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Impairment of branchial and coronary blood flow reduces reproductive fitness, but not cardiac performance in paternal smallmouth bass (*Micropterus dolomieu*)

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ABSTRACT

The capacity to extract oxygen from the water, and the ability of the heart to drive tissue oxygen transport, are fundamental determinants of important life-history performance traits in fish. Cardiac performance is in turn dependent on the heart's own oxygen supply, which in some teleost species is partly delivered via a coronary circulation originating directly from the gills that perfuses the heart, and is crucial for cardiac, metabolic and locomotory capacities. It is currently unknown, however, how a compromised branchial blood flow (e.g., by angling-induced hook damage to the gills), constraining oxygen uptake and coronary blood flow, affects the energetically demanding parental care behaviours and reproductive fitness in fish. Here, we tested the hypothesis that blocking ¼ of the branchial blood flow and abolishing coronary blood flow would negatively affect parental care behaviours, cardiac performance (heart rate metrics, via implanted Star-Oddi heart rate loggers) and reproductive fitness of paternal smallmouth bass (*Micropterus dolomieu*). Our findings reveal that branchial/coronary ligation compromised reproductive fitness, as reflected by a lower proportion of broods reaching free-swimming fry and a tendency for a higher nest abandonment rate relative to sham operated control fish. While this was associated with a tendency for a reduced aggression in ligated fish, parental care behaviours were largely unaffected by the ligation. Moreover, the ligation did not impair any of the heart rate performance metrics. Our findings highlight that gill damage may compromise reproductive output of smallmouth bass populations during the spawning season. Yet, the mechanism(s) behind this finding remains elusive.

1. Introduction

Some fish species have adapted to engage in parental care behaviours as it promotes reproductive fitness (Goldberg et al., 2020; Gross and Sargent, 1985). One such species is the smallmouth bass (*Micropterus dolomieu*, Lacépède, 1802), which exhibit care of their offspring from the egg stage to free swimming fry, whereby the parental care is undertaken by the males (a pattern observed in many teleost fish (Blumer, 1979)). The paternal care behaviours comprise tending to the developing offspring by actively fanning the eggs or larvae with fins to keep them oxygenated and free of debris as well as guarding and actively defending the brood from predators (Cooke et al., 2002; Ridgway, 1988). During the parental care period, paternal bass may swim up to 45–60 km per day chasing away predators, while remaining in close proximity (~3 m) to the nest (Cooke et al., 2002). Thus, it may be postulated that the paternal behaviour in smallmouth bass comprises a metabolically challenging endeavour which is undertaken on limited energy stores as the fish may substantially limit feeding during this period (Brownscombe et al., 2017a; Cooke et al., 2002; Hinch and Collins, 1991; Steinhart et al., 2004).

Smallmouth bass are a popular target species for anglers and the exacerbated aggression during parental care increases their susceptibility to angling (Kieffer et al., 1995; Philipp et al., 1997). Even though most bass (90 + %) are released (Quinn and Paukert, 2009), fishing can

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result in various injuries and alterations in behaviour and physiology. While the harvest of fish from a nest results in near certain reproductive failure, a catch and release event also may negatively impact the capacity of males to defend their nests, which may lead to nest abandonment, reduction in brood success and thus a reduction in reproductive fitness (Cooke et al., 2000; Hanson et al., 2007; Kieffer et al., 1995; Philipp et al., 1997; Suski et al., 2003). One potential negative effect of catch and release angling which remains largely unexplored is whether, and to what extent, hook damage to the gills affects the parental care behaviour or the physiological capacity of paternal bass. Such injuries occur in a variety of fish (Arlinghaus et al., 2008; Lennox et al., 2015; Meka, 2004; Muoneke and Childress, 1994; Stålhammar et al., 2014; Thomé-Souza et al., 2014), including black bass species (*Micropterus* sp.) e.g., largemouth bass (*Micropterus salmoides*) (Clarke et al., 2021; Cooke et al., 2003b; Pelzman, 1978). Beyond hook damage, gills of black bass can also be injured by anglers during handling or hook removal by fingers or hook removal devices (e.g., pliers; Cooke et al. in review) or by retention gear such as stringers that secure fish via the operculum (Cooke and Hogle, 2000). Gill damage or ablation of branchial blood flow through one or several gill arches (each representing 1/8 of total gill surface) could impair branchial oxygen uptake and overall circulatory oxygen transport capacity, e.g., as previously reported in rainbow trout with ligated or physically damaged gill arches (Davis, 1971; Duthie and Hughes, 1987). Furthermore, specific damage to the second pair of gill arches would also impair oxygen supply to the heart, because smallmouth bass, like some other teleost fish species e.g., salmonids, have a coronary circulation that originates from the second pair of gill arches (personal observation). Yet, whether damage to one or several gill arches impacts parental care or cardiac performances in fish is to our knowledge unknown. Moreover, the extent to which the smallmouth bass heart relies on blood supply via the coronaries for cardiac performance or whole animal performance traits is currently unexplored.

