluegills increase with increasing temperature of acclimation. Upper TL_{50} decreases and lower TL_{50} increases with increasing exposure duration.

3. Adult bluegills can be induced to spawn in laboratory aquaria by manipulation of temperature and photoperiod. Injection of female bluegills with carp pituitary induces ovulation and enables experiments to be conducted with eggs of known history from several sets of donor parents simultaneously.

4. Preliminary experiments indicate that fry survive for 96 hrs. at temperatures of 11 to 34C. Fry therefore have greater thermal tolerance than eggs and less thermal tolerance than juveniles, but fry survive poorly in the life support conditions of our testing facility.

5. The range of temperatures for optimal growth and survival of juveniles was less than the range defined by upper and lower TL_{50}. An optimal range was not clearly defined because of inadequate life support conditions.

SECTION II

RECOMMENDATIONS

Preliminary tests conducted with sac-fry and swim-up fry indicate that sac-fry were more resistant to thermal stress than eggs. Swim-up fry were more sensitive to high temperature than any other stage, perhaps because they have a shorter time to feed successfully before starvation. Results of the sac-fry tests should be verified by experiments with larger numbers of test animals and more intermediate temperatures. The test with swim-up fry should be repeated, with more test animals, and with animals that are reared to swim-up stage at higher stock temperatures. Larvae should be fed more frequently (4 to 5 times daily) beginning as soon as they reach swim-up stage.

Tests should be conducted to establish the effects of parental thermal history on the thermal tolerance of offspring. Adults maintained at three stock temperatures, e.g., 22, 26, and 30C should be induced to spawn and thermal bioassays conducted on their progeny.

Hormone injections affect egg viability and hence may affect their observed temperature tolerance. Experiments might be conducted to compare temperature tolerance of eggs from normal spawns with the tolerance of eggs from hormone-induced spawns.

Optimal limits for growth and survival of juvenile bluegills were not well defined for most stocks. This experiment should be repeated, eliminating some of the extreme temperatures that showed large negative changes in biomass, and including more of the intermediate temperatures. In particular, to define the optimal range for summer acclimated animals, the 32.2C stock should be tested between 18 and 35C.

Swim-up fry may be most sensitive to high temperature because the demands for food must be met adequately during a short critical period (Toetz, 1966). Swim-up fry may also be more sensitive than other stages to water quality conditions and the turbulent environment of the culture baskets. Tests with swim-up fry should be repeated with an improved life support system using other kinds of food (besides *Artemia* nauplii and limnoplankton) and with different feeding rates.

The water quality and life support characteristics of the thermal test facility can be improved by developing better methods to control dissolved oxygen and pH, and to eliminate toxic metals and metabolites. Subsequent experiments at ASI have yielded better survival of bluegill fry in tap-water treated by charcoal filtration and in water treated by deionizing resins and reconstituted with reagent grade inorganic salts. Chemicals must be carefully selected to avoid addition of toxic heavy metals present as contaminants.

Confinement of fish in culture baskets is necessary to prevent escape of fish, facilitate counting and permit replicates within a given temperature chamber. Experiments are needed to define optimal container size and fish density within containers in the temperature chambers to prevent crowding.

SECTION III

INTRODUCTION

The disposal of waste heat, arising from industrial processes and the production of electric power, constitutes a major pollution problem with pronounced effects on aquatic organisms (Kennedy and Mihursky, 1967; Raney and Menzel, 1969). The need for electric power is increasing rapidly. Parker and Krenkel (1969) predict that by the year 2000, the equivalent of the total flow of surface waters in the United States will be required for cooling purposes. If in meeting electrical production requirements it is necessary to discharge large quantities of heated water, the effects of this water on aquatic ecology must be assessed so that practical guidelines and restrictions can be developed.

Fish constitute the highest trophic level in the ecology of most fresh waters and are a source of food and recreation for man. A population of fish in a lake or stream, however, is a complex phenomenon. Prediction of the effects of environmental changes on this population requires both laboratory and field experiments. Carefully controlled laboratory experiments are necessary to define precise limits of tolerance, whereas field observations are necessary to determine whether the guidelines developed in the laboratory are relevant to natural populations.

To determine effects of temperature on a fish population, animals must be tested at all stages of their life cycle. The temperature history of the animals prior to testing, as well as the duration of exposure to temperature extremes, influence the thermal tolerance (Brett, 1958). Results of temperature bioassays are thus most meaningful when the acclimation temperature and duration of exposure have been controlled in the laboratory.

The responses of eggs to temperature stress and the limits for survival or the "optimal range" can be determined in several ways (Hokanson, McCormick, and Jones, 1973). The most generally acceptable criterion for determining survival limits is the range over which 50% of the animals survive (American Public Health Association, 1971). To determine egg survival, total percent hatch, percent hatch of normal eggs, percent fertilization (Lindroth, 1946), percent abnormal hatch (Price, 1940), or percent of fertilized eggs surviving to hatch (Hubbs, 1962) may be measured. However, percent normal hatch is the most meaningful of these criteria with respect to natural populations and is the end result of the processes of fertilization and egg survival.

Eggs may be exposed directly to test temperatures or they may be tempered at various rates to the test temperatures. If eggs are tempered to test temperatures, the rate of tempering becomes critical. The embryo then has an opportunity to develop to a stage with a different tolerance, and the rate at which this development occurs adds an experimental variable. Direct exposure is desirable because the eggs are exposed to temperature extremes sooner. Direct exposure to temperature stress (thermal shock) may reduce survival of eggs (Kelley, 1968) but if this stress is applied early in embryonic development, preferably prior to the first cell division, then egg survival is not reduced (Gorodilov, 1969; Tatarko, 1968).

Eggs can be obtained from ripe adult fish collected in the field. However, the temperature history of adults is unknown, collection of eggs or adults produces a stress that may affect egg viability, and collection or holding of large numbers of individuals may be necessary to obtain a few ripe parents. If animals are obtained ripe and "stripped" in the field, it is difficult to precisely control temperature during fertilization and transport of eggs to the laboratory. For these reasons, it is desirable to induce fish to reproduce in the laboratory. Fish can usually be induced to spawn in laboratory aquaria by manipulation of light, temperature, and photoperiod. However, such spawns are unpredictable and require many large aquaria to obtain sufficient eggs for testing. The use of pituitary hormone injections facilitates the production of viable eggs, but the viability of these eggs may be less than viability of eggs that are spawned naturally (Pickford & Atz, 1957).

Testing of larvae and juveniles should be performed with fish that have been acclimated in the laboratory to stock temperatures that represent the range found in the natural environment. Gradual change in temperature during acclimation permits metabolic changes similar to changes that may occur in response to seasonal temperature changes in the environment. Fish that have been acclimated to appropriate temperatures can then be challenged with a direct temperature change to estimate the effects of addition or removal of heated water, which might occur during operation of a power facility.

Proper evaluation of the effects of temperature requires that other variables which may affect survival must be controlled, especially oxygen, food, crowding and water quality (Fry, 1947).

Short-term survival at test temperatures indicates the ability of fish to resist thermal stress but does not indicate whether the population will continue to survive with prolonged temperature alteration. Long-term studies are necessary to measure the effects of temperature on growth rate, mortality, and overall population biomass.

Fish have an optimal physiological range of thermal tolerance (Fry, 1951) within which maximum growth and activity occur. The optimal physiological range may bracket an optimal temperature for various metabolic activities. The temperature for optimal growth is dependent upon the amount of food available (Brett, Shelbourne, and Shoop, 1969). The temperature for optimal growth shifts to lower values as the amount of food is reduced. Measurement of the optimal physiological temperature therefore requires that food be present in excess. The optimal physiological temperature may regulate diurnal and seasonal behavior patterns of some fishes to increase food conversion when the food supply is limited (Brett, 1971).

The bluegill sunfish, Lepomis macrochirus, is an important forage, food, and game fish in North American fresh waters. Bluegills are generally considered a warm water species but they are widely distributed in the United States and southern Canada. In the United States, L. macrochirus ranges from Minnesota to southern Florida and has been introduced into Hawaii (Eddy, 1969) and Puerto Rico (Erdman, 1967). L. macrochirus thus survives in a wide range of environments and may be ideally suited for survival in, or introduction into, heated water effluents in temperate zones.

The thermal tolerance of bluegills has been studied previously to some extent. The relationship between zoogeography and thermal tolerance (Hart, 1952) and the upper limits of thermal tolerance (Cairns, 1956) have been determined for juvenile and adult bluegills. The limit of tolerance for juvenile bluegills exposed briefly to elevated temperatures has also been tested (Iezzi, Filson and Myers, 1952).

The objectives of this study were to determine the optimal temperature ranges and tolerance limits for incubation of L. macrochirus eggs and for survival of juveniles. Techniques were developed to induce spawning of bluegills in the laboratory and to determine favorable conditions for rearing of larvae in our test facilities. Preliminary tests were conducted to determine the approximate temperature tolerance of bluegill fry and the approximate temperature range for optimal growth of juvenile bluegills. A bibliography of the literature relevant to thermal tolerance, spawning induction, ecology, and larval rearing of L. macrochirus is included as an appendix.

SECTION IV

MATERIALS AND METHODS

Field Collections

Adult bluegills were collected from canals near State Road 27, nine miles southwest of Florida City, Florida; from Drainage Canal No. 35B between U. S. Highways 27 and 441 in Water Conservation Area 2A of the Central and South Florida Flood Control District; from Welaka Fish Hatchery, Putnam County, Florida; and from Lake Apopka, Orange County, Florida. Fish were captured by hook and line, by electro-shocking, or by seining, and transported to the laboratory. Newly collected fish were tempered from ambient field temperature to 28C (1C/hr) and transferred to 3.4 kiloliter tanks for quarantine. After one week, females were placed in 2.9 kiloliter aquaria and males were placed in 209-liter aquaria at 28C. Several males and females were sacrificed from each monthly field collection to estimate the gonadosomatic index (GSI = gonad wt/total fish wt x 100) of the wild population.

Temperature Records

During the incubation and bioassay experiments, the temperature of each culture chamber in the thermal testing facility was recorded automatically at two minute intervals. Temperature of each culture chamber was also determined daily, to ±0.1C, with a mercury thermometer. The daily range, daily mean, grand mean, and 95% confidence limits of all means were obtained from the recorded temperature records after correction by reference to the manual readings.

During the bioassay experiments, test containers generally remained within a ±0.3C range of their nominal temperatures. Temperatures reported in the results section represent the grand mean and 95% confidence limits.

Water Quality

Water from the Boca Raton municipal water treatment plant was used during the bioassay tests. This water was dechlorinated by aeration for two weeks prior to the test, and was adequate to support the growth of nauplii and adults of *Daphnia magna* for at least one week.

A detailed analysis of water in the testing facility was performed during the bioassay experiments. Levels of ionic components were measured by techniques described in *Standard Methods for the Examination of Water and Wastewater*, Twelfth Edition, 1965.

In addition to the detailed analysis, water quality was monitored daily during the bioassay experiments using reagents and techniques prepared by the Hach Chemical Company, Ames, Iowa. Water samples were collected daily from test chambers at three temperatures (high, low and intermediate). Each sample was analyzed for changes in pH, ammonia or dissolved oxygen that would result from organic decomposition in the biological filter, autotrophic tanks or test chambers. Levels of nitrites and nitrates were monitored at irregular intervals to check for gradual enrichment of the water.

Incubation Tests

Two egg incubation tests were conducted to determine the upper and lower limits of temperature for hatch of normal bluegill fry (after Hokanson et al., 1973). Upper and lower TL_{50} were defined by incubating eggs at test temperatures ranging from 16 to 38C at 2C intervals.

Thermal History of Adults. For the preliminary incubation test (Appendix D), conducted in June, 1971, adult bluegills collected from the field were held for 8 days at 26C. These adults were pooled with animals that had been maintained in the laboratory for spawning-induction experiments (Appendix A) under a variety of temperature conditions. All animals were held under a 16L-8D photoperiod. Incubation test 1 was conducted in July, and incubation test 2 was conducted in August, 1971. Females used for tests 1 and 2 were held in the laboratory at 23.8 to 26.4C for 20 to 122 days prior to the start of the incubation experiments.

Injection Procedures. The development of the spawning inducation technology is given in detail in Appendix A. Carp pituitary glands (Stoller Fish Industries, Ames, Iowa) were suspended (10 mg/ml) in fish Ringer's solution (Humason, 1967). Carp pituitary suspensions were injected into female bluegills with 1 ml plastic syringes and 24 gauge needles. Egg development was monitored prior to each injection. A 2 mm O.D. glass catheter attached to a syringe (Pennell, 1964) was inserted into the genital pore, eggs were withdrawn, suspended in water, and examined under a microscope.

During the holding period, females were injected intraperitoneally with 2 mg carp pituitary every other day, to induce formation of premature eggs. Injections to induce egg production began 30 to 44 hours before eggs were needed for incubation tests. Twenty females that had premature eggs were injected intramuscularly (below the base of the dorsal fin) with 1 mg carp pituitary every four hours and these injections were continued until eggs matured. The thermal history of females used in the first and second incubation tests is summarized in Table 1.

Fertilization. Fertilization was attempted when eggs reached maturity. When 50% of the eggs from three or more females could be fertilized, eggs from these females were stripped into a shallow dissecting tray (30 x 21 x 5 cm) containing 350 ml of water from the holding tank. The bottom of the tray was covered with 2.5 cm. x 2.5 cm pieces of Plexiglas®, which formed a substrate to which the eggs adhered. Sperm from four or five males were uniformly distributed over the eggs. The tray was floated in a water bath maintained at 25.0 or 26.0C. Eggs and sperm were allowed to mix for two minutes, then were rinsed in water from the holding tank. This served to remove extra sperm and non-adhesive eggs. Conditions under which the eggs were fertilized are given for each test in Table 2.

Table 1. Thermal history for adult female bluegills used in the two incubation tests. All animals were exposed to a 16L-8D photoperiod.

	Test 1	Test 2
Collection Temp (C)	23-28	23-28
Holding		
Duration (days)	20-108	38-122
Mean Temp (C)	24.5	24.7
Egg Production		
Duration (hrs)	24	28
Mean Temp (C)	26.0	25.0

Table 2. Number of parents and the temperature at which parents and eggs were held prior to and during fertilization for the two incubation tests.

	Test 1	Test 2
No. males	4	5
No. females	6	3
Mean Temp (C)	26.0	25.0

Bioassay Procedure. The Plexiglas® squares, with adherent eggs, were immediately transferred to 257-ml glass jars filled with water from the holding tank. Each jar was placed in a randomly assigned culture chamber of the thermal testing facility within ten minutes after fertilization. The eggs in the jars were tempered rapidly (12C/hr) to the temperature of the chamber. The Plexiglas® square was then transferred from the jar to a culture basket in the test chamber (two baskets per chamber). Excess eggs were removed from the plastic squares so that each square held 25 eggs. Duplicate test chambers were maintained at each test temperature giving N = 100 eggs for each temperature. Odd-numbered chambers were assigned to one experimental replicate (A) and even-numbered chambers were assigned to the second replicate (B). Dead eggs and dead, normal, and deformed fry were counted and removed daily. Normal eggs were transparent and yellowish-white. Dead eggs were translucent to opaque. Fry that had noticeably curved spines or swollen bodies (edema) were considered to be deformed.

Analysis of Results. Total percent hatch and percent hatch of normal fry were determined at each test temperature. A given test was considered valid when more than 50% of the eggs hatched in a replicate at any test temperature. Relative percent hatch was then calculated for each test. The hatch at optimal temperature (temperature which gave the maximum percent hatch of normal fry) was assigned the value of 100% and numbers of hatched fry at other temperatures in the same test were designated as proportions of the maximum percent hatch of normal fry (after Hokanson et al., 1973).

Upper and lower TL_{50} values were determined for each experiment. Relative percent hatch of normal fry was first graphed as a function of temperature. A linear equation was fitted to two points (above and below the temperature for 50% hatch) by the method of least squares. The upper and lower TL_{50} values were determined from the linear equations.

Juvenile Bioassay

A bioassay test was conducted to determined the range of thermal tolerance for juvenile bluegills. Juvenile bluegills (mean wt = 0.3 gm, range = 0.1 to 2.1 gm) were obtained from the Federal Hatchery at Richloam, Georgia. Fish were randomly assigned to four groups; acclimated at 0.5C/day to stock temperatures of 12.1, 19.0, 26.0 and 32.9C; and maintained at these temperatures for 8 days. Ten fish were randomly assigned from these stocks and transferred directly to each culture basket. Culture baskets were floated in 75.8-liter fiberglass culture chambers (see Appendix C). Chambers were maintained at various test temperatures in the thermal test facility. The temperature range to which each stock was exposed is given in the results.

For the stocks that were acclimated to 12.1, 19.0, or 26.0C, two replicate culture chambers were held at each temperature from 7 to 33C, and one culture chamber per test temperature was used at 3 to 6C. For the stock acclimated to 32.9C, one culture chamber was used at 39C, while two culture chambers were used at each of the remaining test temperatures.

When two chambers were available at a given temperature, the odd-numbered chamber was designated as one replicate (A) and the even-numbered chamber as the second replicate (B). Each replicate contained two culture baskets (10 animals per basket) with fish from a given stock.

For temperatures at which only a single chamber was available, this chamber contained four culture baskets with fish from a given stock. Two adjacent protectors were assigned as one replicate (A) and the remaining two protectors were assigned as the second replicate (B).

Fish were fed to satiation at 0800, 1200, and 1700 hrs daily with adult Artemia, Daphnia, and Tetramin®. Dead fish were counted and removed after 0.83, 1.10, 24, 48, 72, and 96 hours. Samples taken at 0.83 and 1.10 hrs were averaged to determine mortality at one hour. Results of bioassays were analyzed to determine TL_{50} temperatures, slope function (S) of the TL_{50} curve, and 95% confidence limits for each stock at 1, 24, 48, 72, and 96 hours (Litchfield and Wilcoxon, 1949).

SECTION V

RESULTS

Water Quality

Results of detailed analyses of water in the thermal test facility are presented in Table 3. Results of routine water quality analyses are presented in Table 4. Routine analyses indicated that pH varied between 7.4 and 8.3. Dissolved oxygen (expressed as % air saturation) ranged between 71 and 145%, and ammonia (un-ionized NH_3) reached a maximum of 0.095 ppm. Concentrations of nitrite reached a maximum of .008 ppm while nitrate had a maximum concentration of 2 ppm.

Table 3. Trace-chemical composition of water from the thermal testing facility based on a single determination. This analysis was performed by Precision Analytical Laboratories, Inc., North Miami, Florida.

Component	Sensitivity of Test (mg/l)	Concentration (mg/l)
Calcium	0.01	38.50
Magnesium	0.01	2.84
Potassium	0.01	0.90
Sulfate	0.1	4.5
Sulfide	0.05	< 0.05
Nitrate	0.05	< 0.05
Nitrite	0.05	< 0.05
Ammonia	0.01	< 0.01
Phenol	0.001	< 0.001
Chloride	0.1	38.0
Fluoride	0.01	< 0.01
Cyanide	0.005	< 0.005
Iron	0.001	0.045
Copper	0.01	0.10*
Zinc	0.001	0.001
Cadmium	0.01	0.03*
Chromium	0.01	< 0.01
Lead	0.001	< 0.001
Alkalinity	1.0	51

*These levels may be harmful to fish (see discussion)

Table 4. Results of routine water-quality monitoring during bioassay experiments. Mean (ppm unless otherwise specified) and range are given for N = 3 replicates of each analysis.

Day No.	pH Mean	pH Range	Diss. Oxygen (% Sat.) Mean	Diss. Oxygen (% Sat.) Range	Ammonia (NH_3) Mean	Ammonia (NH_3) Range	Nitrite Mean	Nitrite Range	Nitrate Mean	Nitrate Range
Incubation Test No. 1										
0	7.7	7.5-7.9	105	93-117	.009	.006-.013	.000	0-0	0	0-0
1	7.9	7.8-8.0	110	93-132	.013	.005-.021	.001	0-.001	1	0-2
2	7.7	7.6-7.7	98	82-117	.007	.004-.010	.001	0-.001	0	0-0
3	7.8	7.7-7.8	109	93-118	.019	.008-.029	.001	0-.001	0	0-0
4	7.8	7.7-7.9	109	93-118	.023	.002-.044	.001	0-.001	0	0-0
Incubation Test No. 2										
1	7.5	7.5-7.6	105	93-117	.011	.004-.012	.001	0-.001	0	0-0
2	7.5	7.4-7.6	105	93-117	.008	.006-.012	.000	0-0	0	0-0
3	*		105	93-117	*	*	*		*	
Juvenile Bioassay (26, 33C Stocks)										
1	7.9	7.8-7.9	101	80-119	.027	.018-.042	.004	.003-.005	0	0-0
2	7.7	7.6-7.8	104	80-134	.016	.005-.031	.003	.001-.005	*	
3	7.9	7.8-8.0	106	80-134	.010	.005-.018	.005	.003-.008	*	
4	8.2	8.1-8.3	115	93-132	.047	.015-.095	.001	.000-.003	0	0-0
Juvenile Bioassay (12C Stock)										
0	8.0	7.9-8.1	91	74-108	.012	.006-.028	*		*	
1	8.0	7.9-8.2	88	74-104	.016	.008-.028	.004	.003-.006	0	0-0
2	8.2	8.2-8.2	92	77-102	.018	.011-.028	*		*	
3	7.9	7.9-8.0	90	74-104	.012	.006-.022	*		*	
4	7.7	7.7-7.8	87	71-102	.007	.004-.012	.000	.000-.001	1	0-2
Juvenile Bioassay (19C Stock)										
0	8.0	8.0-8.0	102	80-122	.013	.007-.021	.003	.001-.005	0	0-0
1	7.7	7.6-7.7	99	82-116	.009	.003-.018	.004	.003-.005	0	0-0
2	8.1	8.1-8.2	107	94-125	.017	.009-.029	.005	.001-.005	0	0-0
3	7.9	7.9-7.9	117	82-145	.013	.008-.019	.004	.003-.005	0	0-0
4	7.7	7.7-7.8	99	82-116	.039	.005-.024	*		*	

*Samples not analyzed

Incubation Tests

The total percent hatch, percent normal hatch, and relative percent normal hatch are presented in Table 5. The relative percent normal hatch was calculated by setting the maximum percent normal hatch to 100 and adjusting all other values accordingly. The relative percent normal hatch values are plotted against actual test temperatures in Figure 1. These values were used to calculate the upper and lower temperature TL_{50} values.

During a preliminary incubation test (Appendix D), egg viability was low and hatching rates differed greatly between replicates. The first incubation test showed close agreement between replicates with regard to temperature for maximal hatch. Replicate A had a maximal hatch (greater than 50%) at 27.8C, while replicate B had a maximal hatch (greater than 50%) at 26.0C. When results of both replicates were combined, maximal percent hatch was at 22.2C, but did not reach 50%. There was a 1 to 5% abnormal hatch at all test temperatures. The upper TL_{50} was 34.5C and the lower TL_{50} was 21.2C.

During the second test, maximal percent hatch occurred at 23.9C for replicate A, and at 28.0C for replicate B. Both replicates had no hatch at 18.1C and replicate A had no hatch at 35.9C. When results of both replicates were combined, maximal hatch was at 23.9C, and exceeded 50%. The percent abnormal hatch ranged from 0 to 4% and was observed primarily at extreme temperatures. The upper TL_{50} was 33.2C and the lower TL_{50} was 22.6C.

Juvenile Bioassay

Survival of juvenile bluegills at the various test temperatures is shown in Table 6. Prior to the test, juveniles were acclimated to stock temperatures of 12.1, 19.0, 26.0 or 32.9C. Animals from each stock were then distributed to a series of test temperatures (Table 6) designed to bracket the upper and lower limits of thermal tolerance.

The TL_{50} temperatures for juvenile bluegills, as defined by statistical analyses of the survival data, are presented in Table 7. For the stock acclimated to 12.1C, a lower TL_{50} was not defined at 1, 24, 48, or 72 hours (TL_{50} was less than the minimum test temperature of 3.0C), but TL_{50} was 3.2C (with 95% condifdence limits of $\pm$0.56C) after 96-hour exposure. The upper one-hour TL_{50} for the 12.1C stock was 28.9 $\pm$0.43C. The TL_{50} temperature decreased at 48 and 72 hours and reached a minimum of 27.5 $\pm$ 0.58C after 96 hours.

The stock acclimated to 19.0C had a lower one-hour TL_{50} of 4.0 $\pm$ 0.36C. This value increased, with longer exposure times, to a maximum of 6.3 $\pm$ 0.41C after 96-hour exposure. The upper TL_{50} was 34.7 $\pm$ 0.31C after one hour and decreased to 33.0 $\pm$ 0.26C after 96 hours.

Table 5. Total percent hatch, percent hatch of normal larvae, and relative percent normal hatch (see text) for bluegill eggs. Percentages represent the combined results from two replicates at each test temperature.

