

Cardiovascular performance of six species of field-acclimatized centrarchid sunfish during the parental care period

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SUMMARY

Parental care is an energetically costly period of the life history of many fish species characterized by extended high intensity activity. To date, there have been no studies that have investigated the cardiovascular correlates of extended parental care in fish. Using Doppler flow probes, the cardiovascular performance of six syntopic centrarchid fish species ($N=232$) that provide sole, male parental care was examined across a range of water temperatures that encompass their reproductive periods (14–26°C). Experiments were restricted to males but included both nesting and non-nesting individuals to evaluate the cardiovascular performance of fish during parental care. Resting values for cardiac output (\dot{Q}) and heart rate (f_H) tended to be higher for nesting fish when adjusted for variation in temperature. Both of these cardiac variables also increased with water temperature. Stroke volume (V_S) was similar among nesting and non-nesting fish and was generally thermally insensitive. When exposed to exhaustive exercise, nesting fish took longer to exhaust than non-nesting individuals. The high resting levels found in nesting fish accompanied by only minor increases in maximal values typically resulted in reductions in cardiac scope. Cardiovascular variables recovered more quickly in nesting fish, which could facilitate the high activity and bursting associated with parental care. Interspecifically, several cardiovascular variables were correlated with parental care activity. Parental care investment became more energetically expensive as the degree of cardiac frequency modulation decreased. Additionally, as the duration of parental care increased, so did the time required for fish to become exhausted, although this relationship was probably influenced by the fact that the larger species (e.g. smallmouth bass *Micropterus dolomieu*; largemouth bass *Micropterus salmoides*) provided the lengthiest care. Collectively, these data indicate that fish that provide parental care possess adaptations, including sufficient phenotypic plasticity, such that they can enhance their ability to provide high intensity protracted care, and emphasize the nexus between behavior and physiology.

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Key words: parental care, behavior, physiology, energetics, fitness, sunfish, Centrarchidae, heart rate, cardiac output, cardiovascular performance.

INTRODUCTION

Much has been learned over the past ~40 years related to the form and function of the cardiovascular system of fishes (e.g. Randall, 1968; Jones and Randall, 1978; Satchell, 1991; Farrell and Jones, 1992; Farrell, 2002; Gamperl and Farrell, 2004). However, there have been few advances in linking the cardiovascular physiology of fish with ecological and evolutionary concepts. One aspect of an organism's life history that is directly relevant to its ecology and fitness is the reproductive period (Roff, 1992). This is particularly true for species providing parental care, especially those which provide intense care for protracted periods. In fish, this period of care is often characterized by fanning, maintaining constant vigilance against potential predators and chasing predators (Blummer, 1979; Clutton-Brock, 1991). During exercise or protracted activity, the cardiovascular response is rapid and dramatic to facilitate the delivery of oxygen to tissues (Jones and Randall, 1978) and is clearly important for fish providing parental care. In the present study, we examined the cardiovascular performance of six syntopic centrarchid fishes across a range of water temperatures that encompass their reproductive period (14–26°C).

Species of fish in the centrarchid fish family are excellent models for investigating cardiovascular performance and reproduction. The group includes six common species that often occur syntopically (smallmouth bass *Micropterus dolomieu* Lacépède, largemouth bass *Micropterus salmoides* Lacépède, rock bass *Ambloplites rupestris* Rafinesque, black crappie *Pomoxis nigromaculatus* Lesueur, pumpkinseed *Lepomis gibbosus* L., and bluegill *Lepomis macrochirus* Rafinesque). A common trait that arose in protocentrarchids that helps to define the family centrarchidae is the presence of sole paternal care (Gross, 1980). However, parental investment varies widely among these species (Cooke et al., 2006). For example, bluegill provide care for several days whereas smallmouth bass provide care for several weeks. In addition to variation in energetics, reproduction in centrarchid fishes varies with water temperature (Breder, 1936). For example, black crappie begin to reproduce at ~14°C whereas bluegill reproduction often peaks at ~24°C. Despite numerous ethological studies describing the reproductive characteristics of these species (e.g. Breder, 1936; Miller, 1975; Colgan and Brown, 1988; Cooke et al., 2002), little is known about the physiological correlates of parental care behavior.

A variety of physiological processes including swimming performance and metabolic rate exhibit seasonal patterns. The most prominent component of seasonality is water temperature, which plays an important role in dictating metabolic rates and activity (Beamish, 1964; Fry, 1971; Lemons and Crawshaw, 1985). However, in some instances, metabolic components have been determined to be thermally insensitive (Evans, 1984). For this reason, investigators have examined other seasonal factors such as reproductive phase to explain the observed patterns. For several fish species, including some centrarchid fishes, standard metabolic rate appears to be heightened during the reproductive period, relative to similar water temperatures during other seasons (Roberts, 1964; Burns, 1975; Meakins, 1975; Evans, 1984; Cooke, 2004). In controlled laboratory environments, the ability to detect seasonal changes associated with factors other than water temperatures is regarded as difficult (Fry, 1971) because fish rarely behave normally. Therefore, it is necessary to perform studies on fish field-acclimatized to the specific environmental conditions (e.g. Facey and Grossman, 1990; Adams and Parsons, 1998; Lee et al., 2003). This approach can provide realism and introduce natural variation that is inherent in field-acclimatized fish. Thus, our first objective was to contrast the resting cardiovascular performance of several field-acclimatized centrarchid species, focusing on nesting and non-nesting males across a gradient of temperature. We predicted that across the range of water temperatures that an individual species was engaged in reproductive activities, resting cardiac metrics will be higher for nesting fish than non-nesting fish.

Our second objective was to contrast the response of nesting and non-nesting fish to forced exhaustive exercise across water temperatures that encompass the reproductive period. The predictions that we developed are based on the notion that the parental care period is integral to the fitness of these organisms, and that adaptations should exist to facilitate extended and intense care (Baylis, 1978). Even though fish providing paternal care would have depleted energy stores (e.g. Mackereth et al., 1999), we predicted that they will require longer periods to exhaustion than non-nesting fish. As we predicted that metabolic rates would be higher for nesting fish, we suggest that these fish will have adaptations to allow them to maintain metabolic scope, which is important for sustaining performance (Priede, 1985). Therefore, we predicted that to maintain metabolic scope, nesting fish will increase the maximal cardiac performance relative to non-nesting fish. We also predicted that it would be beneficial to expedite recovery when providing care, so nesting fish will recover more rapidly than non-nesting fish. This would enable them to engage predators presumably more rapidly and thus more effectively.

The third and final objective of this study was to compare and contrast the cardiovascular performance of different species, including degree of frequency modulation [i.e. frequency modulators regulate cardiac output (\dot{Q}) primarily by adjusting the frequency of the heart beat rather than ventricular stroke volume (V_s) (Farrell, 1991b; Thorarensen et al., 1996a)], and evaluate the relationship between parental care investment and cardiovascular performance. Previous research (i.e. Schreer et al., 2001; Cooke et al., 2003b) has revealed that some species within the centrarchid family appear to be cardiac frequency modulators. Frequency modulation has been suggested to be beneficial for delivering oxygen to tissues in species with highly variable activity levels, such as ambush predators (Lucas et al., 1991). Parental care also requires variable activity levels (Cooke et al., 2002). Therefore, we predicted that fish with intense parental care as indicated by high levels of relative energetic expenditure (Cooke et al., 2006) will be correlated with the degree

of frequency modulation. We further predicted that those species with extended care will require more time to exhaust, recover more rapidly, and do so with increased scope, and have increased relative changes in heart rate (f_H) than fish with short duration care.

