Cold Tolerance in Two Subspecies of Bluegill

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Abstract: Two bluegill subspecies (common bluegill *Lepomis macrochirus macrochirus* and coppernose bluegill *L. m. purpurescens*) were subjected to cold tolerance tests in the laboratory. Juvenile and adult bluegills were exposed to water temperature decreases of 1° C/hour from acclimation temperatures of 20° and 30° C until loss of equilibrium. Response temperatures of both subspecies were similar; temperatures at which 50% of test fish lost equilibrium were usually <1.0° C lower for common bluegill and overlapping occurred in fiducial limits.

Bluegill acclimated to 20° C lost equilibrium at 0.6°–4.2° C; those acclimated to 30° C lost equilibrium at 6.4°–10.4° C. Adults were more resistant than juveniles to low temperatures when acclimated to 20° C but juveniles were more resistant at 30° C.

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Bluegill (*Lepomis macrochirus*) are found in waters throughout the United States (Lee et al. 1980). They are a popular sport fish and an important prey item for many predatory species. Culture and stocking of bluegill began in the early 1900s and such practices remain as important components of various fishery management programs (Swingle 1970). Of 3 subspecies, the 2 most often encountered are the common bluegill (*L. m. macrochirus*, Rafinesque 1820) and the southeastern or coppernose bluegill (*L. m. purpurescens*, Hubbs and Allen 1943). The third sub-

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species (*L. m. speciosus,* Baird and Girard 1854) is commonly known as the Texas bluegill.

Coppernose bluegill reportedly have more desirable sportfish characteristics than common bluegill including faster growth, larger size, increased hardiness, and improved sportfishing qualities (Hubbs and Allen 1943, Henry 1979). Coppernose bluegill have been cultured and introduced into impoundments in southeastern states (W. King, unpubl. rep., N.C. Dep. Conserv. Devel., Raleigh, 1947) and California (Henry 1979). Successful introductions and establishment of populations are not well documented. Guest (1984), for unknown reasons, had limited success in establishing coppernose and common bluegill in central Texas impoundments. Genetic intergradation commonly occurs in nature between common and coppernose bluegill (Avise and Smith 1974, Felley 1980, Felley and Avise 1980), therefore recognition of pure coppernose bluegill populations with improved sportfishing success may be masked.

The Texas Parks and Wildlife Department (TPWD) has introduced bluegill into public waters since 1934 to enhance existing fisheries. However, until recently, stocking was irregular and culture efforts were limited. In 1981 a stock of coppernose bluegill brood fish was obtained and a program of fingerling stocking established. Survival of stocked juveniles in some impoundments was uncertain. Fisheries biologists in West and North Texas failed to recover coppernose bluegill in management surveys the year following introduction (B. J. Follis and J. E. Kraai, TPWD, Austin, pers. commun.). Although there is no literature concerning temperature tolerance of this subspecies, it was believed that adaptation to their native environment may not have provided them the ability to withstand colder temperatures encountered in Texas. This study was conducted to determine the cold tolerance of juvenile and adult common and coppernose bluegill.

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Methods

Coppernose bluegill were produced at the A. E. Wood Fish Hatchery (WFH), San Marcos, Texas. Adults were second-generation progeny of stock collected from Lake Okeechobee, Florida; juveniles were third-generation progeny. Fish were transported to Heart of the Hills Research Station (HOH), Ingram, Texas, 1 month before temperature acclimation began.

Common bluegill consisted of 2 stocks, 1 obtained from WFH (of unknown origin) and 1 from Hords Creek Reservoir, Coleman County, Texas. Hatchery stock adults were transported to HOH as juveniles 3 years prior to the experiment. Wild stock adults collected from Hords Creek Reservoir were captured with boat-

mounted electrofishing equipment 6 months before the study. Juveniles of both common bluegill stocks were produced at HOH.

Production of juveniles took place in similar 0.25-ha earthen ponds. Adults were stocked at 60 pairs/ha approximately 1 month before spawning. Ponds were fertilized weekly with inorganic granular fertilizer (16–20–0 N-P-K) at a rate of 1.0 mg/liter as P_2O_5. Supplemental feed (20% crude protein, crumbles) was offered to fish 5 times weekly at an approximate rate of 2% adult body weight. Excessive macrophytes were controlled with applications of 1.5 kg/ha Diuron as needed.