Incubation Test No. 1

Mean Temperature (± 95% CL)	Number Temperature Observations	Replicate	Total Hatch	Normal Hatch	Relative Normal Hatch
17.9 ± 0.20	96	A	0	0	0
17.9 ± 0.20	96	B			
20.1 ± 0.27	96	A	2	0	0
20.1 ± 0.29	96	B			
22.2 ± 0.16	96	A	46	44	100
22.2 ± 0.16	96	B			
24.0 ± 0.77	96	A	27	24	55
24.0 ± 0.91	96	B			
26.0 ± 0.36	96	A	38	36	82
26.1 ± 0.35	96	B			
27.9 ± 0.26	96	A	42	37	84
27.6 ± 1.04	96	B			
29.9 ± 0.28	96	A	34	33	76
30.3 ± 0.37	96	B			
32.0 ± 0.23	96	A	35	32	73
32.1 ± 0.46	96	B			
33.9 ± 0.37	96	A	34	29	66
34.1 ± 0.41	96	B			
35.9 ± 0.37	96	A	6	4	9
35.9 ± 0.36	96	B			
38.2 ± 0.48	96	A			
38.2 ± 0.45	96	B	0	0	0

TL_{50} (based on relative percent normal hatch)
Upper 34.5C
Lower 21.2C

Table 5 (cont'd)

Incubation Test No. 2

Mean Temperature (± 95% CL)	Number Temperature Observations	Replicate	Total Hatch	Normal Hatch	Relative Normal Hatch
18.1 ± 0.38	96	A			
18.1 ± 0.62	96	B	0	0	0
20.2 ± 0.36	96	A			
20.1 ± 0.48	96	B	5	5	9
22.1 ± 0.34	96	A			
22.3 ± 0.40	96	B	24	21	36
23.8 ± 0.65	96	A			
24.0 ± 0.15	96	B	59	58	100
26.1 ± 0.12	96	A			
26.2 ± 0.22	96	B	44	43	74
28.0 ± 0.20	73	A			
28.0 ± 0.18	73	B	53	53	91
30.0 ± 0.20	96	A			
29.9 ± 0.32	96	B	41	41	71
31.7 ± 0.82	96	A			
31.7 ± 1.12	96	B	49	49	85
34.0 ± 0.19	96	A			
33.9 ± 0.41	96	B	24	20	34
35.8 ± 1.08	96	A			
35.9 ± 1.03	96	B	1	1	2
38.0 ± 0.50	96	A			
38.0 ± 0.32	96	B	0	0	0

TL_{50} (based on relative percent normal hatch)
Upper 33.2C
Lower 22.6C

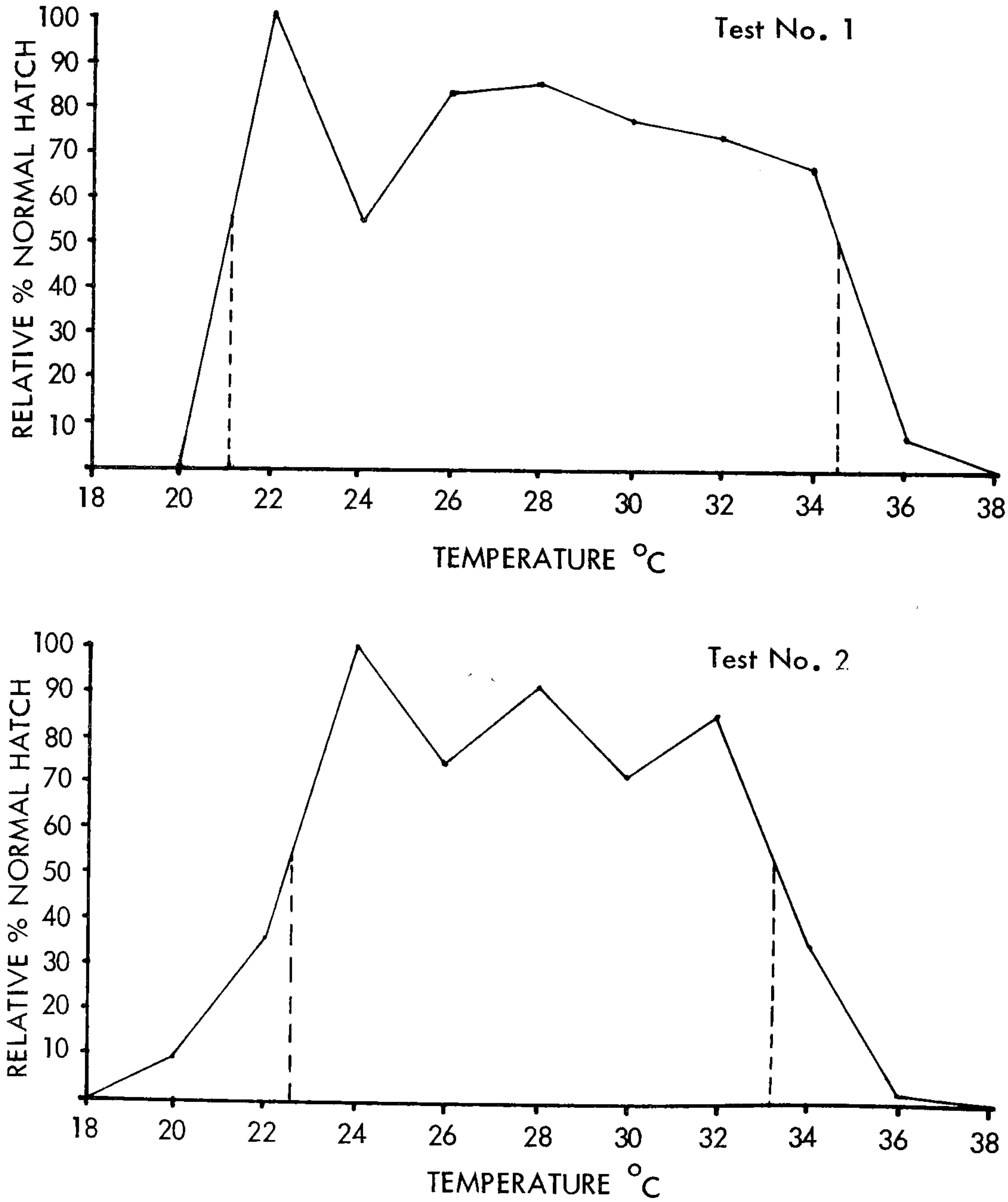

Figure 1. Relative percent normal hatch (see text) of bluegill eggs at various temperatures during two incubation tests. Each temperature included two replicate culture chambers with 50 eggs per chamber. Dashed lines indicate TL_{50} limits.

Table 6. Percent survival of juvenile bluegills at various test temperatures. Survival at 0.83 and 1.10 hrs were averaged to determined the 1-hr TL_{50}.

Stock Acclimated to 12.1C

Temperature Mean ± 95% CL	No. Temperature Observations	Replicate	Time (hrs) 0.83	1.10	24	48	72	96
3.0 ± 0.32	96	A	95	95	85	75	65	50
	96	B	95	95	80	65	60	40
4.0 ± 0.42	96	A	100	100	70	65	55	50
	96	B	95	95	90	80	75	75
5.2 ± 0.80	96	A	100	100	85	85	80	80
	96	B	100	95	85	85	75	70
6.1 ± 0.40	96	A	100	100	75	70	70	60
	96	B	100	100	80	70	70	60
6.9 ± 1.31	96	A	95	95	95	95	95	75
	96	B	100	100	90	90	85	75
7.9 ± 0.26	96	A	100	100	95	80	80	65
7.9 ± 0.36	96	B	100	100	85	85	85	70
26.1 ± 0.22	96	A	65	65	60	60	60	60
25.9 ± 0.40	96	B	80	80	75	70	65	60
27.0 ± 0.28	96	A	50	50	50	50	50	50
26.9 ± 0.40	96	B	70	55	55	55	55	55
28.0 ± 1.40	96	A	70	70	60	60	55	55
27.8 ± 1.32	96	B	60	50	45	45	40	40
29.1 ± 0.20	96	A	65	65	60	60	55	55
28.9 ± 0.24	96	B	45	40	35	35	35	30
29.9 ± 0.26	96	A	15	10	5	5	5	5
30.0 ± 0.27	96	B	40	35	10	10	10	10
31.0 ± 0.13	30	A	5	5	0	-	-	-
31.1 ± 0.12	30	B	15	5	0	-		
32.1 ± 1.80	30	A	5	5	0	-		
32.2 ± 0.12	30	B	0					

Table 6. (cont'd)

Stock Acclimated to 19.0C

Temperature Mean ± 95% CL	No. Temperature Observations	Replicate	Time (hrs) 0.83	1.10	24	48	72	96
3.2 ± 0.50	30	A	0	-				
		B	0	-				
4.1 ± 0.78	96	A	75	60	25	25	15	10
		B	45	45	10	5	5	5
4.8 ± 0.73	96	A	95	95	80	55	45	20
		B	85	80	25	15	10	10
6.0 ± 0.19	96	A	90	90	80	65	65	55
		B	100	100	90	45	30	20
6.8 ± 0.84	96	A	100	100	70	60	55	55
7.0 ± 0.28	96	B	95	95	95	60	55	55
7.8 ± 0.34	96	A	100	100	90	80	80	80
8.0 ± 0.56	96	B	90	90	80	75	75	70
9.1 ± 0.30	96	A	100	100	95	90	90	90
8.9 ± 0.36	96	B	100	100	95	95	90	90
9.8 ± 0.28	96	A	100	100	100	95	90	80
9.8 ± 0.28	96	B	100	100	95	90	85	85
30.1 ± 0.32	96	A	100	100	100	100	100	100
30.1 ± 0.50	96	B	100	100	100	100	100	100
30.9 ± 0.20	96	A	100	100	85	80	80	80
30.8 ± 1.11	96	B	100	100	90	90	85	80
31.8 ± 0.47	96	A	100	100	95	95	95	95
31.9 ± 0.31	96	B	100	100	70	65	65	65
32.9 ± 0.48	96	A	95	95	75	70	70	60
32.9 ± 0.54	96	B	100	100	75	70	70	65
33.9 ± 0.21	96	A	60	55	15	15	10	10
33.8 ± 0.23	96	B	85	65	10	10	10	10
34.9 ± 0.51	96	A	50	10	10	10	10	10
34.7 ± 0.09	96	B	30	10	0	-		

Table 6. (cont'd)

Stock Acclimated to 26.0C

Temperature Mean ± 95% CL	No. Temperature Observations	Replicate	Time (hrs) 0.83	1.10	24	48	72	96
5.6 ± 0.81	30	A	10	5	0	-		
6.0 ± 0.19	30	B	0	-				
6.8 ± 0.84	24	A	65	65	0	-		
7.0 ± 0.28	24	B	65	50	5	0		
7.8 ± 0.34	72	A	85	85	60	5	5	0
8.0 ± 0.56	72	B	80	80	35	0	-	
9.1 ± 0.30	96	A	100	100	70	20	10	10
8.9 ± 0.36	96	B	100	100	80	45	20	15
9.8 ± 0.28	96	A	100	100	95	80	80	80
9.8 ± 0.27	96	B	100	100	90	85	80	75
10.9 ± 0.38	96	A	100	100	95	95	95	90
11.0 ± 0.16	96	B	100	100	100	95	90	85
12.0 ± 0.44	96	A	100	100	95	95	95	95
11.8 ± 0.64	96	B	100	100	95	95	90	90
13.0 ± 0.28	96	A	100	100	100	100	100	100
13.0 ± 0.26	96	B	100	100	100	100	95	90
26.0 ± 0.22	96	A	100	100	100	100	100	100
26.0 ± 0.20	96	B	100	100	100	90	90	90
33.9 ± 0.76	96	A	100	100	100	90	90	90
33.9 ± 0.40	96	B	100	100	95	90	90	85
34.9 ± 0.68	96	A	100	100	85	75	55	50
34.8 ± 0.82	96	B	100	100	100	100	55	90
36.0 ± 1.02	96	A	100	100	90	85	85	85
35.8 ± 1.20	96	B	100	100	70	60	50	45
37.0 ± 0.53	96	A	100	13	5	5	5	5
37.2 ± 0.49	96	B	90	90	25	20	15	15
37.7 ± 0.04	96	A	10	5	0	-		
37.8 ± 1.43	96	B	50	15	0			

Table 6 (cont'd)

Stock Acclimated to 32.9C

Temperature Mean ± 95% CL	No. Temperature Observations	Replicate	Time (hrs) 0.83	1.10	24	48	72	96
10.0 ± 0.18	30	A	25	0	-			
10.0 ± 0.19	30	B	75	5	-			
11.2 ± 0.16	24	A	100	75	0			
11.1 ± 0.16	24	B	95	85	0			
11.9 ± 0.40	24	A	100	100	0	-		
11.9 ± 0.28	24	B	100	100	0	-		
13.0 ± 0.24	72	A	100	65	35	5	0	-
12.8 ± 0.46	72	B	100	70	50	0	-	
13.9 ± 0.13	48	A	100	90	90	0	-	
13.9 ± 0.14	48	B	100	75	0	-		
14.8 ± 0.49	96	A	100	100	90	40	25	25
14.9 ± 0.30	96	B	100	100	80	25	25	25
15.9 ± 0.23	96	A	100	100	90	85	85	85
15.9 ± 0.20	96	B	100	100	100	95	95	95
17.0 ± 0.18	96	A	95	95	90	85	85	85
17.0 ± 0.70	96	B	100	100	95	95	95	95
19.9 ± 0.20	96	A	100	100	100	100	90	90
20.2 ± 0.32	96	B	100	100	95	90	90	90
32.9 ± 0.28	96	A	100	100	80	80	75	75
32.9 ± 0.20	96	B	100	100	95	95	95	95
36.0 ± 0.86	96	A	100	100	100	100	100	100
35.7 ± 1.17	96	B	100	100	70	45	35	15
37.0 ± 0.59	96	A	100	100	85	85	75	70
37.0 ± 0.61	96	B	100	100	85	80	80	80
37.8 ± 0.50	96	A	100	95	25	15	10	10
38.0 ± 0.36	96	B	100	95	0	-	-	
38.9 ± 0.15	30	A	65	15	0	-		
		B	80	55	0	-		

Table 7. The upper and lower TL_{50} values for bluegills (Replicates combined). Confidence limits (95% CL) and slope function of the mortality curve (S) were calculated for each TL_{50}. Animals were acclimated to 12.1, 19.0, 26.0 or 32.9C before the experiment.

Exposure Time	1 hr	24 hr	48 hr	72 hr	96 hr
		Stock Acclimated to 12.1C			
Upper TL_{50}	28.9	28.5	28.5	27.7	27.5
95% CL	±0.43	±0.37	±0.37	±0.57	±0.58
S	1.06	1.05	1.05	1.11	1.11
Lower TL_{50}	(not defined,	(not defined,	(not defined,	(not defined,	3.2
95% CL	TL_{50} < 3C)	TL_{50} < 3C)	TL_{50} < 3C)	TL_{50} < 3C)	±0.56
S					1.89
		Stock Acclimated to 19.0C			
Upper TL_{50}	34.7	33.0	33.0	32.9	33.0
95% CL	±0.31	±1.67	±1.04	±0.88	±0.26
S	1.03	1.04	1.04	1.04	1.03
Lower TL_{50}	4.0	4.7	5.8	6.1	6.3
95% CL	±0.36	±0.41	±0.48	±0.50	±0.41
S	1.22	1.19	1.39	1.40	1.35
		Stock Acclimated to 26.0C			
Upper TL_{50}	37.4	36.4	36.1	36.1	36.1
95% CL	±0.30*	±1.27	±0.34	±1.00	±1.10
S	1.02	1.03	1.02	1.03	1.04
Lower TL_{50}	7.0	8.2	9.5	9.6	9.8
95% CL	±0.20	±0.25	±0.27	±0.26	±1.00
S	1.12	1.12	1.09	1.09	1.09
		Stock Acclimated to 32.9C***			
Upper TL_{50}	39.0	37.5	37.4	37.3	37.3
95% CL	±0.23*	**	±0.18*	±0.18	±0.19
S	1.01	1.01	1.01	1.01	1.01
Lower TL_{50}	10.3	13.7	15.0	15.3	15.3
95% CL	±0.20*	±1.60	±1.55	±0.22*	±0.22*
S	1.05	1.09	1.05	1.03	1.03

* Plot of Two points, no X^2 test for "goodness of fit"

** Plot of two points, no values between 16 and 84%, CL could not be calculated

*** One replicate at 36C excluded (see text)

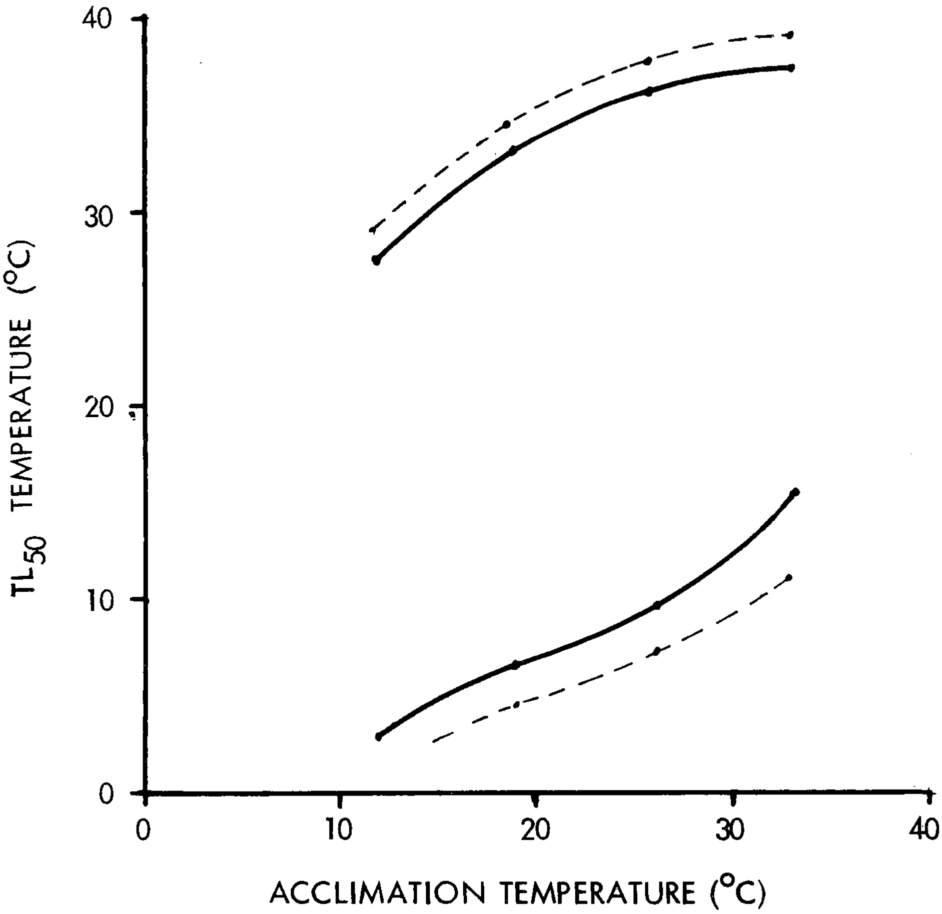

Figure 2. The 1 hour (----) and 96 hour (——) TL_{50} temperatures of juvenile bluegills acclimated to stock temperatures of 12.1°, 19.0°, 26.0° or 32.9°C.

Juveniles acclimated to 32.9C prior to testing had a one hour lower TL_{50} of 10.3 ± 0.20C and a 96-hour TL_{50} of 15.3 ± 0.22C. Fish in one replicate at 36.0C had 70% survival by 24 hours and 15% survival after 96 hours, while fish in the second replicate had no mortality for 96 hours. There is no clear indication why the replicates differed so markedly in survival. The replicate with poor survival was ignored during calculation of upper TL_{50}. The upper TL_{50} after one hour was 39.0 ± 0.23C and decreased to 37.3 ± 0.19C after 96 hours.

As the temperature of acclimation increased, juvenile bluegills showed greater tolerance of high temperatures and reduced tolerance of low temperatures. Figure 2 indicates the relationship between one hour and 96-hour TL_{50} temperatures and temperature of acclimation. The TL_{50} temperatures for 24, 48 and 72 hour exposures were between these values. Within a given stock, the high-temperature TL_{50} temperature decreased and the lower TL_{50} increased with greater exposure duration.

SECTION VI

DISCUSSION

Incubation Tests

The percent hatch of bluegill eggs at each temperature, and the temperature for optimal embryonic development varied between experiments, but differences between replicates were greater than differences between tests. Genetic differences among parents, if they existed, were masked by the larger variation within and between experiments and the procedure of pooling gametes from several parents for each test. Differences in hatching rate between replicates decreased in the first test relative to the preliminary test and in the second experiment relative to the first, suggesting that the source of variability was controlled in the latter tests.

The relationship between temperature and incidence of abnormal fry was not clearly defined. Dead and abnormal fry were less abundant during the second incubation experiment than during the first experiment (Fig. 1). In the first experiment, abnormal fry occurred at both intermediate and extreme temperatures. During the second experiment, dead and abnormal fry were absent at temperatures where total hatch was less than 10% or at 28 to 30C. Differences in the incidence of abnormal fry and in optimal temperature between experiments were probably due to variables within the experimental design.

The dosage and timing of carp pituitary injections, and the time selected for egg removal affect egg viability (Pickford and Atz, 1957) and hence may affect the observed temperature tolerance. Variations in water quality may also have contributed to the observed differences. The second incubation test was conducted two weeks after the first test, when water in the thermal testing facility was conditioned by previous test animals. Concentrations of cadmium and copper were above safe limits (Table 3). Cadmium is acutely toxic to bluegills at 1.94 ppm (Pickering and Henderson, 1965) and copper is acutely toxic at 1.25 ppm (Cairns and Scheier, 1968) after 96-hour exposure. The chronic toxicity of cadmium and copper for bluegills is not known. Chronic toxicity tests of cadmium with the fathead minnow indicate a maximum acceptable toxicant concentration between 37 and 57 μg/l (Pickering and Gast, 1972). Chronic toxicity tests of copper with brook trout indicate a maximum acceptable toxicant concentration between 9.5 and 17.4 μg/l (McKim and Benoit, 1971). Assuming that these values are similar to values for bluegills, the level of cadmium in our water supply is marginally acceptable while the level of copper could have had an effect on egg survival. Oxygen was super-saturated in some parts of the system, but there was no evidence of gas embolism. Ammonia was present at levels that cause gill damage in some fish (Burrows, 1964) and reduced growth in others (Kawamoto, 1961).

Parent bluegills used in the two tests differed in thermal history. Adult fish which supplied eggs for the second incubation test were held longer under laboratory conditions and maintained at a more constant temperature than were the fish for the first incubation test.

During the incubation tests, several females and males were stripped simultaneously. Attempts were made to distribute eggs uniformly on plastic squares, but lack of a completely random distribution was a source of error. Distribution of eggs was more uniform when fewer donors were used, as in experiment 2.

The TL_{50} values observed in these tests correspond with the spawning temperatures observed in natural populations. Adult bluegills in laboratory aquaria spawned at temperatures from 22C to 30C (Appendix A), which corresponds to the temperature range for optimal hatch during the incubation tests. In the field, L. macrochirus spawns at temperatures from 20 to 30C (Breder, 1936; James, 1946; Morgan, 1951; Miller, 1963; and Clugston, 1966). The upper TL_{50} for bluegill eggs exceeds the temperatures at which spawning normally occurs, while the lower TL_{50} corresponds with the lower temperature limit for spawning observed in the laboratory.

Juvenile Bioassay

Juvenile bluegills are more mobile than larvae and may range over the same environments as those preferred by adults. However, juvenile bluegills frequently inhabit shallow inshore waters where food is abundant and predators are less effective. Juveniles might be expected to have thermal tolerance limits that are the same as or greater than adults.

Iezzi, Filson, and Myers (1952) indicated that the rate of temperature change and the duration of exposure to high temperature have significant effects on observed TL_{50} values for juvenile bluegills, especially during short-term bioassays. In the experiments conducted at Aquatic Sciences, Inc., the one hour TL_{50} temperatures for animals acclimated to 32.9C and transferreddirectly to test temperatures were within 0.7C of the maximum sublethal temperature following a 1-hr exposure, as determined by Iezzi, et al. (1952), for animals acclimated to 32.2C and tempered at 3.3C/min. Cairns (1956) has shown that if bluegills are tempered to test temperatures at 2C/day, they can tolerate temperatures up to 39.2C (80% survival after one day).

During the experiments conducted at Aquatic Sciences, Inc. juvenile bluegills that were obtained during the summer from Richloam, Georgia and acclimated to 19.0C had an upper 24-hr TL_{50} of 33.0C. These fish were less sensitive to high temperature than juvenile bluegills from Welaka, Florida that were acclimated to 20C (Hart, 1952). Lower TL_{50} for the fish tested at Aquatic Sciences were similar to those determined by Hart (1952) at all acclimation temperatures.

Differences in acclimation, photoperiod (Roberts, 1967) or size are probably the most important factors that influence temperature tolerance. Hart (1952) did not indicate the rate at which fish were tempered to acclimation temperatures, but acclimation generally required 3 or 4 days. In the experiments conducted at Aquatic Sciences, fish were adjusted to acclimation temperature at 0.5C/day and maintained at that temperature for 8 days prior to testing, so that acclimation lasted from two to six weeks. Increased acclimation time may explain the apparent increase in temperature tolerance of bluegills collected from Richloam, Georgia.

