Physiological Disturbances and Overwinter Mortality of Largemouth Bass from Different Latitudes

Matthew M. VanLandeghem[1,2,*]
Curtis P. Wagner[1,†]
David H. Wahl[1]
Cory D. Suski[2]
[1]Kaskaskia Biological Station, Illinois Natural History Survey, University of Illinois at Urbana-Champaign, 1235 CR 1000N, Sullivan, Illinois 61951; [2]Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, W401-C Turner Hall, 1102 South Goodwin Avenue, Urbana, Illinois 61801

Accepted 6/1/2013; Electronically Published 9/20/2013

ABSTRACT

Thermal conditions associated with winter can influence the distribution of a species. Because winter severity varies along latitudes, populations of temperate fish located along a latitudinal gradient may display variation in both sublethal and lethal responses to cold stressors. Sublethal physiological disturbances were quantified in age 1 largemouth bass (*Micropterus salmoides*) from populations originating from Alabama and Illinois but raised in a common environment. Fish were exposed to 6 h of rapid cold shock from 20° to 8°C (controls were held at 20°C) and then sampled for white muscle, whole blood, and plasma. After cold shock, glucose concentrations were elevated in Alabama but not Illinois fish. Sodium was lower and chloride was higher in Alabama largemouth bass, but fish from Illinois had a greater propensity for potassium loss during cold shock. In Illinois ponds, Alabama largemouth bass exhibited lower overwinter survival (adult: 10%; age 0: 22%) than did those from Illinois (adult: 80%; age 0: 82%). Latitudinal variation in physiological responses to cold stressors may therefore influence overwinter survival of largemouth bass and the ability of a fish species to exist over large geographic areas.

* Corresponding author. Present address: Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Agricultural Sciences Room 218, 15th and Boston, Lubbock, Texas 79409; e-mail: matt.vanlandeghem@ttu.edu.
† Present address: Ohio Department of Natural Resources, Division of Wildlife–District Three, 912 Portage Drive, Akron, Ohio 44319.

Physiological and Biochemical Zoology 86(6):716–726. 2013. 1522-2152/2013/8606-2154$15.00. DOI: 10.1086/673181

Introduction

Many species distributed over large geographic areas can be subjected to clines of environmental conditions throughout their range (Hillebrand 2004; Conover et al. 2009). More specifically, variation in temperature across large geographic areas can be extreme and can induce differences in thermal tolerance for individuals from different areas. For example, *Drosophila melanogaster* from southern populations exhibit lower resistance to cold temperatures than do those from northern populations, which have adapted cold resistance to survive low temperatures present at higher latitudes (Hoffman et al. 2002). Southern populations of eastern fence lizards (*Scleoporus undulates*) have lower metabolic rates at high temperatures than do individuals from northern populations, presumably to reduce metabolic energy costs and increase reproductive output at warm southern temperatures (Angilletta 2001). Furthermore, tolerance of heat and cold extremes determines the northern and southern extent of the latitudinal range of European diving beetles (*Deronectes* spp.; Calosi et al. 2010). Clearly, latitudinal patterns in physiology are prevalent, are strongly influenced by thermal gradients, and can impact the range and distributions of organisms.

Many temperate freshwater fishes are distributed across large geographic ranges and can experience thermal regimes that vary on a variety of scales. Rapid, extreme changes in temperature (i.e., temperature "shocks") can occur due to natural and anthropogenic sources, and fish generally are able to withstand these fluctuations by employing a host of physiological and behavioral responses (reviewed in Donaldson et al. 2008). When considered across broad geographical areas, high latitudes are susceptible to large temperature variation within days and across seasons; this thermal variability can influence intraspecific variation along latitudes (Conover and Present 1990; Conover et al. 2009). Growth, survival, and food consumption have been shown to differ among latitudinally separated fish populations (Clapp and Wahl 1996; Fullerton et al. 2000; Galarowicz and Wahl 2003). Physiological processes, such as metabolic rates and cardiac variables, have also shown similar division among populations (Clapp and Wahl 1996; Cooke et al. 2001; Galarowicz and Wahl 2003; Cooke and Philipp 2005). For example, at high (25°C) temperature, the metabolic rate of walleye (*Sander vitreus*) from southern populations is approximately 50% greater than that of walleye from northern populations (Galarowicz and Wahl 2003). Few studies, however, have quantified latitudinal variation in the stress response of fishes. For example, DeKoning et al. (2004) showed that hepatic enzyme activities differed between a northern and a southern population of killifish (*Fundulus heteroclitus*), likely because of latitudinal differences in stress hormone physiology. Given that

temperature and thermal regimes can vary greatly along latitudes, the physiological response of fish to thermal stressors may also vary with latitudinal origin. Latitudinal variability in the physiological response to thermal challenges may ultimately affect the ability of fish to survive extreme environmental events, yet it is currently unknown whether these differences exist and whether they can influence mortality.

One extreme thermal event encountered by temperate fish populations is winter, which can negatively impact the growth and survival of fishes (reviewed in Suski and Ridgway 2009). Low food availability, predation, osmoregulatory challenges, reduced energy reserves, and tolerance to low temperatures have all previously been shown to influence survival of fishes over winter (Fullerton et al. 2000; McCollum et al. 2003; Hurst 2007; Suski and Ridgway 2009). In addition, experiments subjecting transplanted individuals to a common-garden environment have shown that latitudinal origin can affect overwinter survival. High-latitude populations of Atlantic silverside (*Menidia menidia*) exhibited greater survival than did low-latitude populations when exposed to high-latitude winter conditions, presumably because of an adaptive response (e.g., greater cold tolerance, energy accumulation) to the more severe winters present at high latitudes (Schultz et al. 1998). Previous work has demonstrated strong latitudinal effects of winter severity on largemouth bass (*Micropterus* spp.), illustrated by poor survival of low-latitude (Alabama) fish when exposed to mid-latitude (Ohio) and high-latitude (Wisconsin) winters (Fullerton et al. 2000). The genetic stock of low-latitude largemouth bass used in Fullerton et al. (2000), however, was largely (96%) comprised of the Florida largemouth bass (*Micropterus floridanus*) genome, suggesting that some of the observed patterns could have been reflective of interspecific variation rather than intraspecific (latitudinal) variability. Specifically, high mortality of low-latitude largemouth bass utilized by Fullerton et al. (2000) could be due to the strong genetic influence of *M. floridanus*, which is not subjected to severe winters in its natural range of peninsular Florida, rather than latitudinal variability of the more ubiquitous *Micropterus salmoides*, which is distributed over a much larger range of winter severities. Thus, a comparison of pure *M. salmoides* from low and high latitudes would provide insight into the intraspecific variability in the response of fish to thermal challenges.

Therefore, the objectives of this study were to (1) quantify the physiological impacts of an acute cold stressor on two latitudinally distinct populations of pure largemouth bass (exclusively *M. salmoides*) reared in a common environment, (2) quantify differences in overwinter mortality of two latitudinally distinct populations of largemouth bass using a common-garden approach, and (3) determine whether population-specific physiological properties are a potential mechanism for differences in overwinter mortality. The hypothesis we are testing is that local adaptation influenced by latitudinal origin will have a significant impact on the physiological response of largemouth bass to both short- and long-duration cold stressors, with northern populations displaying increased performance relative to southern populations. To accomplish this, we conducted a laboratory-based thermal challenge experiment with a complementary mortality experiment with two populations of largemouth bass. Largemouth bass were used as a model organism because they are latitudinally ubiquitous within North America and can experience a range of winter severity throughout their native range.