MATERIALS AND METHODS

Study animals

Experiments were performed at the Queen's University Biology Station (QUBS) on Lake Opinicon in eastern Ontario, Canada. The six species of centrarchids that we investigated were syntopic and generally spawn over several weeks in the spring. Physiological assessments were undertaken across the range of temperatures within which all of these fish reproduce (~14–26°C) (Wismer and Christie, 1987). Data for each species were collected across the range of water temperatures. However, most efforts focused on the specific temperature range during which each species provided parental care.

All fish used in this study were angled using rod and reel. We focused our study on males, the only sex that provides parental care among these species. Nesting males were identified by snorkelers and then captured from their nest. We restricted sampling to those fish that had offspring that were not free swimming and were usually in the egg or wriggler (i.e. embryo in the nest) stage. Herein we refer to these fish as 'parental-care-providing' or 'nesting'; however, it is important to note that fish that were used in the study were freshly removed from nests so parental care was technically terminated upon their removal. Non-nesting fish were targeted by angling in areas away from the littoral zone where there was an absence of suitable spawning habitat. We included only sexually mature fish and attempted to collect fish of equal sizes for the two groups (i.e. nesting and non-nesting). All fish were landed within 20 s to minimize stress and were then transported to the laboratory in large, aerated coolers (75 l). In the laboratory, all fish were held in large common tanks (300 l). Fish were marked with binary dorsal spine clips to identify nesting and non-nesting fish. Flow-through lake water was provided for the fish so that they remained acclimatized to field conditions such as slight diel temperature variation even though there were briefly held in the lab (i.e. we were trying to avoid acclimation). Natural light through windows provided seasonally appropriate photoperiods. While in captivity, food was withheld. All fish used in this experiment were handled under an identical schedule. Fish were angled on day one and surgery was conducted 24 h later. Fish were then given 24 h to recover from surgery and then chased until exhaustion. Post-mortem calibrations were conducted the following morning as the next batch of surgeries was being completed. All surgeries were conducted by one of three trained individuals and the same individual conducted all calibrations.

Surgical procedure and \dot{Q} apparatus

Each fish was anesthetized prior to surgery with 60 p.p.m. clove oil (emulsified with ethanol, 9:1 ethanol:clove oil) for approximately eight minutes, until the fish had lost equilibrium and was non-responsive. An anesthetized fish was placed on its side on a wetted sponge. Anesthesia was maintained during surgery by irrigating the gills with a solution of clove oil at 30 p.p.m. The surgical procedure was identical to that described by Schreer et al. (Schreer et al., 2001). Briefly, a flexible silicone cuff-type Doppler flow probe (subminiature 20 MHz piezoelectric transducer: Iowa Doppler Products, Iowa City, IA, USA), sized to match the diameter of the vessel, was secured around the ventral aorta with a single suture and additional sutures were used to secure the wire to the body wall. The transducer was hard-wired to a receiver unit *via* a cable

positioned directly above the tank. This allowed the fish to swim freely within the confines of the tank.

Recording of blood flow was described in Schreer et al. (Schreer et al., 2001). Briefly, a flowmeter (545C-4 Directional Pulsed Doppler Flowmeter: Bioengineering, The University of Iowa, Iowa City, IA, USA) and a digital strip-chart recorder (LabVIEW, Version 4.0.1, National Instruments Corporation, Austin, TX, USA) were used to record the velocity of the blood. Peaks in velocity indicate individual heart beats, and peaks per unit time yields heart rate (f_H). The mean velocity per unit time is an index of flow or cardiac output (\dot{Q}). Flow can also be calculated directly in ml time^{-1} (see Post-mortem calibration section). \dot{Q} divided by f_H yields stroke volume (V_S).

Exhaustive exercise protocol

Following surgery, individual fish were placed into a 70 l tank (50 cm \times 50 cm) and monitored until they had regained equilibrium. Fish were allowed to recover from surgery and to acclimate to the tank for 24 h. A darkened area covering $\sim 30\%$ of the tank provided cover and ensured that the fish were not disturbed by general laboratory activity. To simulate exercise, fish were chased in the tank by hand, which involved repeated touching and/or squeezing the caudal peduncle region (Kieffer, 2000). Exhaustion was determined when fish would no longer swim in response to this stimulation and began to lose equilibrium. Cardiac variables were recorded continuously for at least one hour prior to the exercise simulation (the resting period), during the exercise simulation and for at least six hours post-exercise (the recovery period). During the experimentation, access to the laboratory was restricted.

Post-mortem calibrations and measurements

The post-mortem calibration was described in Cooke et al. (Cooke et al., 2001). Briefly, to convert Doppler shift (in volts) to blood flow (ml min^{-1}), fish were killed with an overdose of anesthetic (180 p.p.m. clove oil), and the head, including the pericardial cavity, was separated from the rest of the fish. The sinus venosus, atrium and ventricle were removed and the bulbus arteriosus was catheterized with tubing (PE 120, VWR Canada, Mississauga, ON, Canada) for perfusion of the ventral aorta. Using a constant infusion pump (Harvard Apparatus, South Natick, MA, USA), anticoagulated pig's blood was perfused through the aorta to calibrate the probes over a range of flow rates encompassing those recorded during the trials. For this paper, we have only included fish for which we had acceptable calibrations that permitted the determination of actual flow rates. It is possible that the cuff shifted during calibration so the values presented here should be regarded as estimates. For the larger fish, more than 90% of calibrations were successful. For bluegill and pumpkinseed, fewer than 80% were successful. Reference flow rates were analyzed with linear least-squares regression. Of those 232 calibrations that were successful, more than 90% had $R^2 > 0.90$. The ventricles were patted dry and weighed to the nearest 0.001 g. Cardiac mass in fish scales in almost direct proportion to body mass (Poupa and Lindström, 1983) so we reported values as both absolute (AVM) and relative (RVM) ventricular mass. Ventral aorta diameter was determined from the Doppler cuff lumen (to the nearest 0.1 mm) that was used for each fish.

Parental care activity

To address objective three, to compare and contrast the cardiovascular performance of different species and evaluate the relationship between parental care investment and cardiovascular

performance, we used data collected in a parallel field study that contrasted the parental care energetics of the same six species of centrarchid fish (Cooke et al., 2006). That study was conducted at the same time in the same water body. Parental care metrics that we used to evaluate relationships with cardiovascular performance were: mean nesting swim speed in body lengths per second; mean caudal fin, pectoral fin and turning rates in beats per second; mean duration of care in days; mean total metabolic costs of care for the entire duration in mg O_2 ; and mean total metabolic costs of care for the entire duration mass adjusted to $\text{mg O}_2 \text{kg}^{-1}$. Cardiovascular and exercise parameters included in the analyses were: adjusted mean rate of exhaustion; adjusted mean recovery time for all variables; adjusted mean scope for f_H and \dot{Q} ; adjusted mean relative change in f_H ; and degree of frequency modulation [ranked from highest (1) to lowest (6)]. Prior to ranking, the degree of frequency modulation was calculated using the formula in Thorarensen et al. (Thorarensen et al., 1996a) and modified by Cooke et al. (Cooke et al., 2003a). For all other cardiac parameters we used the adjusted mean value derived from analysis of covariance (ANCOVA) analysis to control for water temperature.

