Hybridization among Divergent Stocks of Largemouth Bass (*Micropterus salmoides*) Results in Altered Cardiovascular Performance: The Influence of Genetic and Geographic Distance

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Accepted 8/17/2005; Electronically Published 2/3/2006

ABSTRACT

Animal populations exhibit wide ranges of divergence associated with both geographic and genetic distances. Here, we examined the role of crossing distance on the cardiovascular response to exhaustive exercise among differentiated stocks of largemouth bass *Micropterus salmoides* at 10°C and 20°C. Stocks of 2+ fish were produced using adults from three regions in the midwestern United States (southeastern Wisconsin, northwestern Wisconsin, and west central Minnesota) and were crossed with fish from central Illinois. Doppler flow probes were used to quantify cardiac output, heart rate, and stroke volume. Cardiac variables (both resting and maximal) were consistently lowest in pure Illinois fish relative to the F_1 interstock hybrids. Additionally, when exposed to exercise, cardiac variables for F_1 interstock hybrids required ~40% longer to return to resting levels compared with the pure Illinois stock. However, the time required to exhaust fish was similar across stocks. Interestingly, all of the stocks (including the interstock hybrids and pure Illinois) maintained cardiac scope. In general, the patterns observed in cardiovascular performance were consistent for both water temperatures. Multiple regression analysis was used to determine which of the divergence metrics contributed to variation in cardiovascular performance in interstock hybrids. Mitochondrial DNA data (genetic distance) were infrequently identified as a significant source of variation in cardiovascular performance. However, genetic distance data for the neutral allozyme markers revealed that these stocks have experienced significant divergence. Latitude (geographic distance) accounted for between 31% and 45% of variation observed in the recovery parameters. This study suggests that the magnitude of stock divergence is an important determinant in the degree to which cardiovascular performance of bass is altered from interstock hybridization and associated breakdown of coadapted gene complexes. Furthermore, these data suggest that translocating bass among even geographically discrete regions or among stocks with reasonable genetic similarity can result in substantial performance alterations at the F_1 generation.

Introduction

Intraspecific translocations of organisms (i.e., when individuals from one population/stock are introduced into a population/stock of that same species at a different location) are common among a variety of taxa (Tallmon et al. 2004) but are perhaps most common in teleost fish (Philipp et al. 1983; Ryman et al. 1994; Cross 2000). This activity can result from unintentional aquaculture escapement (Hindar et al. 1991) or from intentional stocking by management agencies to supplement stocks (Cowx 1994). Translocations can also occur as part of scientific experimentation to study local adaptation and intraspecific variation among populations (e.g., Thornhill 1993). Irrespective of the reason or method, the translocation of organisms facilitates the interbreeding of previously isolated individuals. Earlier studies on the impacts of translocations indicate that in general, translocated organisms perform more poorly than their locally adapted conspecifics, and when crossed, the hybridized individuals typically exhibit impaired performance at the F_2 generation (Templeton 1986; Burke and Arnold 2001). Despite some examples of heterosis (see Emlen 1991), the F_1 hybrids also frequently exhibit performance impairments indicative of outbreeding depression (Thornhill 1993). Since researchers have observed differences in the performance and fitness of different stocks when translocated from their point of origin

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Physiological and Biochemical Zoology 79(2):400–410. 2006. © 2006 by The University of Chicago. All rights reserved. 1522-2152/2006/7902-5038$15.00

(e.g., Philipp and Claussen 1995; Philipp et al. 2002), there is evidence that each population has evolved an adaptive suite of genotypic and phenotypic characters for its specific environment (i.e., local adaptation; Wright 1978). However, several important questions remain. For example, how is outbreeding depression manifested in hybrids, and what proximate mechanisms result in fitness alterations? Also, what aspects of divergence among stocks are most important for determining the severity of outbreeding depression?

Outbreeding depression is defined as the loss in fitness among offspring of parents that are too distantly related to each other, either through loss of adaptive capability or the disruption of coadapted gene complexes (Templeton 1986; Thornhill 1993; Hallerman 2003). In fish, empirical examples of outbreeding depression are more common than in many other taxa (Waldman and McKinnan 1993). Among the fishes, research has focused on two groups that are frequently used as classic examples of local adaptation and outbreeding depression in animal biology: the Pacific salmon (*Oncorhynchus* spp.) and the black bass (*Micropterus* spp.; e.g., Waples 1995; Hallerman 2003). Among salmonids, examples of local adaptation are widespread (e.g., Taylor 1991), although instances of outbreeding depression resulting from the mixing of stocks are less common. Some of clearest data on local adaptation and outbreeding are derived from research conducted on pink salmon *Oncorhynchus gorbuscha* (e.g., Gharrett and Smoker 1991; Gharrett et al. 1999). Using adult returns as a surrogate for fitness, these authors determined that although few differences were observed at the F_1 generation, in the F_2 generation, returns of hybridized stocks showed poorer return rates than the unhybridized local control stocks. Studies on black bass have indicated that the introgression of closely related individuals can result in differential fitness as evidenced by reductions in growth and survival (Philipp and Claussen 1995) as well as reproductive output (Philipp et al. 2002). Furthermore, translocated largemouth bass *Micropterus salmoides* and interstock hybrids exhibit reduced swimming performance as well as differential efficiency compared with locally adapted stocks (Cooke et al. 2001). Recent research has revealed that there was a suite of cardiovascular differences between two differentiated stocks of largemouth bass (Cooke and Philipp 2005). Furthermore, when mixed, both reciprocal interstock hybrids at the F_1 generation exhibited altered cardiovascular performance (Cooke and Philipp 2005). Aside from these recent examples, there have been few attempts to use physiological techniques to assess the variation in physiological performance that may underlie intraspecific diversity or the energetic consequences of mixing divergent populations (but see Burton 1990 and Burton et al. 1999 for an example of using physiological performance of interpopulation hybrids in copepods as evidence of coadapted gene complexes).