Seasonal changes in temperature tolerance of fishes result in increased tolerance of high temperature during summer months and decreased tolerance of high temperature during winter (Fry, 1947). The experiments by Hart (1952) were conducted with fish collected and tested during the winter months at Welaka, Florida. The fish tested at Aquatic Sciences were collected in the late summer and tested during early fall.

Hart (1952) observed an apparent increase in thermal tolerance with increasing size. The fish used in our experiments were considerably smaller, and yet showed a greater tolerance of high temperature than the fish tested by Hart (1952). Subsequent tests (Appendix D) suggested that small bluegills were less resistant than large bluegills to low temperature stress.

Water quality and life support conditions during our experiments were less than optimal. The concentrations of cadmium and copper in our water supply may have had adverse effects on juveniles. Ammonia at the levels measured in our system has been claimed to have adverse effects on the growth and survival of other fish (Burrows, 1964; Kawamoto, 1961).

Fish densities of 10 animals per 0.96 liter culture basket were shown to have adverse effects on survival of juveniles at high temperatures when tested for 16 days (Appendix B). This crowding level probably had little effect on survival of bluegill during the bioassay test, but may have significantly reduced growth and survival during the growth test (Appendix D). Larger culture baskets should be used for the determination of growth and long-term survival. A density of 10 animals per basket was necessary to obtain sufficient experimental animals to allow for natural mortality and sampling during the growth study.

SECTION VII

ACKNOWLEDGMENTS

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SECTION VIII

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SECTION IX

GLOSSARY

acclimation - compensatory changes occurring in an organism in response to variation of a single environmental factor (usually in the laboratory).

acclimatization - compensatory changes that occur in an organism undergoing multiple natural changes in its climatic, physical and biotic environment.

adaptation - any alteration or response of an organism which favors survival in a changed environment.

autotrophic - needing only inorganic compounds for nutrition.

biomass - the weight of living matter.

catheter - tubular device for insertion into canals, vessels or body cavities to permit injection or withdrawal of materials.

95% confidence limits - a range of values surrounding a sample mean within which 95% of future measurements will fall.

culture basket (protector) - 0.96 liter cylindrical polystyrene vessel. Openings on the side of the basket are covered with 253 micron mesh screen to permit passage of water.

culture chamber - a 75.8 liter fiberglass container, filled with water and fitted with brackets to support eight culture baskets.

electro-shocking - a technique used in collecting fish by which fish are stunned with an electric current and netted from the water.

endogenous food source nutrient source obtained by metabolizing reserve energy sources within the body.

exogenous food source - food obtained from outside the body.

food conversion - the ratio of increase in dry body weight of an organism to the dry weight of food consumed.

gametogenesis - the process by which egg and sperm cells are produced within the body.

gonadosomatic index (GSI) - the ratio of gonadal weight to whole body weight, an index of gonadal maturity.

goodness-of-fit - a statistical test to determine the degree to which a given set of data approximates some hypothetical distribution, such as a straight line.

incubation - the period of egg development between fertilization and egg hatching.

induced spawning - manipulation of external and/or internal environmental factors to cause fish to spawn.

instantaneous biomass change - the difference between rates of instantaneous growth and instantaneous mortality.

instantanteous growth (g) - defined as the natural logarithm of the ratio of final weight over initial weight divided by the time period. $g = [Ln(W_t/W_o)]/t$, where Ln = natural logarithm. W_t = weight after time t and W_o = initial weight.

instantaneous mortality (i) - defined as the natural logarithm of the ratio of the number of surviving animals over initial number of animals, divided by the time period. $i = [Ln(N_t/N_o)]/t$, where N_t = number of surviving animals after time, t and N_o = initial number of animals.

intramuscular within the muscle tissue.

intraperitoneal - within the peritoneum or internal body cavity.

juvenile a stage in the life history of an animal when morphological characters of an adult are present but it is not sexually mature.

mature eggs - refers to eggs that are capable of fertilization to produce viable offspring.

metabolic rate the rate of energy expenditure of an animal, usually expressed per unit weight.

method of least squares - a method of fitting a curve to a set of data so that the sum of the squares of the distance of the points from the curve is minimized.

nauplius - the first free-living stage in the development of some crustaceans, e.g., anostracans, cladocerans, barnacles.

pituitary - endocrine gland beneath the floor of the brain of vertebrates. Anterior lobe controls hormone production by thyroid, gonads, and adrenal glands.

premature eggs - are eggs that have increased in size to a point where they are almost mature but are still not capable of fertilization.

random assignment - placement of animals into an experiment in such a manner as to simulate a chance distribution and hence to produce unbiased statistical data.

relative percent hatch the number of animals that hatch at the optimal temperature is assigned the value 100%. Hatch at the remaining temperatures are assigned as relative proportions of the hatch at the optimal temperature.

respiration - the sum of enzymatic reactions, both oxidative and non-oxidative, by which energy is made available for biological work.

sac-fry - the just-hatched larvae of fishes in which the external yolk-sac is still present.

slope function - expresses the ratio between dosages and mortality. The dosages necessary to produce 84%, 50% and 16% mortality are defined as ED84, ED50 and ED16 respectively. Slope function (S) = $[(ED_{84}/ED_{50}) + (ED_{50}/ED_{16})]/2$.

standard length - the length of fishes measured from the mouth to the end of the caudal peduncle (exclusive of caudal fin).

stock - a group of fish that has been maintained in the laboratory under carefully-defined environmental conditions.

stripping - a method of removing gametes from fish by gently squeezing the abdomen, forcing eggs or sperm out of the gential pore.

swim-up fry - stage in fish development when larvae are first capable of actively swimming. The yolk sac has generally been absorbed by this point.

thermocline - a sharp temperature discontinuity in a fresh water lake, where warm surface waters grade rapidly into cold waters in the deeper areas.

TL_{50} - median tolerance limit. The amount or level of toxicant causing 50% mortality in a population at some specified time, e.g., 96 hours.

ventilation - circulation or movement of fluid medium (air or water) of the external environment across respiratory exchange surfaces.

yolk-sac - sac containing a store of food material that hangs from the ventral surface of vertebrate embryos (elasmobranchs, teleosts, reptiles, birds).

zoogeography - branch of biogeography concerned with the geographical distribution of animals and especially the determination of areas characterized by specific groups of animals.

SECTION X

APPENDICES

APPENDIX A

Development of Spawning Induction Techniques

Phase 1. Effects of External Environment on Gonad Maturation

Procedures

Adult bluegills were collected from canals near State Road 27, nine miles south of Florida City, Florida, on 17 November, 1970, and quarantined in the laboratory for two weeks. Mean GSI values were determined for 5 female fish (0.56%) and four males (0.40%). All fish were sampled, but failed to release gametes. Three conditions were tested for their effects on gonadal maturation:

1) the presence of males on development of ovaries

2) a 16L-8D photoperiod at 25C relative to a 8L-16D photoperiod

3) population density, i.e., one male with one female, versus one male with two females.

Fish were held in 95, 190, or 333 liter aquaria. All fish were fed three times daily with trout chow (Ralston Purina Co.), earthworms (*Lumbricus* sp.) and freshwater shrimp (*Palaemonetes paludosus*). Behavioral observations were made daily. After two months, all females were sacrificed to determine the GSI. Males were stripped to determine whether sperm could be obtained.

Results

Results of this experiment are summarized in Table A-1. Exposure to 16L-8D photoperiod at 25C led to gonadal development and nest digging and increased aggressive behavior among males.

Gonadal development of females was induced in the presence or absence of males. When one male was present with one female, gonadal development of the female was less than with more than one female. When one male was present with two females at 16L-8D photoperiod and 25C, gonads of one female developed (dominant female) while those of the second fish did not. Males maintained at 16L-8D photoperiod and 25C released sperm when stripped, while males held at 8L-16D photoperiod and 22C did not release sperm. Females maintained at 8L-16D photoperiod and 22C showed no increase in GSI.

Discussion

Males reached sexual maturity when maintained in the presence of females at 25C and 16L-8D photoperiod. Optimal maturation of females, however,

Table A-1. Effects of 16L-8D photoperiod at 25C on gonadal maturation of bluegill sunfish, relative to controls that received 8L-16D photoperiod at 22C.

Test Animals						
Test Conditions					Results	
No. Males	No. Females	Tank Size (L)	Photoperiod	Temp. (C)	Females*** (GSI)	Males**** (Sperm Release)
0	2	95	16L-8D	25	3.3 2.0	
1	2	95	16L-8D	25	4.1 0.5	+
1	2	95*	16L-8D	25	1.0**	+
1	1	333	16L-8D	25	1.8	+
1	1	333	16L-8D	25	0.8	+
Controls						
0	2	95	8L-16D	22	0.5 0.4	
1	2	190	8L-16D	22	0.7 0.7	-

* Male separated from the females by an opaque partition.

** The second female released eggs when stripped, and was presumed to have a GSI of about 4.0. This female was saved for further experimentation.

*** GSI = gonadosomatic index = (ovary wt/body wt) x 100.

**** + = released sperm, (-) = failed to release sperm.

might require that females be kept together, away from males, to reduce the effects of social hierarchies and to permit uniform development. Hierarchy formation among females was a factor that needed further study. In addition, there was no indication yet whether spawning could be induced out of season, or how spawning and the formation of hierarchies might be affected by hormonal injections. The following experiments were designed to gather data on these points and extend spawning technology.

Phase 2. Induction of Spawning in the Laboratory

Procedures

Adult female and male bluegills were collected from the field in October, 1971. Thirteen fish sampled from this group included seven females (Mean GSI = 0.27) and six males (GSI = 0.1). Fifty animals were maintained for three weeks in the laboratory with simulated summer environment (16L-8D photoperiod at 27-29C). Twenty-three fish were selected from this group after three weeks and placed in a separate 2.9 kiloliter aquarium with 16L-8D photoperiod at 28C. Six weeks after the initial collection, four fish from the tank were sampled. Two males released sperm when squeezed and were not sacrificed. Two females had GSI values of 0.15 and 0.50.

Males had thus achieved some degree of maturity, but females were undeveloped. For some spring and summer-spawning fishes, spawning may be triggered by a sharp increase in temperature (Prosser and Brown, 1961). Since a sharp increase to perhaps 35 or 36C would probably harm bluegills, the fish were first acclimated to a lower temperature (ambient = 24C) for three weeks and then the temperature was rapidly increased (1C/hr) to 30-32C.

After the temperature was increased (now nine weeks since the fish were collected) the population density in the test aquarium was reduced to 10 fish, 5 males and 5 females. The aquarium substrate was covered with a thin layer of black gravel to make nest-digging activity more conspicuous. In a further attempt to stimulate spawning, six of the ten fish were injected with carp pituitary (1.5 mg) combined with 0.25 cc of Combistrep® antibiotic solution (Pfizer Pharmaceutical Co.). A second injection of carp pituitary and Combistrep® was administered two days later, and a third injection of 0.3 mg of carp pituitary (without Combistrep®) was given two days after the second injection.

Results

On the day following the first injection, one female control fish was observed digging a nest. Three male fish died following the second injection (Mean GSI = 0.22). Two females died following the third injection (GSI = 4.7 and 7.2). A large nest (0.6 m diameter) was dug by one male fish in the aquarium one week after the third injection. Spawning occurred on 28 December 1970, approximately 10.5 weeks after the fish were first collected. Several thousand viable eggs were present in this spawn.

The five fish in the tank now included one dominant male, which maintained the nest and one dominant female (the same fish that dug a nest on the day after the first injection). Nest-digging by centrarchids under natural conditions is generally confined to males; this activity was frequently engaged in by female bluegills in the laboratory, both in heterosexual and homosexual aggregations. Another female fish stayed in a corner of the aquarium near the water surface. Two other fish dug small nests in the corners but did not engage in spawning. Once again, hierarchy was established.

These five remaining fish were maintained on a 16L-8D photoperiod at a mean temperature of 30.1C for four weeks. After four weeks, temperature in the tank was reduced to ambient ($<$ 24C) for one week and again elevated to 29.5C for 10 days. Spawning occurred 6.5 weeks after the first spawn, within 10 days of the temperature increase, and without the aid of hormones.

Discussion

Spawning of bluegills was induced in the laboratory by manipulation of temperature and photoperiod. These spawns may be triggered by cycled temperature. The conditions under which the animals were held were not conducive to egg maturation in more than one female at a time. Eggs were difficult to collect from spawns that occurred in large aquaria, and removal of the adhesive eggs from gravel required more than the ten minutes following fertilization.

The dosages of carp pituitary were lethal to five of the six fish. Three injected males died after the second injection and did not show increased gonadal development. Two females which died following the third injection had nearly mature gonads with GSI values of 4.5 and 7.2.

At this point, it was known that viable eggs were not continually available from a given female. Further experiments were necessary to determine whether simultaneous ripening of several females could be triggered by temperature manipulation. In addition we needed to define the periodicity of the ovarian cycle and to systematically define the course of final egg development on the basis of observable changes in egg appearance. These observations would then enable us to predict when fish were capable of producing ripe eggs so that several fish could be stripped simultaneously.

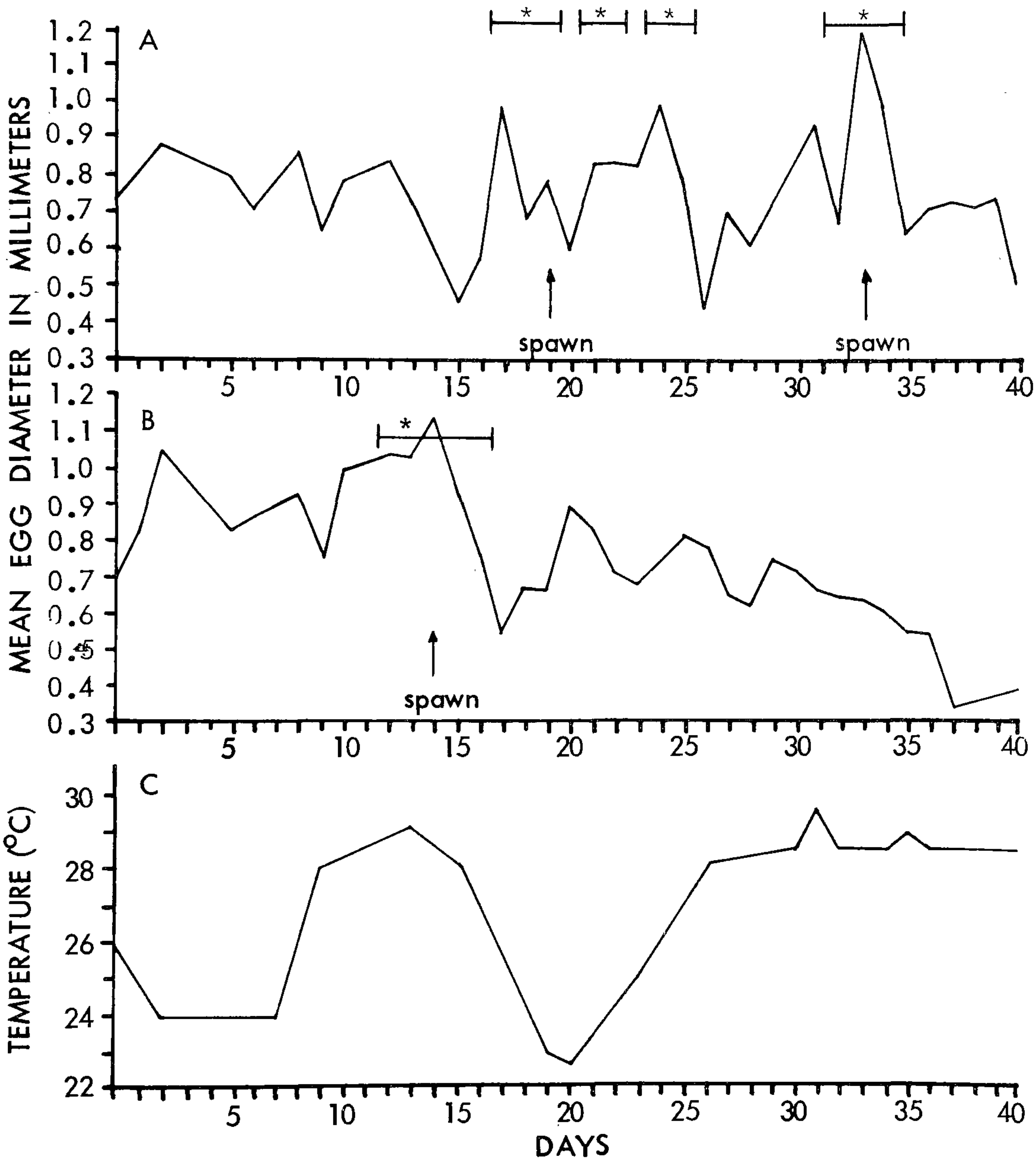


Figure A-1. Mean diameter of eggs sampled each day from two female bluegills (A, B) maintained for forty days in an aquarium with three males at 16L-8D photoperiod and cycled temperature (C). * Oil globules.

Phase 3. Periodicity of the Ovarian Cycle and the Course of Egg Development in Female Bluegills

Procedures

The 2.9 kiloliter aquarium containing five adult bluegills was maintained with 16L-8D photoperiod. Temperature was cycled between 23 and 28.5C for six weeks. The two apparent females were checked every 1-4 days for the release of viable eggs, or sampled for eggs at various stages of development. Females were carefully netted from the tank and handled without the use of anesthetics. Eggs were removed from the genital opening with a catheter and observed under a microscope. Three to four eggs were measured with an ocular micrometer to ± .01 mm and the mean of these values was determined. Eggs were observed for the presence and appearance of an oil globule, coloration, texture, and appearance of the cytoplasm.

Results

Fish spawned three times during the six week experiment (Fig. A-1). Two spawns (on days 14 and 33) occurred within 5 to 7 days of the return to elevated temperature. One spawn, on day 19, occurred during a temperature minimum. Mean egg diameters ranged between 0.34 and 1.22 mm. Lack of a consistent pattern of egg diameter in relation to spawning may reflect inadequate frequency of sampling during periods of rapid development, or small numbers of eggs collected with the catheter and used in the calculation of each mean.

The ovarian cycle, as monitored by egg diameter, appears to be characterized by a series of peaks at intervals of 2 to 11 days. The mean periodicities and standard deviations were 4.11 ± 1.54 days for one fish and 6.35 ± 2.42 days for the second fish. Egg diameter by itself was a poor predictor of spawning activity. Eggs were at least 0.70 mm diameter on the day prior to spawning. In all cases, however, distinct oil globules appeared in the eggs at least two days prior to spawning. The complete cycle, from one spawn to the next, was completed in 14 days.

Discussion

The results of Phase 3 substantiate previous observations (Phases 1 and 2) that, when two females are placed in a tank with one dominant male, one or the other female will spawn, but not both females simultaneously.

From the eggs sampled during these and subsequent experiments, and from animals collected from the field, the cycle of egg development was determined from observed characteristics of the eggs. Immature (prespawning) eggs were less than 0.85 mm in diameter, dark brown, and granular. Premature eggs, which appeared two to three days before spawning, were 0.85-0.92 mm in diameter with central "submerged" or multiple oil globules and had a dense granular appearance. As eggs matured, the oil globules coalesced and migrated to the egg surface, while the egg diameter generally increased to 0.92-1.10 mm. Mature eggs (capable of fertilization) were slightly granular, perfectly spherical and strongly adherent to surfaces. Mature eggs ranged from 0.85-1.36 mm diameter. Eggs became over-ripe within 24 hours after becoming mature. Over-ripe eggs were completely clear and non-spherical.

Cycled temperature may be conducive to spawning, but a sharp rise in temperature does not in itself trigger a spawn. Controlled temperature alone could not be used to produce several ripe females simultaneously.

Phase 4. Use of Hormone Injections to Induce Egg Maturation in Female Bluegill Sunfish

Human chorionic gonadotropin (HCG) has been used to induce ovulation in bluegills during the spawning season (Pennell, 1964) and carp pituitary glands (CP) have been used to induce ovulation and spawning in a variety of fish (Pickford and Atz, 1957). Daily injections of HCG and carp pituitary at various dosage levels, were tested for their effects on egg development. In most fish, levels of HCG and CP were increased with time, following the recommendation of Garland Pardue, Auburn University (personal communication). Results from injected fish were compared with a control fish (uninjected) and a fish that received 100 IU of HCG throughout the experiment.

Procedures

Hormones and pituitary gland suspensions were injected into bluegills with 1 ml plastic syringes and 24 gauge needles. Injections were given intraperitoneally. Fish in each experimental group were marked by injection of approximately 0.1 ml of India ink beneath the dermis.

Human chorionic gonadotropin (HCG) was obtained from Sigma Chemical Co., St. Louis, Mo. HCG was suspended in fish Ringer's solution (1000 IU/ml) Lyophilized carp pituitary glands (CP) were purchased from Stoller Fish Industries, Ames, Iowa, and were suspended (10 mg/ml) in fish Ringer's solution. Injections were administered each day for 27 to 31 days. Egg development of each fish was checked daily with a catheter. Adult bluegills (150-350 gm) were maintained in 2.9 kiloliter aquaria with a 16L-8D photoperiod and temperature was cycled between 23 and 29C. The following series of injections were administered intraperitoneally:

Carp pituitary

1) 1 mg/day for nine days, 2 mg/day for eight days, 4 mg/day for 14 days
2) 1 mg/day for seven days, 2 mg/day for 23 days

HCG

1) 100 IU/day for 27 days
2) 100 IU/day for 3 days
3) 100 IU/day for 5 days, 300 IU/day for 4 days,

500 IU/day for 1 day, 800 IU/day for 5 days,
900 IU/day for 1 day, 1000 IU/day for 7 days

Carp pituitary followed by HCG

1) 1 mg CP for 7 days, 2 mg CP for 16 days,
400 IU HCG for 8 days

Control fish (not injected)

Results

The mean diameter of eggs sampled from all fish ranged from 0.4 to 1.10 mm during the experiment (Fig. A-2). Injection of 1 mg CP/day for 7 days followed by 2 mg CP/day for 23 days induced production of premature eggs in one fish. This fish produced eggs above 0.70 mm in mean diameter for 70.4% of the test period and oil globules were present for 55.6% of the test period. The fish that received increasing dosages of HCG released eggs that were larger than 0.70 mm during 75% of the test period (100 IU HCG followed by 200 IU) and 71.4% of the test period (100 IU to 1000 IU in increasing dosages), but none of these eggs developed oil globules. The fish that received 100 IU of HCG/day throughout the experiment had eggs above 0.70 mm for 67.9% of the test period and these eggs had oil globules present on two days (7.1%). The fish that received 1, 2 and 4 mg of CP failed to release eggs through much of the experiment and the eggs did not develop oil globules. The fish that was injected with CP followed by HCG maintained eggs above 0.70 mm for 75.9% of the time. Oil globules were present in the eggs 34.4% of the time, but oil globules were not seen after the fish received injections of HCG.

Discussion

Previous research (Phases 2, 3) established that eggs were 0.7 mm or larger in mean diameter on the day prior to spawning, while the smallest fertilized eggs that were collected from nests were 0.85 mm in diameter. Oil globules were present in the eggs for two to four days before spawning occurred. These criteria could thus be used to evaluate the effects of hormone injections on egg maturation.

Since eggs within the ovary do not develop uniformly, a sample with a mean diameter of 0.70 generally contains a mixture of eggs ranging from 0.85 mm down to 0.60 in diameter. Fish were considered premature when the diameter of more than half of the eggs in a sample exceeded 0.85 mm.

Hormone injections of 1 mg CP followed by 2 mg CP were best suited to the induction of egg maturation, but this alone did not induce complete maturation. Injections of HCG led to an increase in egg size but were not conducive to the formation of oil globules. In fact, when HCG injections were administered after CP, oil globules diminished.