Material and Methods

Adult largemouth bass were collected by boat electrofishing from November 2006 through March 2007 from Pierce Lake, located in northern Illinois (42°20′38″N, 88°58′58″W; $N = 64$; mean total length [TL] ± SE = 325 ± 4.8 mm), and from Hastie Lake, located in southern Alabama (30°57′41″N, 87°52′40″W; $N = 61$; mean TL ± SE = 351 ± 5.8 mm), and transported to the Sam Parr Biological Station, Kinmundy, Illinois (38°42′36″N, 88°44′39″W). No evidence of hybridization with Florida largemouth bass has been observed for either population (Pierce Lake, IL, and Hastie Lake, AL, hereafter termed IL and AL, respectively; Hallerman et al. 1986; Nedbal and Philipp 1994). On arrival at the Sam Parr Biological Station, fish were evenly dispersed into 0.4-ha earthen ponds (two ponds per population) supporting moderate aquatic vegetation and naturally colonized invertebrates. Established bluegill (*Lepomis macrochirus*) populations and supplemental fathead minnow (*Pimephales promelas*) introductions provided prey resources. Adult largemouth bass were allowed to spawn naturally. All procedures conformed to the University of Illinois Institutional Animal Care and Use Committee and complied with the current laws of the United States.

Rearing ponds were drained in November 2007 to collect adult and age 0 largemouth bass. Equal numbers of age 0 fish from both populations (IL: $N = 55$, mean TL ± SE = 162 ± 3.1 mm; AL: $N = 55$, mean TL ± SE = 162 ± 3.0 mm) were individually marked with passive integrated transponder tags (Harvey and Campbell 1989; Wagner et al. 2007) and introduced into a 0.4-ha earthen pond at the Sam Parr Biological Station to assess overwinter survival differences. Furthermore, adult largemouth bass (IL: $N = 34$, mean TL ± SE = 339 ± 8.9 mm; AL: $N = 30$, mean TL ± SE = 365 ± 7.9 mm) were batch-marked and stocked in a separate pond (0.4 ha). Ponds were drained in March 2008 to collect remaining fish and determine overwinter survival rates.

During spring 2008, age 1 offspring from these same two populations were collected from two additional 0.4-ha ponds when water temperatures ranged between 18° and 20°C. Once collected, fish were transported to the Kaskaskia Biological Station, Sullivan, Illinois, where physiological experiments were conducted during spring 2008. Fish were held for at least 7 d in laboratory holding tanks before use in experiments, allowing for recovery from transport-induced stressors, acclimation to laboratory conditions, and resumption of feeding. During holding, largemouth bass were fed fathead minnows ad lib. but were starved for 48 h before use in experiments. Holding tanks were maintained at 20° ± 1°C and were continuously aerated (dissolved oxygen = 7.8 ± 0.6 mg O_2/L; YSI 85 temperature and

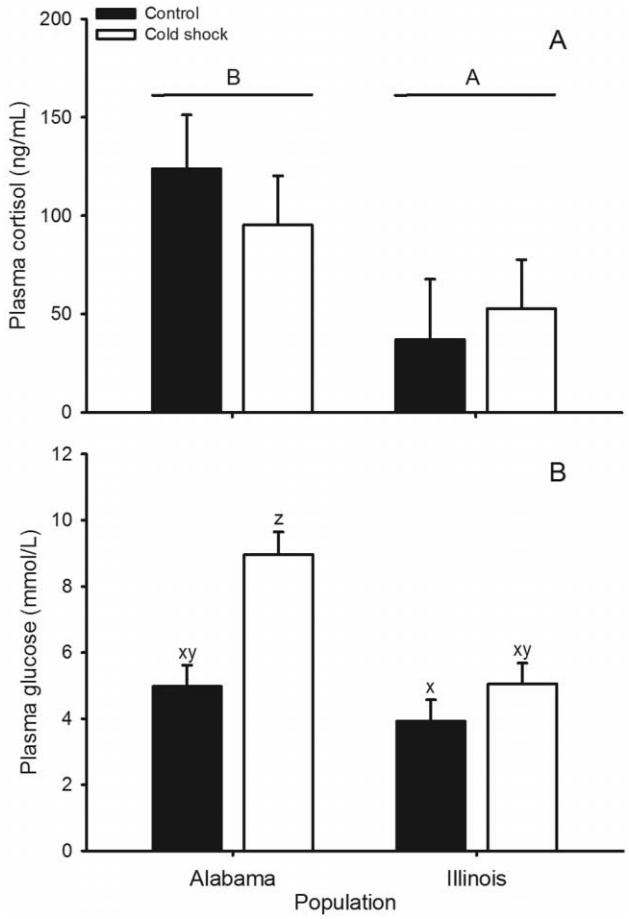

Figure 1. Concentrations of plasma cortisol (*A*) and glucose (*B*) of largemouth bass originating from Alabama and Illinois. Fish were either held for 6 h at 20°C (controls; filled bars; $n = 6$ per population) or subjected to rapid cold shock from 20° to 8°C and held at 8°C for 6 h (cold shock; open bars; $n = 7$ per population). Mean separation is indicated by lowercase letters; statistically different means do not share letters. Uppercase letters separate population means when the main effect of population was significant and the main effect of temperature and the interaction of the main effects (population × temperature) were not significant.

dissolved oxygen meter; YSI, Yellow Springs, OH), and total ammonia nitrogen was maintained at or below 1 mg/L (model 33D; Aquarium Pharmaceuticals, Chalfont, PA).

Once acclimated to laboratory conditions, largemouth bass from each population were placed into individual, aerated, opaque boxes containing 20°C water continuously supplied from a central basin (Suski et al. 2006). Water was pumped from the basin into individual chambers, allowed to overflow from the chambers, and then drained back to the central basin, forming a closed circuit. Fish were allowed to acclimate to conditions within the opaque boxes for 24 h before the start of the experiment. After the 24-h acclimation time, one group of 14 largemouth bass (seven from each population) was subjected to a rapid (<5 min) temperature change from 20° to 8°C by pumping chilled water from a separate basin into the chambers (VanLandeghem et al. 2010); fish were held at 8°C for 6 h. A second group of 12 largemouth bass (six from each population) was held at 20°C (no temperature change) for 6 h as a control group.