Identifying the underlying genetic differences among populations/stocks and the spatial boundaries that define them requires genetic analyses, specifically the use of allozyme and RFLP-mtDNA analyses. To date, however, there are few empirical assessments of the populations/stocks that have been defined using these genetic techniques. Only by explicitly testing whether or not biologically meaningful differences exist among identified populations/stocks can we critically assess the criteria used to identify the number and location of these units or provide a better understanding of the physiological diversity of fishes and the factors that promote intraspecific variation. To our knowledge, the only studies to address that issue are the Cooke et al. (2001) and Cooke and Philipp (2005) articles discussed above. In those studies, however, the researchers only evaluated two defined stocks. Additional studies are required that involve numerous stocks with different levels of genetic and spatial separation, as the geographic and genetic scale at which organisms may be translocated without causing deleterious changes is a contentious issue in conservation biology (Edmands 2002; Tallmon et al. 2004). This issue is also of interest to evolutionary physiologists (Garland and Carter 1994) and to physiologists with a fundamental interest in the intraspecific variation in animal performance (Spicer and Gaston 1999).

Here, we build on the earlier research of Cooke and Philipp (2005), which focused on the cardiovascular performance of two stocks of largemouth bass (both parental stocks and reciprocal hybrids). Since cardiovascular performance and morphology are intimately linked to the energetics and physical performance of fish (Keen and Farrell 1994; Kolok and Farrell 1994; Thorarensen et al. 1996), cardiovascular physiology served as a sensitive and logical approach for assessing intraspecific variation (e.g., Gamperl and Farrell 2004). For this study, we hybridized three genetically distinct stocks of largemouth bass collected from different geographical regions in the upper midwestern United States with a fourth stock of fish from Illinois to assess the physiological basis of adaptation to different climatic conditions by monitoring the cardiovascular responses to exhaustive exercise. Using these data, we relate the magnitude of differences in physiological performance to the genetic (allozyme and mtDNA) distance and geographical distance (latitude) of these translocated stocks from the pure Illinois stock. The stocks that we examine in this article were identified by a large-scale study designed to develop management units for warm-water fish in the midwestern United States (Fields et al. 1997). To our knowledge, this is the first experimental study of the role of genetic and geographic distance in affecting the physiological performance of wild vertebrate populations/stocks posthybridization.

Material and Methods

Production of Experimental Stocks

Based on a molecular genetic study of largemouth bass in the upper midwest (Fields et al. 1997) that proposed geographic

boundaries for 15 putative stocks of largemouth bass in Illinois, Minnesota, and Wisconsin, fish from the following four sites were collected for use as broodstock (Fig. 1): IL—from central Illinois (Lake Shelbyville, Kaskaskia River Drainage, Mississippi River basin); BC—from southeastern Wisconsin (Big Cedar Lake, Lake Michigan Drainage, Great Lakes basin); LP—from northeastern Wisconsin (Lipsett Lake, St. Croix River drainage, Mississippi River basin); and DD—from west central Minnesota (Dead Lake, Red River drainage, Hudson Bay basin).

In March 1998, adult fish from the appropriate stock were put into 0.04-ha clay-lined, earthen ponds at the Illinois Natural History Survey Aquatic Research Field Laboratory in Champaign, Illinois, to produce four different experimental lines of largemouth bass using male IL largemouth bass and females from each of the four stocks (pure parental Illinois stock, IL × IL, as well as the F_1 interstock hybrids, BC × IL, LP × IL, and DD × IL). Production ponds were drained in September 1998, and age-0 fish from each stock were given differential fin clips for external identification before being stocked into common rearing ponds. Fish fed on natural invertebrate forage as well as fathead minnows *Pimephales promelas* and juvenile bluegill *Lepomis macrochirus* produced in the ponds. Data for the IL × IL stock and the BC × IL stock are also presented in an earlier publication (Cooke and Philipp 2005) along with data on other stocks that are not presented here (i.e., IL × BC and BC × BC). All experiments were conducted simultaneously to maximize use of these unique and valuable resources. The University of Illinois Office of Laboratory Animal Research approved all husbandry practices and experimental procedures.