Phylogenetic relatedness poses a potential problem for comparative studies because closely related species can share a certain character state through common ancestry rather than through independent evolution (Felsenstein, 1985; Harvey and Pagel, 1991). Therefore, we conducted our analysis with and without controlling for phylogenetic effects. To control for phylogenetic effects, data were converted to phylogenetically independent standardized contrasts (PIC) using the PDTREE module of the Phylogenetic Diversity Analysis Package (PDAP, V.6.0, Riverside, CA, USA) (Garland et al., 1992; Garland et al., 1993), which is based upon the methods of Felsenstein (Felsenstein, 1985). Branch lengths were transformed using Grafen's (Grafen, 1989) method for arbitrary branch lengths prior to analyses. The phylogeny for the independent contrasts was obtained primarily from a molecular genetic analysis by Roe et al. (Roe et al., 2002). This phylogeny included all of the species we examined except for pumpkinseed. A previous phylogeny for the centrarchids based upon morphology (Mabee, 1993) suggests that pumpkinseed are closely related to bluegill, the other *Lepomis* species. This is also supported by recent genetic evidence (Neff et al., 1999). Therefore, pumpkinseed were considered to be a separate branch, originating at the *Lepomis* node. The branch length for pumpkinseed was assumed to be the same as bluegill. Branch lengths provided for several other *Lepomis* in Roe et al. (Roe et al., 2002) were all of similar length.

Data analysis

To determine recovery times, cardiac traces for each fish were adjusted to resting (100%) and evaluated visually. A fish was considered to be recovered when values returned to resting and became stable (within 10% of resting values) (Schreer et al., 2001). Maximal disturbance was determined as the greatest change in a cardiac parameter (either positive or negative) during the recovery period. Using the GLM procedure in Systat (V.10.0, Systat Systems, Inc., Point Richmond, CA, USA), we first tested for homogeneity of slopes. If the homogeneity of slope assumption had not been violated, we then applied ANCOVA to the data. First, we used the nesting status of the fish as the treatment, temperature as a covariate and grouped by species. Next, we used the species as the treatment, temperature as a covariate and grouped by nesting status. Wherever significant differences were noted using the procedure, we used Tukey's test for least-squares means to determine where the differences occurred. Least-squares linear regressions and analysis

of variance (ANOVA) were used to generate mathematical relationships among appropriate variables in the ANCOVA models (e.g. non-nesting black crappie across a range of temperatures). Pearson's correlations were used to evaluate the relationship among cardiovascular performance and the parental care metrics. Tests were conducted using Systat V.10.0 and JMP IN V.4.1 (Carey, NC, USA) and significance was evaluated at $\alpha=0.05$ unless otherwise noted.

RESULTS

Here we present data for 232 fish that were monitored for \dot{Q} during the spring of 2001 (Table 1). Since these species have substantially different body morphology, the sizes of fish (both total length and mass) vary widely, so we only present summary statistics (Table 1). Relative ventricle masses were similar when adjusted for temperature among nesting and non-nesting fish for all species, and were all $\sim 0.1\%$ of the body mass (P values > 0.30 ; Table 1). Note that all individual species-specific test statistics are presented separately in the supplementary material.

Variation in resting values across water temperatures

For each of the six species, resting slopes for \dot{Q} and f_H were generally similar among nesting and non-nesting fish as a function of temperature. However, in most instances the intercepts differed (Fig. 1; Table S1 in supplementary material). Resting \dot{Q} and f_H were generally higher for nesting fish than non-nesting fish for all species that we examined (Fig. 1). Conversely, the resting V_S did not vary with nesting status for each of the species that we examined (Fig. 1). Resting V_S for nesting and non-nesting fish also generally had homogenous slopes. However, none of the intercepts were significantly different (Fig. 1; Table S1 in supplementary material).

Substantial interspecific differences in resting cardiac variables were noted when fish were grouped as either nesting or non-nesting (Fig. 2; Table 2). For non-nesting fish, the homogeneity of slopes assumption was violated, suggesting that the rate of change associated with temperature differed among species. Nesting fish exhibited this pattern for \dot{Q} . However, the slopes for f_H and V_S did not differ among species even though the intercepts did (Table 2). In general, \dot{Q} and f_H increased with increasing water temperature whereas V_S was static or decreased (Fig. 2).

Variation in response to exercise

The time required to exhaust fish varied by nesting status (Fig. 3; Table S2 in supplementary material). Nesting fish consistently took longer to exhaust than did non-nesting conspecifics for all species

that we examined (Fig. S1 in supplementary material). Substantial species differences in time until exhaustion were noted when fish were grouped as either nesting or non-nesting (Table 2). When adjusted for temperature, the time required for fish to become exhausted was similar among species for nesting fish but differed for non-nesting individuals (Fig. 2).

Maximal cardiac variables recorded following exercise were characterized by increased levels of \dot{Q} and f_H , and decreased levels of V_S for all species relative to resting values (Fig. 4). Similar to resting values, maximal \dot{Q} and f_H generally increased with increasing water temperatures, whereas V_S was static or decreased (Fig. 3; Table S3 in supplementary material). Maximal values were generally similar between nesting and non-nesting fish for each species (Fig. 3). Maximal \dot{Q} values for nesting and non-nesting fish generally increased at the same rate with temperature; however, the nesting fish usually had higher intercepts indicating differences among nesting status (Table S3 in supplementary material). For \dot{Q} , only smallmouth bass, rock bass and bluegill had higher maximal values during the nesting phase (Fig. 2). Maximal f_H and V_S also typically changed at the same rate for nesting and non-nesting fish, and rarely did the intercepts differ (Fig. 3; Table S3 in supplementary material). Maximal cardiac parameters varied by species for each of the nesting phases. Non-nesting maximal \dot{Q} and V_S slopes differed by species whereas nesting f_H and V_S slopes differed by species (Table 2; Fig. 2). For other variables, the intercepts differed significantly.

The percentage change in cardiac variables generally did not differ among nesting phases for \dot{Q} and V_S but did for f_H (Table S4 and Fig. S2 in supplementary material). For all species, the maximal change in f_H was greater for non-nesting fish than nesting conspecifics (Fig. S2 in supplementary material). The maximal percentage change in cardiac variables varied widely among species for all cardiac parameters and both nesting phases (Table 2; Fig. 2). For every variable except non-nesting f_H , the slopes were similar; however, the intercepts differed significantly (Table 2). The greatest change in \dot{Q} was observed for pumpkinseed and largemouth bass.