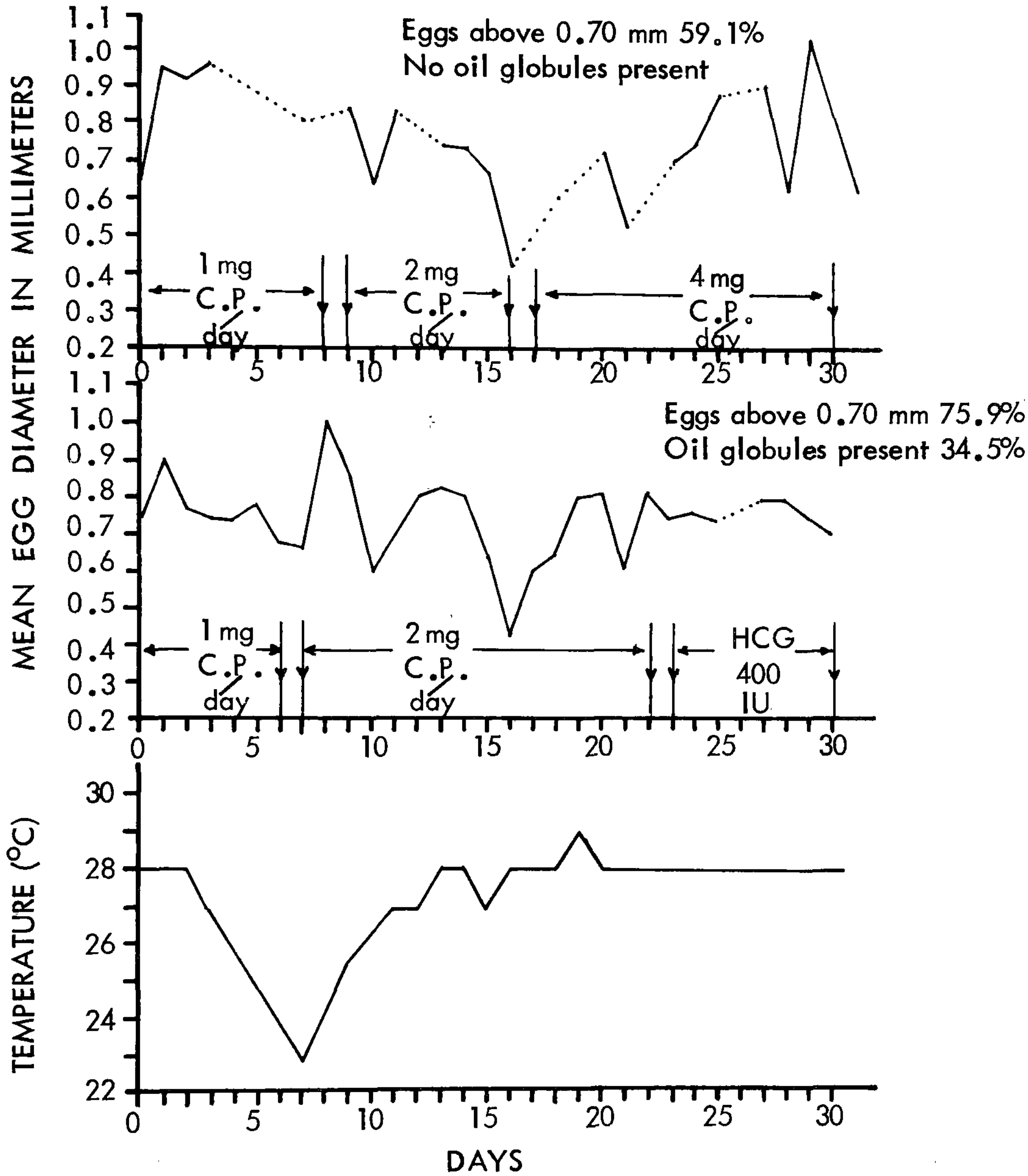


Figure A-2. Mean egg diameter of samples from female bluegills injected (↓) daily with carp pituitary (C.P.) or human chorionic gonadotropin (HCG). Fish were maintained in a 1.9 kiloliter aquarium, with a 16L-8D photoperiod, at the indicated temperature cycle for 30 days.

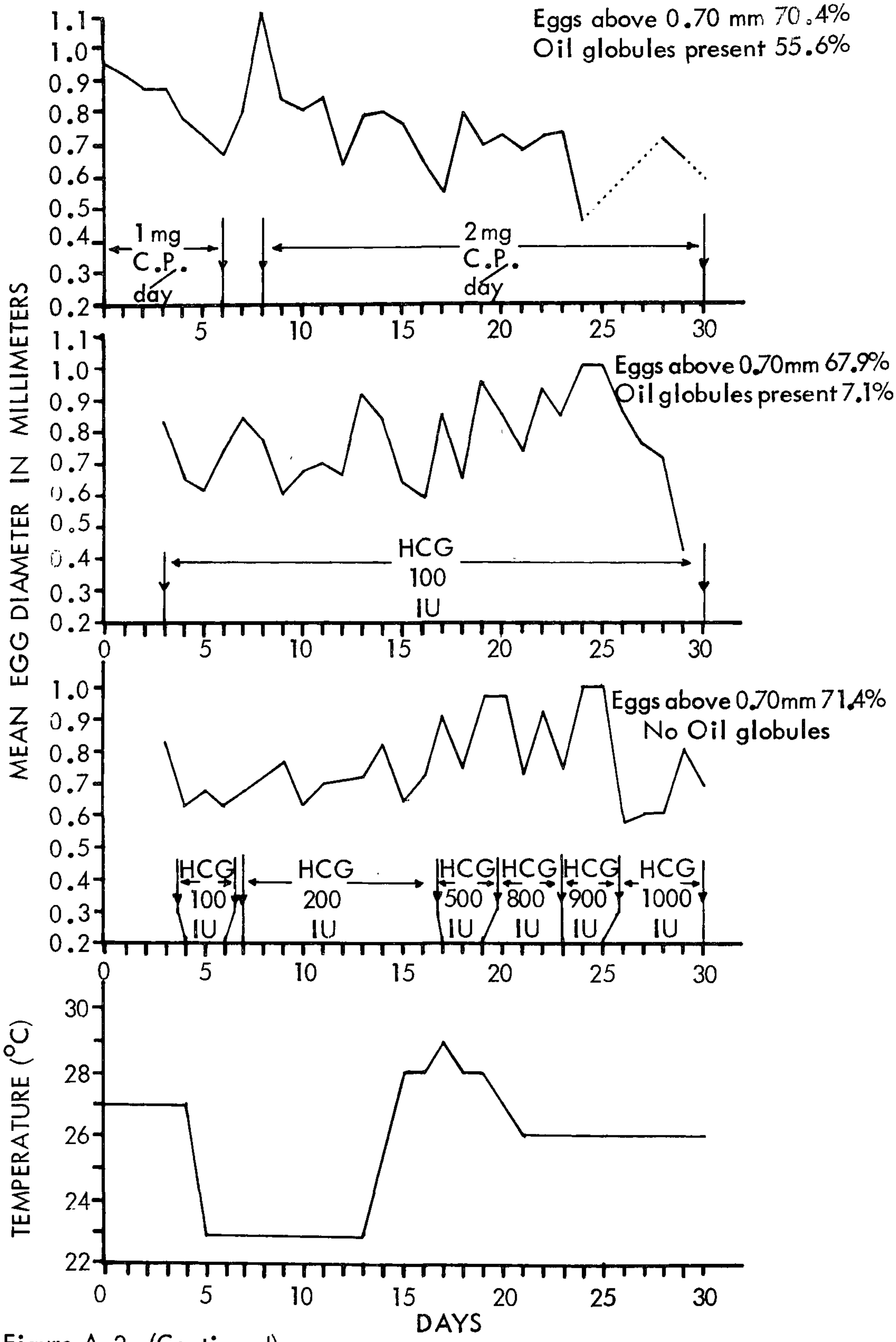


Figure A-2. (Continued)

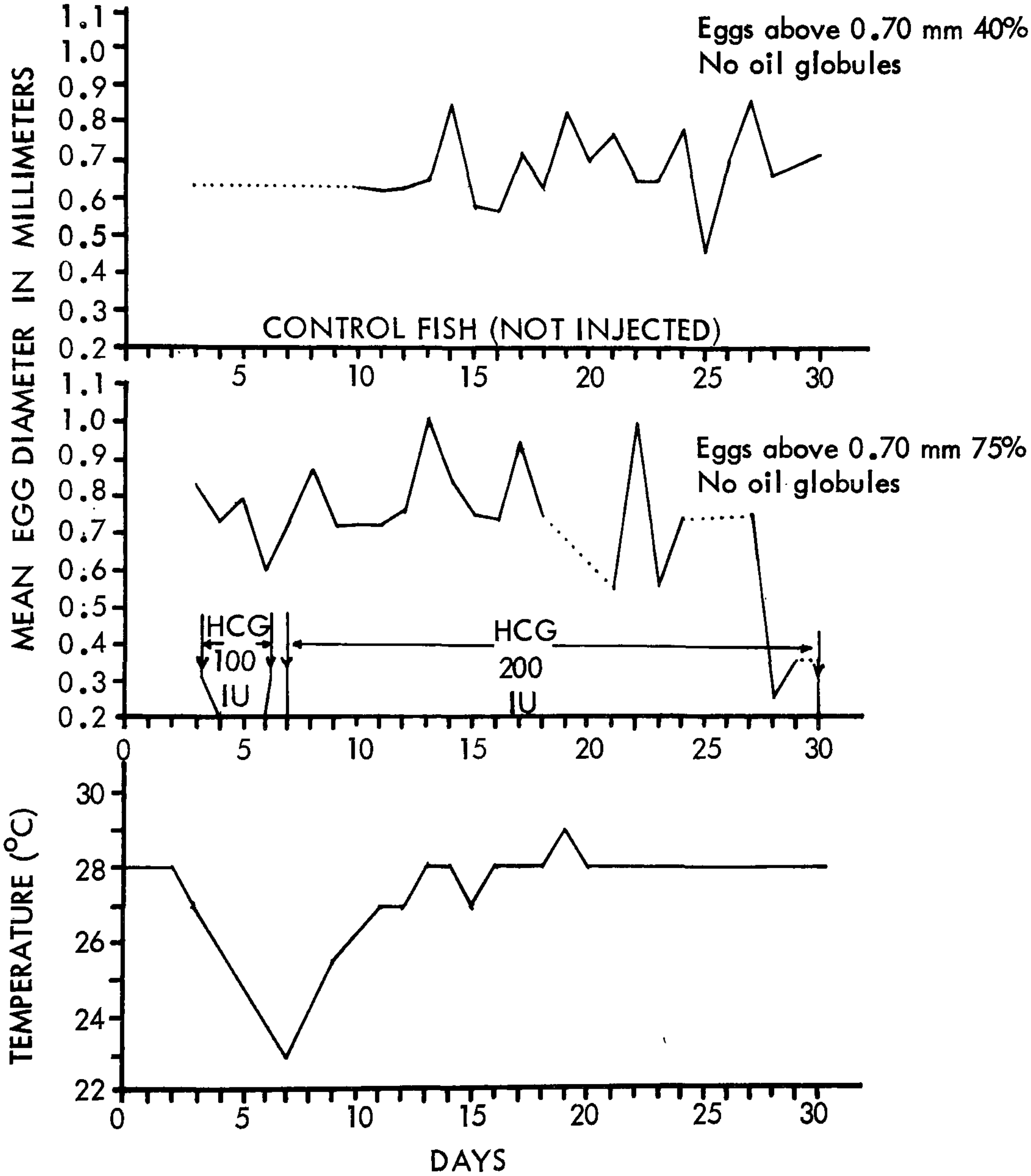


Figure A-2. (Continued)

Further experiments were still needed to refine the use of hormone and pituitary injections for inducing gonadal ripening and to develop reliable methods for inducing the final stages of egg maturation. Also, fish used in previous experiments spawned at temperatures from 23 to 30C, but the optimum temperature conditions were still not defined.

Phase 5. Effects of Carp Pituitary, HCG, and Manipulation of Temperature on the Development of Premature Eggs

Procedures

Twelve female bluegills were placed in each of five 2.9 kiloliter aquaria with 16L-8D photoperiod. Fish were given a cycled temperature (20-28C) or were held at relatively constant temperature within the following ranges: 27-31C, 27-29C, 28-30C, and 23-26C. Injections of carp pituitary (1, 2 or 4 mg), HCG (400 IU, or fish Ringer's solution (0.8 cc, sham) were given once every two days. Uninjected controls were also held in each tank. Eggs were sampled daily and fertilization was attempted with all mature eggs.

Results

Results of this experiment are summarized in Table A-2. No mature eggs were obtained from any of the fish during this experiment. Fish in all experimental and control groups developed premature eggs with oil globules, and fish that received 2 mg carp pituitary per day maintained premature eggs longer than did the remaining fish. HCG, once again, induced eggs to increase in size but was not conducive to oil globule formation. Fish held at fairly constant temperatures (26-29C) maintained premature eggs and eggs with oil globules for longer than did fish at lower or higher temperatures or fish that were exposed to a temperature cycle.

Discussion

A temperature cycle was not necessary to induce egg maturation, at least to the premature stage. The optimal temperature for egg maturation was 26-29C. Injections of 2 mg CP/day produced better egg development than injections of 4 mg/day or injections of 400 IU/day of HCG. Injections of 2 mg every other day was less damaging to the fish than were the daily injections used in Phase 3.

Tests were still needed to determine reliable techniques for inducing the final phases of egg maturation.

Table A-2. Effects of bi-daily hormone injections on egg production by female <u>L. macrochirus</u>. Two fish were given each injection (see text for code), a sham injection (0.8 ml Ringer's), or were uninjected (control) and observed daily for 20 days. The percentage of observations was recorded in which eggs with oil globules (A) or eggs that were 0.85 mm or greater in diameter (B) were obtained.

Temperature Range (C)	Egg Condition	HCG	CP 2 mg	CP 4 mg	Sham	Control	Mean Percent
27-31	A	35.0	32.5	5.0	2.5	17.5	18.5
26-29	B	32.5	57.5	30.0	5.0	37.5	32.5
26-29	A	12.5	57.5	32.5	2.5	0	31.0
	B	72.5	92.5	52.5	32.5	97.5	69.5
20-28	A	30.0	55.0	40.0	10.0	10.0	29.0
	B	77.5	67.5	80.0	47.5	62.5	67.0
28-30	A	12.5	45.0	40.0	12.5	12.5	24.5
	B	52.5	60.0	57.5	40.0	37.5	49.5
23-26	A	27.5	15.0	15.0	10.0	10.0	15.5
	B	75.5	47.5	47.5	65.0	31.0	53.2
Mean Percent	A	23.5	41.0	26.5	7.5	10.0	33.7
	B	62.0	65.0	53.5	38.0	53.2	54.3

Phase 6. Induction of Ovulation by Injections of Carp Pituitary

Procedures

One female, held in the laboratory at 23C, had large (0.87 mm), premature eggs. This fish was given three intramuscular injections with 1.0 mg carp pituitary at 0800, 1200 and 1700 for one day in an attempt to induce development of mature eggs. At each injection, eggs were sampled and mixed with sperm.

Additional females were held at 26C and given bi-daily intraperitoneal injections of 2 mg carp pituitary to induce development of premature eggs. Six females with premature eggs were given the following intramuscular injections of carp pituitary (two fish per type of injection): a) 1 mg every four hours; b) 1 mg every eight hours; and c) 2 mg every eight hours. One of the females in each regime was given 1000 units of Penicillin G with each carp pituitary injection to reduce bacterial infection. Eggs were sampled prior to each injection. Percent fertilization, when fertilization was attempted, was recorded after one hour. Series a) thru c) were replicated. After females produced fertile eggs, they continued to be sampled at two-hour intervals to determine the length of time that eggs remained viable.

Results

The single female, injected at 0800, 1200 and 1700, ovulated, producing mature eggs within 30 hours of the first injection. The hatch from these eggs was 92%. All females injected with 1 mg/4 hours (Fig. A-3) produced mature eggs within 26-44 hours (from 7 to 11 injections). Viable eggs were obtained for 8 to 16 hours after ovulation.

Carp pituitary injections of 1 mg/8 hr (Fig. A-4) produced mature eggs in 3 of 4 females. Eggs matured within 32 to 48 hours following the first injection and remained viable for up to 20 hours. Greater than 50% hatch was obtained from eggs released during the first six hours.

Injection of 2 mg carp pituitary/8 hr (Fig. A-5) induced the production of mature eggs in 3 of 4 females within 24 to 36 hours after the first injection. The eggs remained fertile for 12 to 18 hours. Percent hatch was greater than 50% for eggs released during the first six hours.

Discussion

The final stages of egg maturation could thus be induced by frequent intramuscular injections of carp pituitary. Eggs ripened in 24 to 44 hours after the first injection. Adults did not survive more than two days after they were injected and stripped. Fish secreted excessive mucous. The animals developed ulcerated lesions at the injection sites, perhaps due to secondary bacterial infection or perhaps as an allergic reaction to carp pituitary. In any case, concomitant injection of antibiotics did not improve survival.

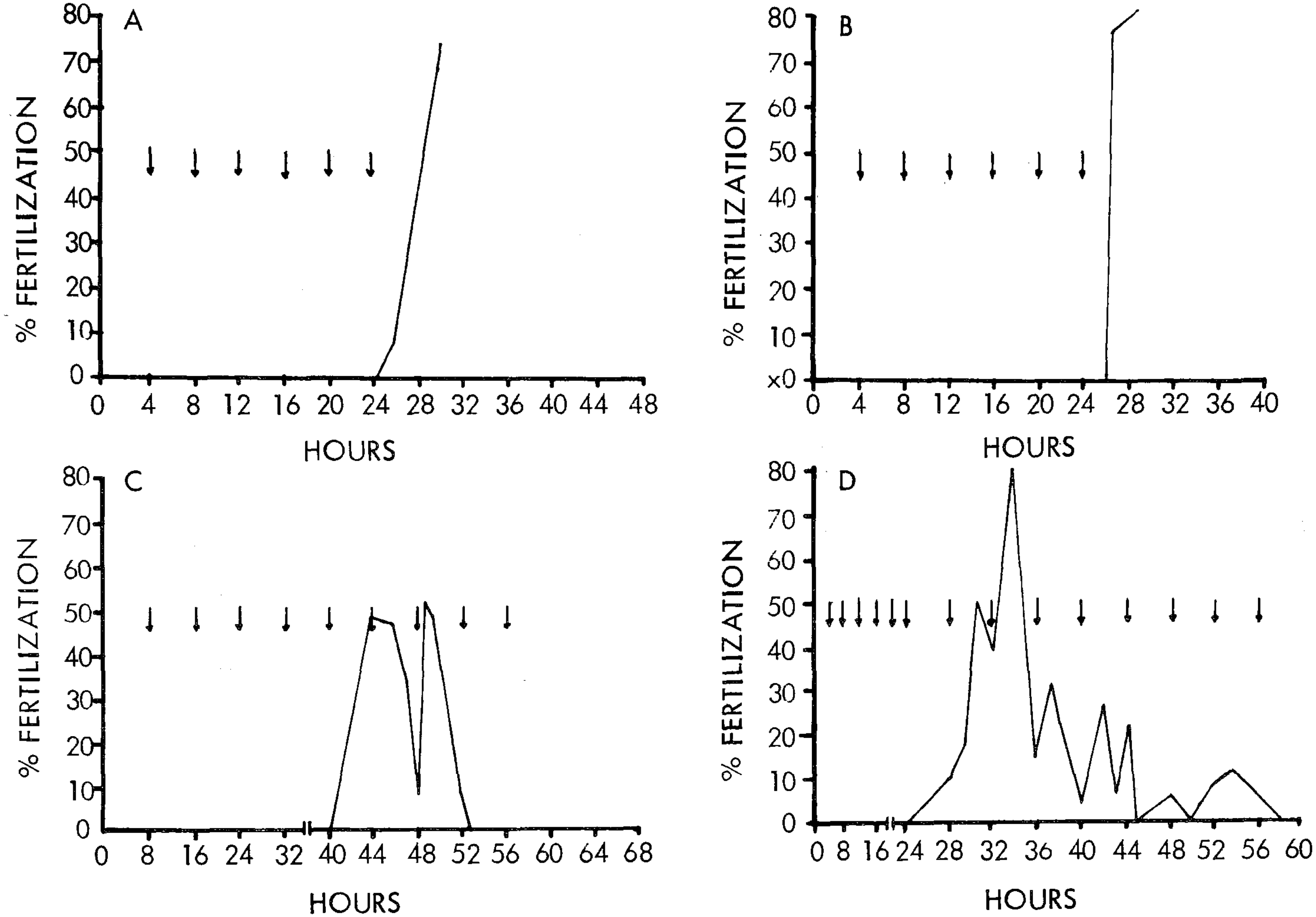
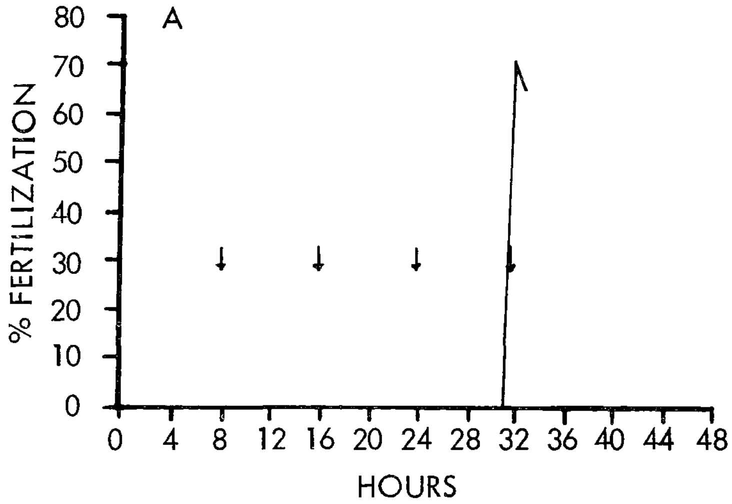


Figure A-3. Percent fertilization (——) of eggs obtained from four female *Lepomis macrochirus* injected (↓) with carp pituitary (1 mg/4 hrs.). Female A also received 1000 IU of Penicillin-G with each injection.



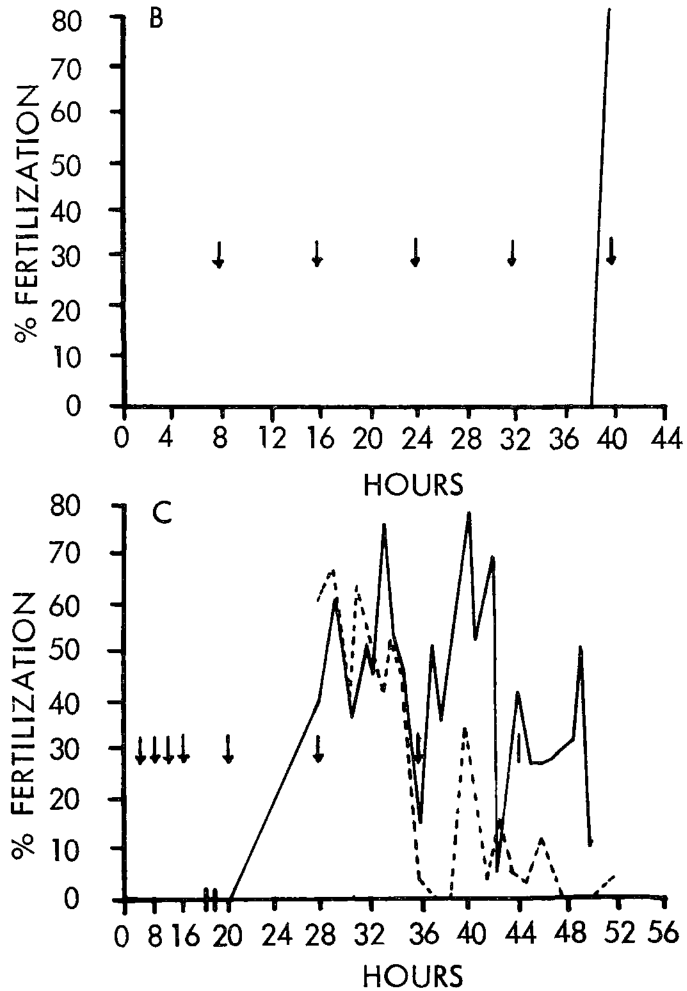
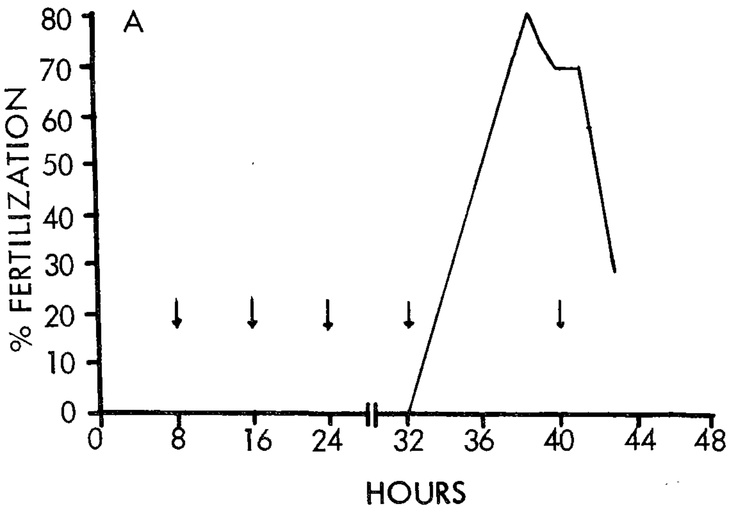
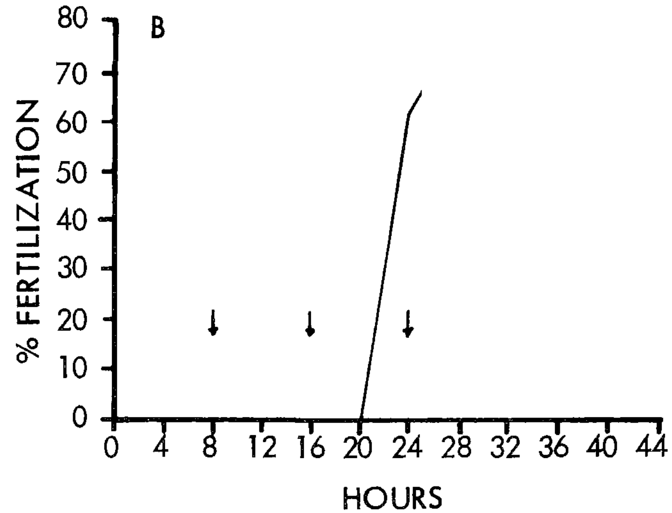


Figure A-4. Percentages of fertilization (——) and hatch (- - -) of eggs from three female Lepomis macrochirus injected (↓) with carp pituitary (1.0 mg/ 8 hrs.). Female B received 1000 IU of Penicillin-$\bar{G}$ with each injection. One female in this group (not shown) failed to produce eggs.