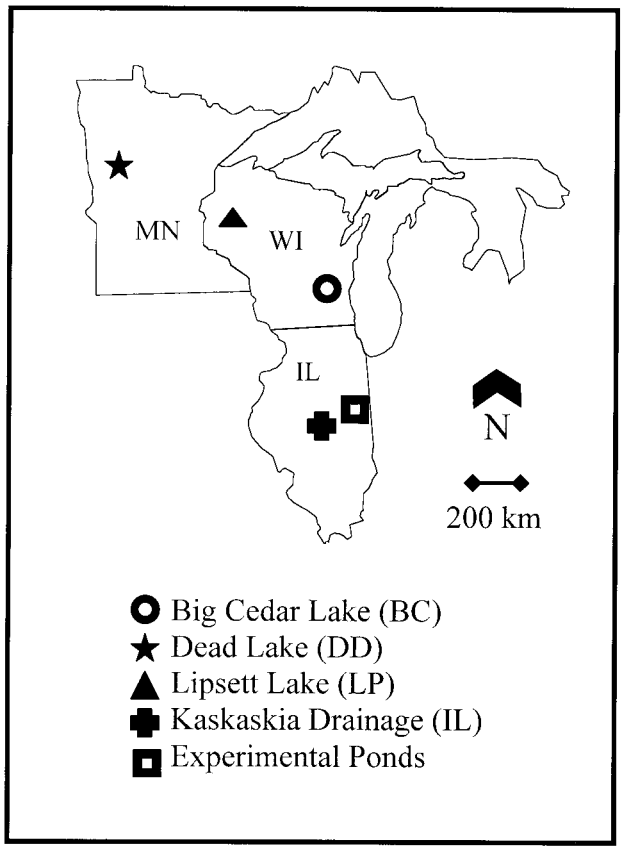

Figure 1. Map of the midwestern Unites States, including Illinois, Wisconsin, and Minnesota. The symbols indicate the origin of the different stocks used in this experiment as well as the location of the experimental ponds where experiments were conducted.

Genetic and Geographic Distance Calculations

Genetic and geographic distances among source populations were determined using several techniques. Allele frequencies, percent polymorphic loci, mean heterozygosity, and *F* statistics (Wright 1965) for each polymorphic locus and all loci pooled were calculated using BIOSYS-1 (Swofford and Selander 1981). Genetic distances of allozyme data for the Rogers coefficient (Rogers 1972) were calculated using BIOSYS-1 (Swofford and Selander 1981). Mitochondrial DNA (mt DNA) restriction fragment presence-absence data matrices for estimation of sequence divergence (Nei and Li 1979) and nucleon diversity (Nei and Tajima 1981) were analyzed as described by Nedbal and Philipp (1994) using REAP, version 4.0 (McElroy et al. 1991). A distance matrix for mtDNA was subsequently calculated from haplotype frequency data and nucleon sequence divergence data using NTSYS, version 1.50 (Rohlf 1989). Geographic distance was determined from the latitude of each stock (Table 1) by calculating the degrees separating them and transforming these data into linear kilometers. All distance data were calculated in reference to the pure Illinois stock.

Experimental Testing

Twice during the autumn of 2000, when ambient water temperatures corresponded to 10° and 20°C, some of each stock were seined from the ponds and held in raceways at various times 1 wk before experimentation. Although all fish used in the experiments were age 2 and generally of similar size (Table 2), there were some specific differences in total length (Table 3). There was also variation in mass; however, it was not consistent, as revealed by a significant interaction (Table 3). Water temperatures were relatively stable for a period of approximately 2 wk before experimentation but varied up to 3°C on a diel basis. During the experiments, water temperatures were controlled so that they varied by no more than 1°C. All experiments were conducted between 1000 and 1600 hours. Fish were exposed to natural photoperiods of Champaign, Illinois, during residency in pond and raceway environments and during experimentation. Experiments were conducted between September 18 and December 4, 2000.

Descriptions of the surgical procedure are provided elsewhere (Cooke et al. 2003), including specific details of this study

Table 1: Summary of stock characteristics for hybridized largemouth bass

Source Population	Latitude and Longitude	Rogers Genetic Distance	mtDNA Genetic Difference
IL (Lake Shelbyville, IL)	39°59′N, 88°61′W	NA	NA
BC (Big Cedar Lake, WI)	42°57′N, 87°54′W	.08	.03
LP (Lippsett Lake, WI)	44°52′N, 92°29′W	.04	.001
DD (Dead Lake, MN)	46°50′N, 95°72′W	.04	.004

Note. Rogers genetic distance was calculated for allozymes, and mtDNA genetic distance was calculated for RFLP-mtDNA data. All genetic distances are relative to the stock with which all fish were hybridized (i.e., IL).

(Cooke and Philipp 2005). Briefly, each fish was anesthetized before surgery with 60 ppm clove oil (emulsified with ethanol, 9 : 1 ethanol : clove oil) for approximately 8 min, until the fish had lost equilibrium and was nonresponsive. An anesthetized fish was placed on its side on a wetted sponge. The anesthetized state was maintained during surgery by irrigating the gills with water containing a maintenance concentration of anesthetic (30 ppm clove oil). A flexible silicone cuff-type Doppler flow probe (subminiature 20 MHz piezoelectric transducer, Iowa Doppler Products, Iowa City, IA) was placed around the aorta. The cuff was secured around the vessel, and the lead wire to the probe was sutured to the body of the fish. A flowmeter (545C-4 Directional Pulsed Doppler Flowmeter, Bioengineering, University of Iowa, Iowa City, IA) and a digital strip-chart recorder (LabVIEW, version 4.0.1, National Instruments, Austin, TX) were used to record real-time data on cardiac performance (i.e., heart rate, f_H; cardiac output, Q; stroke volume, SV_H).

Following surgery, individual fish were placed immediately into a 70-L tank (50 cm × 50 cm) and monitored until they had regained equilibrium. Fish were allowed to recover from surgery and to acclimate to the tank for at least 18 hr. A darkened area covering approximately 30% of the tank provided cover and ensured that the fish were not disturbed by general laboratory activity. The tanks were continuously supplied with pond water at either 10° or 20°C. To simulate exercise, fish were chased around the tank by hand (Kieffer 2000) until they reached exhaustion (i.e., stopped swimming and lost equilibrium). Cardiac parameters were recorded continuously for at least 1 hr before the exercise simulation (the resting period), during the exercise simulation, and for at least 6 hr postexercise (the recovery period).