At the end of 6 h of exposure to the temperature change or control temperature, largemouth bass were terminally sampled by adding an overdose of anesthetic (250 mg/L 3-aminobenzoic acid ethyl ester methanesulphonate [MS-222] buffered with 500 mg/L sodium bicarbonate) directly to each chamber. After cessation of ventilation, each fish was quickly weighed (nearest g) and measured (nearest mm). Approximately 1 mL of blood was then drawn directly from the gill arch using a 21-G hypodermic needle and 1-mL syringe rinsed with lithium heparin (Houston 1990). Immediately after withdrawal, a small portion (about 100 μL) of whole blood was placed into a 1.5-mL centrifuge tube and flash-frozen in liquid nitrogen for later determination of hemoglobin. Hematocrit values for whole blood (% packed cell volume) were then determined by inducing a small amount of whole blood into two heparinized microcapillary tubes (about 20 μL each) that were then puttied and centrifuged at 16,000 rpm (13,700 *g*) for 3 min using a hematocrit centrifuge and read using a digital reader (CritSpin models CS22 and CSD2). The remaining whole blood (about 800 μL) was placed in a 1.5-mL microcentrifuge tube and centrifuged for 2 min at 2,000 *g* for 2 min. Plasma was immediately separated from erythrocytes using a transfer pipette, divided into three aliquots of approximately 100 μL in 1.5-mL centrifuge tubes, and flash-frozen in liquid nitrogen before being transferred to an ultracold freezer (below −80°C) until processing (Suski et al. 2003). In addition to blood and plasma, a portion of white epaxial musculature (about 5–10 g) posterior to the operculum and above the lateral line was excised, freeze-clamped in aluminum tongs precooled in liquid nitrogen, and stored in liquid nitrogen or at −80°C until further processing (see Suski et al. 2003). Total length (IL mean TL ± SE = 226 ± 5.1 mm; AL mean TL ± SE = 213 ± 8.6 mm; $t = -1.11$, df = 24, $P = 0.28$), weight (IL mean ± SE = 128.8 ± 1.0 g; AL mean ± SE = 93.0 ± 9.7 g; $t = -2.04$, df = 24, $P = 0.052$), and Fulton's condition factor (Ricker 1975; IL mean ± SE = 1.084 ± 0.06; AL mean ± SE = 1.128 ± 0.05; $t = 0.54$, df = 24, $P = 0.60$) of largemouth bass in the temperature challenge experiment were not significantly different between populations.

Laboratory Analyses

Analyses of plasma parameters are described in detail in Suski et al. (2003). Briefly, plasma cortisol, whole-blood hemoglobin, and plasma hemoglobin concentrations were determined using commercially available kits (cortisol: kit 900-071, Assay Designs, Ann Arbor, MI; hemoglobin: QuantiChrom Hemoglobin Assay kit, DIHB-250, BioAssay Systems, Hayward, CA). Plasma sodium and potassium concentrations were determined using a flame photometer (model 2655-00; Cole-Parmer Instrument, Chicago, IL), and plasma chloride concentrations were determined using a chloride titrator (model 4435000; Labconco Corporation, Kan-

sas City, MO). Plasma activities of lactate dehydrogenase (LDH; enzyme 1.1.1.27) were quantified using standard kinetic spectrophotometric techniques on the basis of the methods of Wroblewski and LaDue (1955). Plasma glucose concentrations and concentrations of lactate, adenosine triphosphate (ATP), and phosphocreatine (PCr) in white muscle were determined enzymatically, after the methods of Lowry and Passonneau (1972), in a 96-well microplate with a commercially available spectrophotometer (Spectra Max Plus 384, model 05362; Molecular Devices, Union City, CA). Water content of white muscle was determined by drying preweighed tissue in an 80°C oven until a constant mass was attained.

Statistical Analyses

For physiological experiments, a two-way ANOVA was used to test for the interaction of the main effects of population and water temperature on blood, plasma, and white-muscle parameters. When the interaction was significant ($P < 0.05$) or when the interaction was not significant ($P > 0.05$) but at least one of the main effects was significant ($P < 0.05$), a Tukey-Kramer HSD post hoc test was used for mean separation. Differences in overwinter survival rates between populations were compared with χ^2 tests obtained from logistic regression analysis of variance models (GENMOD procedure; Littell et al. 2002) for both the age 0 and the adult largemouth bass. Statistical analyses were performed using SAS (ver. 9.1; SAS Institute, Cary, NC), and the level of significance (α) for all tests was 0.05.

Results

Exposure of largemouth bass from both populations to cold shock from 20° to 8°C resulted in significant differences in plasma and white-muscle parameters relative to control fish held at 20°C. The physiological response to cold shock differed, however, between populations, and significant physiological differences between populations were also observed among control fish. Specifically, plasma cortisol concentrations of control AL largemouth bass were twofold greater than those of IL fish; however, plasma cortisol concentrations of cold-shocked largemouth bass were not significantly different from those of controls for either population (fig. 1*A*; table 1). Plasma glucose concentrations of cold-shocked IL largemouth bass did not differ from those of IL control fish, but glucose concentrations of cold-shocked AL largemouth bass were double those of AL controls (fig. 1*B*; table 1). Cold shock had no significant effect on sodium concentrations for either population, but plasma sodium concentrations of AL largemouth bass were about 10% lower than those of IL largemouth bass (fig. 2*A*; table 1). Similar to plasma sodium, exposure to a temperature shock from 20° to 8°C had no significant effect on plasma chloride concentrations relative to controls for either population, but chloride concentrations were about 5% greater for the AL population than for the IL population (fig. 2*B*; table 1). After cold shock, AL largemouth bass did not exhibit significant differences in plasma potassium concentrations relative to AL controls (fig. 2*C*; table 1). Plasma potassium concentrations of cold-shocked largemouth bass from the IL population, however, were 40% lower than those of IL controls (fig. 2*C*; table 1).

Similar to plasma constituents, significant differences between populations were also observed for white-muscle parameters. White-muscle PCr concentrations of cold-shocked AL largemouth bass were double those observed in controls, whereas PCr concentrations of cold-shocked and control IL fish were not significantly different (fig. 3*A*; table 1). Concentrations of ATP in white muscle did not significantly differ relative to control values after cold shock for either population (fig. 3*B*; table 1). Although the white-muscle lactate concentrations did not significantly differ between cold-shocked and control fish for either population, the muscle lactate concentration of the control group of the AL population of largemouth bass was about five times greater than that of the cold-shock group of the IL population of largemouth bass (fig. 3*C*; table 1). No significant effects of population, temperature, or their interaction were observed for whole-blood hemoglobin concentration, hematocrit, plasma hemoglobin concentration, or plasma LDH activity (tables 1, 2). White-muscle water content ranged from 80.5% to 82.3%, and no significant effects of population, temperature, or their interaction were observed, indicating no fluid shifts into or out of white muscle after thermal challenges (tables 1, 2).

Overwinter survival of age 0 largemouth bass in Illinois ponds differed significantly between populations ($\chi^2 = 42.9$, df = 1, $P < 0.0001$). Specifically, survival of IL largemouth bass (mean ± SE = 82% ± 5%) was almost fourfold greater than that of AL largemouth bass (mean ± SE = 22% ± 6%). Differential size-selective mortality (Garvey et al. 1998, 2004) between populations likely did not explain the overwinter survival differences between IL and AL age 0 largemouth bass because both populations were similar in size at the time of fall stocking (mean TL = 162 mm for both populations) and during spring draining (IL: mean TL ± SE = 168 ± 3 mm; AL: mean TL ± SE = 174 ± 6 mm; $P = 0.39$). Adult largemouth bass exhibited a survival pattern similar to that of the age 0 fish, with adults from the IL population showing significantly higher overwinter survival (mean ± SE = 80% ± 7%) compared with AL largemouth bass (mean ± SE = 10% ± 5%; $\chi^2 = 34.4$, df = 1, $P < 0.0001$).