For both scope for \dot{Q} and f_H , the rate of change with temperature for nesting and non-nesting fish were similar (Fig. S3 and Table S5 in supplementary material). The scope for \dot{Q} and f_H also varied with nesting status but not consistently (Table S5 in supplementary material; Fig. 2). Variation in intercepts among nesting and non-nesting fish was more variable among species (Table 2; Fig. 2). There was a general trend towards non-nesting fish having larger scopes for f_H (Fig. 2). This was especially evident for pumpkinseed. When fish were grouped as either nesting or non-

Table 1. Meristics of fish used for experimentation including cardiac morphometrics

Species	Nesting status (Y or N)	N	Total length (mm)	Mass (g)	Aorta diameter (mm)	AVM (g)	RVM
Black crappie (BC)	N	22	246 (9)	191 (19)	1.1 (0.04)	0.15 (0.02)	0.08 (0.004)
	Y	12	265 (7)	268 (24)	1.2 (0.04)	0.21 (0.02)	0.08 (0.003)
Smallmouth bass (SMB)	N	17	323 (9)	477 (53)	1.6 (0.04)	0.51 (0.05)	0.11 (0.005)
	Y	13	336 (16)	659 (124)	1.6 (0.10)	0.59 (0.10)	0.10 (0.006)
Rock bass (RB)	N	22	226 (6)	228 (17)	1.2 (0.04)	0.28 (0.02)	0.12 (0.006)
	Y	15	234 (5)	247 (12)	1.3 (0.03)	0.28 (0.01)	0.11 (0.005)
Largemouth bass (LMB)	N	39	318 (7)	513 (42)	1.5 (0.04)	0.42 (0.03)	0.09 (0.003)
	Y	22	336 (11)	621 (75)	1.6 (0.07)	0.50 (0.05)	0.09 (0.005)
Pumpkinseed (PS)	N	21	193 (4)	151 (8)	1.0 (0.05)	0.20 (0.02)	0.13 (0.006)
	Y	16	194 (3)	150 (8)	1.0 (0.02)	0.21 (0.02)	0.14 (0.008)
Bluegill (BG)	N	21	195 (3)	130 (5)	1.1 (0.02)	0.14 (0.01)	0.10 (0.005)
	Y	12	190 (4)	126 (8)	1.0 (0.04)	0.14 (0.01)	0.11 (0.009)

Values are presented as means (\pm s.e.m.). Actual ventricular mass (AVM) and relative ventricular mass (RVM) were determined by weighing the ventricles after they had been patted dry. Statistical analyses were only conducted on RVM. RVM did not differ significantly among nesting and non-nesting fish for any of the species we examined (all ANCOVAs, $P > 0.30$).

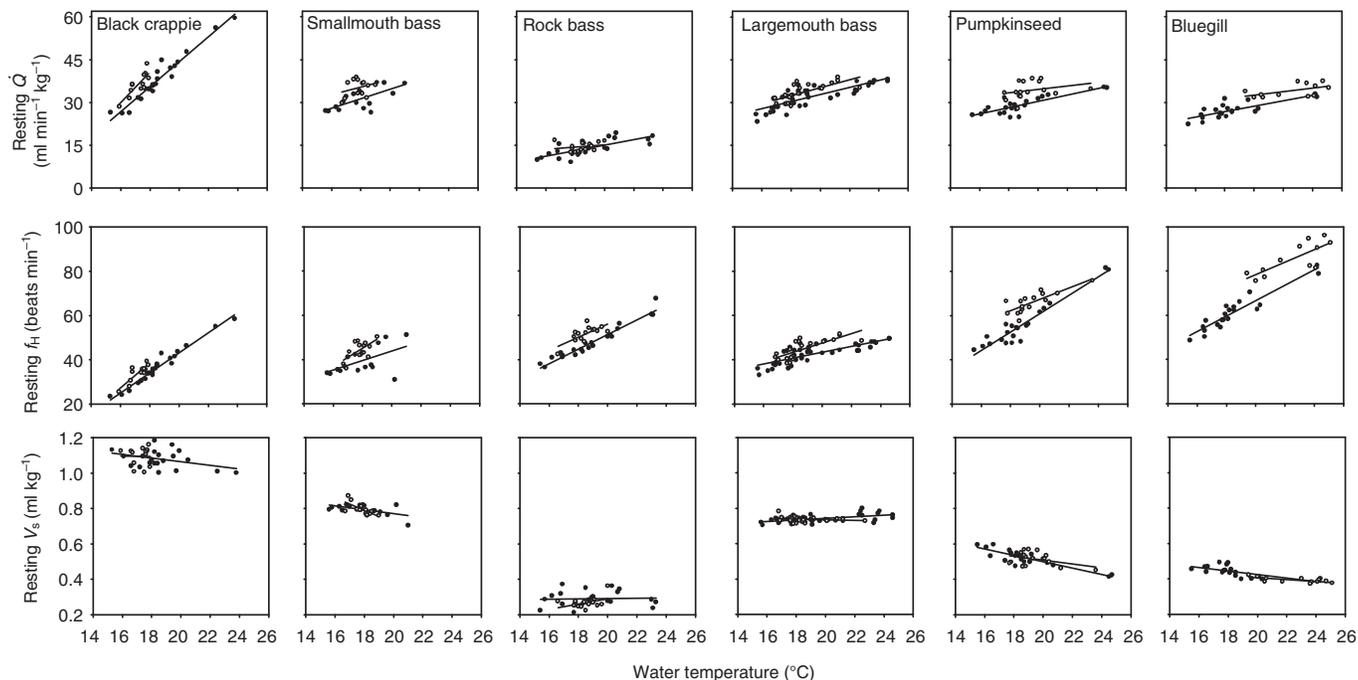


Fig. 1. Scatterplots and regressions for resting cardiovascular variables of nesting (open circles) and non-nesting (filled circles) fish across a range of water temperatures. Details on the equations, correlation coefficients and significance are presented in Table S1 in supplementary material. \dot{Q} , cardiac output; f_H , heart rate; V_S , stroke volume.

nesting, substantial differences were noted among species. Scope for \dot{Q} had similar slopes among nesting phases but differed by intercepts. Conversely, scope for f_H had substantial differences in slope for both nesting and non-nesting fish among species (Table 2). Scope for \dot{Q} was highest for smallmouth bass and largemouth bass and scope for f_H was highest for smallmouth bass (Fig. 2).

For most species, recovery times changed at the same rate across temperatures for nesting and non-nesting fish, and the intercepts differed significantly (Fig. 4; Table S6 in supplementary material). For all the species that we examined, cardiac recovery times for nesting fish were usually 50% faster than non-nesting fish (Fig. 2). For all species, recovery time for f_H and \dot{Q} were similar; however, V_S typically recovered ~10% more rapidly. Few species-specific trends in cardiac recovery time were noted for both non-nesting and nesting fish (Fig. 2). The slopes were significantly different for all cardiac variables for nesting and non-nesting fish (Table 2). In general, for all cardiac parameters, nesting largemouth bass and bluegill recovered before the other species. No species-specific differences were noted for cardiac recovery of non-nesting fish (Table 2).

The rate of change in the time of peak cardiac parameters during recovery generally did not differ among nesting and non-nesting fish and only about half of the intercept values differed by nesting status (Fig. S4 and Table S7 in supplementary material). There were consistent trends of the peak in cardiac variables occurring ~15% earlier in nesting fish than non-nesting fish for all the species and cardiac variables we examined (Fig. 2). However, only for rock bass was this trend consistently significant. When assessing species differences, non-nesting fish typically had significantly different slopes precluding additional analyses (Table 2). Peak cardiac recovery times were slower during the non-nesting stage (Fig. 2).