Following experimentation, fish were killed with an overdose of anesthetic (180 ppm clove oil), and a postmortem calibration was conducted to convert Doppler shift (in V) to actual blood flow (mL min^{-1}) using the techniques described in Cooke et al. (2003). Reference flow rates were analyzed with linear least squares regression (mean $R^2 = 0.971$). Ventricles were patted dry, weighed to the nearest 0.001 g, and mass corrected for the size of the fish to represent relative ventricular mass (RVM). To examine the temperature dependence of stock-specific resting cardiac parameters, we calculated Q_{10} rates (Schmidt-Nielsen 1997) using the formula

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2 - T_1)},$$

where R_1 and R_2 are the rates of cardiac activity at temperatures 1 (T_1) and 2 (T_2).

Analysis

To determine recovery times, resting values for Q, f_H, and SV_H were adjusted to 100%, and the cardiac parameters were plotted and evaluated visually. A fish was considered to be recovered when values returned to resting and became stable (within 10% of resting values; Cooke et al. 2003). Maximal disturbance, either positive or negative, was determined as the greatest change (i.e., maximum value recorded) in a cardiac parameter during the recovery period. For largemouth bass, maximal values are reached several minutes after the termination of the exercise (i.e., chasing activity; Cooke et al. 2003; Cooke and Philipp 2005). Cardiac scope (i.e., the difference between resting and maximal cardiac activity) for both f_H and Q was calculated and used to provide insight into performance capabilities of the fish (Farrell 1991). Data were assessed for normality using quantile plots and homogeneity of variance using residual plots (SYSTAT, version 8.0, SAS Institute). The premise of all analyses was to test two null hypotheses: first, that there were no differences among the stocks at each of the two temperatures, and second, that there were no differences within each of the stocks across the two different temperatures (JMPIN, version 4.01, SAS Institute). All tests were conducted using two-way ANOVA with stock being the main effect and water temperature the secondary effect. We used Dunnett's test (Dunnett 1955) to determine when values from interstock hybrids differed from the pure Illinois fish. Stepwise multiple regression (Allison 1998) was used to assess the contribution of environmental distance (latitude) and genetic distance (mtDNA and Rogers protein) to the variation we observed in cardiovascular performance among different interstock hybrids (JMPIN, version

Table 2: Meristics for the experimental stocks of largemouth bass at two water temperatures

Temperature (°C) and Experimental Stock (M × F)	N	Total Length (mm)	Mass (g)	RVM	Chase Time (s)	Scope for Q (mL min^{-1} kg^{-1})	Scope for f_H (beats min^{-1})
10:							
IL × IL	12	177 ± 3	63 ± 4	.06 ± .005	142 ± 8	8.4 ± 1.6	25.9 ± 1.7
BC × IL	8	186 ± 2[a]	72 ± 2	.08 ± .007	151 ± 11	11.6 ± 2.2	22.0 ± 3.4[b]
LP × IL	10	176 ± 2	60 ± 3	.06 ± .005	148 ± 9	10.4 ± 1.9	23.3 ± 2.9[b]
DD × IL	11	189 ± 4[a]	76 ± 4	.07 ± .005	145 ± 8	8.0 ± 1.2	25.8 ± 1.9[b]
20:							
IL × IL	9	176 ± 2	66 ± 6	.07 ± .009	145 ± 16	7.8 ± 1.7	25.3 ± 3.3
BC × IL	9	179 ± 2	64 ± 3	.07 ± .003	135 ± 14	7.2 ± .8	27.6 ± 1.9
LP × IL	9	194 ± 1[a]	87 ± 5	.08 ± .005	122 ± 15	10.4 ± 1.1	30.9 ± 2.2
DD × IL	8	184 ± 2	73 ± 3	.08 ± .004	141 ± 14	11.7 ± 1.4	30.6 ± 2.6

Note. All values reported are means (±1 SEM). Additional detail on statistical tests are available in Table 3. Relative ventricular mass (RVM) is body-mass corrected. Cardiac scope (i.e., the difference between resting and maximal cardiac activity) is presented for heart rate (f_H) and cardiac output (Q).

[a] Significantly different ($P < 0.05$) from the IL × IL stock at each temperature.

[b] Significantly different ($P < 0.05$) between water temperatures for each stock.

4.01, SAS Institute). All values reported are means ± SE, and significance was evaluated at $\alpha = 0.05$.

Results

Relative ventricular mass did not vary significantly by stock or temperature (Tables 2, 3). However, both stock and temperature explained significant variation in resting cardiac variables (Table 3; Fig. 2). Cardiac output and f_H values were significantly lower at 10° than at 20°C for all four stocks, whereas for SV_H, only the LP × IL stock differed significantly between 10° and 20°C (Fig. 2). Resting Q and f_H were consistently lowest for the IL × IL stock and uniformly higher for the other three stocks. The only departure from this pattern was that at 10°C, resting Q and f_H were similar between the IL × IL and the DD × IL fish (Fig. 2). Resting SV_H values did not vary significantly among stocks (Table 3; Fig. 2). Q_{10} values for resting Q and f_H ranged from 1.37 to 1.65 (Fig. 2), whereas resting SV_H Q_{10} values ranged from 0.89 to 1.01.