The capacity of the heart to drive blood flow and thus tissue oxygen delivery is a key determinant of aerobic metabolic energy production, which in turn dictates aerobic metabolic scope and the capacity for sustained or repeated physical activity (Eliason et al., 2013; Farrell et al., 2009). Cardiac output, which is the total blood flow generated by the heart per time unit, is the product of heart rate and stroke volume (see Farrell and Smith, 2017). Both smallmouth bass and largemouth bass are known to regulate cardiac output predominately through "frequency modulations" during conditions which require an increased tissue oxygen supply, e.g., during and when recovering from brief exercise events which smallmouth bass exhibit frequently during their parental care period (Cooke et al., 2003a; Schreer et al., 2001). Therefore, recordings of heart rate is likely a good proxy for metabolic performance and scope in smallmouth bass (Schreer et al., 2001). For example, Prystay et al. (2019) assessed heart rate metrics in nesting bass using surgically implanted heart rate loggers (DST milli HRT, Star-Oddi, Gardabaer, Iceland). While they did not find any significant relationships between heart rate performance metrics and parental care behaviours in paternal smallmouth bass, they did find that heart rate correlated positively with the aggression level of the fish. However, it is currently unknown whether or to what extent compromised branchial oxygen uptake capacity and impaired cardiac oxygen supply affect the relationship between cardiac performance (which is negatively impacted by coronary ligation, as outlined below) and parental care behaviour and fitness in bass (i.e., the metrics assessed by Prystay et al., 2019), or in fish in general, and this is therefore the topic of inquiry in the current research.

Cardiac performance is dependent on the heart's own oxygen supply, which in all fish is predominately delivered via the venous blood that oxygenates the inner spongy myocardium (i.e., luminal supply). However, some teleost species also have a coronary circulation that perfuses the outer compact layer of the heart with fully oxygenated arterial blood directly from the gills (Farrell et al., 2012; Farrell and Smith, 2017; Icardo, 2017). The coronary circulation is predominantly beneficial when fish are exposed to environmental or physical challenges, which raises the myocardial oxygen demand and/or constrain luminal oxygen supply (Ekström et al., 2016; Farrell and Clutterham, 2003; Steffensen and Farrell, 1998). For example, in salmonids in which ~20–60% of the heart is comprised of compact myocardium (Brijs et al., 2016; Ekström et al., 2017; Farrell et al., 2009), coronary blood flow increases during exercise (Gamperl et al., 1995), acute warming (Ekström et al., 2017) and hypoxia (Axelsson and Farrell, 1993; Gamperl et al., 1994; Gamperl et al., 1995). In resting rainbow trout (*Onchorhynchus mykiss*), experimental abolishment of the coronary supply to the heart by coronary artery ligation decreases stroke volume, while routine cardiac output is sustained due to compensatory elevations in heart rate (Ekström et al., 2019; Morgenroth et al., 2021). Moreover, the heart rate scope (Maximum (active) heart rate – resting heart rate), as well as the maximum metabolic rate and aerobic scope, are significantly reduced following coronary ligation as demonstrated in rainbow trout instrumented with Star-Oddi heart rate loggers (Ekström et al., 2018). Coronary ligation also lowers the maximum sustained swimming speed in chinook salmon (*Onchorhynchus tshawytscha*) (Farrell and Steffensen, 1987) and reduces tolerance to acute environmental warming and hypoxia in rainbow trout (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021). Thus, while it is clear that the coronary circulation plays a crucial role for whole animal performance and environmental tolerance in salmonids, no study to date has assessed whether coronary supply to the heart influences traits directly related to reproductive fitness in fish. Moreover, most previous research studying the relevance of the coronary circulation has been conducted in salmonids, and similar investigations in other teleost species with a coronary circulation (e.g., smallmouth bass) remain largely unexplored.