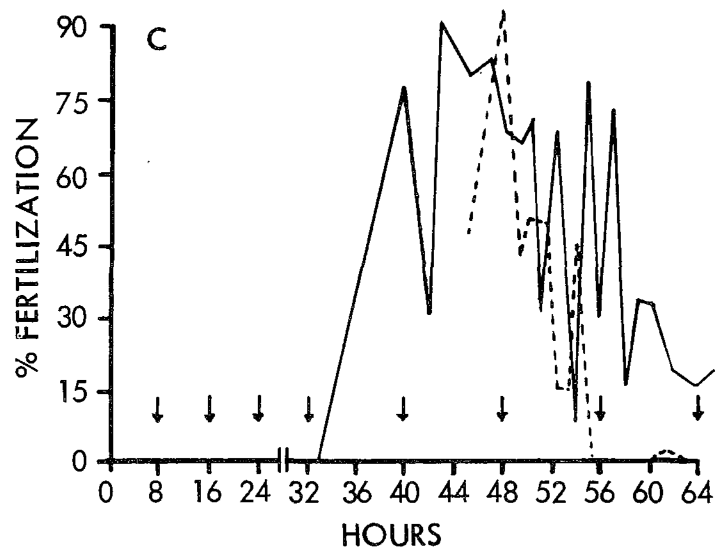


Figure A-5. Percentages of fertilization (——) and hatch (- - -) of eggs from three female Lepomis macrochirus injected (↓) with carp pituitary (2 mg/8 hrs.). Female B received 1000 IU of Penicillin-G with each injection. One female (not shown) failed to produce eggs.

The time needed to obtain fertile eggs ranged from 24 to 40 hours after injection. This indicated that considerably more than 4 animals should be selected in order to have several females with fertilizable eggs available simultaneously. Depending upon the number of fish available with mature eggs, 20-33 females were injected to ripen 3 to 7 females simultaneously.

A reliable technique was now available for obtaining mature eggs from several female bluegills simultaneously. The final procedure consists of six steps.

1) Fish collected from the field are returned to the laboratory, acclimated to captivity, and sexed as well as possible on the basis of morphology, coloration, and behavior.

2) Males are kept at 16L-8D photoperiod at 26C, with as many as 20 fish per 2.9 kiloliter aquarium. Ripening requires up to two months for fish collected outside the spawning season.

3) Females are kept isolated from males and given a 16L-8D photoperiod at a temperature of 26-28C. The production of premature eggs may require up to three months.

4) Females are injected intraperitoneally with 2 mg carp pituitary every other day, and egg development is checked daily.

5) When a sufficient number of females have developed premature eggs, 20-30 such females are selected and injected intramuscularly with 1 mg carp pituitary every four hours. Eggs are sampled at each injection interval and examined for size, appearance, adhesiveness, and fertilization-ability until several females have mature eggs simultaneously.

6) Females producing eggs with greater than 50% fertilization at the previous sampling interval are stripped and eggs are fertilized with milt from four or more males.

APPENDIX B

Rearing and Life Support

Growth and survival of young bluegills were determined under a variety of culture conditions. Studies were conducted with eggs spawned in the laboratory. Juveniles were collected in the field near Florida City, Florida or in Conservation Area 2A of the Florida Flood Control District or were obtained from Federal Fish Hatcheries at Welaka, Florida and Richloam, Georgia.

Phase 1. Air Supply, Water Exchange, Feeding and Temperature

Procedures

Sac-fry were obtained from eggs spawned in the laboratory at 23C. Rearing tests were conducted for 15 days in 20.3 cm diameter culture bowls; each treatment had two replicates. Combinations of the following treatments were tested: aeration (through 2.5 cm-long airstones) versus no aeration; 10% water change daily versus 60% water change daily; food (newly-hatched *Artemia salina* nauplii) offered at time of swim-up stage versus food offered two days later; temperature of 20C, 23C (ambient), or 25C.

Results

The best conditions for survival of *L. macrochirus* fry (Table B-1) were: 1) absence of a supplementary air supply, 2) 10% water change daily, 3) ambient temperature (23C), and 4) introduction of food two days after swim-up stage.

Phase 2. Diet and Temperature

Procedures

Eggs were collected from a natural spawn in the laboratory at 26C and tempered at 8C/hour to temperatures of 15, 20, 25, and 30C. Sac-fry were maintained at these temperatures and fed at 0800, 1200, and 1600 daily when they reached swim-up stage. Survival at the various temperature levels was compared using two diets: 1) nauplii of *Artemia salina* (360 microns by 135 microns); and 2) a mixed diet of *Artemia* nauplii, cultured rotifers, and limno-plankton (65-270 micron fraction) collected from the field.

Results

Larvae that were fed a mixed diet of rotifers, *Artemia*, and limnoplankton died within two days at 25C and within three days at 20C (Fig. B-1). In contrast, more than 90% of the larvae that were fed only *Artemia* at these temperatures survived after eight days. At 15 and 30C,

Table B-1. Percent survival of L. macrochirus sac-fry after 15 days. Fry were reared in 20.3 cm bowls with aeration (A); no aeration (NA); 10% (10WC) or 60% (60WC) water changes daily at 20, 23, or 25C. Animals were fed at time of swim-up stage (FO) or two days later (F2).

Primary Variable	Other Conditions	No. Fry Tested	Percentage Survival
NA	10WC, FO, 20C	600	16.9
A	10WC, FO, 20C	600	3.6
FO	NA, 10WC, 23C	150	19.5
F2	NA, 10WC, 23C	150	24.0
10WC	NA, FO, 23C	300	21.5
60WC	NA, FO, 23C	300	12.0
20C	NA, FO, 10WC	100	21.0
23C	NA, FO, 10WC	100	42.0
25C	NA, FO, 10WC	100	3.0

Combined Variables V1	V2	Other Conditions	No. Fry Tested	Percentage Survival
FO	20	NA, 10WC	100	21.0
	23	NA, 10WC	100	42.0
	25	NA, 10WC	100	3.0
F2	20	NA, 10WC	50	22.0
	23	NA, 10WC	50	46.0
	25	NA, 10WC	50	19.5
10WC	FO	NA, 23C	150	46.0
	F2	NA, 23C	50	12.0
60WC	FO	NA, 23C	150	32.0
	F2	NA, 23C	50	

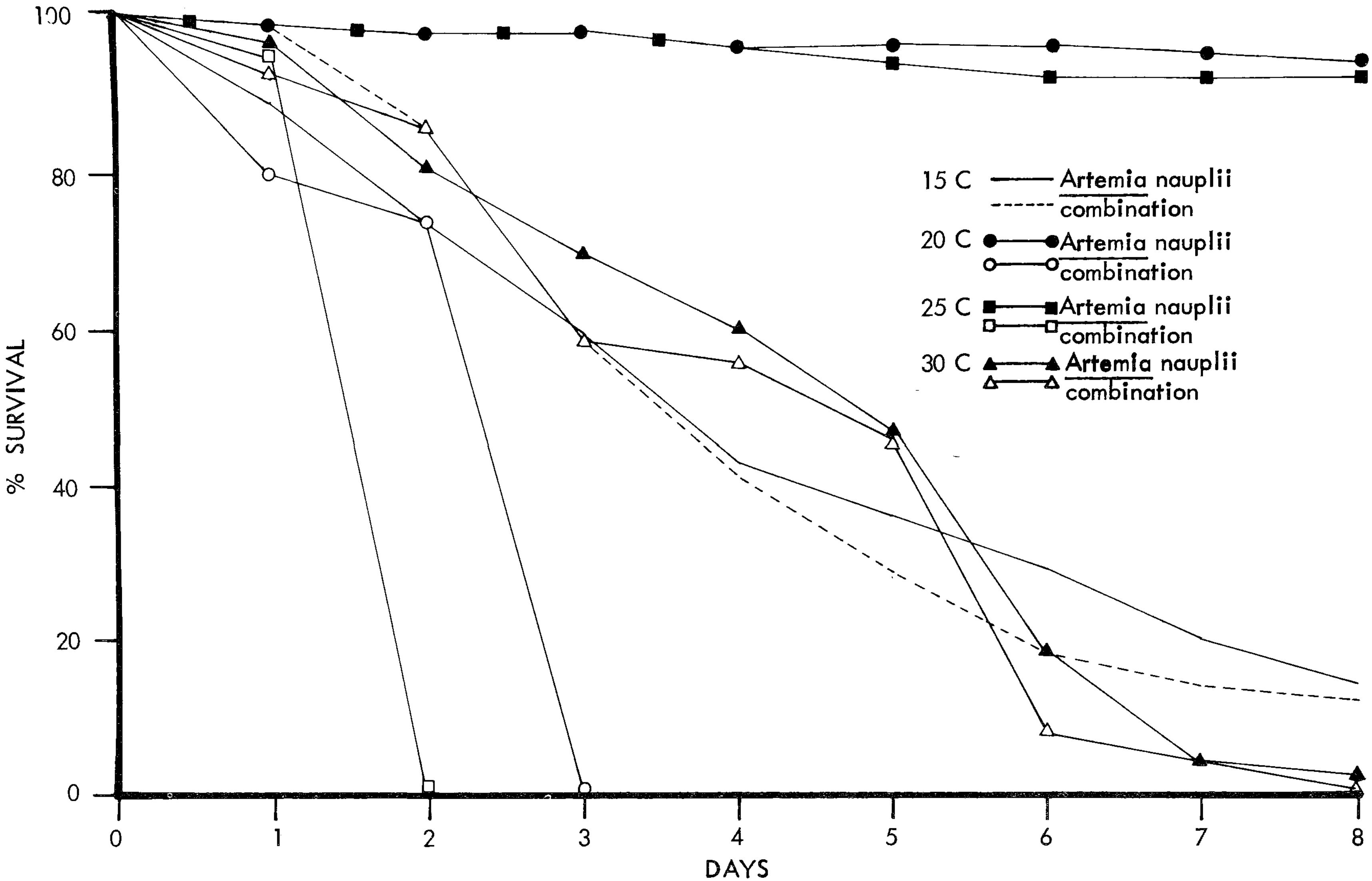


Figure B-1. Survival of bluegill sac-fry at four temperatures. Diets consisted of mixed *Artemia* nauplii, limnoplankton, and rotifers (combination) or *Artemia* nauplii.

survival of larvae that were fed *Artemia* alone was not significantly greater than survival of larvae that were fed the mixed diet.

Phase 3. Population Density

Procedures

Population densities of 5, 10, 15, and 20 juvenile bluegills per 0.96 liter culture basket were evaluated to determine acceptable densities for maintenance of animals during the bioassay tests. Juvenile bluegills ranging from 2.5 to 5 cm in total length were obtained in February, 1971, from the Federal Hatchery at Richloam, Georgia. Fish were placed in culture baskets and transferred directly from 27C to aquaria maintained at temperatures ranging from 10 to 40C at 5C increments. Fish were fed frozen adult *Artemia* three times daily. The experiment was ended after 16 days.

Results

Survival of juvenile bluegills (Table B-2) at high population densities (15 and 20 per culture basket) was significantly lower at 30 and 35C than survival at low densities (10 and 5 per culture basket. Mortality at 5 and 10 animals per protector and high temperature was greater than expected, and may indicate an effect of crowding. The sampling frequency and sample size required by the growth experiment made it necessary to select a density of 10 animals per protector for use during the bioassays despite the possible adverse effect of density.

Table B-2. Percent survival of juvenile *L. macrochirus* at various temperatures with 5, 10, 15 or 20 animals per test container (0.96 liter protectors). Fish were acclimated to 25C, transferred directly to test temperatures, and observed for mortality for the next 16 days.

Temp.	No. of Animals per Protector			
	5	10	15	20
	Percentage Survival			
10	0	0	0	0
15	100	100	93	90
20	100	100	80	80
25	100	100	80	45
30	80	90	47	65
35	80	50	0	0
40	0	0	0	0

Phase 4. Prototype of the Thermal Testing Facility

Procedure

A prototype of the thermal testing facility was constructed to test the system design (Fig. B-2). Water was recirculated at the rate of 1.5-liters per minute through the 75.8-liter system. Water was treated by passing it through a biological filter and an autotrophic compartment containing *Anacharis*. From these conditioning tanks, water flowed into two 75.8-liter culture chambers containing eight 1-liter culture baskets. Each culture basket contained 25 newly-hatched sac-fry and received water at a rate of 250 ml/min. Water was delivered through the bottom of protectors in one chamber and through the top in the other. Larvae were fed *Artemia* nauplii three times daily, beginning two days after they reached swim-up stage.

Results

Survival of sac-fry in the prototype facility after 15 days was greater when water was delivered into the top of the culture basket (Fig. B-3) than when water was delivered through the bottom of the protector. The top-delivery system was selected for use in the culture chambers of the thermal test facility.

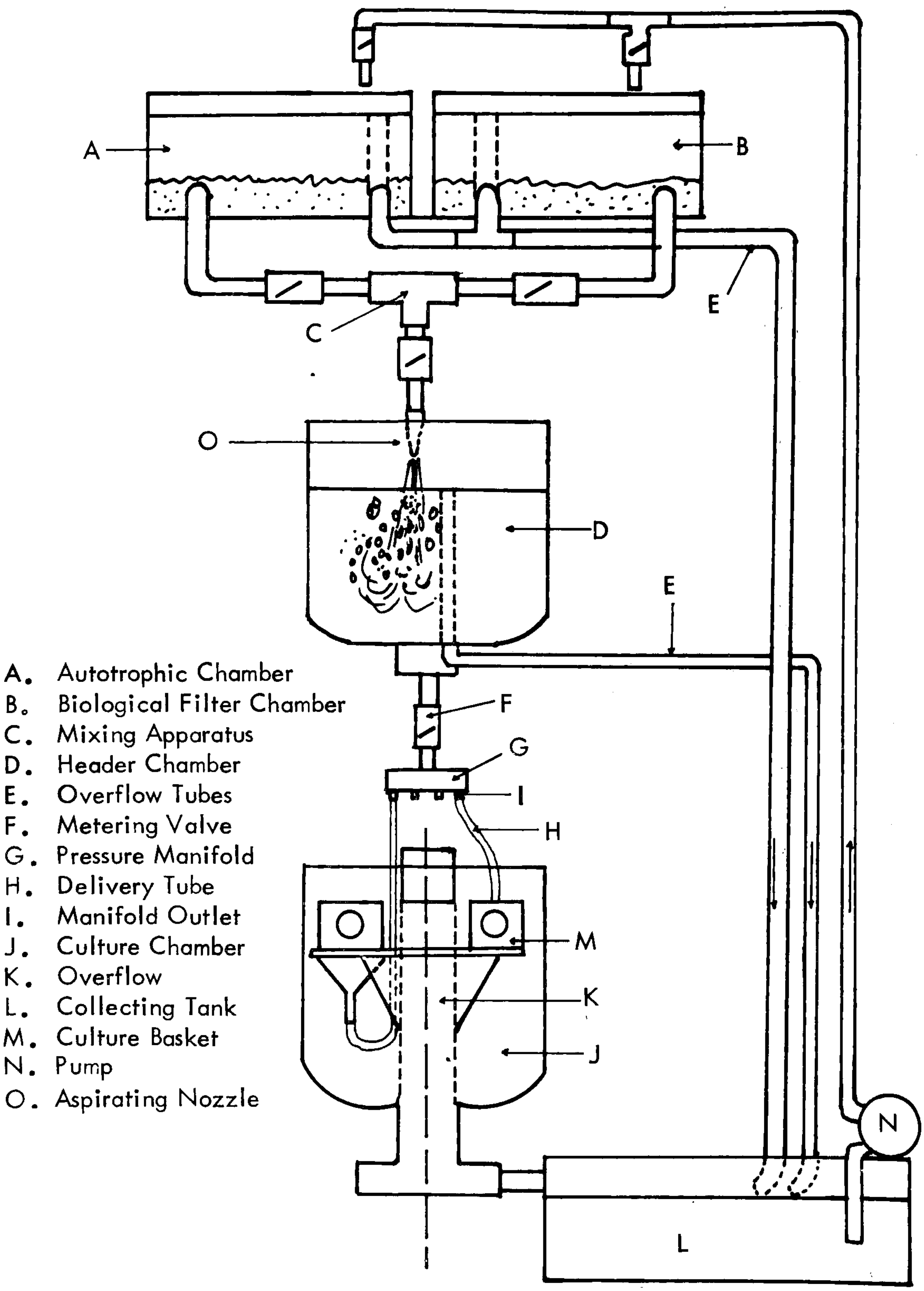


Figure B-2. Prototype of Bluegill thermal testing facility.

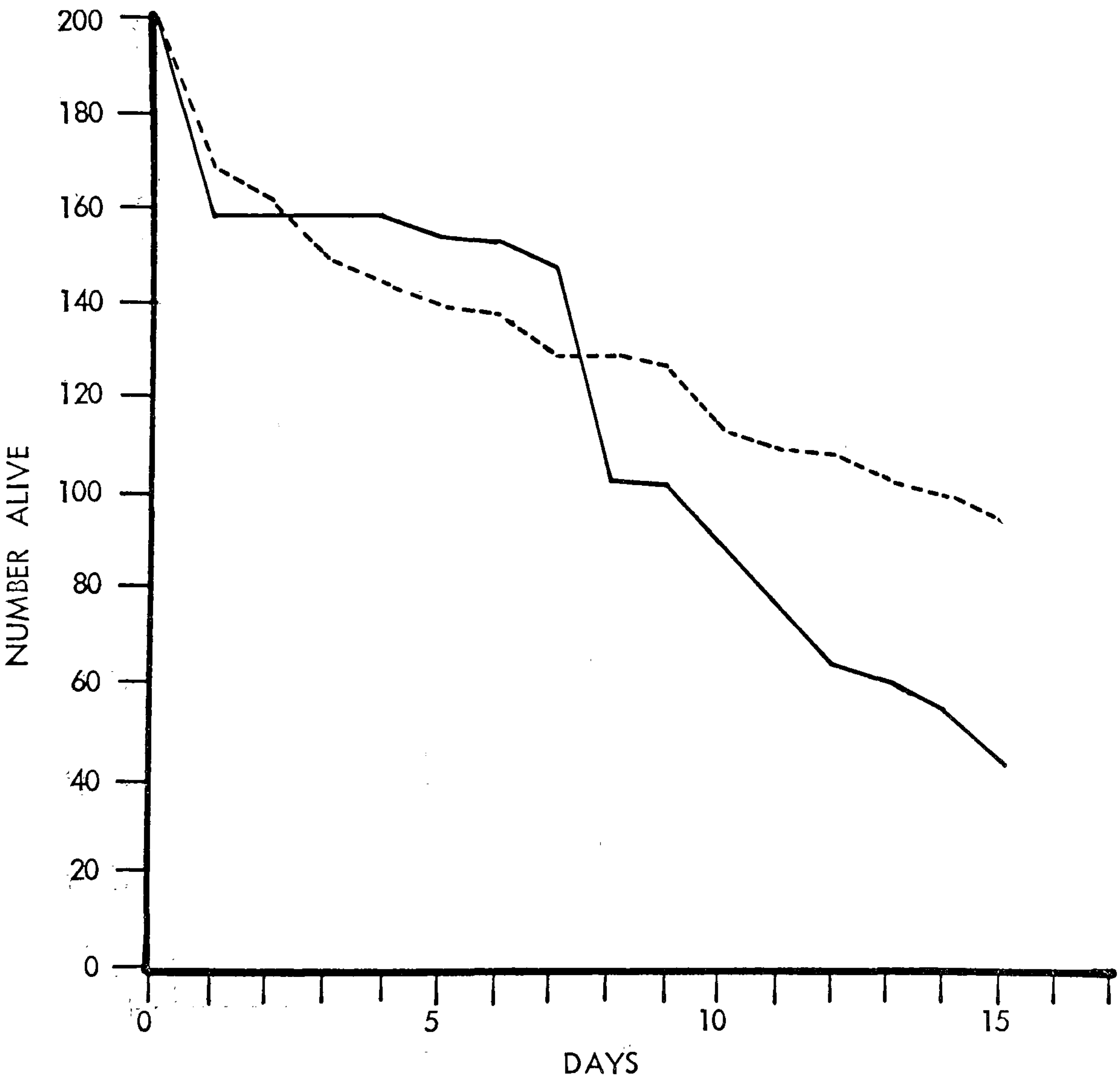


Figure B-3. Survival of Lepomis macrochirus sac-fry at 26 C in the prototype of the thermal testing facility. Protectors received 250 ml/min of water from the top (– – –) or the bottom (——).

APPENDIX C

Thermal Test Facility

Design Specifications. A special testing facility was designed and constructed to perform the bluegill bioassays. Design specifications for the facility were:

1. Capability to establish the temperature in each culture vessel at any specified temperature over the range from 3 to 40C;

2. Capability to maintain the temperature in a given chamber within ±0.3C of the specified temperature for extended periods of time (up to 6 weeks);

3. Continuous recording of the temperature in each culture vessel;

4. Capacity to provide suitable life support conditions for eggs, larvae, and juveniles of the test species allowing adequate survival, growth, and monitoring;

5. Capacity to maintain consistent water quality over extended periods of time (up to 6 weeks);

6. Controlled flow rate of water into each culture basket;

7. Use of simulated natural daylight (Duro-Test Corp. Vita-Lite® fluorescent tubes) with photoperiod corresponding to the light cycles for the area from which the adult specimens were collected.

Theoretical Basis. Several alternative designs were considered to meet the design specifications, but the need to precisely control the temperature of a relatively large volume of moving water led to the development of a temperature-adjustment mechanism based on mixing water of different temperatures. Water from reservoirs maintained at 4C, ambient (25C), and 40C could be combined in a known ratio within a header tank above each culture basket by means of precision mixing valves, to produce water temperatures that are about 1C below the desired test temperatures. Temperatures below 6C require additional cooling supplied by a refrigeration coil set in the individual header tanks.

Approximate temperatures in the header tanks can be achieved by maintenance of constant temperatures in the reservoirs and by maintenance of a constant room temperature. Precise control of temperature in each culture chamber can be regulated by a heater located in the header tank and controlled by a thermoregulator in the culture vessel.

To prevent mixing of test animals within the system and to facilitate observation and sampling of test animals, eggs, larvae and juveniles can be confined in small containers. This confinement, however, requires an adequate exchange of water to maintain oxygen levels and to remove waste products. Mesh-covered culture baskets immersed in a large water bath would provide confinement and have the thermal-stability of a large test container. Several baskets (protectors) in a given culture chamber can be used to expose fish of different origin to identical temperature conditions.

Natural or municipal water supplies generally vary in chemical composition, even over short periods of time. A closed, recirculating culture system reduces the problem of outside contamination. However, water in a closed system exhibits changes as a result of biological activity, including increases in suspended solids, biological oxygen demand, and nitrogenous compounds. Accumulation of these materials can be prevented by various water-treatment methods such as mechanical filters, biological filters, and tanks containing growing plants (autotrophic tanks).

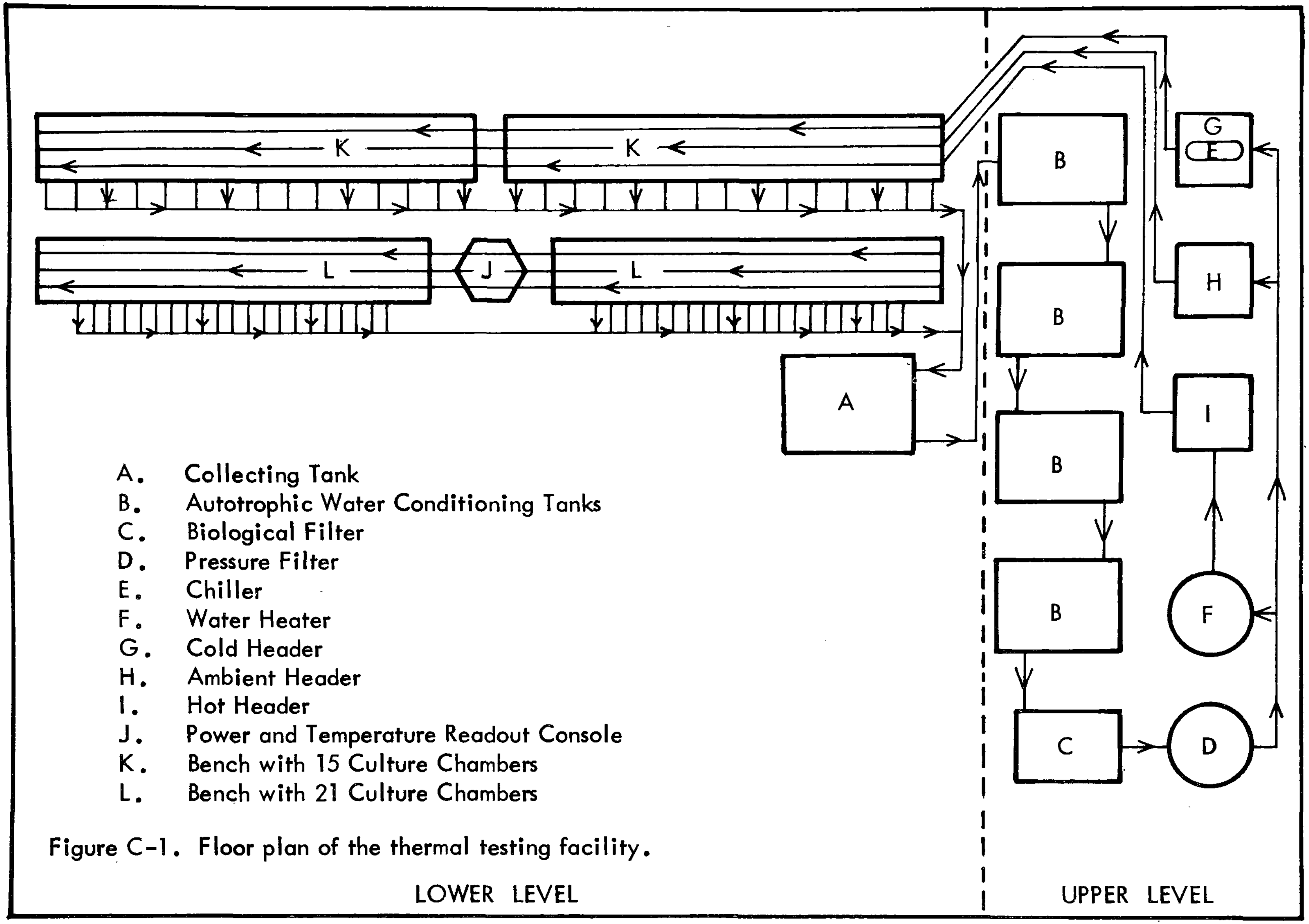
Temperature of each thermal test unit can be continuously monitored by thermistor probes connected to electronic recorders, while photoperiod can be controlled by a single timer connected to lights above each test unit.