No significant differences were observed in the amount of time it required to exhaust different stocks of fish at both temperatures (Tables 2, 3). Cardiovascular parameters changed in response to exercise and varied by water temperature and stock. In general, maximal Q and f_H were significantly higher at 20°C than at 10°C, whereas SV_H was only significantly different among IL × IL fish between the two temperatures (Table 3; Fig. 3). Maximal Q values were lower for IL × IL fish than for LP × IL fish at both temperatures, for BC × IL fish at 10°C, and for DD × IL fish at 20°C. For maximal f_H, no significant differences were observed among stocks at 10°C; however, at 20°C, all interstock hybrids had significantly higher maximal f_H than did the pure IL × IL stock (Fig. 3). Maximal change in SV_H did not differ among stocks at either temperature (Table 3; Fig. 3). Although scope for Q did not vary by temperature or stock (Tables 2, 3), scope for f_H did differ by water temperature (Tables 2, 3).

Cardiac recovery times did not vary by temperature for all three cardiac variables and for all stocks (Table 3; Fig. 4). However, temperature-specific recovery times did vary extensively by stock. At both temperatures, Q, f_H, and SV_H recovered most rapidly for the pure stock (IL × IL) but significantly slower for all interstock hybrids (Fig. 4). Recovery times were ~70 min for IL × IL fish Q and f_H and ~60 min for IL × IL SV_H. The interstock hybrids generally required ~100 min for Q and f_H to recover and ~80 min for SV_H to recover.

Mitochondrial DNA were never considered to be the primary source of variation in cardiovascular performance and were only considered to be significant secondary contributors in three resting cardiac variables (Table 4). Allozymes were determined to be the primary source of variation for resting and maximal Q at 10°C and resting f_H at both temperatures. Allozymes were only a secondary source for resting Q at 20°C (Table 4). Latitude was the most frequent and generally highest primary source of variation among the three distance measures we assessed, indicating the importance of direct environmental selection. Latitude accounted for between 31% and 45% of variation observed in the recovery parameters and was the only parameter that we tested that accounted for a significant amount of variation in recovery parameters (Table 4). Latitude also explained a significant amount of the variation in maximal cardiac parameters at 20°C and for resting Q at 20°C. Latitude was never a significant secondary contributor to variation in the cardiac parameters. For several of the cardiac parameters, none of the three distance measures significantly accounted for any of the observed variation. For all but one instance, the cardiac parameter was SV_H.

Table 3: ANOVA summaries for all tests performed

Parameter and Source	*F*	df	*P*
Total length:			
Stock	5.3	3	.003
Temperature	.2	1	.674
Stock × temperature	5.7	3	.002
Mass:			
Stock	2.6	3	.064
Temperature	2.6	1	.110
Stock × temperature	5.7	3	.002
RVM:			
Stock	1.9	3	.144
Temperature	3.6	1	.062
Stock × temperature	1.8	3	.168
Resting Q:			
Stock	7.3	3	<.001
Temperature	123.0	1	<.001
Stock × temperature	.5	3	.670
Resting f_H:			
Stock	8.4	3	<.001
Temperature	219.1	1	<.001
Stock × temperature	.1	3	.956
Resting SV_H:			
Stock	.5	3	.717
Temperature	4.8	1	.032
Stock × temperature	1.2	3	.304
Chase duration:			
Stock	.2	3	.875
Temperature	1.6	1	.205
Stock × temperature	.6	3	.588
Max Q:			
Stock	5.2	3	.003
Temperature	42.7	1	<.001
Stock × temperature	1.9	3	.133
Max f_H:			
Stock	3.5	3	.020
Temperature	107.8	1	<.001
Stock × temperature	1.1	3	.350
Max SV_H:			
Stock	.1	3	.984
Temperature	2.2	1	.139
Stock × temperature	.7	3	.578
Q scope:			
Stock	58.3	3	.470
Temperature	2.3	1	.751
Stock × temperature	145.9	3	.104
f_H scope:			
Stock	.7	3	.538
Temperature	6.1	1	.016
Stock × temperature	1.1	3	.368
Recovery time Q:			
Stock	18.6	3	<.001
Temperature	1.6	1	.206
Stock × temperature	.1	3	.998
Recovery time f_H:			
Stock	18.1	3	<.001
Temperature	1.9	1	.175
Stock × temperature	.1	3	.999
Recovery time SV_H:			
Stock	13.5	3	<.001
Temperature	.1	1	.857
Stock × temperature	.3	3	.795

Note. For all parameters, the main effect was stock and the secondary effect was water temperature.

Discussion

Our objective was to evaluate the cardiovascular consequences of interstock hybridization of largemouth bass and evaluate the relative importance of different divergence measures (e.g., genetic and geographic distance). Our data clearly illustrate that there are substantial differences in the cardiovascular performance of interstock hybrids relative to a nonhybridized locally adapted stock, consistent with earlier research on two stocks of largemouth bass (Cooke and Philipp 2005). Specifically, compared with the pure IL × IL lines, we observed that interstock hybrids exhibited higher resting cardiovascular levels, higher maximal cardiac disturbance resulting from exhaustive exercise, and delayed cardiac recovery times. More interestingly, for the first time we presented experimental data on the role of genetic and geographic distance in affecting the physiological performance of wild vertebrate populations/stocks posthybridization. We revealed that the magnitude of population diver-