Electrophoretic evaluation was undertaken to determine genetic composition of bluegill stocks. The liver and eyes were excised from 20 adults of each stock and processed according to methods described by Harvey et al. (1980). Horizontal starch gel electrophoresis was conducted according to procedures found in Selander et al. (1971). The enzymatic proteins, esterase (EST-3), glutamate oxaloacetate transaminase (GOT-1, -2), and phosphoglucomutase (PGM) were analyzed. Buffer systems, enzyme stains, and scoring techniques were those used by Kulzer and Greenbaum (1986).

Fishes were collected from production ponds 1 month before temperature acclimation and transferred into indoor 800-liter circular fiberglass tanks. Thirty adults of each group (coppernose, wild stock common, and hatchery stock common) were placed into each of 2 tanks. They were marked using a paper punch according to group in either the dorsal, anal, or caudal fin. Seventy juveniles of each group were placed into separate partially submerged wooden frame wire mesh boxes (45 × 40 × 40 cm) in each of the 2 tanks. Water temperature was maintained at 25° ± 1° C for 2 weeks before temperature acclimation began. Thermostatically operated submersible heating units controlled water temperature. Water quality in tanks was maintained throughout the experiment with recirculating gravel filters and periodic siphoning of particulates.

Adults were offered artificial feed *ad libitum* twice daily, 5 times weekly. Juveniles were fed live brine shrimp nauplii (*Artemia* sp.) in a similar manner during the initial 2-week holding period before artificial feed was offered. Fishes were not fed during the temperature decline portion of the experiment.

Acclimation to constant temperatures (20° and 30° ± 1° C) took place in holding tanks at a rate of 1.0° C/day. Water was warmed or chilled as required with portable thermostatically controlled heating and chilling units. Once at the desired acclimation temperature, fish were held for 21 days before testing.

Low temperature tests took place in 2 adjacent identical 800-liter circular fiberglass tanks (91.4-cm diameter). Each tank was partitioned in thirds with netted frames to separate groups. Fish groups were randomly assigned tank sections. Ten adults or 30 juveniles (Table 1) of each group and acclimation temperature were tested in each of 2 replicates. Adults were placed directly into the tank while juveniles were held in partially submerged wire mesh baskets (25 × 18 × 18 cm). Ten fish of each group served as controls for each acclimation temperature.

Experiments took place in September and October. Replicate tests occurred 2 days apart; experiments with adults took place 1 month before tests with juveniles.

Table 1. Mean total length (mm ± SD) of juvenile and adult common and coppernose bluegill used in cold tolerance experiments. Common bluegill stocks are designated as WS for wild stock and HS for hatchery stock.

Acclimation temperature (°C)	*N*	Common HS	Common WS	Coppernose
		Juveniles		
20	30	44 ± 4.8	44 ± 3.3	43 ± 3.4
30	30	44 ± 4.9	47 ± 3.9	45 ± 3.7
		Adults		
20	10	165 ± 10.6	171 ± 10.2	173 ± 7.9
30	10	159 ± 9.2	168 ± 10.9	174 ± 6.7

At the onset of each replicate, the test tank was warmed to 30° C and test groups acclimated to 30° C were transferred into the test tank. Water temperature was decreased 1° C/hour until 20° C, then test groups acclimated to 20° C were placed into the test tank. Water was again cooled 1° C/hour using chilling units controlled with an electrical system modified from Abell et al. (1977) until each fish lost equilibrium. Fish condition and water temperature (measured to the nearest 0.1° C) were checked each half hour. Once equilibrium loss started, observation of fish was continuous to note exact temperature of equilibrium loss. Equilibrium was considered lost when specimens were unable to maintain an upright position and laid flat on the tank or basket bottom. After individual response temperatures were recorded, affected fish were removed from test tanks.

We evaluated resistance to low temperatures by calculating and comparing ET_{50}'s, the point at which 50% of the fish from 1 test group (replicates combined) lost equilibrium. ET_{50}'s were determined from regressions of probit transformations of numbers of fish that lost equilibrium on natural logs of temperatures at equilibrium loss (SAS 1985). Fiducial limits (95%) around each ET_{50} were calculated. Methods for statistical analysis were adopted from Finney (1978) and Sokal and Rohlf (1969).

Results

Electrophoretic analyses indicated pure common bluegill alleles at all 4 enzyme-encoding gene loci for both common bluegill stocks. Alleles from coppernose bluegill samples indicated pure coppernose subspecies characters at EST and PGM loci; however, an intergrade genotype was noted in 2 fish (10%) at the GOT-2 locus.