Discussion

Previous studies have shown that resting (prestress) physiological parameters of fish—such as metabolic rate (Galarowicz and Wahl 2003), cardiac function (Cooke et al. 2001; Cooke and Philipp 2005), and activity rates of hepatic enzymes (DeKoning et al. 2004)—can vary across populations from different latitudes, illustrating that physiological process for fishes can be influenced by geographical origin. In our study, physiological differences between populations of latitudinally separated largemouth bass were observed in the control groups but were apparent only for a few of the physiological parameters after acute cold shock. More specifically, concentrations of cortisol and

Table 1: Results from two-way ANOVA for plasma, whole-blood, and white-muscle parameters

Response variable, source	SS	df	*F*	*P*
Plasma cortisol (ng/mL):				
Population	5.3	1	4.9	**.042**
Temperature	.5	1	.5	.495
Population × temperature	.1	1	.1	.74
Error	18.5	17		
Plasma glucose (mmol/L):				
Population	65.6	1	14.5	.001
Temperature	69.3	1	15.4	.001
Population × temperature	20.9	1	4.6	**.044**
Error	87.3	19		
Plasma sodium (mEq/L):				
Population	3,298.5	1	10.34	**.004**
Temperature	12.7	1	.04	.844
Population × temperature	158.5	1	.5	.489
Error	6,485.7	20		
Plasma chloride (mEq/L):				
Population	235.8	1	4.5	**.048**
Temperature	107.4	1	2	.17
Population × temperature	39.2	1	.7	.4
Error	1,028.9	19		
Plasma potassium (mEq/L):				
Population	1.16	1	5.6	.029
Temperature	.7	1	3.4	.082
Population × temperature	1.23	1	5.9	**.025**
Error	4.17	20		
White-muscle PCr (μmol/g wet mass):				
Population	591.5	1	12.2	.002
Temperature	529.2	1	11	.004
Population × temperature	363.4	1	7.5	**.013**
Error	966.6	20		
White-muscle ATP (μmol/g wet mass):				
Population	4.4	1	2.1	.164
Temperature	16.4	1	7.8	**.011**
Population × temperature	1.4	1	.7	.428
Error	42.4	20		
White-muscle lactate (μmol/g wet mass):				
Population	7.4	1	4.4	**.048**
Temperature	11.9	1	7.2	**.014**
Population × temperature	1.5	1	.9	.356
Error	33.2	20		
Whole-blood hemoglobin (g/dL):				
Population	3.1	1	1.2	.293
Temperature	1.5	1	.6	.456
Population × temperature	.9	1	.3	.571
Error	52.6	20		
Whole-blood hematocrit (% PCV):				
Population	63.5	1	2.69	.142
Temperature	18.7	1	.79	.401
Population × temperature	.2	1	.01	.931
Error	175.6	7		
Plasma hemoglobin (mg/dL):				
Population	72	1	.01	.909
Temperature	576.3	1	.11	.747
Population × temperature	5,478.1	1	1.02	.326
Error	91,111	17		

Table 1 (*Continued*)

Response variable, source	SS	df	*F*	*P*
Plasma LDH activity (U/L):				
Population	637.9	1	.04	.836
Temperature	581.8	1	.04	.843
Population × temperature	11,836	1	.82	.378
Error	275,623	19		
White-muscle water content (%):				
Population	.5	1	.05	.825
Temperature	3	1	.33	.57
Population × temperature	22.2	1	2.48	.13
Error	197.3	22		

Note. Plasma, whole blood, and white muscle were collected from largemouth bass from two populations (Alabama and Illinois) subjected to rapid cold shock from 20° to 8°C and held at 8°C for 6 h. A group of largemouth bass for each population was held at 20°C (no temperature change) for 6 h as controls. Statistical significance is indicated by boldface type. ATP = adenosine triphosphate, LDH = lactate dehydrogenase, PCr = phosphocreatine, PCV = packed cell volume, SS = sum of squares.

chloride in plasma were greater for control largemouth bass from the southern (AL) population, while concentrations of sodium in plasma were greater for the northern (IL) population. Although cortisol values of control IL largemouth bass (~50 ng/L) were similar to cortisol values reported for resting largemouth bass in other studies from our laboratory (e.g., ~50 ng/L [Suski et al. 2006], ~25–50 ng/L [Gingerich et al. 2010], ~20–50 ng/L [VanLandeghem et al. 2010], and ~50–80 ng/L [Gingerich and Suski 2012]), cortisol levels of control AL largemouth bass were much higher (~100–125 ng/L). This discrepancy in control cortisol levels is unlikely due to differences in holding conditions. Specifically, IL and AL largemouth bass were placed into adjacent containers and simultaneously exposed to experimental temperatures. Because AL fish were exposed to an Illinois winter before use in experimentation, elevated cortisol levels may have been a response to a long-term thermal stressor; high cortisol could also be due to interpopulation variation in response to laboratory confinement (e.g., Volckaert et al. 2012). Many physiological parameters of largemouth bass, including cortisol, return to prestress levels within a few hours of recovery (Suski et al. 2006). The 24-h acclimation period should have provided adequate time for both IL and AL largemouth bass to recover from handling stress at the beginning of each experimental run. Although high cortisol levels observed in the control AL largemouth bass may not be indicative of undisturbed fish and therefore precludes a direct comparison of cortisol between populations, the difference between control IL and AL largemouth bass was unlikely due to differences in holding or experimental conditions.

Cortisol is typically released in fish after exposure to acute stressors, whereas the role of constitutive concentrations in resting (nonstressed) fish is less clear. In mammals, constitutive levels of cortisol are responsible for the maintenance of baseline glucose levels and adequate blood pressure (Bamberger et al. 1996) and may play a similar role in teleosts (Mommsen et al. 1999). Interestingly, cardiac output has been shown to be lower in southern largemouth bass relative to those from northern populations (Cooke and Philipp 2005), highlighting potential relationships among cortisol, cardiac variables, and latitudinal origin. Cortisol production is also linked to hydromineral balance of fish, and elevated cortisol can result in sodium loss via the gills (Gonzalez and McDonald 1992; Wendelaar Bonga 1997). Sodium concentrations in the plasma of AL largemouth bass were lower than those in IL fish, and this may have resulted from elevated cortisol levels. Alternatively, northern fish may have higher rates of reaction (i.e., Q_{10} effects) at 20°C for active transport mechanisms, ion exchangers, and channels responsible for sodium regulation (Hochachka 1988; Metz et al. 2003), resulting in higher sodium concentrations than in southern fish. For example, ion-regulatory rates have been shown to be higher in killifish from a northern population than from a southern population when examined at the same temperature (Scott et al. 2004). Similar to sodium, temperature-specific differences in exchangers responsible for chloride regulation may explain the observed differences in plasma chloride between populations. The differences in sodium and chloride concentrations between populations were small in magnitude (≤10%), and even larger differences in sodium and chloride (>10%), such as those associated with acute stressors, were not directly related to mortality of rainbow trout (Wood et al. 1983) or largemouth bass (Thompson et al. 2008). Ion regulation is costly for fish, as it consumes between 2% and 20% of resting metabolism (Febry and Lutz 1987). Because the activity rates of ion pumps, exchangers, and channels are strongly influenced by temperature, evolutionary adaptation of these processes to function optimally within a specific thermal regime may minimize costs associated with ion regulation. Clearly, baseline differences in sodium and chloride concentrations between populations are small relative to ionic disturbances associated with acute stressors, but they may be related to population-level differences in baseline cortisol levels or activities of processes controlling ion regulation.