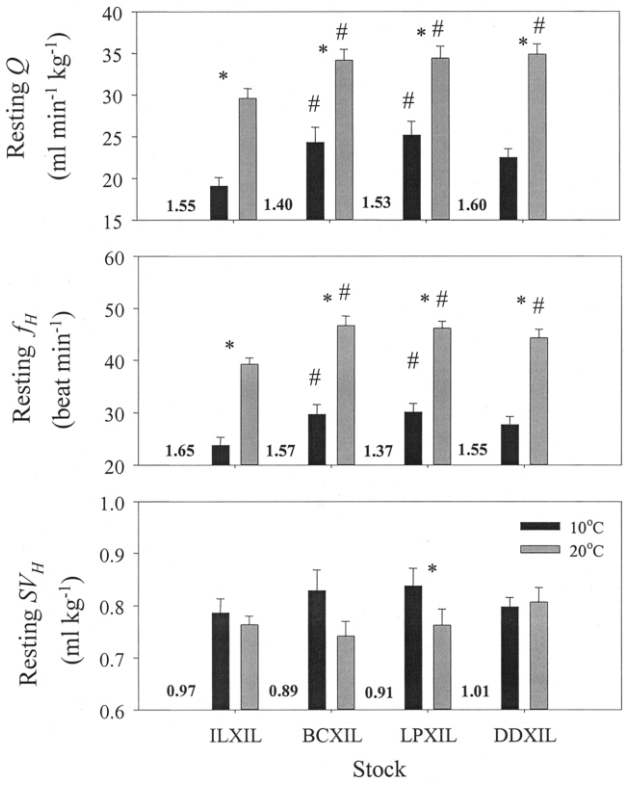

Figure 2. Effects of water temperature and stock on the resting cardiac performance of largemouth bass. Asterisks indicate significantly different ($P < 0.05$) values within a stock at the two different temperatures. Number symbols indicate significantly different ($P < 0.05$) values between IL × IL fish and the three interstock hybrids at each temperature. Numerical values on the panels to the left of the 10°C bars represent stock-specific Q_{10} values for all three cardiac parameters. Additional details on statistical tests are available in Table 3. Data presented are means ± 1 SE.

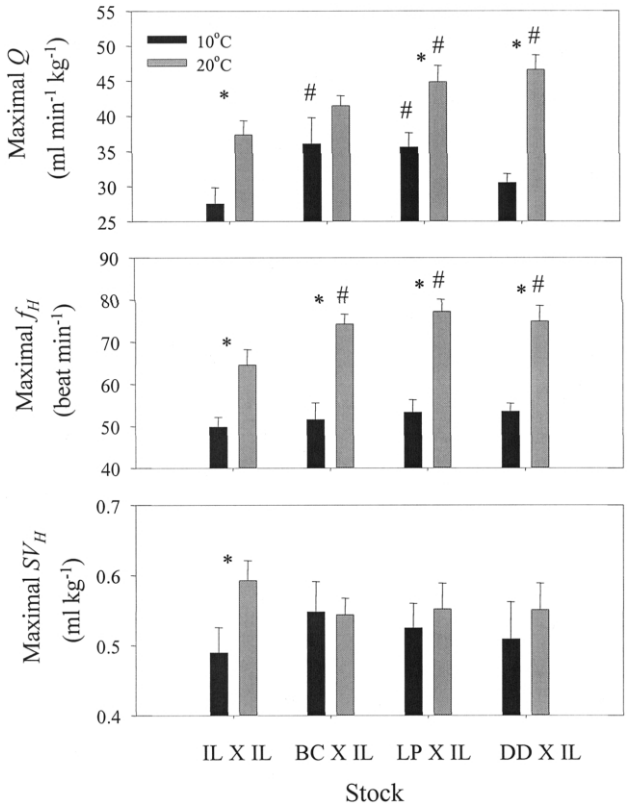

Figure 3. Effects of water temperature and stock on the maximal disturbance (i.e., maximal value recorded, either positive or negative, in cardiac variables from resting during the experimentation) of largemouth bass. Asterisks indicate significantly different ($P < 0.05$) values within a stock at the two different temperatures. Number symbols indicate significantly different ($P < 0.05$) values between IL × IL fish and the three interstock hybrids at each temperature. Additional details on statistical tests are available in Table 3. Data presented are means ± 1 SE.

gence is an important determinant in the degree to which cardiovascular performance of bass is altered from interstock hybridization and associated breakdown of coadapted gene complexes. Using multiple regression analyses, we determined that different measures of divergence contributed differentially to the observed variation in cardiovascular performance metrics. Below, we briefly discuss the consequences of interstock hybridization on cardiovascular performance of largemouth bass, and focus the majority of the discussion on understanding the effects of divergence on the cardiovascular performance observed among crosses.

In this study we extended the earlier work of Cooke and Philipp (2005), who only compared IL × IL with fish from Big Cedar Lake, Wisconsin. In that study the researchers compared both reciprocal crosses (IL × BC and BC × IL) as well as the pure BC stock (BC × BC). Here, we used two additional stocks (Lipsett Lake, Wisconsin, and Dead Lake, Minnesota) to evaluate the cardiovascular consequences of interstock hybridization in multiple populations. We observed that interstock hybrids generally exhibited higher resting cardiovascular levels and higher maximal cardiac disturbance resulting from exhaustive exercise compared with pure IL × IL fish. Furthermore, after being exposed to a performance challenge, cardiovascular recovery times were consistently ~40% longer for interstock hybrids than for the IL × IL fish. All of these findings are in general agreement with the earlier work by Cooke and Philipp (2005) that focused only on the BC × IL stock. Cooke and Philipp (2005) determined that the performance of the interstock hybrids in their study (i.e., BC × IL and IL × BC) was not intermediate to the two parental groups (BC × BC and IL × IL), providing further evidence of outbreeding depression at the F_1 level. Although we did not explicitly assess the performance of the parental stocks from Lipsett Lake or Dead Lake, the findings from Cooke and Philipp (2005) support the conclusion that the interstock hybrids are indeed experiencing performance alterations (potentially impairments) relative to