Cardiovascular performance and parental care

As we predicted, there was a strong correlation among the degree of frequency modulation and the total mass adjusted energy expenditure during the duration of parental care ($r_s=0.967$, $P=0.002$; $r_{SPIC}=0.995$, $P<0.001$). Also consistent with our predictions, the duration of care was positively correlated with duration required to

Table 2. Analysis of covariance table for cardiovascular performance of non-nesting and nesting fish of six species

Variable	Non-nesting fish		Nesting fish	
	Slope	Intercept	Slopes	Intercepts
Resting \dot{Q}	<0.001		<0.001	
Resting f_H	<0.001		0.278	<0.001
Resting V_S	<0.001		0.070	<0.001
Chase time	0.010		0.129	0.004
Maximal \dot{Q}	<0.001		0.309	<0.001
Maximal f_H	0.202	<0.001	0.007	
Maximal V_S	0.015		0.008	
Change \dot{Q}	0.210	<0.001	0.102	<0.001
Change f_H	<0.001		0.454	<0.001
Change V_S	0.130	<0.001	0.065	<0.001
Scope for f_H	<0.001		0.017	
Scope for \dot{Q}	0.056	<0.001	0.165	<0.001
Peak in \dot{Q}	0.014		0.459	0.016
Peak in f_H	<0.001		0.482	0.897
Peak in V_S	<0.001		0.288	0.089
Recovery for \dot{Q}	0.012		<0.001	
Recovery for f_H	0.015		<0.001	
Recovery for V_S	0.004		<0.001	

For all analyses, the treatment was species and the covariate was water temperature. When the homogeneity of slopes assumption (slope) was met and when no significant interaction was present, the covariance model was fit (intercept). \dot{Q} , cardiac output; f_H , heart rate; V_S , stroke volume.

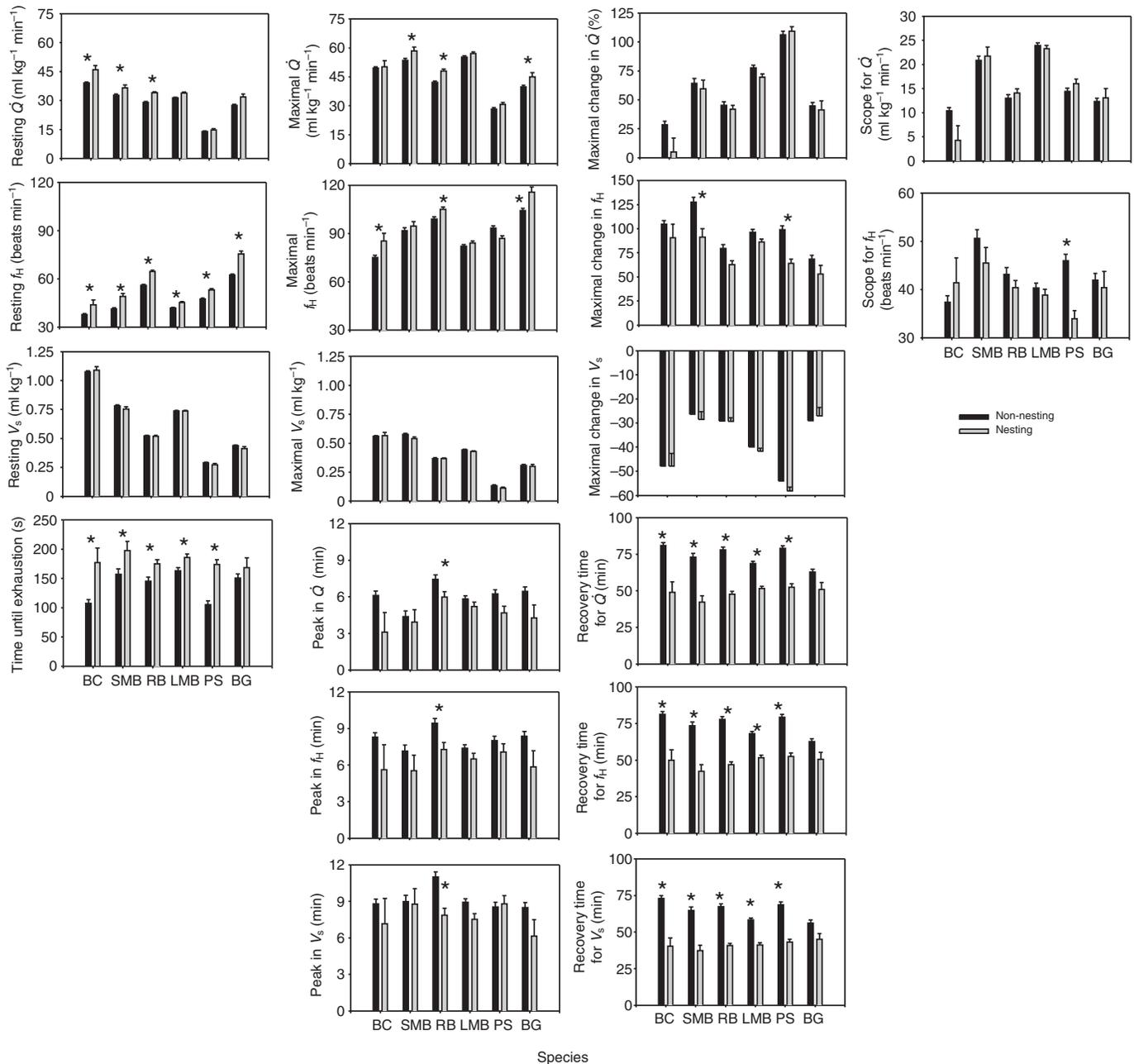


Fig. 2. Comparison of temperature standardized cardiovascular variables for nesting and non-nesting fish of six species. Bars represent least squares means \pm s.e.m. Significantly different values ($P < 0.05$) among nesting status for an individual species are indicated by an asterisk. Statistical details associated with ANCOVAs is provided in Table 2. \dot{Q} , cardiac output; f_H , heart rate; V_S , stroke volume; BC, black crappie; SMB, smallmouth bass; RB, rock bass; LMB, largemouth bass; PS, pumpkinseed; BG, bluegill.

exhaust fish ($r_s = 0.945$, $P = 0.005$; $r_{SPIC} = 0.896$, $P = 0.039$). The duration of care was not correlated with scope (f_H , $r_s = 0.336$, $P = 0.515$; $r_{SPIC} = 0.364$, $P = 0.547$; \dot{Q} , $r_s = 0.677$, $P = 0.140$; $r_{SPIC} = 0.676$, $P = 0.211$) or relative changes in f_H ($r_s = 0.737$, $P = 0.094$; $r_{SPIC} = 0.4632$, $P = 0.432$). We also failed to observe significant relationships between the duration of care and recovery time for \dot{Q} ($r_s = -0.520$, $P = 0.291$; $r_{SPIC} = -0.572$, $P = 0.313$), f_H ($r_s = -0.446$, $P = 0.376$; $r_{SPIC} = -0.574$, $P = 0.310$) and V_S ($r_s = -0.705$, $P = 0.118$; $r_{SPIC} = -0.506$, $P = 0.322$). Although not components of our predictions, we noted several other relationships that are worthy of reporting. The relative increase in f_H was correlated (negatively) with swimming speed ($r_s = -0.952$, $P = 0.004$) and with pectoral fin rate ($r_s = -0.916$, $P = 0.010$)

whereas scope for \dot{Q} was positively correlated with total energy expenditure ($r_s = 0.848$, $P = 0.033$).