When exposed to rapid cold shock, the responses of glucose, ionic systems, and white-muscle parameters varied between largemouth bass from different latitudes. For example, largemouth bass from the southern population exhibited an increase

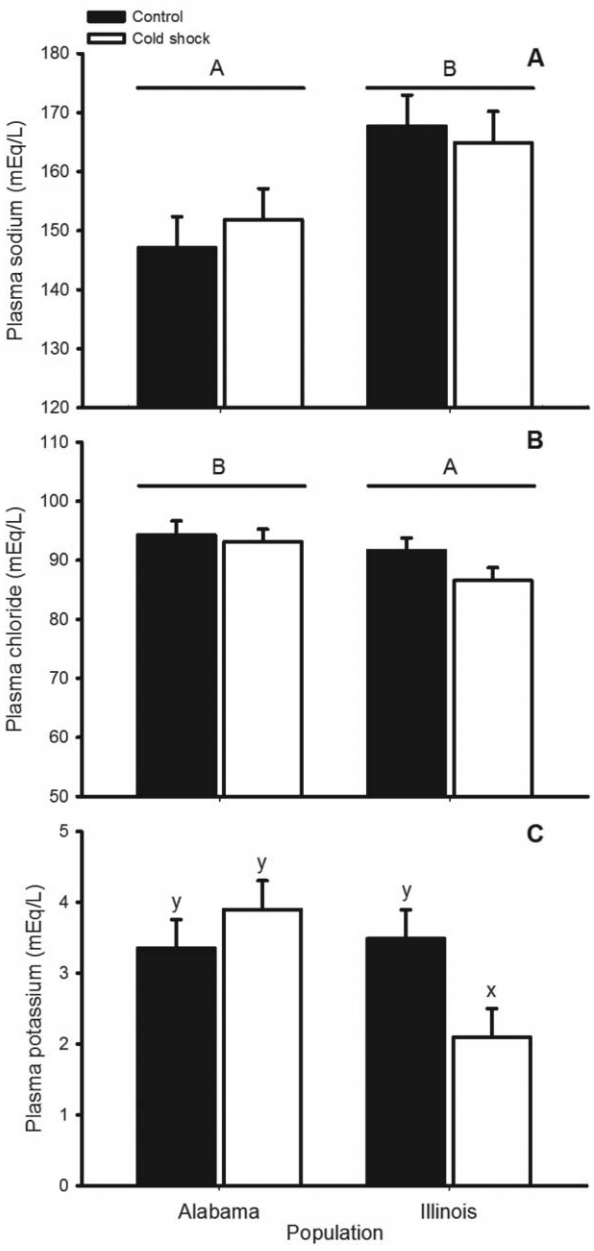

Figure 2. Concentrations of plasma sodium (*A*), chloride (*B*), and potassium (*C*) of largemouth bass originating from Alabama and Illinois. Fish were either held for 6 h at 20°C (controls; filled bars; $n = 6$ per population) or subjected to rapid cold shock from 20° to 8°C and held at 8°C for 6 h (cold shock; open bars; $n = 7$ per population). Mean separation is indicated by lowercase letters; statistically different means do not share letters. Uppercase letters separate population means when the main effect of population was significant and the main effect of temperature and the interaction of the main effects (population × temperature) were not significant.

in plasma glucose concentrations, whereas fish from the northern population did not. For fish, glucose is released as part of the secondary stress response as fuel for aerobic tissues, such as the heart and gills (Wendelaar Bonga 1997). Elevated glucose levels can also indicate an increase in metabolic rate (Moon 2001), suggesting possible metabolic differences between populations at low temperatures. Previous studies have shown that, at cool temperatures, metabolic rates of walleye and largemouth bass from southern populations were greater than rates of those from northern populations, likely as an evolutionary adaptation to maximize growth rates across thermal regimes (Cooke et al. 2001; Galarowicz and Wahl 2003). Alternatively, the high glucose levels observed in AL largemouth bass could be indicative of slower glucose utilization at cold temperatures (Sun et al. 1994) rather than reflective of metabolic status. Elevated glucose levels in largemouth bass typically recover within 2 h after the cessation of a stressor, even if fish are allowed to recover at cool temperatures (Suski et al. 2006). Concentrations of plasma glucose for AL fish remained elevated 6 h after the onset of an acute cold shock, however, suggesting that prolonged exposure to low temperature likely acts as a stressor for largemouth bass from southern latitudes but not northern latitudes.

After cold shock, potassium concentrations of IL largemouth bass decreased relative to control values, but no differences in potassium concentrations were observed for AL fish. Excessive potassium loss is of interest, as it can impair muscle function (Sjøgaard 1991) and has been linked to decreased swimming performance (Jain and Farrell 2003). Potassium can be lost at the gills, as hormones released during acute stress increase gill surface area for improved oxygen uptake but also increase gill permeability and the area by which passive diffusion of ions can occur (Gonzalez and McDonald 1992; Wendelaar Bonga 1997). Cortisol is typically elevated after the primary and secondary stress response (Wendelaar Bonga 1997), but cortisol levels of IL largemouth bass did not increase relative to those of controls. This suggests that stress hormones had only minor effects on gill structure and permeability and that the observed potassium decline in IL fish was due to other mechanisms. Decreases in plasma potassium concentrations could be related to activity of the sodium-potassium pump (Na^+-K^+-ATPase) at the gills. Activity of gill Na^+-K^+-ATPase can be upregulated at low temperature (Metz et al. 2003), but the temperature at which upregulation occurs may differ between populations. Potassium can also decrease if absorbed by red blood cells; however, hematocrit levels did not increase and hemoglobin did not change, indicating no change in red blood cell concentrations or cell size that would be indicative of potassium uptake (Nielsen and Lykkeboe 1991). Regardless of the mechanism responsible for potassium loss, largemouth bass from the northern population showed a greater propensity for potassium loss than did fish from the southern population during acute cold shock, suggesting that latitudinal origin may influence ionic regulation during acute stressors.

In addition to changes in glucose and potassium, PCr concentrations in white muscle doubled after cold shock in largemouth bass from the southern population, whereas they did not change in fish from the northern population. PCr is consumed under anaerobic conditions, such as exhaustive exercise, and PCr recovery occurs via oxidation of lipids under aerobic conditions (Richards et al. 2002). Temperature has been shown to influence the recovery of PCr in largemouth bass. After 1