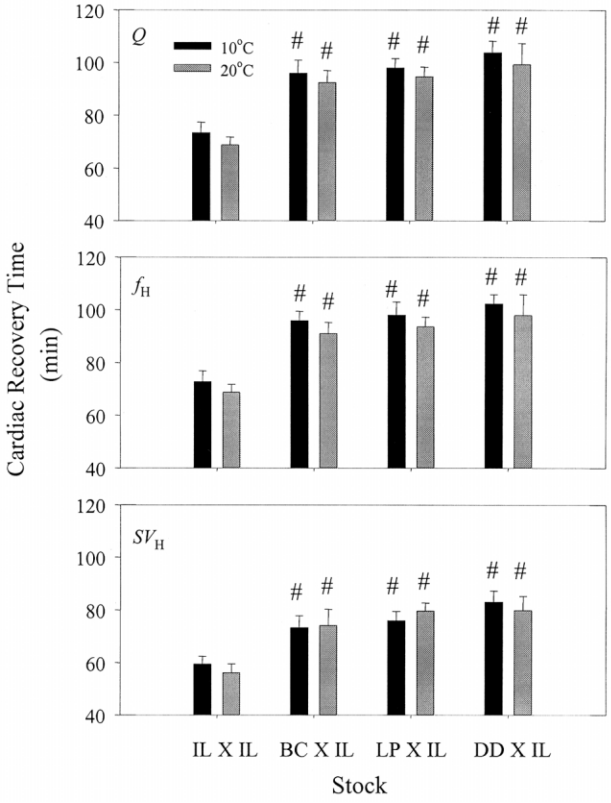

Figure 4. Effects of water temperature and stock on the recovery of cardiac parameters of largemouth bass following exhaustive exercise. Number symbols indicate significantly different ($P < 0.05$) values between IL × IL fish and the three interstock hybrids at each temperature. Additional details on statistical tests are available in Table 3. Data presented are means ± 1 SE.

Table 4: Output from stepwise multiple regressions to evaluate the relative variance contribution of three measures of distance to observed cardiovascular values

Cardiac Parameter and Temperature (°C)	R^2 (% variation explained)	Mean Square (MS)	Probability of Total Model (P)	Variation Explained by Distance Measures (%)		
				Allozymes	mtDNA	Latitude
Resting Q:						
10	25.2	78.39	.013	**16.6**	22.6	NS
20	27.1	54.15	.019	26.7	NS	**21.7**
Resting f_H:						
10	21.9	86.67	.026	**15.8**	20.5	NS
20	33.7	104.72	.005	**26.4**	32.9	NS
Resting SV_H:						
10	5.6	.006	.730	NS	NS	NS
20	9.1	.006	.389	NS	NS	NS
Maximal Q:						
10	20.5	173.27	.035	**14.8**	NS	NS
20	28.9	143.21	.013	NS	NS	**28.6**
Maximal f_H:						
10	3.5	33.27	.720	NS	NS	NS
20	26.7	278.59	.020	NS	NS	**19.3**
Maximal SV_H:						
10	2.9	.005	.772	NS	NS	NS
20	3.1	.004	.803	NS	NS	NS
Recovery Q						
10	48.9	2,031.47	<.001	NS	NS	**44.7**
20	42.0	1,611.49	<.001	NS	NS	**36.3**
Recovery f_H						
10	47.8	2,007.36	<.001	NS	NS	**42.8**
20	41.5	1,495.10	<.001	NS	NS	**35.9**
Recovery SV_H						
10	40.1	1,130.10	<.001	NS	NS	**39.0**
20	36.3	1,085.43	.003	NS	NS	**31.4**

Note. Bold values represent the primary source of variation, and normal font indicates the secondary sources of variation. Nonsignificant variance contributors (NS, $P > 0.05$) are also noted.

both parental stocks. An alternative explanation for the altered performance of interstock hybrids relative to the pure IL × IL fish could be that fish were exposed to different intensities of exercise using our manual chasing approach. We feel that this was unlikely because the same individual chased all the fish and had a set of clear criteria for judging exhaustion. In addition, a previous study of critical swimming speed (in a swimming flume) using some of these same stocks (i.e., IL × IL and BC × IL; Cooke et al. 2001) revealed stock-specific patterns of exhaustion similar to what we observed here by chasing the fish manually.

The consequences of the performance alterations we observed in our study may include heightened metabolic costs associated with higher resting cardiovascular rates. Interestingly, all crosses were able to maintain cardiac scope by elevating maximal cardiac values, suggesting no direct effect on physical performance at these temperatures. However, earlier work by Cooke et al. (2001) revealed that swimming performance of BC × IL fish was impaired relative to pure IL × IL or BC × BC, suggesting that other physiological alterations not evident in cardiac scope are probably present. Fish that exhibit delayed recovery, such as the interstock hybrids in our study, remain at an elevated cardiac stage for a longer period and thus have less scope for activity to escape predators or deal with other stressors (Priede 1977, 1985). In fact, a conceptual theory presented by Priede (1977) suggests that the more time metabolic rates exceed the normal range, the greater the long-term impact on fitness and survival. In this scenario, the protracted recovery period we observed in interstock hybrids could result in fitness impacts either through increased mortality risks or from reductions in energy for growth or reproduction. Although we have no direct empirical evidence of such an effect,

a parallel study (i.e., Philipp et al. 2002) using the same stocks clearly shows reduced growth and survival in all of the interstock hybrids that we used in the current study. Our current research may provide a mechanistic understanding of the fitness impacts observed in interstock largemouth bass hybrids by Philipp et al. (2002) and Philipp and Claussen (1995) as well as the swimming performance impairments observed by Cooke et al. (2001).