Bluegill acclimated to 30° C had higher ET_{50}'s than those acclimated to 20° C, regardless of subspecies or stock (Table 2). However, effects due to fish size varied; ET_{50}'s for adults (1.2°–1.9° C) were lower than for juveniles (2.0°–2.7° C) when

Table 2. Temperatures (°C) at which 50% of test populations lost equilibrium (ET_{50}) with 95% fiducial limits around ET_{50} values (in parenthesis), and temperature range at loss of equilibrium for juvenile (N = 30 for each group) and adult (N = 10 for each group) common and coppernose bluegill. Fish were acclimated to 20° and 30° C and subjected to a 1° C/hour temperature decline. Common bluegill stocks are designated as WS for wild stock and HS for hatchery stock.

Subspecies	20° C		30° C	
	Temperature range	ET_{50}	Temperature range	ET_{50}
		Juvenile		
Common, HS	4.1 - 1.8	2.1 (1.7, 2.3)	9.4 - 6.9	7.3 (6.3, 7.8)
Common, WS	3.3 - 1.3	2.0 (1.2, 2.3)	9.4 - 6.4	6.8 (6.5, 7.1)
Coppernose	4.2 - 2.1	2.7 (2.3, 2.9)	9.4 - 8.1	8.3 (7.0, 8.6)
		Adult		
Common, HS	2.7 - 0.8	1.4 ([a], 1.9)	9.1 - 8.1	8.4 ([a], 8.7)
Common, WS	1.5 - 0.6	1.2 ([a],[a])	9.1 - 7.0	7.6 (6.9, 7.9)
Coppernose	3.8 - 1.4	1.9 (0.8, 2.4)	10.4 - 8.2	8.5 (7.4, 8.9)

[a]Indicates data did not allow calculation of fiducial limits.

acclimated to 20° C, but juveniles had lower ET_{50}'s (6.8°–8.3° C) than adults (7.6°–8.5° C) when acclimated to 30° C.

Although ET_{50}'s for common bluegills were consistently less than those for coppernose bluegills, differences were usually <1° C. In cases where ficucial limits around ET_{50}'s could be estimated (Table 2), overlapping occurred between subspecies and fish sizes for each acclimation temperature. However, overlapping did not occur between acclimation temperatures.

Discussion

Historically, bioassays conducted to determine relative temperature tolerance in fish involved death of specimens (Fry et al. 1946, Brett 1956). Unfortunately, in some instances death may be difficult to achieve (Guest 1982). We agree with Becker et al. (1977) that determining the impact of cold shock should be based on equilibrium loss rather than death. We chose loss of equilibrium because we could achieve this response and it is biologically significant. In waters where bluegill may be exposed to severe and sudden temperature declines, and equilibrium loss occurs, chances of predation are increased (Coutant et al. 1974). In addition, if feeding ceases and condition of fish weakens, they may become vulnerable to disease.

Acclimation temperature appeared positively related to bluegill cold tolerance. This is consistent with results of other temperature tolerance tests with this species (Christianson and Tichenor 1968, Banner and Van Arman 1973, Hickman and Dewey 1973, Murphy et al. 1976, Peterson and Schutsky 1976).

The affect of fish size on response temperature was inconsistent. This is in

contrast to Cox (1974) who determined there were differences in critical thermal maximum and loss of equilibrium relative to bluegill size; large fish (14.0 cm) lost equilibrium sooner but died at higher temperatures than small fish (7.0 cm). His results suggested larger fish had more stamina in resisting death due to heat stress.

Values of ET_{50} were low compared to lower lethal temperatures (LT_{50}) reported by Banner and Van Arman (1973) who conducted experiments with juvenile bluegill (mean weight = 0.3 g). Although they did not identify subspecies of their bluegill collected in Florida and Georgia, it is probable they were coppernose bluegill considering geographic distribution of that subspecies (Avise and Smith 1974). Banner and Van Arman's (1973) fish acclimated to 19.0° and 32.9° C had LT_{50}'s of 4.0° and 10.3° C, respectively. The probable reason for differences in study results is their test involved immediate placement of bluegill from acclimation to test temperatures.

Two fish (10%) from the sample of coppernose bluegill were determined to be intergrades. However, this intergradation was evident at only one locus. Therefore, we believe test results for coppernose bluegill represent true reactions for this subspecies.

Water temperature declines found normally in Texas should not be detrimental to coppernose bluegill. Becker et al. (1977) found a closely related species (*Lepomis gibbosus*) showed significant temperature adaptation when cooling rate was reduced. Temperature declines in our study were probably more severe than those which occur naturally. Even from an acclimation temperature of 20° C with a decline rate of 1° C/hour, coppernose bluegill had ET_{50}'s of 1.9°–2.7° C, similar to those of common bluegill. Therefore, temperature tolerance should not be a factor when considering further introductions of coppernose bluegill.

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