In the current study, we examined the effects of potential gill vasculature damage, e.g., following catch and release angling, on cardiac performance, parental care behaviours and reproductive success in paternal smallmouth bass by experimentally blocking blood flow through the pair of second gill arches, from where the coronary artery in this species originates (i.e., branchial/coronary ligation). We also instrumented a subset of fish with heart rate bio-loggers to record the heart rate in free-swimming fish during their parental care period. We hypothesized that the branchial/coronary ligation would lead to elevations in resting heart rate (indicative of an impaired stroke volume) and lowered heart rate scope, and that the capacity to defend or tend to the brood would be compromised in ligated fish, thus leading to reduced reproductive fitness due to lower brood survival and a higher incidence of nest abandonment.

2. Materials and methods

The experiments described below were conducted between May 13th and June 6th 2017 in the Rideau River watershed, Ontario, Canada. The fish collection was conducted with a Scientific Collection Permit for FMZ 18 (#1079390) issued by the Ontario Ministry of Natural Resources and Forestry. All experimental procedures described below were approved by an Animal Care Protocol (BT-026) issued by the Carleton University animal care committee.

2.1. Study animals, study sites and identification of nest sites

We examined parental male smallmouth bass (*M. dolomieu*, $n = 73$) of varying age and body size in Opinicon (44.5600°N, 76.3278°W), Indian (44.5930°N, 76.3265°W) and Sand (44.5678°N, 76.2636°W) lakes. All three lakes are interconnected via a canal system and thus function in many ways as a single system. Nest sites were located and assessed by snorkelers as previously described by Prystay et al. (2019). Briefly, nest sites occupied by a nesting male were rated based on brood size (or egg score, rating from 1 to 5, see (Algera et al., 2017b; Kubacki, 1992; Philipp et al., 1997; Prystay et al., 2019; Suski et al., 2003; Zuckerman et al., 2014). Egg scores of 1 to 2 indicated few eggs sparsely scattered over nesting sites, where a score of 1 involved the eggs covering less area than a score of 2. An egg score of 3 was an average nest with many eggs scattered throughout the nest with patches that were slightly more densely packed. A score of 4 was a brood with a lot of eggs densely packed and covering a large area of the nest, and an egg score of 5 was when the nest had an exceptional egg density throughout the nest. Only nests with an egg score of 3 or higher were used, as these ratings are known to reduce the likelihood of the male abandoning the nest (Zuckerman et al., 2014). See Table 1 for fish size and egg score metrics.

2.2. Fish capture and surgical procedures

On the first day of the experimental protocol, the fish were captured on their nests by angling using artificial fishing lures. The angler was directed by a snorkeler positioned by the nest who communicated the behaviour of the fish to the angler via hand signals to facilitate angling and ensure a rapid and superficial hookset. The fish were taken onboard a boat using a rubberized capture net, and were immediately placed on a surgery table. The fish were immobilized by electro-anesthesia using fish handling gloves (10 mA, Smith-Root, Inc., Washington, USA) contacting the snout and the caudal peduncle (Reid et al., 2019), and the gills were continuously irrigated with aerated lake water during the subsequent surgical procedure (see below).

A subset of fish (from now on referred to as the “heart rate logger fish”) were instrumented with heart rate and temperature loggers (DST milli HRT, Star-Oddi, Gardabaer, Iceland). The logger was inserted into the abdominal cavity via a ~ 3-5 cm incision between the pelvic and pectoral fins, and was positioned vertically posterior to the pericardial wall. The logger was anchored to the ventral abdominal musculature using a 3–0 suture (PDS II polydioxanone monofilament). The incision was then closed using 4 to 5 square knot sutures (3–0, PDS II polydioxanone monofilament). Another subset of fish (“non-logger fish”) were not implanted with heart rate loggers but were instead treated as described below immediately upon capture.

Fish in both the non-logger and heart rate logger treatment groups were then divided into two new groups. In one group (branchial/coronary ligated), two pieces of braided fishing line (0.35 mm, placed ~1 cm apart) were placed in between the gill rakers and filaments around the entire second gill arch on both sides (See Fig. S1, available online), from which the hypobranchial and coronary artery originate in this species (personal observation). The braided lines were tied tightly to constrict the branchial blood flow through the gill arches and thus the hypobranchial and coronary artery, thus ensuring an abolishment of coronary blood flow to the heart. In a second group (sham operated), the gill arches were touched and manipulated to mimic the ligation procedure, but no braided line was tied around the gill arches in this group. There were no significant differences ($P > 0.05$, *t*-test) in surgery time between the sham operation (Average: 8 min (′) and 24 s (′), ± SD: 2′,18″) and coronary ligation procedure (Average: 8′,6″, ± SD: 1′,54″). The total body length of the fish was recorded to represent fish size (to the nearest mm), and the fish was then released close to the nest. During the time for the surgical procedures, the snorkeler guarded the nest from predators (mostly bluegill (*Lepomis macrochirus*), rock bass (*Ambloplites rupestris*), pumpkinseed (*Lepomis gibbosus*) and smallmouth and largemouth bass, *M. salmoides*)) by temporarily placing a chicken wire cover over the nest. Upon release of the parental male, the snorkeler removed the cover and monitored the return of the fish to the nest, which typically occurred within 1–3 min.