Component Descriptions. The system occupies two floors of an air conditioned warehouse. Experimental apparatus and monitoring equipment are located on the lower level. Water conditioning units are located on the upper level. This arrangement provides for easy access to all components and permits control of experimental apparatus without disruption of the life-support elements of the system. An auxiliary diesel generator was available in the event of electrical failure. The entire system exchanges 9.5 kiloliters of water per hour. Components of the system are illustrated in Figure C-1.

A. Collecting Tank. Water from all culture chambers drains into a common fiberglass collecting tank. The temperature of the resulting mixture approximates ambient temperature (24C). Water is pumped from the collecting tank by an electric pump with a polyvinyl-chloride (PVC) impeller to autotrophic tanks on the upper level.

B. Autotrophic Tanks. Four autotrophic tanks (in series) receive water from the collecting tank. The tanks are constructed of marine plywood coated with polyester resin. Each tank is 3.61 x 1.17 x 0.5 meters, contains 2.27 kiloliters of water and 5.7 kg (wet weight) of Anacharis sp. Each tank is lighted by 24 full-spectrum Vita-Lite® fluorescent bulbs. Water flows from the autotrophic tanks to the biological filter. Plants in the autotrophic tank removed dissolved nutrients, especially nitrite, nitrate and phosphate, from the water.

C. Biological Filter. The biological filter is 2.4 x 1.2 x 1.2 meters and contains coarse (4-6 mm) calcareous (oolite) gravel as a substrate for the growth of nitrifying bacteria. These bacteria metabo-



A. Collecting Tank
B. Autotrophic Water Conditioning Tanks
C. Biological Filter
D. Pressure Filter
E. Chiller
F. Water Heater
G. Cold Header
H. Ambient Header
I. Hot Header
J. Power and Temperature Readout Console
K. Bench with 15 Culture Chambers
L. Bench with 21 Culture Chambers

Figure C-1. Floor plan of the thermal testing facility.

lize ammonia and other toxic wastes to less toxic materials. When filled with gravel substrate, the filter contains 1.4 kiloliters of water.

D. Pressure Filter. Water is pumped from the biological filter to the pressure filter by a direct-drive stainless steel pump. The pressure filter is constructed of fiberglass, 0.74 meters in diameter, 0.96 meters in height, and contains diatomaceous earth capable of removing particles 1.0 micron or larger. The pressure filter removed suspended solids and large parasites from the water. Water from the pressure filter flows by three routes: 1) through the chiller to the cold header tank, 2) through the heater to the hot header tank, or 3) directly to the ambient header tank.

E. Chiller. The chiller cools water for the low-temperature reservoir. Chilling 2.27 kiloliters of water per hour to 1C is accomplished with a thirty-ton (90,720 kg-cal/hr) stainless steel chiller.

F. Hot Water Heater. The hot water heater raises the temperature of the water for the high-temperature reservoir. Approximately 1.89 kiloliters of water per hour is heated to 40C in a 63,000 kg-cal/hr gas water heater.

G. Cold, Ambient, Hot Water Headers. Three 1.143-kiloliter fiberglass tanks provide a constant head of water pressure for hot, ambient, and cold water supplied to the tank racks. Water in the headers receives extensive aeration. Water is piped to the racks and metered to the thermal control units above individual culture chambers. Air temperature, water flow rate and hence rate of temperature change in the delivery pipes are constant.

H. Tank Racks. Seventy-two culture chambers and temperature control units are arranged on two rows of tank racks. Each culture chamber is lighted by full spectrum Vita-Lite® fluorescent lamps (Duro-Test Corp.), regulated by an electric timer, to deliver a controlled photoperiod.

I. Temperature Control Units and Culture Chambers. Temperature control units and culture chambers (Figure C-2) have the following components:
   A. Valve for mixing waters of various temperatures and for regulating water flow.

   B. Header tank.

   C. 500 watt heater

   D. Heater controller

   E. Flow-control outlets

   F. 75.8-liter fiberglass culture chamber

A. Valve
B. Header Chamber
C. 500 Watt Heater
D. Heat Controller
E. Header Flow Control Outlets
F. Culture Chamber
G. Culture Basket
H. Thermal Monitor Sensor
I. Incoming Water
J. Header Overflow Standpipe
K. Bench Supports
L. Culture Chamber Standpipe
M. Unit Outflow
N. To Collecting Tank
O. Water Level
P. Delivery Tube

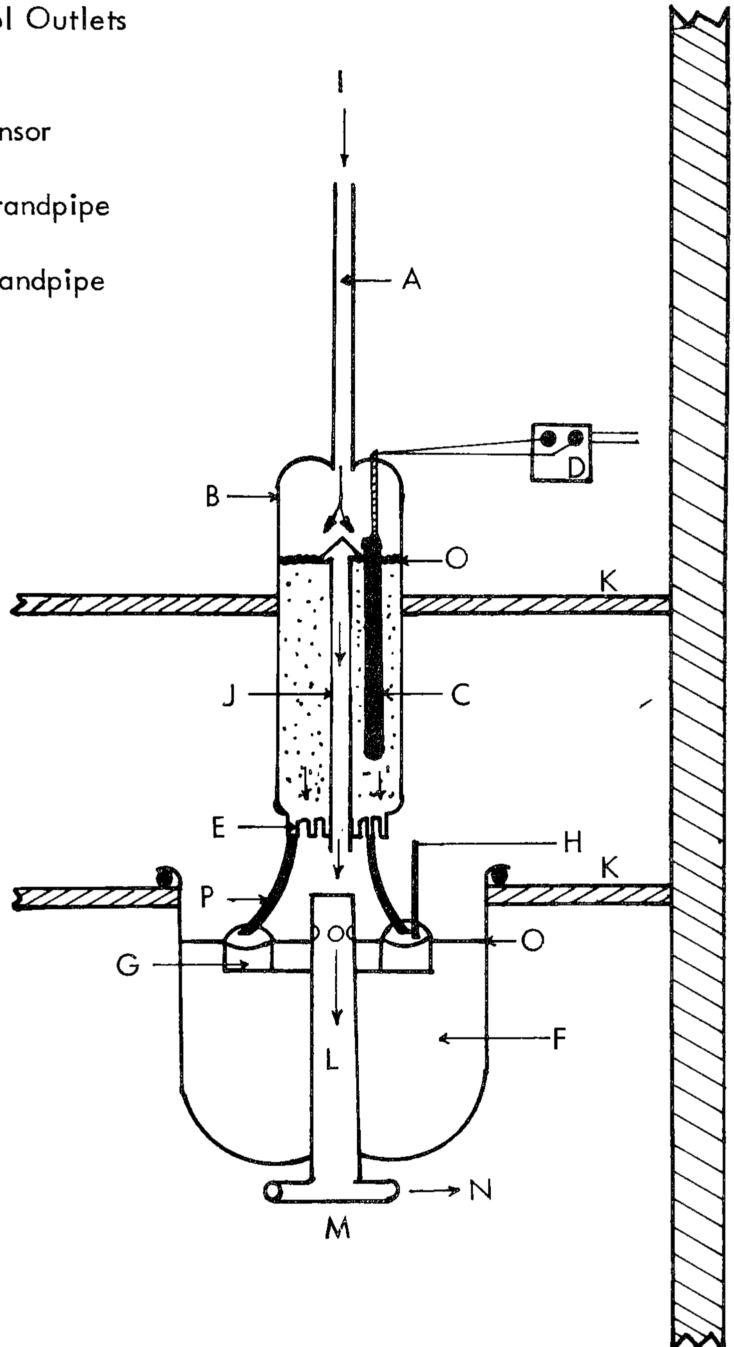
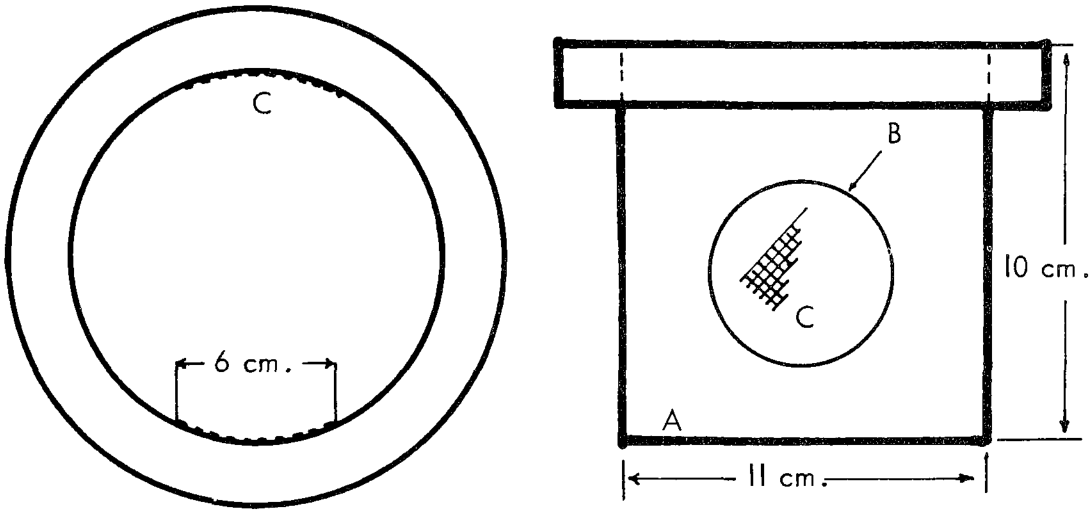


Figure C-2. Culture Chamber and Temperature Control Apparatus.

G. 0.96-liter, 253 micron mesh culture baskets (Figure C-3)

H. Temperature monitor sensor

Waters of different temperatures are mixed to provide water approxi mately 1C below the required test level. High temperatures are achieved by mixing hot with ambient water and low temperatures by mixing cold with ambient water. The mixture is sprayed into the header chamber to provide oxygen or to reduce supersaturation. The procedure was not adequate to reduce super-saturation at the highest temperatures, but was effective at lower temperatures. A 500 watt heater in the thermal control header is linked by a feedback mechanism to the heater control to provide the final adjustment to the desired temperature level. Water flows from the header chamber through the flow-control outlets at 250 ml/min to each of the eight 0.96-liter culture baskets containing the test animals. Culture baskets (Figure C-3) consisted of a polystyrene plastic cylinder (r=5.5 cm, h=10 cm) with two 6 cm diameter holes drilled in opposite sides and covered with 253 micron mesh Nytex® screen. Water overflows from the culture chamber via a central standpipe and returns to the collecting tank. Honeywell Electronik-16 Multipoint Recorders, located on the central console (Figure C-1, J), provide chart records of temperatures measured by the thermal sensor in each of the seventy-two culture chambers.



A. Polystyrene frame
B. Holes drilled in sides
C. Nytex® screen

Figure C-3. 0.96 liter culture basket (protector) for confinement of eggs and larvae during the bioassay tests.

APPENDIX D

PRELIMINARY BIOASSAY EXPERIMENTS

INTRODUCTION

The thermal test facility (Appendix C) was used to conduct a series of preliminary temperature bioassay experiments. A preliminary egg-incubation experiment was performed as a test of the thermal facility, to familiarize personnel with adult and egg handling procedures, and to define the approximate range over which bluegill eggs would hatch.

Preliminary tests were conducted with bluegill sac-fry and swim-up fry to determine whether the water quality, feeding schedule, diet, and amount of food were adequate and to define approximate ranges for TL50 determinations and growth experiments.

A growth test was conducted with juvenile bluegills to define the temperature limits at which growth and survival were sufficient to maintain a population. These limits could then be used in subsequent tests to define the optimal temperature and the temperature range for optimal growth and survival.

MATERIALS AND METHODS

The preliminary incubation test was conducted using the techniques described in the main report. The adults used for the preliminary incubation test were maintained under the temperature conditions shown in Table D-1. Eggs from seven females were fertilized with the sperm from four males at 25.1C. Results were analyzed as described in the main report, except that replicates were treated separately.

Table D-1. Thermal history for adult bluegills used in the preliminary incubation test. All animals were exposed to 16L/8D photoperiod.

Collection Temperature (C)	23-26
Holding	
Duration (Days)	8-82
Mean Temperature (C)	24.5
Egg Production	
Duration (hrs)	24
Mean Temperature (C)	25.1

Bioassay Tests on Fry

Preliminary tests were conducted to determine maximum and minimum thermal tolerances of bluegill sac-fry and swim-up fry. For these tests, only a few animals were available for testing at any temperature. During these experiments, temperatures were maintained to ± 1C (Means not calculated) and water chemistry measurements were not made. Eggs obtained from females in the laboratory were incubated at 22, 26, and 34C. Newly hatched fry, and fry reared to the swim-up stage at 22C were transferred directly to test temperatures in the thermal test facility. After larvae had reached swim-up stage (See Appendix B), they were fed Artemia nauplii (360-450 microns in length) three times daily. Dead fry were counted and removed from test chambers after 24, 48, 72, and 96 hours. Percent survival was calculated at each test temperature.

Juvenile Growth Test

Juvenile bluegills (mean wt = 0.29 gm, range = 0.1 to 2.0 gm) from stocks acclimated to 12.1, 19.0, 26.0 and 32.9C were transferred directly to a series of test temperatures (Table D-6) in the thermal test facility. Test temperatures were measured as during the juvenile bioassay. Mean temperature ± 95% confidence limits are given for each replicate culture chamber. Animals were maintained at various test temperatures for three to six weeks. Juvenile bluegills were fed to satiation three times daily (0800, 1200, 1600) with adult Artemia, Daphnia, and Tetramin®. These feedings constituted a maximum ration, as some uneaten Artemia and Daphnia remained in the protectors between feedings and overnight. Dead animals were counted and removed daily. Prior to the bioassay test, an initial sample of 28 to 115 animals was selected randomly, along with the test animals at each stock temperature. Ten animals from each test temperature were sampled randomly at bi-weekly intervals for up to six weeks. These fish were preserved in Bouin's solution (Humason, 1967), and were later measured to determine growth rates. Standard length was determined with calipers to the nearest 0.1 mm. Fish were weighed with an analytical balance to the nearest 0.1 gm. Whenever five or fewer animals survived at any test temperature, these animals were removed, preserved and measured. Instantaneous mortality, instantaneous growth (weight), and instantaneous change in population biomass (Ricker, 1958) were calculated for each stock and test temperature.

RESULTS

Water Quality

The trace-chemical composition of the water used during these tests is described in the main report. Routine water quality tests were not performed during the preliminary egg incubation and fry bioassay tests. Results of routine monitoring during the growth experiment are presented in Table D-2. Dissolved oxygen levels ranged from 61% saturation to supersaturation (145%). As mentioned before, improved control of oxygen levels in the test system is necessary (see Appendix C). Ammonia levels (un-ionized NH_3) were between .003 and .095 ppm. The sensitivity of the ammonia test was 0.25 ppm of NH_4, which represented as much as 0.039 ppm of NH_3 at high temperature and pH levels. The levels of NH_3 measured were, in general, unacceptable to achieve optimal growth and development (see Discussion in main report). Nitrite levels ranged from 0 to 0.010 ppm; pH ranged from 7.60 to 8.34 (except one reading of 6.94) and nitrate ranged from 0 to 5 ppm.

Incubation Test

The percent hatch of bluegill eggs at various test temperatures is pre-

Table D-2. Routine water chemistry data: juvenile growth test.

Day	pH Mean	pH Range	Dissolved Oxygen (% Saturation) Mean	Dissolved Oxygen (% Saturation) Range	Ammonia (Un-ionized NH_3) Mean	Ammonia (Un-ionized NH_3) Range	Nitrite Mean	Nitrite Range	Nitrate Mean	Nitrate Range
1	7.9	7.8-7.9	101	80-119	.027	.018-.042	.004	.003-.005	0	0-0
2	7.68	7.64-7.75	104	80-134	.016	.005-.031	.003	.001-.005	*	
3	7.9	7.8-8.0	106	80-134	.010	.005-.018	.005	.003-.008	*	
4	8.19	8.13-8.27	115	93-132	.047	.015-.095	.001	.0 -.003	0	0-0
5	7.64	7.60-7.69	110	88-134	.006	.004-.009	.002	.001-.003	0	0-0
6	7.83	7.72-7.93	108	88-122	.006	.004-.018	.002	.001-.003	0	0-0
7	8.00	7.95-8.03	102	80-122	.013	.004-.022	.002	.001-.005	0	0-0
8	8.01	7.98-8.04	102	80-122	.013	.007-.021	.003	.001-.005	0	0-0
9	7.68	7.64-7.73	99	82-116	.009	.003-.018	.004	.003-.005	0	0-0
10	8.13	8.07-8.22	107	94-125	.017	.009-.029	.004	.001-.005	0	0-0
11	7.86	7.86-7.87	117	82-145	.013	.008-.019	.004-	.003-.005	0	0-0
12	7.75	7.71-7.82	99	82-116	.039	.005-.024	*		*	
13	8.14	8.09-8.23	97	80-116	.027	.011-.052	*		*	
14	8.10	8.07-8.12	98	80-116	.023	.010-.035	.009	.008-.010	*	
15	7.93	7.88-7.99	102	87-120	.012	.006-.019	*		*	
16	7.09	6.94-7.23	93	74-107	.002	.001-.004	*		*	
17	8.06	7.99-8.12	103	87-122	.015	.008-.025	*		*	
18	7.97	7.93-8.02	112	95-131	.012	.007-.020	.004	.004-.005	0	0-0
19	8.18	8.14-8.25	111	86-127	.030	.011-.044	*		*	
20	8.12	8.05-8.22	98	82-117	.018	.009-.031	*		*	
21	8.06	7.99-8.14	93	80-110	.017	.008-.029	.003	.003-.003	0	0-0
22	8.02	7.91-8.14	91	74-108	.012	.006-.028	*		*	
23	8.12	8.03-8.18	88	74-104	.016	.008-.028	.003	.003-.006	0	0-0
24	8.15	8.15-8.16	92	77-102	.018	.011-.028	*		*	
25	7.93	7.87-8.03	90	74-104	.012	.006-.022	*		*	
26	7.74	7.70-7.78	87	71-102	.007	.004-.012	.000	.0 -.001	1	0-2
27	8.05	7.99-8.14	92	80-108	.018	.008-.033	*		*	
28	8.03	7.91-8.12	83	69-96	.015	.007-.025	.002	.001-.003	2	0-3
29	8.08	8.04-8.15	82	66-102	.018	.009-.032	*		*	

Table D-2. (cont'd)

Day	pH Mean	pH Range	Dissolved Oxygen (% Saturation) Mean	Dissolved Oxygen (% Saturation) Range	Ammonia (Un-ionized NH_3) Mean	Ammonia (Un-ionized NH_3) Range	Nitrite Mean	Nitrite Range	Nitrate Mean	Nitrate Range
30	7.83	7.78-7.91	82	65-102	.010	.005-.017	*		*	
31	7.97	7.88-8.08	83	64-99	.013	.007-.023	.002	.001-.005	2	0-3
32	7.85	7.80-7.94	84	70-102	.010	.005-.017	*		*	
33	7.96	7.89-8.06	82	64-102	.016	.006-.033	.002	.001-.003	0	0-0
34	7.98	7.91-8.08	83	66-102	.013	.006-.023	*		*	
35	8.01	7.97-8.05	80	65-99	.014	.008-.022	*		*	
36	8.09	8.05-8.16	85	72-96	.017	.009-.029	.001	.0 -.001	1	0-2
37	8.13	8.08-8.21	76	61-89	.022	.009-.041	*		*	
38	8.11	8.03-8.21	80	67-93	.014	.009-.020	.003	.003-.003	0	0-0
39	8.27	8.22-8.34	88	72-107	.024	.013-.039	*		*	
40	8.06	8.01-8.12	86	66-104	.016	.008-.025	*		*	
41	8.09	8.03-8.16	86	67-107	.022	.009-.041	.002	.001-.003	2	0-5
42	7.90	7.84-8.01	81	64-102	.012	.006-.020	*		*	
43	7.91	7.86-7.98	77	64-93	.011	.005-.020	*		*	
44	8.10	8.06-8.15	81	70-95	.018	.009-.029	.002	.001-.003	0	0-0
45	8.21	8.08-8.30	82	67-102	.028	.010-.054	*		*	
46	8.25	8.20-8.31	88	74-104	.023	.013-.036	*		*	

*Samples not analyzed

sented in Table D-3. Eggs hatched at temperatures from 18.7 to 33.8C, but failed to hatch at 16 or 35.8C. The temperatures for maximum hatch were 22.6C (replicate A) and 30.6C (replicate B), when both replicates were combined, optimal temperature for hatch was 30.6C. The combined results of the replicate A temperature chambers differed greatly from the combined results obtained from replicate B chambers with respect to hatching rate and optimal temperature for hatch. Both replicates had a low percent hatch at 26.2C. Egg viability was low during this experiment, so that a lower TL50 was not defined in replicate B. Temperatures were recorded manually at 6-hr intervals. Temperature control was not adequate since the confidence limits ranged from ± 1.32 to ± 4.46C during the experiment.

Replicate A had an upper TL50 of 32.4C and a lower TL50 of 21.4C. Replicate B had an upper TL50 of 31.6C, but lower TL50 was not defined. When both replicates were combined, upper TL50 was 31.6C and lower TL50 was 22.6C. Upper TL50 values determined during the preliminary test were slightly lower than the TL50 temperatures subsequently defined in incubation tests 1 and 2 (see main report).

Bioassay Tests on Fry

Results of the preliminary tests were not intended to define TL50 temperatures for sac-fry (Table D-4) from the three stocks, but rather to define the temperature ranges necessary for subsequent testing. Maximum survival of fry that were hatched at 22C was 83% at 29C. Larvae from this stock were exposed to temperatures from 9 to 37C, but only survived for 96 hours at temperatures from 16 to 32C.

Sac-fry that were hatched at 26C were exposed to temperatures from 8 to 38C survived for 96 hours at 12, 16 and 34C. These fry survived 96-hour exposure to temperatures that were 4C lower and 2C higher than the temperatures tolerated by the 22C stock. Sac-fry from the stock at 34C survived for 96 hours at temperatures from 18 to 24C. Sac-fry did not survive at 14 and 16C and thus had poorer survival at low temperatures than the stock at 26C. Larvae from the stock at 34C survived better at 38C than did larvae from the stock at 26C.

Preliminary tests with swim-up fry hatched at 22C (Table D-5) indicated survival for 96 hours at temperatures from 11 to 26C. The low-temperature TL50 was between 11 and 12C. The upper 96-hour TL50 was not defined because of poor survival of the fry at all test temperatures above 12C.

Juvenile Growth Tests

The juvenile growth test was conducted with fish that were acclimated to 12.1, 19.0, 26.0 or 32.9C. Instantaneous rates of mortality, growth (weight of preserved fish), and change in population biomass (Ricker, 1958) for juvenile bluegills are presented in Table D-6 and Figure D-1. The calculated rates were multiplied by 100 to express the rates as percentages.

Table D-3. Percent hatch of bluegill eggs at various temperatures.