Recognition that hybridization of different populations/stocks results in performance alterations generates additional questions. For example, what aspects of divergence among populations/stocks are most important for determining the severity of outbreeding depression? The current data set provides the first opportunity to address this question on a wild vertebrate. In general, the effect of genetic distance, and in particular, allozymes, appeared to be important in accounting for variation in resting cardiac variables. There was no consistent source for variation in the maximal values. However, for recovery variables, geographic distance (latitude) was the most important contributor to cardiac recovery duration. Genetic distance did not contribute significantly to any of the observed variation in cardiac recovery. These findings are interesting for the fact that genetic distance seems to play an important role in determining baseline variation in cardiac rates, whereas geographic distance seems to play an important role in determining the ability of organisms to respond to and recover from stress. Using modeling simulations parameterized with data for diploid unisexual invertebrates, Edmands and Timmerman (2003) suggested that the magnitude of outbreeding depression is positively correlated with genetic distance, although this pattern was not clearly evident in our empirical data.

The data that we present suggest that genetically defined population/stock management units are able to delineate populations/stocks that are sufficiently different to warrant such designation. However, these genetic differences were not as important contributors to variation in physiological performance as latitude, suggesting that geographic distance should also play an important role in defining populations/stocks. Indeed, if we consider geographic distance to reflect a surrogate of environmental differentiation, it is possible that fish are locally adapted to a specific locale, yet because they have not been isolated for sufficient periods or have been derived from the same founder populations, there is no clear genetic pattern. Geographic distance, or more frequently, watershed boundaries, are used by managers to spatially separate populations/stocks (see Nielsen 1995). This approach may be a simplistic means of rapidly identifying management units in situations where genetic data are unavailable. Since F_1 generations are unpredictable in their response to interstock hybridization (Emlen 1991), it is difficult to characterize all of the variation we observed with the three distance measures. Indeed, individual F_1 fish may exhibit heterosis, thus masking variation in performance. Additionally, other environmental factors, such as degree days and photoperiod, as well as genetic factors, such as duration of time separated and amount of gene flow, may all contribute to variation in cardiac performance, although at present this is speculative. Research focused on the physiological consequences of interstock hybridization on F_2 generations should be conducted to address this concern. In copepods, such a study has been conducted (i.e., Edmands 1999), revealing that F_1 interpopulation hybrids (both genetically and geographically separate) exhibited heterosis, whereas F_2 hybrids exhibited clear outbreeding depression. Interestingly, when sequence divergence among populations was <5%, there was little evidence of breakdown at the F_2 generation; however, the closest populations in that study (1 km, 0.2% sequence divergence) did exhibit such a trend.

Patterns of glacial recolonization may help to explain the observed differences in genetic and geographic variation among populations/stocks. Specifically, the creation of multiple refugia may have separated Big Cedar (BC) from the other stocks much earlier. The BC stock probably was based on a stock from Indiana and used this as an invasion route (Field et al. 1997). It is thought that the other stocks, including IL, were derived from fish that recolonized up the Mississippi River (Fields et al. 1997). Due to complicated colonization patterns such as those described here, it is clear why adjacent stocks are not always the most closely related. Selection relies on a combination of the selection forces, the degree of gene flow, and the duration of separation.

In conclusion, even for fish geographically separated by only several degrees of latitude, substantial variation in cardiac performance was observed. Furthermore, although the genetic distances were relatively small (range from 0.001 to 0.03 for mtDNA and 0.04 to 0.08 for allozymes), they (especially proteins) contributed a substantial amount of variation to resting cardiac values. Moreover, the duration required for fish to recover from exercise was affected by the distance of the fish from the source population. Collectively, these data suggest that both genetic distance and geographical distance are important determinants of the degree to which the physiological performance of interstock hybrids will be compromised or altered. Indeed, evidence suggests that the level of outbreeding depression or hybrid vigor cannot be predicted from parental divergence alone (Edmands 2002; Edmands and Timmerman 2003). Because of the important link between cardiovascular physiology and energetics (Thorarensen et al. 1996), it is possible that differences in efficiency will translate into differences in fitness. Perhaps greater inclusion of physiological approaches into the study of interpopulation/interstock hybridization will provide conservation biologists with better understanding of pressing conservation issues and provide animal physiologists with greater appreciation for the intraspecific diversity in the physiological performance of animals.

Acknowledgments

We thank K. Deters, C. Koschman, E. Grant, T. Kassler, and E. De La Corpuz for expert field assistance. This work was conducted in accordance with the University of Illinois Office of Lab Animal Research. A. Weckworth and J. Schreer kindly edited early versions of this manuscript. Several anonymous reviewers also provided comments that greatly improved the manuscript. The University of Illinois Research Board provided funding for purchase of equipment through support to D.P.P and S.J.C. Additional funding for the project was provided by the Illinois Natural History Survey. S.J.C. was supported by a Natural Sciences and Engineering Research Council of Canada Julie Payette Postgraduate Fellowship.

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