Preliminary experiments in euthanized fish ($n = 4$) showed that the ligation successfully occluded the blood flow at the site of ligation. Immediately following euthanization, fish were injected with saline (0.9% NaCl containing heparine (5000 IU ml^{-1}) into the caudal vessels to prevent blood clotting. A colored silicone casting material (Microfil MV-122, Flowtech, Inc. Carver, MA, USA) was injected into the hypobranchial artery (which stems from the branchial circulation and from which the coronary artery originates) in a retrograde (upstream) direction towards the branchial circulation, which demonstrated that no fluid passed the site of ligation. Additionally, a downstream injection into the hypobranchial artery, i.e., towards the heart, was utilized to ensure the presence of a coronary circulation which perfused the heart in smallmouth bass (and also largemouth bass).

2.3. Behavioural monitoring and assessment

On day 2 of the experimental protocol, snorkelers placed a GoPro camera (HERO 3 or 5, GoPro, Inc.), mounted on a ~ 30 cm tall stand, close to the nest (~1 m) to record the behaviour of the bass for at least 1 h as previously described by Prystay et al. (2019), after which the camera was retrieved. This procedure was repeated on day 3, however, this time, after ~50 min a 4 L transparent glass jar containing a nest predator (i.e., pumpkinseed; *L.gibbosus*, body length: ~130–150 mm, see more details below) was placed on the outer rim of the nest perimeter in view of the camera for 10 min, after which both the camera and jar was retrieved.

The behavioural analyses were conducted using BORIS software (version 6.2.2, (see Friard and Gamba, 2016). The behavioural assessments started on minute 20 after the camera was positioned at the nest, and 20 min of behaviours were recorded on both days 2 and 3. During this time, the amount of time the fish spent on either of three behaviours was assessed; i) nest tending, maintaining the eggs or nest by fanning the eggs or removing debris from the nest area, ii) nest guarding, hovering above the nest (sometimes with an erect dorsal fin) or swimming close to the nest or iii) chasing, actively chasing away predators from the nest. The total time of nest defending was then calculated as the sum of nest guarding and chasing. During the predator exposure on day 3 (i.e., pumpkinseed in a jar), the number of strikes on the jar (i.e., mouth to jar), and the number of threatening displays (i.e., yawns, the fish opening its mouth towards the jar) was recorded. The sum of number of strikes and yawns was determined as an aggression score. The time from the deployment of the jar to the first aggression behaviour was also recorded. The number of naturally occurring predators (conspecifics or other species) were noted during the 20-min behavioural assessments and during the predator exposure.

2.4. Fish recapture, assessment of maximum heart rate and logger removal

On day 4, the two experimental groups with heart rate bio-loggers were recaptured either by conventional angling using artificial lures from a boat with the assistance of a snorkeler, or by underwater angling by the snorkeler. The fish were immediately released into a circular

Table 1
Body length of paternal smallmouth bass (*Micropterus dolomieu*) and the scoring of their nests.

	Opinicon lake		Indian lake		Sand lake	
Average surface temperature at nest sites (°C)	16.1 ± 1.6		13.9 ± 0.5		15.8 ± 1.5	
	Sham operated	Ligated	Sham operated	Ligated	Sham operated	Ligated
Sample size	9	10	11	11		
Body length (mm)	428 ± 11	414 ± 11	461 ± 14	469 ± 14	382 ± 39	409 ± 11
Egg score (i.e., brood size)	3.9 ± 0.7	3.9 ± 0.2	3.6 ± 0.2	3.5 ± 0.2	3.6 ± 0.2	3.7 ± 0.2

Egg score range from 1 (few eggs) to 5 (thousands of eggs). There were no statistically significant differences ($P \leq 0.05$) between treatment groups for either variable.