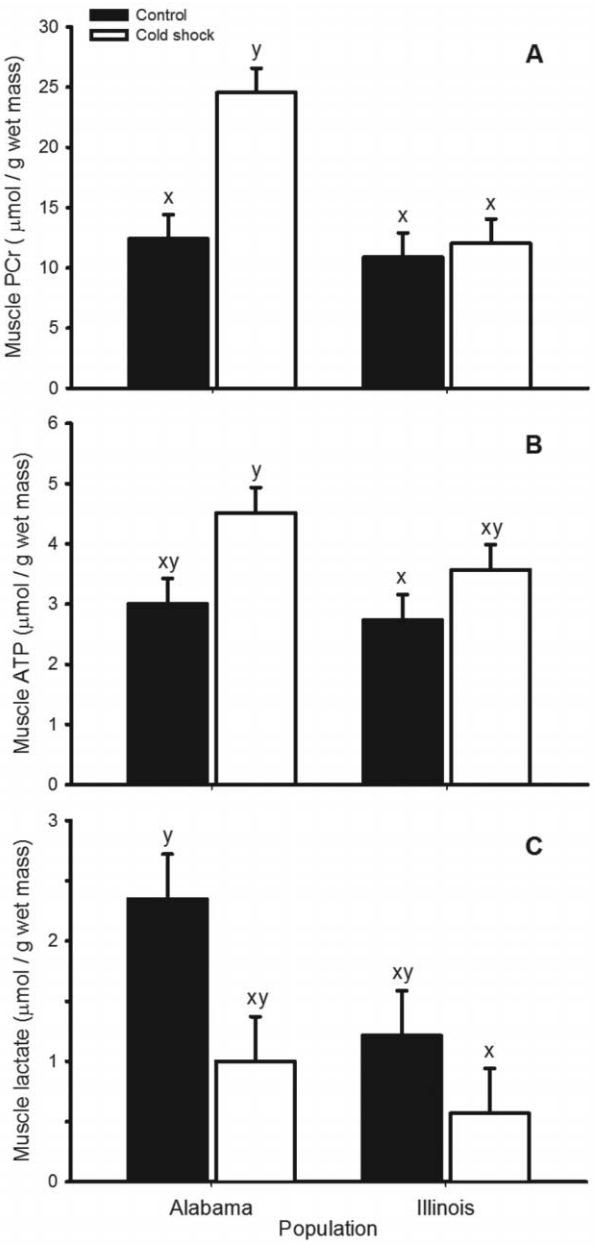

Figure 3. Concentrations of white-muscle phosphocreatine (PCr; *A*), adenosine triphosphate (ATP; *B*), and lactate (*C*) of largemouth bass originating from Alabama and Illinois. Fish were either held for 6 h at 20°C (controls; filled bars; $n = 6$ per population) or subjected to rapid cold shock from 20° to 8°C and held at 8°C for 6 h (cold shock; open bars; $n = 7$ per population). Mean separation is indicated by lowercase letters; statistically different means do not share letters.

min of exhaustive exercise, PCr recovers faster at 20° than at 32°C (Suski et al. 2006). In chain pickerel (*Esox niger*), the activity of white-muscle creatine phosphokinase (CPK), an enzyme responsible for the conversion of creatine to PCr (Hochachka and Mossey 1998), is greater in cold-acclimated fish than in warm-acclimated fish (Kleckner and Sidell 1985). The activity of CPK at low temperature may be greater in southern fish than in northern fish, resulting in higher PCr concentrations after 6 h of reduced temperatures. Although we cannot identify the exact mechanism responsible for the increase in PCr concentration, this result demonstrates that processes associated with production and consumption of PCr are influenced by latitudinal origin when largemouth bass are exposed to acute cold shock. Taken together, our results suggest that cold shock had different effects on multiple physiological systems across latitudinally separated populations of largemouth bass, possibly due to differences in metabolism or glucose utilization rates and activity of enzymes at the reduced temperature, resulting in a stronger propensity of northern fish to lose potassium ions and persistent hyperglycemia and increased anaerobic potential of the southern population.

Overwinter mortality is a factor that has a strong influence on the abundance and distribution of temperate freshwater fishes (Hurst 2007; Suski and Ridgway 2009), and several factors can influence overwinter mortality rates. Latitudinal origin had a strong effect on overwinter morality, as both adult and age 0 IL largemouth bass exhibited higher overwinter survival than did AL fish during common-garden experiments at high latitudes. Size-selective processes are a common driver of overwinter mortality in several fishes, including some populations of largemouth bass, with the smallest fish in a cohort experiencing elevated mortality rates relative to larger individuals (Post et al. 1998; Fullerton et al. 2000). Size-selective mortality, however, was not apparent for either population we examined, as no significant differences were observed in fish size before or after winter. Differences in overwinter mortality between populations were therefore likely driven by factors other than size-selective mortality, such as differences in physiological characteristics (Hurst 2007). For instance, overwinter mortality of white crappie (*Pomoxis annularis*) in an Ohio reservoir was not size selective but was suspected to be due to osmoregulatory failure (McCollum et al. 2003). Physiological characteristics influencing overwinter mortality may vary over latitudes, as sodium concentrations in largemouth bass from southern populations were lower than those in northern fish. The lower sodium concentrations of the southern fish may result in a greater propensity for osmoregulatory failure and could ultimately lead to overwinter mortality higher than that of northern fish. Similarly, elevated glucose and PCr concentrations after acute cold shock occurred in AL but not in IL largemouth bass, suggesting differences in the metabolic responses of northern and southern fish to acute cold shocks. The ability of fish to metabolically compensate for reduced temperatures present during winter may be an adaptation to achieve greater overwinter survival. More specifically, increased aerobic and anaerobic capacity, via compensatory adjustments in activation temperatures of several enzymes, may conserve locomotor capacity and reduce the risk of starvation by allowing low levels of feeding at reduced temperatures (reviewed in Guderley 1990; Tschantz et al. 2002). Furthermore, metabolic compensation may also increase the capacity for lipid oxidation (Guderley 1990). Because overwinter survival has been positively linked to accumulation of lipid reserves before the onset of winter

Table 2: Physiological parameters that were similar between northern and southern largemouth bass

	Alabama		Illinois	
	Control	Cold shock	Control	Cold shock
Whole-blood hemoglobin (g/dL)	7.51 (.47)	6.88 (.47)	7.75 (.47)	7.66 (.47)
Hematocrit (% PCV)	36.4 (2.6)	34.0 (3.3)	31.9 (2.2)	29.4 (3.1)
Plasma hemoglobin (mg/dL)	49.3 (25.9)	80.5 (23.2)	75.4 (21.1)	60.3 (21.1)
Plasma LDH activity (U/L)	156.4 (34.8)	181.6 (38.1)	180.8 (34.8)	141.8 (34.8)
White-muscle water content (%)	80.8 (.9)	81.6 (.8)	82.3 (.8)	80.5 (.9)

Note. Shown are means (SE) of plasma, whole-blood, and white-muscle parameters that were similar (all $P > 0.13$) between two populations of largemouth bass (Alabama and Illinois) subjected to rapid cold shock from 20° and 8°C and held at 8°C for 6 h ($n = 7$ per population). A group of largemouth bass for each population was held at 20°C (no temperature change) for 6 h as controls ($n = 6$ per population).

(Suski and Ridgway 2009), an enhanced capacity for lipid oxidation may promote greater overwinter survival by providing a metabolic pathway for use of this energy reserve. Short-term thermal stressors may trigger an early onset of this metabolic compensation in AL fish when exposed to high-latitude winters, potentially leading to greater depletion of energy reserves and poor overwinter survival. The response of IL fish to short-term thermal stressors, however, may be attenuated to ensure that energy reserves are available and utilized over long winters. In short, AL largemouth bass exhibited osmoregulatory and metabolic disturbances that may have resulted in lower overwinter survival of this population.

Factors other than physiological responses may have been partly responsible for overwinter mortality differences between the two populations. Mortality patterns could be the result of different feeding rates over winter (Fullerton et al. 2000), predation, starvation associated with decreased food availability, or disturbances to physiological systems that were not indicated by any of the parameters measured in this study (Hurst 2007). For example, the swimming ability of AL largemouth bass may have been reduced during winter due to low temperatures and could have reduced predation success of the AL population, whereas the IL largemouth bass may have been relatively unaffected. Furthermore, the established bluegill populations in the ponds may have been adapted to relatively severe winters and avoided AL largemouth bass better than those from the IL population. Fathead minnows, however, were stocked in the ponds to provide an alternative prey resource for both populations. These fathead minnows were obtained from a hatchery and were naive of predators, and they were likely susceptible to largemouth bass from both populations (e.g., Wahl and Stein 1988; Einfalt and Wahl 1997). Furthermore, Fullerton et al. (2000) showed that low-latitude largemouth bass had high mortality when exposed to mid- and high-latitude winters even when supplied with ample prey. Because ample prey was available and the pond did not contain predators, food availability and predation likely were not contributing factors to overwinter mortality.