DISCUSSION

In this paper, the cardiovascular performance of six syntopic centrarchid fishes was examined across a range of water temperatures that encompassed their reproductive periods (14–26°C). The premise of this paper is that because parental care is an important but metabolically challenging activity in the life of centrarchid fishes, cardiovascular adaptations or phenotypic plasticity should exist to facilitate the heightened and protracted period of care. The results of this study demonstrate that parental-

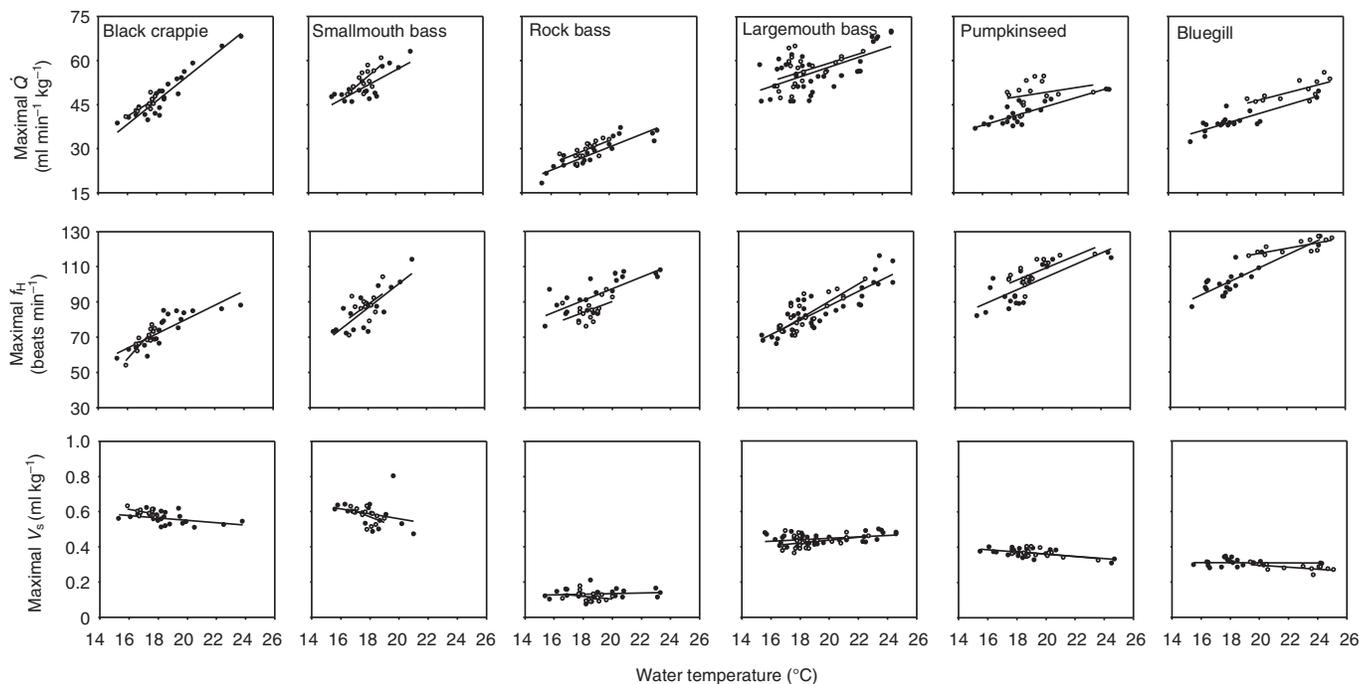


Fig. 3. Scatterplots and regressions for maximal cardiovascular variables of nesting (open circles) and non-nesting (filled circles) fish across a range of water temperatures. Details on the equations, correlation coefficients and significance are presented in Table S3 in supplementary material. \dot{Q} , cardiac output; f_H , heart rate; V_S , stroke volume.

care-providing fish exhibit increased resting f_H and \dot{Q} relative to non-nesting fish. Nesting fish recovered from exhaustive exercise ~30% more rapidly than non-nesting individuals. Furthermore, two cardiovascular factors were strongly correlated with parental care metrics, indicating the important link between cardiovascular performance and reproductive activities.

Variation in resting values

Consistent with our prediction, nesting male fish consistently exhibited higher resting \dot{Q} and f_H than non-nesting conspecifics. Hormones represent the most plausible proximate cause for the changes in cardiovascular performance during the reproductive period (Mooradian et al., 1987). For centrarchids (Kindler et al., 1989; Kindler et al., 1991; Porter, 1996; Gross et al., 2002), as well as numerous other teleost fishes (Liley and Stacey, 1983), 11-ketotestosterone is a reproductive hormone that is directly linked to maturation and reproductive behaviors. Recently, this hormone has also been shown to be correlated with cardiac hypertrophy [perhaps hyperplasia, see Bailey et al. (Bailey et al., 1997)] in wild maturing male rainbow trout (*Oncorhynchus mykiss*) (Thorarensen et al., 1996b). However, our data lacked major trends in RVM between nesting and non-nesting fish. As such, although hormones may influence cardiac performance of centrarchids during parental care, it is not through hormonally mediated changes in cardiac morphology.

\dot{Q} and f_H were both thermally sensitive, increasing positively with increasing water temperatures. However, the rate of change differed among species. The strongest relationship between water temperature and both f_H and \dot{Q} was for black crappie. This species spawns at the lowest water temperature (Cooke et al., 2006) and also has the lowest preferred temperature (Wisner and Christie, 1987). Reflecting this, the rate of change was faster (i.e. steeper slope) for this species. The resting values that we report for f_H and

\dot{Q} fall within the ranges of many other fish species studied to date (Satchell, 1991) and are consistent with previous studies on centrarchid fishes by our lab.

V_S was generally static across a range of water temperatures and for nesting and non-nesting fish. Similar observations of static V_S 's have been observed for centrarchid fish across a range of temperatures (Schreer et al., 2001; Cooke et al., 2003b); however, departures have been noted at low temperatures [i.e. 3°C for largemouth bass, increased V_S (Cooke et al., 2003a)] and high temperatures [>26 °C for smallmouth bass, decreased V_S (Schreer and Cooke, 2002)]. Interspecific differences in V_S were quite extreme, ranging from over 1 ml kg⁻¹ in black crappie to ~0.3 ml kg⁻¹ in rock bass. Interestingly, the range in values among these closely related species is similar to the range in values observed among fish of many different families and orders (e.g. Satchell, 1991).

Variation in response to exercise

A number of cardiovascular performance variables examined differed among nesting and non-nesting fish when exposed to exhaustive exercise. The time required for fish to become exhausted was consistently longer for nesting fish. Superficially, this appears contrary to what one might expect based upon the extreme energetic costs of parental care and the depletion of energy reserves accompanying this period. However, there may be other physiological alterations that actually mobilize energy reserves making them available for exercise (Moyes and West, 1995) or improve the cardiovascular performance of fish leading to our prediction that non-nesting fish would reach exhaustion more rapidly than nesting fish.