basin (Diameter: 90 cm, Height: 30 cm) on the boat containing ~115 L lake water. The fish were subsequently subjected to a chase protocol for 3 min to elicit maximum heart rate, during which the fish was encouraged to swim by carefully prodding the caudal peduncle and fin (see Little et al., 2020; Prystay et al., 2019). The fish were then placed in a cooler (66 * 34 * 31 cm, 70 L) containing lake water for one hour of post-chase maximum heart rate recordings, during which the water was partially exchanged several times to replenish water oxygen levels, remove deleterious metabolic by-products and to maintain a stable water temperature. The heart rate logger fish were then subjected to a second surgical intervention, using the same surgical setup and method of electro-anesthesia as described above. The incision in the abdomen was re-opened, the heart rate logger was removed, and the incision was then closed using 4 to 5 square knot sutures (3–0, PDS II polydioxanone monofilament). The ligations were left in place following the experiments. The fish were then released close to their nests and monitored as explained above. Similarly, the nest was protected from predators during the entirety of these procedures as previously described. Seven heart rate logger fish (5 sham and 2 ligated fish) were euthanized after the post-chase recovery by cranial percussion, and the heart ventricle was excised and placed and stored in 70% ethanol for at least 24 h for subsequent determinations of the proportions of ventricular compact and spongy myocardium. The ventricles were cut in two and the two myocardial layers were separated and left to air dry in a covered container until completely dry, and were then weighed as detailed in Farrell et al. (2007).

2.5. Monitoring of egg development

The nests of the two groups without heart rate bio-loggers (i.e., non-logger fish) were subsequently monitored, on several occasions for 3–14 days following the initial detection of the nest (the egg stage is approximately 6 days in smallmouth bass (Cooke et al., 2002)), by snorkelers to assess brood development until the appearance of hatched free-swimming fry, which indicated a successful nest. The presence or non-presence of the male at the nest was also noted.

2.6. Programming of heart rate loggers and heart rate analyses

The heart rate loggers were programmed to begin sampling at midnight of the day of surgical implantation (i.e., the transition between day 1 and 2) and each logger was programmed to record heart rate for 6 s every 2 min at 100 Hz, and an electrocardiogram (ECG) was recorded every 1.5 h which was later used to validate the quality of heart rate recordings. Only data with a quality index (QI) of 0 (QI range: 0–3) were included for the heart rate analyses, as this ensures a high accuracy and reliability of the heart rate recordings using these loggers (see Brijs et al., 2019). Resting heart rate was determined as the 10th percentile (Prystay et al., 2019), and routine heart rate was calculated as the average heart rate from the data acquired during a given data collection period. To assess post-surgical recovery of heart rates (as indicated by declining heart rates), resting and routine heart rates were assessed using the data collected during the first, second and third day of heart rate data recordings, respectively (i.e., days 2, 3 and 4 of the experimental protocol, Fig. S2, available online, see *Statistics* section below). As both resting and routine heart rate was found to be lowest on day 4 in both groups (see Fig. S2), only resting and routine heart rate data for each respective treatment group from day 4 were used in subsequent analyses. Maximum heart rate was determined as the highest observed heart rate following the chase protocol (on day 4) or during the predator exposure (on day 3). Heart rate scope was calculated as: maximum heart rate (post-chase) – resting heart rate (day 4). We also calculated the percentage of heart rate scope utilized by routine heart rate (day 4) during the routine brooding behaviours ($R\%_{\text{Heart rate}}$) as: (Routine heart rate – resting heart rate) / Heart rate scope *100 (see Prystay et al., 2019).