For species distributed over large geographic areas, local thermal regimes drive intraspecific variability among populations (Conover and Present 1990). Winter severity can vary considerably across latitudes, and intraspecific variability in thermal adaptations along latitudinal gradients has been observed in many species. For example, intraspecific variability in physiological parameters (e.g., metabolic rate, cardiac variables, conversion efficiency, growth) along latitudinal gradients has been suggested to be due to adaptation to local thermal conditions (e.g., Cooke et al. 2001; Galarowicz and Wahl 2003; Garvey and Marschall 2003; Cooke and Philipp 2005). In addition to the physiological parameters listed above, variation in the physiological response of fish to acute cold stressors may be due to evolutionary adaptation to local thermal conditions. Physiological differences between northern and southern largemouth bass suggest that northern largemouth bass are more tolerant of low temperatures. This advantage may be realized over winter, as survival of age 0 and adult northern fish were four- and eightfold greater than that of age 0 and adult southern fish, respectively. Adaptations to local conditions allow populations to optimize growth, survival, and reproduction based on available resources and environmental constraints (Garvey and Marschall 2003). Evolutionary adaptation of physiological responses to acute thermal stressors may be one mechanism specific to local thermal regimes that allows a species to survive in a wide range of thermal environments and occupy large geographic areas.

In conclusion, largemouth bass from northern and southern origins exhibited differences in both sublethal physiological responses to a short-term cold stressor as well as differences in overwinter mortality when exposed to an Illinois winter. We examined lethal and sublethal responses of largemouth bass originating from only two populations due to logistical limitations, which somewhat limited our inference space. Our results, however, are consistent with previous studies that have documented intraspecific variation in the physiology of largemouth bass (e.g., Cooke et al. 2001; Cooke and Philipp 2005), killifish (DeKoning et al. 2004), and eastern fence lizards (Angilletta 2001) collected from only two latitudinally separated populations. In summary, largemouth bass from northern and southern origins displayed differences in both pre- and post-cold-shock physiological parameters, indicating that latitudinal origin affected baseline physiological parameters and the magnitude and nature of the physiological response to acute cold stressors. These differences in the response to cold stress may have been scaled up to impact the ability to survive winter, as southern populations exhibited higher overwinter mortality than did northern conspecifics. Physiological adaptations to

specific thermal regimes allow fish species to survive a range of winter severity throughout their distribution (Schultz et al. 1998; Hurst 2007), and adaptations for overcoming acute cold stressors may provide further physiological mechanisms by which a fish species can survive over large latitudinal gradients.

Acknowledgments

We thank D. DeVries, R. Wright, and B. Martin from the Aquatic Ecology Group, Ireland Center, Auburn University, and J. Garvey, R. Colombo, and A. Lohmeyer from the Fisheries and Illinois Aquaculture Center, Southern Illinois University, for assistance in collecting Alabama fish. In addition, J. Clark, M. Diana, A. Larsen, K. Schnake, E. Smolik, and J. Wisher at the Kaskaskia Biological Station, Illinois Natural History Survey, assisted with collection of Illinois fish. A. Gingerich assisted with laboratory work. We are grateful to L. Einfalt for her assistance with fish care in the wet laboratory. The Ron Ward Memorial Scholarship awarded by the Champaign-Urbana Bass Club and the Graduate Student Conservation Scholarship awarded by the Bass Anglers Sportsman's Society (B.A.S.S.) and Costa del Mar provided financial support to M.M.V. This project was supported in part by the US Department of Agriculture Cooperative State Research Education and Extension Service by McIntire-Stennis funds through project ILLU-875-328.

Literature Cited

Anglilletta M.J. 2001. Variation in metabolic rate between populations of a geographically widespread lizard. Physiol Biochem Zool 74:11–21.

Bamberger C.M., H.M. Schulte, and G.P. Chrousos. 1996. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev 17:245–261.

Calosi P., D.T. Bilton, S.C. Votier, and A. Atfield. 2010. What determines a species' geographical range? thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). J Anim Ecol 79:194–204.

Clapp D.F. and D.H. Wahl. 1996. Comparison of food consumption, growth, and metabolism among muskellunge: an investigation of population differentiation. Trans Am Fish Soc 125:402–410.

Conover D.O., T.A. Duffy, and L.A. Hice. 2009. The covariance between genetic and environmental influences across ecological gradients. Ann NY Acad Sci 1168:100–129.

Conover D.O and T.M.C. Present. 1990. Countergradient variation in growth rate: compensation for length of growing season among Atlantic silversides from different latitudes. Oecologia 83:316–324.

Cooke S.J., T.W. Kassler, and D.P. Philipp. 2001. Physiological performance of largemouth bass related to local adaptation and interstock hybridization: implications for conservation and management. J Fish Biol 59(suppl. A):248–268.

Cooke S.J. and D.P. Philipp. 2005. Influence of local adaptation and interstock hybridization on the cardiovascular performance of largemouth bass *Micropterus salmoides*. J Exp Biol 208:2055–2062.

DeKoning A.B.L., D.J. Picard, S.R. Bond, and P.M. Schulte. 2004. Stress and interpopulation variation in glycolytic enzyme activity and expression in a teleost fish *Fundulus heteroclitus*. Physiol Biochem Zool 77:18–26.

Donaldson M.R., S.J. Cooke, D.A. Patterson, and J.S. Macdonald. 2008. Cold shock and fish. J Fish Biol 73:1491–1530.

Einfalt L.M. and D.H. Wahl. 1997. Prey selection by juvenile walleye as influenced by prey morphology and behavior. Can J Fish Aquat Sci 54:2618–2626.

Febry R. and P. Lutz. 1987. Energy partitioning in fish: the activity related cost of osmoregulation in a euryhaline cichlid. J Exp Biol 128:63–85.

Fullerton A.H., J.E. Garvey, R.A. Wright, and R.A. Stein. 2000. Overwinter growth and survival of largemouth bass: interactions among size, food, origin, and winter severity. Trans Am Fish Soc 129:1–12.

Galarowicz T.L. and D.H. Wahl. 2003. Differences in growth, consumption, and metabolism among walleyes from different latitudes. Trans Am Fish Soc 132:425–437.

Garvey J.E. and L.A. Marschall. 2003. Understanding latitudinal trends in fish body size through models of optimal seasonal energy allocation. Can J Fish Aquat Sci 60:938–948.

Garvey J.E., K.G. Ostrand, and D.H. Wahl. 2004. Energetics, predation, and ration affect size-dependent growth and mortality of fish during winter. Ecology 85:2860–2871.

Garvey J.E., R.A. Wright, and R.A. Stein. 1998. Overwinter growth and survival of age-0 largemouth bass (*Micropterus salmoides*): revisiting the role of body size. Can J Fish Aquat Sci 55:2414–2424.