Test Temp Mean ±95%CL	Replicate	No. Temp Readings	Actual Total % Hatch	Actual Normal % Hatch	Combined Actual Total % Hatch	Combined Actual Normal % Hatch	Relative % Normal Hatch	Combined Relative % Normal Hatch
18.9 ± 1.32	A	16	2	2	1	1	4	2
18.5 ± 1.43	B	16	0	0			0	
20.4 ± 1.73	A	16	2	2	6	6	4	11
20.6 ± 1.47	B	16	10	10			14	
22.6 ± 1.77	A	14	46	46	30	29	100	56
22.6 ± 1.76	B	14	14	12			17	
23.6 ± 2.64	A	14	36	26	29	23	57	43
24.0 ± 2.66	B	14	22	20			28	
26.1 ± 2.76	A	14	12	2	11	3	4	6
26.3 ± 2.53	B	14	10	4			6	
27.5 ± 3.87	A	10	32	18	32	25	39	47
28.1 ± 1.90	B	10	32	32			44	
30.4 ± 3.03	A	10	36	34	58	53	74	100
30.7 ± 2.72	B	10	80	72			100	
31.4 ± 4.46	A	10	36	36	23	23	78	43
32.3 ± [illegible].87	B	10	10	10			14	
33.8 ± 2.84	A	10	4	2	11	7	4	13
33.9 ± 2.54	B	10	18	12			17	
35.8 ± 2.96	A	10	0	0	0	0	0	0
35.7 ± 2.17	B	10	0	0			0	

Upper TL_{50}

Replicate A	32.3
Replicate B	31.6
Combined	31.7

Lower TL_{50}

Replicate A	21.5
Replicate B	(not defined)
Combined	22.3

Table D-4. Percent survival of bluegill sac-fry exposed to various temperatures for 96 hours. Eggs were incubated at 22, 26, or 34C until they hatched, and the newly hatched larvae were transferred directly to test temperatures.

Test Temp. (C)	Time (hrs.) 0	24	48	72	96
22C stock N=12 larvae per test temperature					
9	100	100	0		-
11	100	100	25	0	-
12	100	92	83	0	
13	100	83	17	0	
14	100	50	25	8	0
15	100	92	50	33	0
16	100	75	58	58	50
17	100	83	67	50	25
19	100	67	25	8	8
22	100	50	50	25	17
25	100	25	8	0	-
27	100	58	58	42	17
28	100	42	42	42	25
29	100	83	83	83	83
30	100	83	50	50	42
31	100	67	67	50	50
32	100	50	50	25	17
33	100	25	17	8	0
35	100	42	0	-	-
37	100	42	0		-
26C stock N=5 larvae per test temperature					
8	100	100	100	0	-
12	100	80	20	20	10
16	100	100	100	100	80
34	100	100	100	80	80
38	100	0		-	-
34C stock N=10 larvae per test temperature					
14	100	0			
16	100	40	10	10	0
18	100	90	70	70	70
20	100	90	90	90	70
22	100	90	90	80	80
24	100	80	80	80	80
38	100	80	10	0	-
40	100	0	-		-
42	100	0	-	-	-

Table D-5. Percent survival of fry, reared to the swim-up stage at 22C and transferred directly to various test temperatures. (N=20 fry per test temperature)

Test Temperatures	Percent Survival at Time(hrs) 0	24	48	72	96
6	100	0		-	-
7	100	0		-	-
8	100	0		-	-
9	100	0		-	
10	100	20	0	-	-
11	100	60	60	50	15
12	100	100	100	100	100
13	100	55	50	45	45
14	100	55	50	50	50
15	100	70	70	40	30
16	100	50	50	50	50
17	100	50	50	45	45
18	100	45	40	40	40
19	100	55	55	55	55
20	100	50	50	50	50
21	100	45	45	35	35
22	100	50	50	50	35
23	100	50	50	50	20
24	100	45	45	40	35
25	100	30	30	25	5
26	100	40	40	20	20
27	100	0	-	-	
28	100	40	30	25	0
29	100	30	30	25	0
30	100	15	15	15	0
31	100	60	40	0	-
32	100	0	-	-	
33	100	25	5	0	
34	100	5	5	5	-
35	100	0	0	0	0
36	100	0	-	-	
38	100	0	-	-	
39	100	0	-	-	-
40	100	0			
42	100	0			

Table D-6. Instantaneous rates of growth (weight), mortality, and change in population biomass of juvenile bluegills, previously acclimated to four stock temperatures. Instantaneous rates (Ricker, 1958) were multiplied by 100 to obtain percent change.

Stock Acclimated to 12.1C

Test Temp Mean±95%CL	Repli-cate	No. Temp Readings	Mortality (Replicates Combined) Week No. 1	2	3	4	Mean	** Growth	Biomass Change
3.0 ± 0.40	-	284	18.95	26.76	*		-22.86	4.83	-18.03
3.9 ± 0.56	-	333	7.77	9.86	*		- 8.82	1.59	- 7.23
4 9 ± 0.26	-	425	5.79	7.56	4.41	*	- 5.92	3.02	- 2.90
6.0 ± 1.21	-	541	7.85	2.07	0.00	*	- 3.31	0.16	- 3.15
6.9 ± 1.48	A	541							
7.0 ± 1.07	B	541	7.62	1.15	0.00	*	- 2.92	1.21	- 1.71
7.9 ± 1.96	A	541							
8.0 ± 1.98	B	541	9.09	0.46	0.00	*	- 3.18	0.77	- 2.41
12.0 ± 0.68	A	541							
12.0 ± 0.67	B	541	9.92	0.00	0.00	*	- 3.31	0.06	- 3.25
21.7 ± 0.80	A	587							
21.6 ± 1.56	B	587	5.76	0.00	0.00	*	- 1.92	3.40	+ 1.48
24.1 ± 0.46	A	404							
24.0 ± 0.15	B	404	13.24	1.48	*		- 7.36	2.51	- 4.85
26.0 ± 0.23	A	577							
26.0 ± 0.41	B	577	5.73	4.08	18.80	*	- 9.54	4.02	- 5.52
27.0 ± 0.34	A	337							
27.0 ± 0.30	B	337	6.90	1.05	*		- 3.98	2.05	- 1.93
27.9 ± 0.25	A	577							
27.9 ± 0.32	B	577	10.01	0.00	0.00	0.00	- 2.50	2.44	- 0.06
29.0 ± 0.22	A	433							
28.8 ± 0.47	B	433	10.09	3.03	15.32	*	- 9.48	3.79	- 5.69

** Based on an initial mean weight of 0.38 ± 0.55 gm; N=105
* All remaining fish were removed during the previous sampling period

Table D-6. (cont'd)

Stock Acclimated to 19.0C

Test Temp Mean±95%CL	Replicate	No. Temp Readings	Mortality (Replicates Combined) Week No. 1	2	3	4	5	Mean	** Growth	Biomass Change
5.8 ± 1.16	A	263								
6.0 ± 0.22	B	263	21.13	5.47	*			13.30	4.24	- 9.06
7.0 ± 0.45	A	643								
7.0 ± 0.23	B	643	10.44	0.88	1.88	0.00	*	3.30	3.21	- 0.09
8.0 ± 0.85	A	878								
8.0 ± 0.82	B	878	0.41	0.00	0.00	0.00	*	0.10	0.80	+ 0.70
9.0 ± 0.80	A	878								
9.0 ± 0.82	B	878	1.61	0.42	0.00	0.73	0.00	0.55	-0.54	- 1.09
10.0 ± 1.00	A	901								
10.0 ± 0.33	B	901	4.21	0.00	0.00	0.00	0.00	0.84	0.87	+ 0.03
19.0 ± 0.41	A	911								
19.0 ± 0.39	B	911	1.30	0.41	0.00	0.00	0.00	0.34	1.86	+ 1.52
27.0 ± 0.30	A	669								
27.0 ± 0.28	B	669	2.25	2.26	1.80	0.00		1.58	3.94	+ 2.36
27.9 ± 0.28	A	669								
28.0 ± 0.28	B	669	4.02	0.47	4.80	2.61	*	2.98	4.14	+ 1.16
29.0 ± 0.26	A	669								
28.9 ± 0.40	B	669	0.27	2.04	1.96	3.58	*	1.96	3.74	+ 1.78
30.0 ± 0.30	A	669								
30.0 ± 0.39	B	669	0.55	5.82	3.68	10.38	*	5.11	4.17	- 0.94
31.0 ± 0.40	A	669								
30.9 ± 0.40	B	669	3.25	10.33	3.65	0.00	*	4.31	5.53	+ 1.22
31.9 ± 0.39	A	577								
32.0 ± 0.40	B	577	2.61	3.11	3.76	2.36	*	2.96	3.92	+ 0.96
32.9 ± 0.35	A	601								
32.8 ± 0.59	B	601	7.18	2.70	12.61	16.22	*	9.68	6.37	- 3.31

** Based on an initial mean weight of .25 ± 0.31 gm; N=115

* All remaining fish were removed during the previous sampling period

Table D-6. (cont'd)

Stock Acclimated to 26.0C

Test Temp Mean±95%CL	Replicates	No. Temp Readings	Mortality (Replicates Combined) Week No. 1	2	3	4	5	6	Mean	** Growth	Biomass Change
10.0 + 0.36	A	680									
10.0 ± 0.48	B	680	5.17	5.88	2.90	2.10	*		4.01	1.34	- 2.67
11.0 ± 0.41	A	973									
11.0 ± 0.38	B	973	3.01	0.64	0.00	0.00	0.00	0.00	0.61	0.55	- 0.06
12.0 ± 0.38	A	973									
12.0 ± 0.43	B	973	2.63	0.00	0.00	0.46	0.00	0.00	0.52	0.44	- 0.08
13.0 ± 0.32	A	973									
13.0 ± 0.37	B	973	1.52	0.00	0.00	0.00	0.00	0.00	0.25	0.17	- 0.08
26.1 ± 0.28	A	985									
26.0 ± 0.44	B	985	0.92	0.89	1.10	0.00	3.65	3.52	1.68	3.69	+ 2.01
33.9 ± 0.47	A	649									
34.0 ± 0.38	B	649	3.14	0.67	2.98	1.08	*		1.97	3.81	+ 1.84
35.0 ± 0.94	A	649									
35.0 ± 0.84	B	649	6.94	1.10	0.00	4.86	*		3.22	3.56	+ 0.34
36.0 ± 1.78	A	335									
35.9 ± 2.11	B	335	8.56	1.13	16.48	*			8.72	0.55	- 8.17

** Based on an initial mean weight of .23 ± 0.29 gm; N=38

* All remaining fish were removed during the previous sampling period

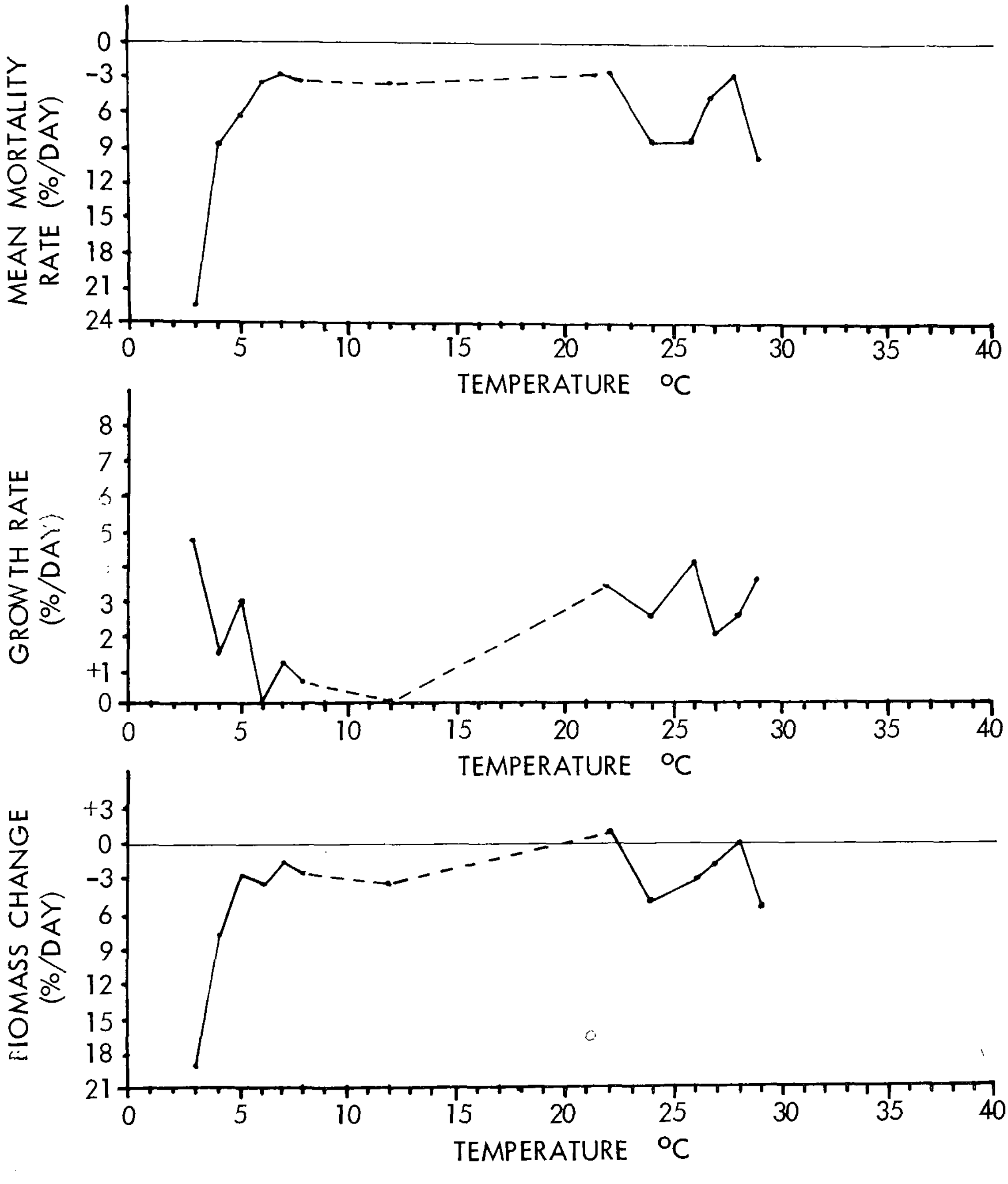
Table D-6. (cont'd)

Stock Acclimated to 32.9C

Test Temp Mean±95%CL	Replicates	No. Temp Readings	Mortality (Replicates Combined) Week No. 1	2	3	4	5	6	Mean	** Growth	Biomass Change
15.9 ± 0.31	A	955									
16.0 ± 0.26	B	955	2.14	0.00	0.00	0.00	0.00	0.00	0.36	0.38	+ 0.02
17.0 ± 0.27	A	1006									
17.0 ± 0.31	B	1006	2.54	1.23	0.52	0.55	0.00	0.00	0.81	0.03	- 0.78
17.9 ± 0.35	A	1006									
18.0 ± 0.25	B	1006	1.32	0.00	0.84	0.00	0.00	0.00	0.36	0.59	+ 0.23
19.0 ± 0.38	A	1006									
19.0 ± 0.18	B	1006	1.42	0.60	0.00	0.00	0.00	0.00	0.34	0.81	+ 0.47
20.0 ± 0.29	A	1006									
20.0 ± 0.33	B	1006	1.31	0.00	0.00	0.00	1.15	0.00	0.41	0.56	+ 0.15
32.9 ± 0.36	A	985									
33.0 ± 0.48	B	985	2.37	1.65	0.76	2.47	5.08	0.00	2.06	1.06	- 1.00
35.9 ± 1.27	A	658									
35.5 ± 1.62	B	658	12.49	1.05	0.00	3.98	*		4.38	0.66	- 3.72
37.0 ± 0.51	A	335									
37.0 ± 0.62	B	335	8.39	8.98	*				8.68	0.39	- 8.29

** Based on an initial mean weight of 0.29 ± 0.39 gm; N=28

* All remaining fish were removed during the previous sampling period



STOCK ACCLIMATED TO 12.1°C

Figure D1. Instantaneous rates of mortality, growth and change in population biomass for juvenile bluegills

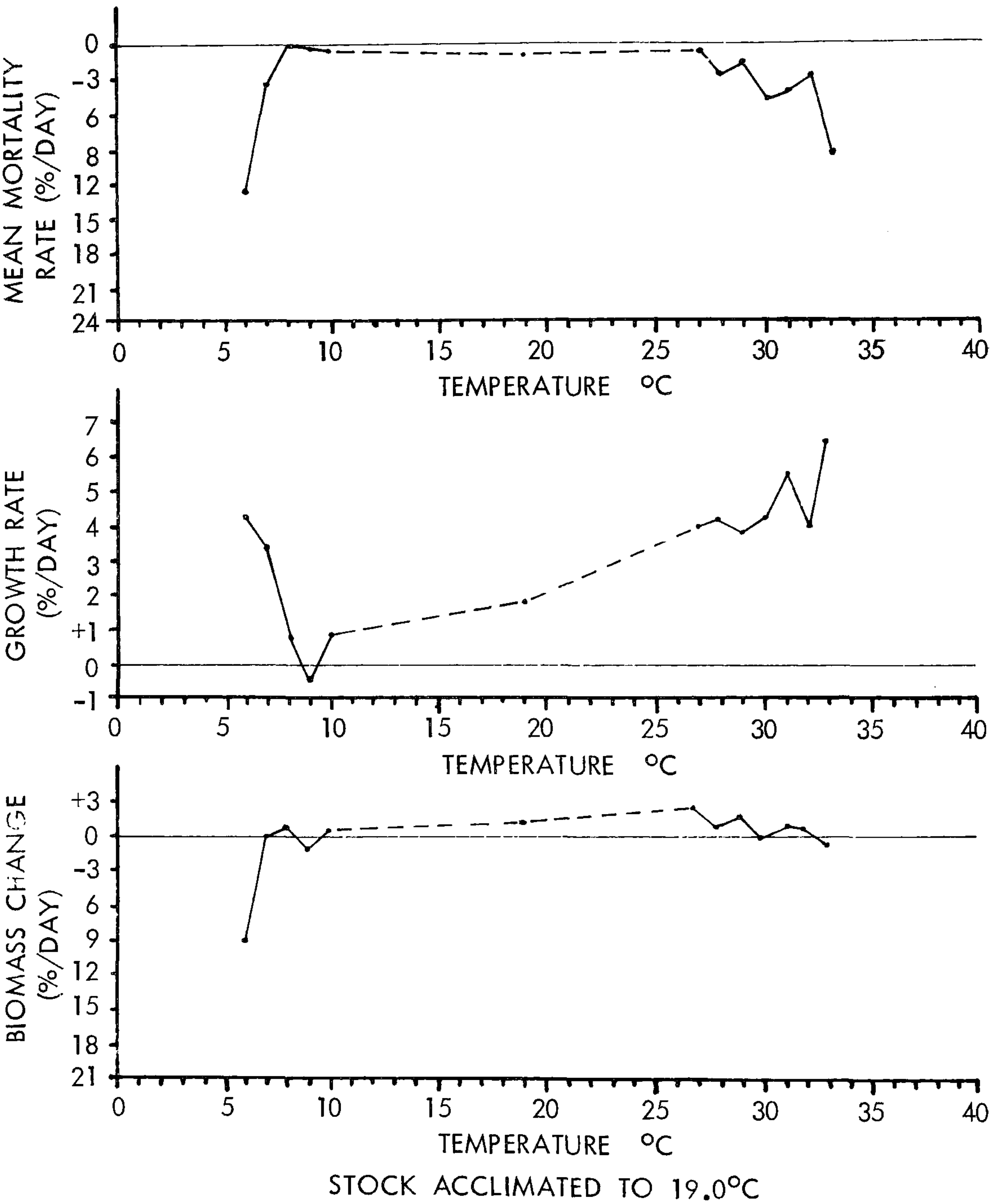
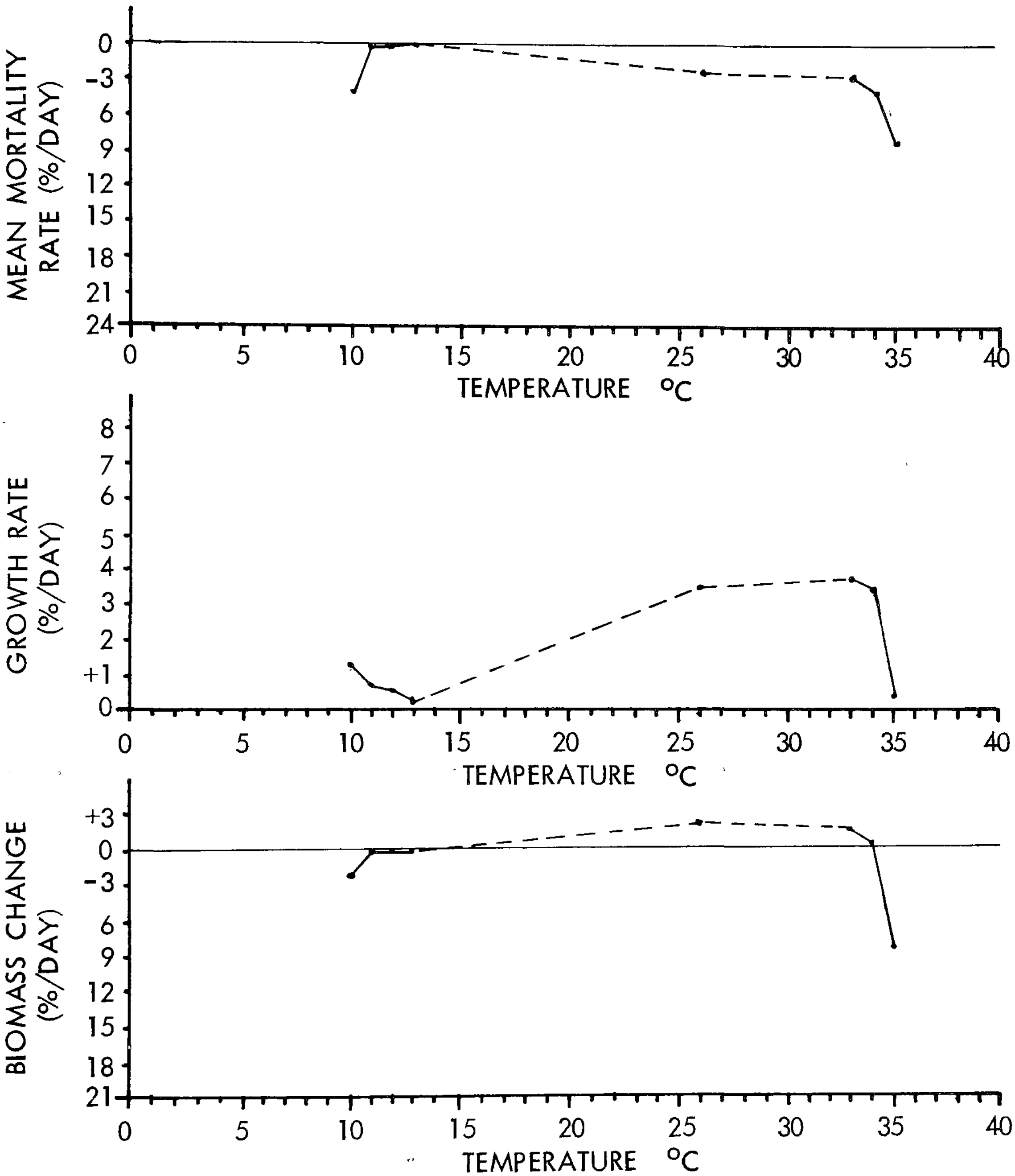
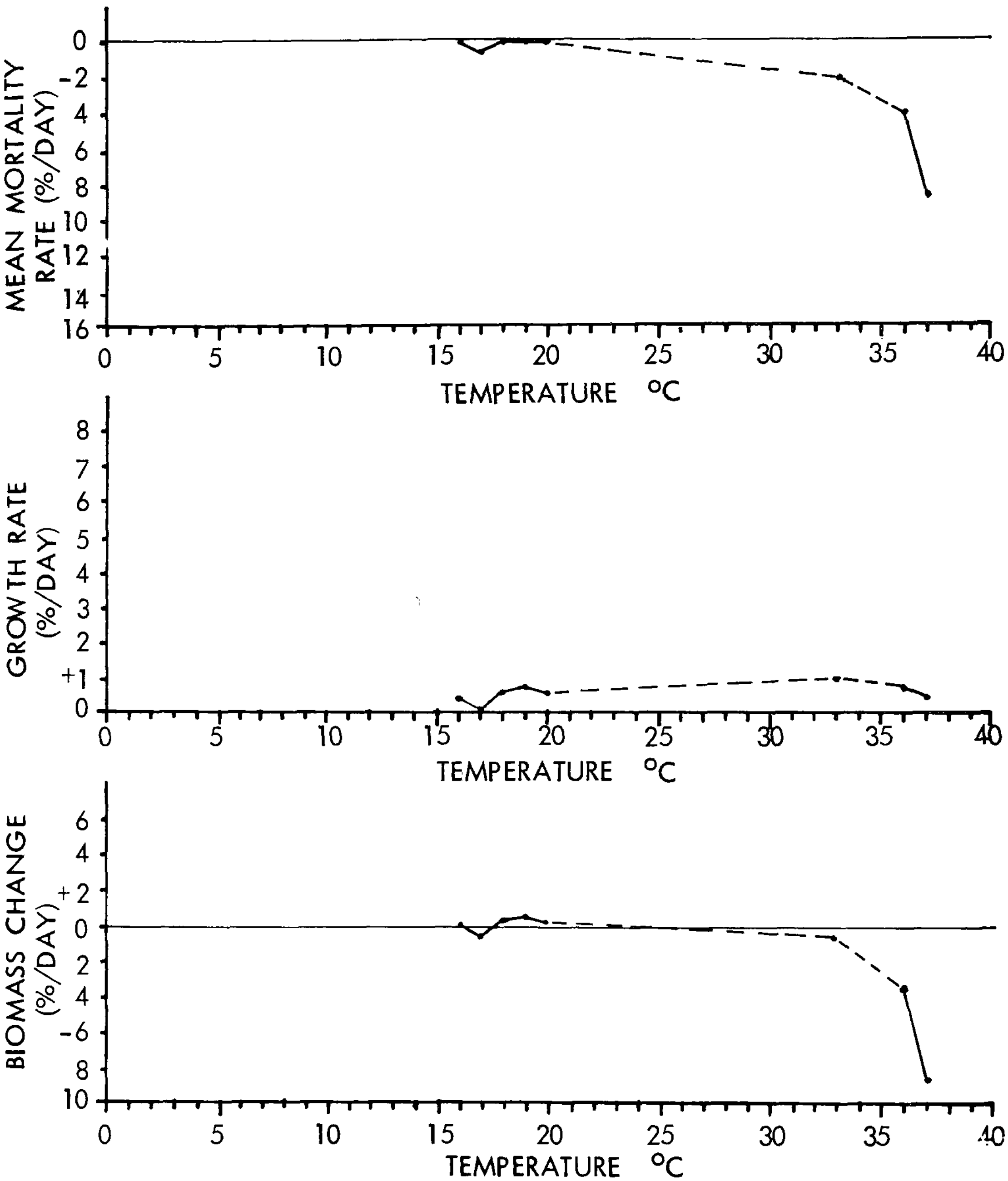


Figure D1. continued



STOCK ACCLIMATED TO 26.0°C

Figure D1. continued



STOCK ACCLIMATED TO 32.9°C

Figure D1. continued

Juvenile stocks were introduced into the experiment on 15 September, 1971 (26.0C and 32.9C); 23 September, 1971 (19.0C); and 7 October, 1971 (12.1C). All experiments were terminated on 26 October, 1971 due to a bacterial disease which may be a reflection of other sources of stress predisposing the fish to infection. Fish that were acclimated to 12.1C prior to testing, were maintained for three weeks in the growth experiment. The 19.0C stock was exposed to test temperatures for five weeks, while the 26.0 and 32.9C stocks were exposed to test temperatures for six weeks.