As noted earlier, reproductive hormones can alter cardiac morphology. However, reproductive hormones can also increase red muscle mass (Thorarensen et al., 1996c). These authors concluded that the collective role of the androgens in stimulating ventricular

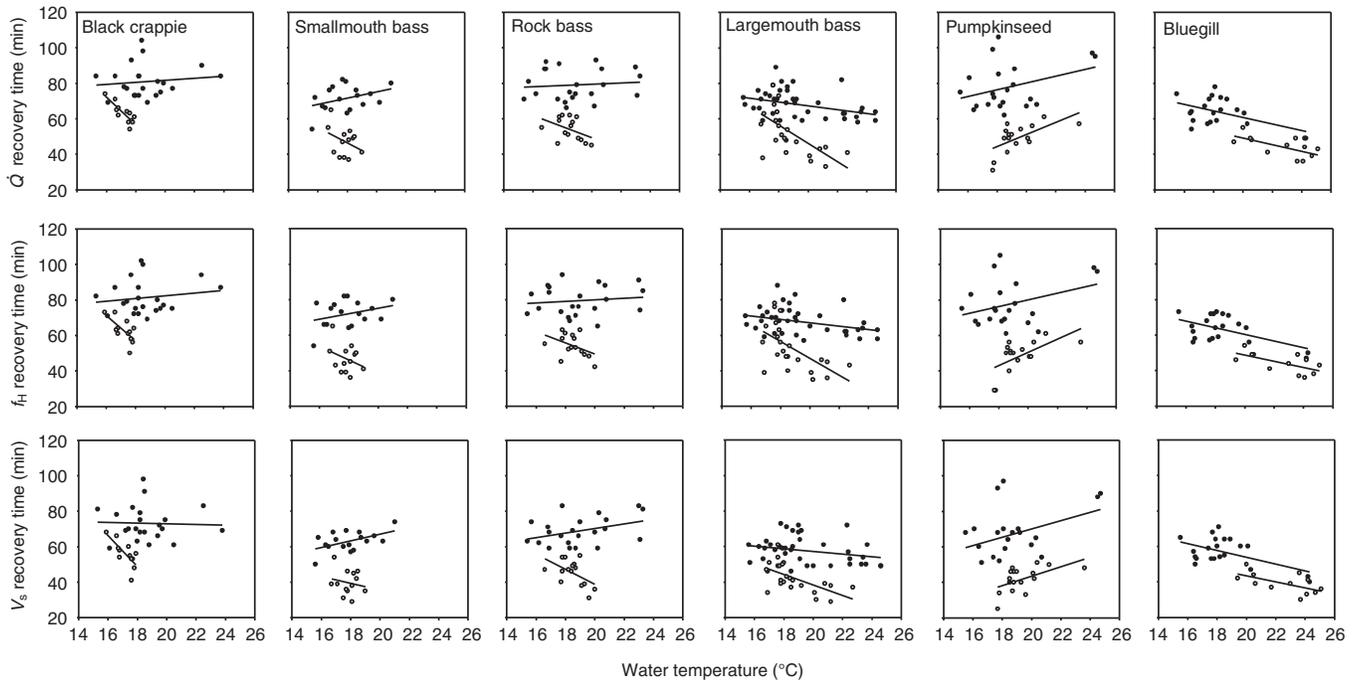


Fig. 4. Scatterplots and regressions for cardiovascular recovery time of nesting (open circles) and non-nesting (filled circles) fish across a range of water temperatures. Details on the equations, correlation coefficients and significance are presented in Table S6 in supplementary material. \dot{Q} , cardiac output; f_H , heart rate; V_S , stroke volume.

and red muscle growth may be to increase the swimming endurance of males during maturation. Although this has not been investigated from a parental care perspective, it is logical to suggest that enhanced aerobic muscle mass would increase exercise capacity and facilitate prolonged heightened care in centrarchid fishes. Parental care in bass involves a combination of aerobic (e.g. fanning, sustained vigilance and patrolling) and anaerobic (e.g. bursting and chasing predators) exercise so although we used an exhaustive exercise protocol, increased aerobic capacity would benefit routine activities and also assist with recovery from oxygen debt. Perhaps the variation in hormones among the centrarchid fishes may play a strong role in ultimately determining the duration of care. Some researchers have documented high corticosteroid levels in fish during reproduction (Barton and Iwama, 1991); however, cortisol levels in smallmouth bass are generally low during this period (Hanson et al., 2009). Although an indicator of stress, for short periods this may be adaptive as it provides energy for the organism (Moyes and West, 1995). Because reproductive hormones appear to influence a variety of behavioral, morphological and physiological traits, this may explain why nesting fish took longer to exhaust than non-nesting fish.

In response to exercise, \dot{Q} increased, despite major reductions in V_S indicating the important role of f_H in regulating \dot{Q} . This is evident not only in actual maximal values but also in the maximal change in % from resting levels. Maximal \dot{Q} and f_H 's increased with increased water temperatures as has been shown in a variety of species including centrarchids (Schreer et al., 2001; Cooke et al., 2003b) whereas maximal V_S generally did not differ with temperature. Few significant differences in maximal \dot{Q} , f_H and V_S were noted among nesting and non-nesting fish, although values for f_H and \dot{Q} were generally slightly higher for nesting fish. The maximal values for f_H and \dot{Q} that we observed were consistent with previous studies on teleost fishes. For example, most fish species'

maximal f_H 's do not exceed $\sim 120 \text{ beats min}^{-1}$ (Farrell, 1991a; Farrell, 1991b) and in our study, this maximal value was rarely exceeded. However, maximal percentage change in V_S values represented decreases of between 30% and 60%. Reductions in V_S in response to exercise can be expected during tachycardia due to reductions in cardiac filling time and therefore end diastolic volume (Farrell, 1991b). Following the peak maximal reduction in V_S , the values slowly returned to resting levels, almost never increasing beyond resting levels during this recovery period. Nesting fish that recovered more quickly achieved the peak maximal changes more rapidly than did the non-nesting fish. The pattern we observed here with decreased V_S relative to resting during the recovery has been previously documented in centrarchid fishes in response to a wide variety of stimuli, including exercise, thermal change and air exposure (Schreer et al., 2001; Cooke et al., 2001; Schreer and Cooke, 2002).

The scope for \dot{Q} and f_H were highly variable, often overlapping for individual species during the nesting and non-nesting stage. In general, scope was either similar among nesting and non-nesting fish or nesting fish had lower scopes. Maintaining or increasing scope during periods of heightened activity such as parental care would seemingly be important and was the basis for our prediction that nesting fish would elevate maximal cardiac performance to maintain scope. Using the framework developed by Priede (Priede, 1977), it is likely that, during parental care, metabolic activity [including cardiovascular performance (Farrell, 2002)] is frequently approaching or surpassing levels that increase risk of mortality. Priede suggests that selection will produce extreme refinement at high power outputs (Priede, 1977). However, Priede also suggests that energy savings should arise largely from reductions in standard metabolic rate (Priede, 1977). This would also serve to maintain or increase scope (Priede, 1985). Our data do not support the notion that the standard metabolic rates of parental-care-providing fish are

depressed during this period of extreme activity. Indeed, the opposite was true. The high resting levels accompanied by only minor increases in maximal values resulted in reductions in scope. Higher activity levels experienced by fish would further compress scope during the parental care period than for non-nesting fish. Selection for savings during these extreme levels of activity (i.e. parental care) may arise not from absolute differences in cardiac values but instead in the ability for fish to recover rapidly (see below).