Gingerich A.J., D.P. Philipp, and C.D. Suski. 2010. The effects of nutritional status on metabolic rate, exercise, and recovery in a freshwater fish. J Comp Physiol B 180:371–384.

Gingerich A.J. and C.D. Suski. 2012. The effect of body size on post-exercise physiology in largemouth bass. Fish Physiol Biochem 38:329–340.

Gonzalez R.J. and D.G. McDonald. 1992. The relationship between oxygen consumption and ion loss in a freshwater fish. J Exp Biol 163:317–332.

Guderley H. 1990. Functional significance of metabolic responses to thermal acclimation in fish muscle. Am J Physiol 259:245–252.

Hallerman E.M., R.O. Smitherman, R.B. Reed, W.H. Tucker, and R.A. Dunham. 1986. Biochemical genetics of largemouth bass in mesosaline and freshwater areas of the Alabama River system. Trans Am Fish Soc 115:15–20.

Harvey W.D. and D.L. Campbell. 1989. Retention of passive integrated transponder tags in largemouth bass brood fish. Prog Fish-Cult 51:164–166.

Hillebrand H. 2004. On the generality of the latitudinal diversity gradient. Am Nat 163:192–211.

Hochachka P.W. 1988. Channels and pumps—determinants of metabolic cold adaptation strategies. Comp Biochem Physiol B 90:515–519.

Hochachka P.W. and M.K.P. Mossey. 1998. Does muscle cre-

atine phosphokinase have access to the total pool of phosphocreatine plus creatine? Am J Physiol 274:868–872.

Hoffman A.A., A. Anderson, and R. Hallas. 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol Lett 5:614–618.

Houston A.H. 1990. Blood and circulation. Pp. 273–334 in C.B. Schreck, P.B. Moyle, eds. Methods for fish biology. American Fisheries Society, Bethesda, MD.

Hurst T.P. 2007. Causes and consequences of winter mortality in fishes. J Fish Biol 71:315–345.

Jain K.E. and A.P. Farrell. 2003. Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. J Exp Biol 206:3569–3579.

Kleckner N.W. and B.D. Sidell. 1985. Comparison of maximal activities of enzymes from tissues of thermally acclimated and naturally acclimatized chain pickerel (*Esox niger*). Physiol Zool 58:18–28.

Littell R.C., W.W. Stroup, and R.J. Freund. 2002. SAS for linear models. 4th ed. SAS Institute, Cary, NC.

Lowry O.H. and J.V. Passonneau. 1972. A flexible system of enzymatic analysis. Academic Press, New York.

McCollum A.B., D.B. Bunnell, and R.A. Stein. 2003. Cold, northern winters: the importance of temperature to overwinter mortality of age-0 white crappies. Trans Am Fish Soc 132:977–987.

Metz J.R., E.H. van den Burg, S.E. Wendelaar Bonga, and G. Flik. 2003. Regulation of branchial Na^+/K^+-ATPase in common carp *Cyprinus carpio* L. acclimated to different temperatures. J Exp Biol 206:2273–2280.

Mommsen T.P., M.M. Vijayan, and T.W. Moon. 1999. Cortisol in teleosts: dynamics, mechanism of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268.

Moon T.W. 2001. Glucose intolerance in teleost fish: fact or faction? Comp Biochem Physiol B 129:243–249.

Nedbal M.A. and D.P. Philipp. 1994. Differentiation of mitochondrial DNA in largemouth bass. Trans Am Fish Soc 123: 460–468.

Nielsen O.B. and G. Lykkeboe. 1991. Changes in plasma and erythrocyte K^+ during hypercapnia and different grades of exercise in trout. J Appl Physiol 72:1285–1290.

Post D.M., J.F. Kitchell, and J.R. Hogson. 1998. Interactions among adult demography, spawning date, growth rate, predation, overwinter mortality, and the recruitment of largemouth bass in a northern lake. Can J Fish Aquat Sci 55: 2588–2600.

Richards J.G., G.J.F. Heigenhauser, and C.M. Wood. 2002. Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. Am J Physiol 282:89–99.

Ricker W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin 191. Bulletin of the Fisheries Research Board of Canada, Ottawa.

Schultz E.T., D.O. Conover, and A. Ehtisham. 1998. The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. Can J Fish Aquat Sci 55:1149–1157.

Scott G.R., J.T. Rogers, J.G. Richards, C.M. Wood, and P.M. Schulte. 2004. Intraspecific divergence of ionregulatory physiology in the euryhaline teleost *Fundulus heteroclitus*: possible mechanisms of freshwater adaptation. J Exp Biol 207:3399–3410.

Sjøgaard G. 1991. Role of exercise-induced potassium fluxes underlying muscle fatigue: a brief review. Can J Physiol Pharmacol 69:238–245.

Sun L.T., G.R. Chen, and C.F. Chang. 1994. Characteristics of blood parameters and gill Na^+-K^+-ATPase in chilled comatose tilapia cultured in various salinities. Comp Biochem Physiol A 107:641–646.

Suski C.D., S.S. Killen, J.D. Kieffer, and B.L. Tufts. 2006. The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. J Fish Biol 68:120–136.

Suski C.D., S.S. Killen, M.B. Morrissey, S.G. Lund, and B.L. Tufts. 2003. Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. N Am J Fish Manag 23:760–769.

Suski C.D. and M.S. Ridgway. 2009. Winter ecology of centrarchid fishes. Pp. 264–292 in S.J. Cooke, D.P. Philipp, eds. Centrarchid fishes: diversity, biology, and conservation. Wiley-Blackwell, West Sussex.

Thompson L.A., S.J. Cooke, M.R. Donaldson, K.C. Hanson, A.J. Gingerich, T. Klefoth, and R. Arlinghaus. 2008. Physiology, behavior, and survival of angled and air-exposed largemouth bass. North Am J Fish Man 28:1059–1068.

Tschantz D.R., E.L. Crockett, P.H. Niewiarowski, and R.L. Londraville. 2002. Cold acclimation strategy is highly variable among the sunfishes (Centrarchidae). Physiol Biochem Zool 75:544–556.

VanLandeghem M.M., D.H. Wahl, and C.D. Suski. 2010. Physiological responses of largemouth bass to acute temperature and oxygen stressors. Fish Manag Ecol 17:414–425.

Volckaert F.A.M., B. Hellemans, C. Batargias, B. Louro, C. Massault, J.K.J. Van Houdt, C. Haley, D. de Koning, and A.V.M. Canario. 2012. Heritability of cortisol response to confinement stress in European sea bass *Dicentrarchus labrax*. Genet Sel Evol 44:15–19.

Wagner C.P., M.J. Jennings, J.M. Kampa, and D.H. Wahl. 2007. Survival, growth, and tag retention in age-0 muskellunge implanted with passive integrated transponders. N Am J Fish Manag 27:873–877.

Wahl D.H. and R.A. Stein. 1988. Selective predation by three esocids: the role of prey behavior and morphology. Trans Am Fish Soc 117:142–151.

Wendelaar Bonga S.E. 1997. The stress response in fish. Physiol Rev 77:591–625.

Wood C.M., J.D. Turner, and M.S. Graham. 1983. Why do fish die after severe exercise? J Fish Biol 22:189–201.

Wroblewski F. and J.S. LaDue. 1955. Lactic dehydrogenase activity in blood. Proc Soc Exp Biol Med 90:210–213.