For the 12.1C stock, mean mortality rates were relatively high at all temperatures. Mortality was highest at the extreme low temperature (22.86%/day at 3.0C) and at 26.0C (9.54%/day). Minimum mortality (1.92%) occurred at 21.6C. Apparent growth rates were high at extreme temperatures. Juvenile bluegills showed an apparent increase in weight of 4.83%/day at 3.0C and 3.79%/day at 28.9C. Initial samples indicated that weight was quite variable within the population. Ten animals did not constitute an adequate sample. Growth rates were lowest (0.06%/day to 1.21%/day) between 6.0 and 12.0C and highest (2.05%/day to 4.02%/day) between 21.6 and 28.9C. Net change in biomass was negative at all temperatures except 21.6C. No population could survive with negative biomass gain and hence this result must be considered atypical, reflecting stress from crowding and low water quality.

The stock that was acclimated to 19.0C showed maximum mortality at extreme low and extreme high temperatures (13.30%/day at 5.9C and 9.68%/day at 32.8C). Juveniles again showed an apparent increase in weight at extreme temperatures (4.24%/day at 5.9C and 6.37%/day at 32.8C). Growth rates were least between 8.0C and 10.0C (-0.54 to 0.87%/day). Net change in biomass was low (-9.06 to 0.70%/day) at 10.0 or less, 30.0C and 32.8C. Bluegills maintained at temperatures of 8.0, 10.0 to 29.0, 30.0, 31.0 and 32.0C showed a net increase in biomass. Maximum rate of biomass increase was 2.36%/day at 27.0C.

The stock that was acclimated to 26.0C had maximum mortality at 36.0C (8.72%/day) and at 10.0C (4.01%/day). Change in weight was 1.34%/day at 10.06. Minimal growth (0.17 to 0.55%/day) occurred at 11.0 to 13.0C and at 36.0C. Maximum growth was 3.81%/day at 34.0C. Net change in biomass was 0.06% to 2.67%/day at temperatures of 10.0 to 13.0C. Rate of biomass change was negative (-8.17%/day) at 36.0C and was positive (0.34%/day) at 34.0C. The highest rate of biomass increase was 2.01%/day at 26.0C.

The stock that was acclimated to 32.9C showed highest mortality (2.06 to 8.68%/day) at 33.0 to 37.0C. Mortality was low (0.34 to 0.81%/day) at temperatures from 16.0 to 20.0C. Maximum growth rate was 1.06%/day at 33.0C. Lowest growth rates were 0.03%/day at 17.0C, 0.38%/day at 16.0C, and 0.39%/day at 37.0C. Net change in biomass was negative (-0.78%/day) at 17.0C and positive (0.17%/day) at 18.0C. At high temperatures (33.0C and above) the rate of biomass change was negative.

Changes in body length with time (Figures D-2 to D-5) did not show a consistent pattern of response with increasing temperature. Some error due to sampling was present, since at several test temperatures, fish showed a decrease in body length (Figure D-2b).

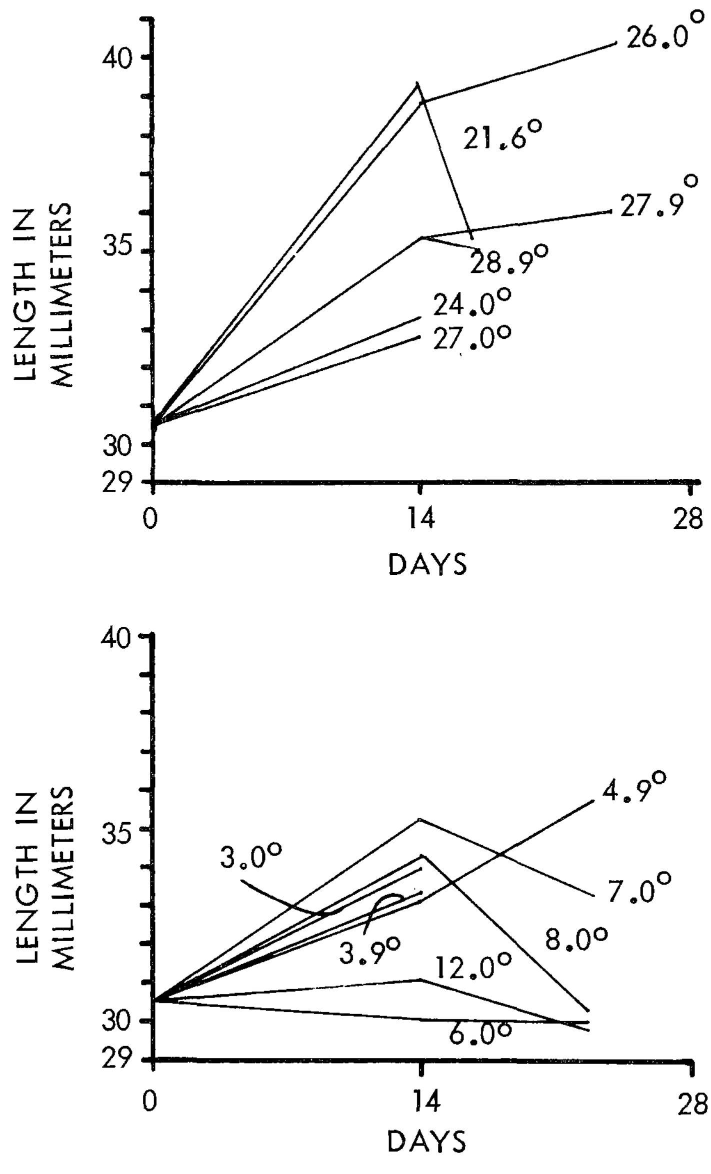


Figure D2. Growth in length of juvenile bluegills. Fish were acclimated to 12.1°C and then exposed to various test temperatures.

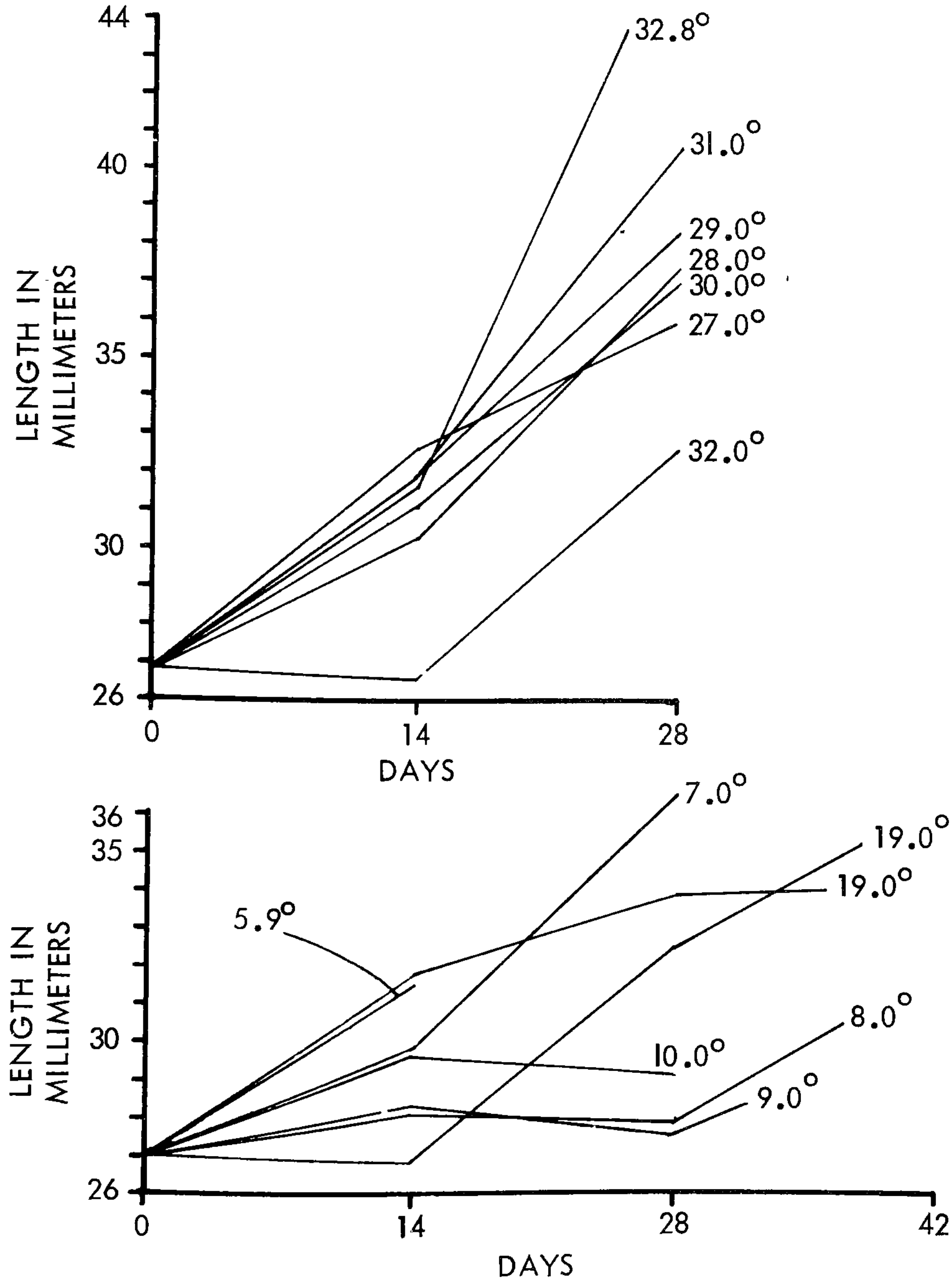


Figure D3. Growth in length of juvenile bluegills. Fish were acclimated to 19.0°C and then exposed to various test temperatures.

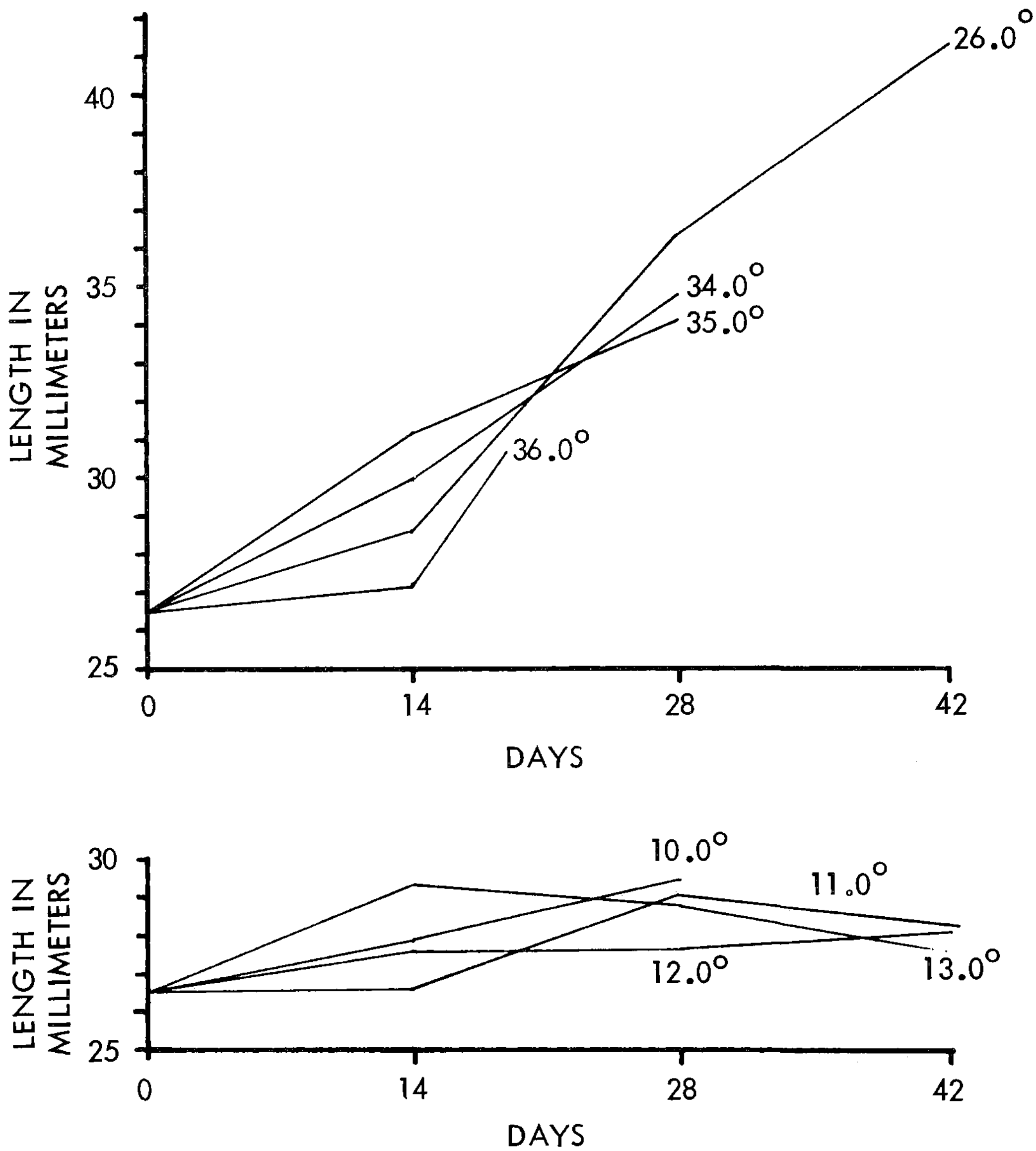


Figure D4. Growth in length of juvenile bluegills. Fish were acclimated to 26.0° and then exposed to various test temperatures.

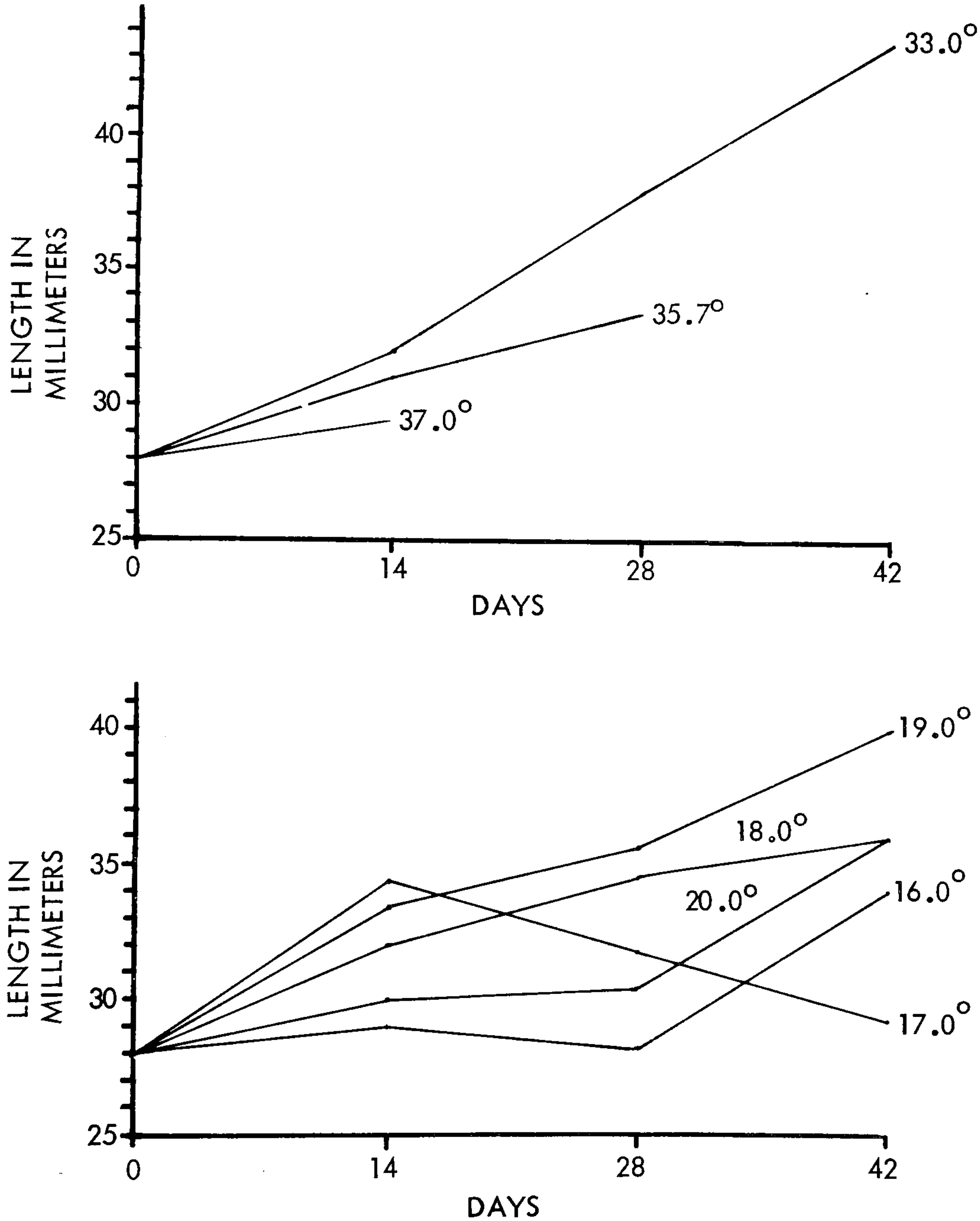


Figure D5. Growth in length of juvenile bluegills. Fish were acclimated to 32.9°C and then exposed to various test temperatures.

For fish acclimated to 12.1C (Figure D-2) and exposed to test temperatures at the upper limits of survival, change in length was maximal at 21.6 during the first two weeks. Increase in length was greater at 21.6 and 26.0C than at 27.9 or 28.9C. Growth was least at 24.0 and 27.0C. At the lower thermal limits, fish acclimated to 12C increased in length for the first two weeks, but decreased in length at 7.0, 8.0, and 12.0C during the second two weeks.

The stock that was acclimated to 19.0C (Figure D-3) showed an increase in length for all temperatures at the upper limits of survival except for the first sample taken at 32.0C. Growth rate increased with increasing temperature except for animals tested at 30.0 and 32.0C. At the lower temperatures, changes in length were erratic. Two replicates at 19.0 reached the same size after 42 days, but the time-course of growth was distinctly different.

The stock that was acclimated to 26.0C (Figure D-4) increased more rapidly in length at 26.0C than at the higher temperatures tested. At low temperatures, the stock that was acclimated to 26.0C showed no appreciable increase in length.

Fish acclimated to 32.9C (Figure D-5) prior to the growth test increased more rapidly in length at 33C than at 35.7 or 37.0C. Animals exposed to low temperatures increased consistently in length at 19.0, 18.0 and 20.0C, while animals at 16.0, and 17.0C showed a decrease in length at one or more sampling intervals.

DISCUSSION

Results of the preliminary bioassay tests indicated several problems with the life support capabilities of the thermal test facility, and sampling for the juvenile growth study.

The preliminary incubation test was not considered adequate to define upper and lower TL50. Egg viability was low. The percent normal hatch was 5% at 26C and lower TL50 was not defined for replicate B because less than 50% of the eggs hatched at all temperatures below 30C. Comparison of the two replicates indicated large differences in TL50 and temperature for optimal hatch that could not be accounted for by the experimental design. Finally, temperature control in the experimental chambers was not adequate. In spite of the large differences in optimal temperature between replicates, and TL50 range for the two replicates was quite similar, which agrees with observations by Hokanson, et al, 1973.

Poor egg viability and variation in hatching may have been due to poor water quality, improper fertilization techniques, differences in handling by personnel or variable tempering rates to test temperatures (see Discussion in main report). This variability, however, was reduced during the subsequent incubation tests.

Preliminary experiments with bluegill fry indicated that mortality rates, especially at optimal temperatures, were too high to attribute to the effects of temperature alone. Poor water quality (see main report), poor feeding, or improper handling techniques contributed significantly to fry mortality.

Bluegill sac-fry exhibited a slightly greater range of thermal tolerance than eggs. Sac-fry leave the nest as swim-up fry approximately four days after hatching at 23C and require an exogenous food supply within six days after hatch. The period between initial swim-up and and yolk-sac absorption is the "critical period" (Toetz, 1966). Thermal tolerance of bluegill swim-up fry should be intermediate between tolerance of sac-fry and tolerance of juveniles. However, an increase in temperature increases metabolic rate, and may shorten the critical period for swim-up fry, and intensify the need for these fry to have adequate food at the proper time. For these reasons, fry should be fed frequently (at least 4 to 5 times per day) during the critical period.

During the juvenile growth test, the span of temperatures tested was not adequate to define an optimal range or optimal temperature, but was sufficient to eliminate some of the extreme temperatures. However, the 12C and 33C stocks showed high mortality during the course of the experiment. Growth rates for most stocks were low.

Measurements of instantaneous growth showed large increases in weight at low temperatures and negative growth at 10C for the 19C stock. These results indicate some error due to sampling. The initial population was quite variable in weight (0.1 to 2.1 gm) so that samples of 10 animals were not representative. However, this type of sampling error does not account for apparent weight increase at low temperatures for the 12, 19 and 26C stocks. The length data show that increase in weight at low temperature is associated with an increase in body length. If animals were not growing at extreme temperature, random error would produce as many negative growth rates as positivie, and growth rates would not be significantly different from zero. Large positive changes in weight would occur at intermediate and high temperatures. The data suggests instead that selective mortality of small juveniles was occurring at the low temperatures. Animals that died of natural causes were not measured. If the small animals died between sampling intervals, only the larger animals remained to be sampled and measured at the next two-week interval.

The 32.9C stock should be retested at temperatures of 18 to 35C to define optimal temperatures and optimal physiological range for the acestival period when modification of thermal regimes by man's activity would be most detrimental.

APPENDIX E

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27 Abstract

Bioassay experiments were conducted to determine thermal tolerance of early life history stages of bluegill sunfish. Bluegill eggs hatched at temperatures from 18 to 36C during two incubation tests. Maximal hatch occurred at 22.2 and 23.9C. Lower TL_{50} temperature for hatch of normal fry was 21.9C and upper TL_{50} temperature was 33.8C.

Juvenile bluegills acclimated to 12.1C had a lower 96-hour TL_{50} of 3.2C and an upper 96-hour TL_{50} of 27.5C. Juveniles acclimated to 32.9C had a lower 96-hour TL_{50} of 15.3C and an upper 96-hr TL_{50} of 37.3C. TL_{50} increased with increasing temperature of acclimation. For juveniles acclimated to a given temperature, upper TL_{50} decreased with longer exposure

A preliminary test determined ranges of thermal tolerance for sac-fry and swim-up fry. In another preliminary test, juvenile bluegills were acclimated to 12.1, 19.0, 26.0 or 32.9C, and reared at a series of test temperatures for three to six wks. to define optimal temperature ranges for growth and survival.

Additional research determined conditions for the culture of Lepomis macrochirus, including spawning induction, hatching, and growth of larvae and juveniles.

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