Consistent with our prediction, recovery following exhaustive exercise was enhanced in nesting fish relative to non-nesting fish when adjusted for temperature. For all cardiac variables, the magnitude of the difference in recovery rates between nesting and non-nesting fish was ~50%. Cooke et al. determined that up to 15% of the time for nesting smallmouth bass and largemouth bass was devoted to burst-type swimming associated with engaging potential predators [speeds >80% of U_{crit} (critical swimming speed)] (Cooke et al., 2002). Additionally, the parental males also exhibit high levels of locomotory activity as they patrol the nest, fan the eggs and maintain vigilance during both day and night. Cooke et al. estimated that overall swimming activity (distance swam) for nesting largemouth bass and smallmouth bass was between 150% and 400% higher than non-nesting fish during the same period (Cooke et al., 2002). As nest-guarding fish are clearly engaged in high levels of activity (both aerobic and anaerobic), adaptations that facilitate recovery are clearly important. Recent research on parental-care-providing three-spined sticklebacks (*Gasterosteus aculeatus*) suggests that the enzymes lactate dehydrogenase and citrate synthase may be involved in ATP generation for sustained activity or in recovery from repeated bursts of activity (Cubillos and Guderley, 2000).

Interesting interspecific differences in cardiac recovery were also noted. For some species recovery did not differ with temperature, for some recovery was enhanced by temperature and for some it was retarded by temperature. The two species that recovered more quickly at warmer temperatures were bluegill and largemouth bass. As noted earlier, these species have the highest thermal preferences (Wisner and Christie, 1987). For many species, the recovery time increased with increasing temperature for non-nesting fish but decreased with increased temperatures for nesting fish. This result is difficult to explain but may represent the interplay of cardiac performance, water temperature and hormones. This may also be adaptive as at warmer temperatures, there are increased predator burdens in the littoral zone; thus, rapid recovery would be advantageous. Several researchers have reported that optimal temperatures for cardiovascular performance and recovery probably exist (Farrell, 1996; Schreer et al., 2001; Farrell, 2002). Indeed, Farrell (Farrell, 2002) reports that the critical swim speed in salmonids has an optimal temperature, and coincident with this is maximum aerobic scope and cardiac scope. These thermal optima for performance may be linked to reproduction (Beitinger and Fitzpatrick, 1979; Schreer et al., 2001; Farrell et al., 2008). Schreer et al.'s (Schreer et al., 2001) study of non-reproductive-phase smallmouth bass determined that cardiac recovery times were more rapid at 16°C than at 12°C or 20°C, providing evidence of an optimal temperature. This temperature coincided with the peak reproductive period for smallmouth bass. In our study, there was some overlap between non-nesting and nesting fish across their reproductive period; however, in general, rapid recovery for fish seemed to be linked more with reproductive status than water temperature.

Because of the repeated bursting activity and sustained aerobic swimming during parental care, it is possible that the patterns

observed among nesting and non-nesting centrarchids are a function of phenotypic plasticity and, in particular, training. The fish training literature is extensive and it is clear that when subjected to sustained exercise training, it is possible to improve cardiac capacity to some extent (Davison, 1997). Aerobic training has been demonstrated to induce cardiac growth and increase maximum cardiac output, cardiac enzyme activity, haematocrit, arterial oxygen content, skeletal muscle capillarity and tissue oxygen extraction (reviewed in Gamperl and Farrell, 2004). The literature regarding sprint training (i.e. anaerobic exercise) and its effect on cardiac performance is less clear (Davison, 1997). Recent work on larval zebra fish (*Danio rerio*) revealed that interval training (including anaerobic components) did not result in changes in \dot{Q} or f_H relative to untrained controls (Pelster et al., 2003). Both Davison (Davison, 1997) and Gamperl and Farrell (Gamperl and Farrell, 2004) note that although there are many cardiovascular changes that arise from sustained exercise training, the effects are often subtle and the cumulative changes may only have a small overall influence on cardiac performance and aerobic capacity. With respect to nesting sunfish, it is unclear whether the duration of exercise during the care period would be sufficient to represent 'training'. Indeed, we captured most nesting fish within 24–48 h of spawning so they would have only participated in 1/3rd to 1/12th of the total parental care period, depending on the species, so it is unlikely that significant physiological changes would be noted during that time frame.

Cardiovascular performance and parental care

As predicted, the degree of frequency modulation was correlated strongly with the overall parental care investment of centrarchid fishes. Previous studies on cardiac function suggest that life history characters that require extended activity such as the pelagic lifestyle of the tuna (Farrell, 1991b) or intense activity such as the ambush feeding mode of the pike (Lucas et al., 1991) usually correlate with an evolutionary trend towards frequency modulation. Frequency modulation is thought to be a more efficient means of facilitating rapid changes in \dot{Q} and thus delivery of oxygen to tissues (Lucas et al., 1991). Because parental care requires heightened and variable activity during an extended period of time, we might expect that fish with extended and intense parental care might also exhibit such cardiac adaptations as we observed. Additionally, we found a moderate positive correlation between time to exhaustion and duration of care. Smallmouth bass and largemouth bass provide lengthier care than the other four species studied and they took significantly longer to exhaust than fish with shorter duration care. However, this relationship may also be a reflection of the fact that those are the largest two species that we studied. We also had predicted that fish with extended care would recover more rapidly than fish with shorter duration care but found little support for that prediction. Collectively, the relationships between cardiovascular performance and parental care metrics indicate that there is a clear nexus between physiology and behavior. It is important to note that numerous other factors, including egg size (Sargent et al., 1987), sexual selection (Clutton-Brock, 1991), competition for mates and resources (Gross and Sargent, 1985), and individual condition (Mackereth et al., 1999; Hanson and Cooke, 2009) may also contribute to variation in parental care activity and parental care strategies.

Conclusions

The data that we present here provide evidence that suggests that the cardiovascular performance and reproductive activity of centrarchid fishes are correlated. Parental care investment was less

energetically expensive for species that exhibited strong cardiac frequency modulation, and species with longer durations of parental care took longer to become exhausted. However, it is not possible to determine the directionality of the correlation. For example, we are unable to resolve whether longer, more vigorous parental care has selected for better cardiovascular performance or if better cardiovascular performance increases the potential for longer, more vigorous parental care. Moreover, there may also simply be sufficient phenotypic flexibility such that cardiac performance can be adjusted in the short-term (days to weeks) as a result of participating in parental care activities, similar to what occurs when fish are 'trained' in laboratory environments. However, given that nesting fish were captured within 24–48 h of spawning it is unlikely that the nesting fish had been exercising for sufficient periods of time to alter the cardiac variables measured here. Because most existing data on cardiovascular performance are based upon laboratory-acclimated fish, seasonal patterns associated with reproduction are difficult to detect and may in fact be removed if acclimation is sufficiently long (Burggren, 1997). Field-acclimated fish were used in this study to provide information that is relevant to the reproductive ecology of centrarchid fishes. Although all fish were held in a laboratory for ~48 h prior to data collection, fish were provided with ambient flow-through lake water and natural photoperiods. We advocate conducting comprehensive studies that yield information on the physiological and energetic bases of parental care and that combine laboratory and field components where possible (e.g. Ricklefs and Wikelski, 2002; Costa and Sinervo, 2004). Given the important role of the endocrine system in both fish behavior and cardiac physiology, future studies should also explore the potential influence of reproductive hormones on cardiac performance.

LIST OF ABBREVIATIONS

ANCOVA	analysis of covariance
ANOVA	analysis of variance
AVM	absolute ventricular mass
f_H	heart rate
PIC	phylogenetically independent contrast
\dot{Q}	cardiac output
rs	Spearman's correlation coefficient
RVM	relative ventricular mass
V_S	stroke volume

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