2.7. Statistics

The statistical analyses were performed using SPSS (v. 25, SPSS Inc., Chicago, IL, USA), and statistical significance was accepted at $P \leq 0.05$. Values are presented as means ± S.E.M unless otherwise stated. Normality and homogeneity of variances were determined using Shapiro-Wilk's and Levene's tests, respectively. Data failing these assumptions was transformed for the statistical tests (log10: Aggression score (Non-logger fish) and time to attack (Heart rate logger and non-logger fish), reflect and log10: nest tending/defending day 3, Non-logger fish and heart rate logger fish). Between treatment group (i.e., sham operated and brancial/coronary ligated fish) differences in body length and nest egg score were assessed by independent two-tailed *t*-tests. One-way ANCOVAs were used to test for differences between sham operated and branchial/coronary ligated fish in the percentage of time spent on nest tending and nest defending behaviours. The number of predators present during the behavioural assessments was included as a covariate. Two-way repeated measures ANOVAs was used to assess the effects of time (days 2–4) and treatment (sham operated vs. branchial/coronary ligated) on post-surgical recovery of resting and routine heart rates. If significant interactions (time*treatment) was detected, one-way repeated measures ANOVAs was conducted to assess the effects of time (days 2–4) on resting and routine heart rates within treatment groups. Significant effects of time within treatment groups was further explored by pairwise comparisons between days, and Bonferroni adjustments were applied to correct for multiple comparisons. A one-way ANCOVA was also used to assess for the effects of temperature (covariate, recorded by the heart rate loggers) on resting and maximum heart rate post-chase and during the predator exposure. However, temperature did not impose a significant influence on heart rate over the narrow temperature range assessed here (range: 15.6–17.1 °C, average: 16.2 ± 0.4 °C, Fig. S3, available online). Therefore, between group differences for resting, routine and maximum heart rate post-chase and during the predator exposure, as well as heart rate scope and $R\%_{\text{Heart rate}}$ were instead assessed by independent two-tailed t-tests. Pearson's correlations were used to assess relationships between parental care behaviours, the number of predators approaching the nest during the behavioural assessments, the aggression score and time to first attack, as well as the heart rate variables. A binominal logistic regression was used to assess the likelihood of the presence of free-swimming fry (i.e., the capacity of males to rear their broods to free-swimming fry) or nest abandonment (i.e., presence of males at the nest) between treatment groups. In the model, presence of fry (yes/no) or male abandonment (yes/no) was included as dependent factors, and treatment (sham operated vs. branchial/coronary ligated, categorical variable), fish length (i.e., size), the location/lake (Indian or Opinicon lake), average number of predators (assessed during day 1 and 2 of the behavioural assessments) or the initial brood score as independent factors. Of the five predictive variables, only treatment was significant for presence of fry ($P = 0.05$, see supplementary table 1) and close to significant ($P = 0.058$, see supplementary table 2) for nest abandonment. However, the model was not significant for either variable ($\chi^2_5 = 5.290$; $P = 0.382$ and $\chi^2_5 = 8.966$; $P = 0.110$, respectively). Only when removing all independent variables except for treatment, the model was significant ($\chi^2_1 = 4.302$; $P = 0.038$) for the presence of fry and for male abandonment ($\chi^2_1 = 3.919$; $P = 0.048$). Surprisingly, however, treatment was not significant, yet close to significant, as a predictor for either variable ($P = 0.051$ and $P = 0.070$, respectively; see supplementary tables 3 and 4, respectively). Therefore, to further explore these trends, we used a Chi-square test of two proportions to determine whether the sham operated and ligated fish differed in the capacity to rear their broods to free-swimming fry (Free-swimming fry present: Yes/No), and a Fisher's exact test to assess between groups differences in regards to the number of males abandoning their nests (nest abandoned: Yes/No). We also conducted another binominal logistic regression as described above, but this time only including fish that did not abandon their nest, to assess the likelihood of presence of free-swimming fry in sham operated ($n = 15$) and branchial/coronary ligated ($n = 18$) fish.

3. Results

There were no differences among groups in terms of body length or nest egg score in fish from any of the study lakes (Table 1). The ventricles of the paternal smallmouth bass in the current study ($n = 7$) were comprised of 9.4 ± 0.9% compact myocardium (mean ± SD, ranging from 4.6 to 12.4%).

3.1. Effects of branchial/coronary ligation on reproductive fitness in parental bass

In non-logger fish, ligated males tended ($P = 0.076$) to abandon their nest to a higher extent (8 out of 23, 35%) relative to sham operated fish (2 out of 20, 10%) (Fig. 1A). Moreover, significantly fewer broods reared by ligated males reached the free-swimming fry stage (13 out of 23, 57%, $\chi^2_1 = 4.113$; $P = 0.043$, Fig. 1B) relative to broods reared by sham operated fish (14 out of 20, 85%). In branchial/coronary ligated fish, 2 out of 15 (13.3%) males that did not abandon their nest failed to brood their eggs to free-swimming fry, compared to 1 out of 18 (5.6%) sham operated fish. However, neither of the predictive variables included in the binominal logistic regression contributed significantly to explain the likelihood of the capacity of the males to rear their offspring to free-swimming fry ($\chi^2_5 = 7.017$; $P = 0.219$).

3.2. Effects of branchial/coronary ligation on parental care behaviours in paternal smallmouth bass

In non-logger fish, the percentage of time spent on both nest tending and nest defending were significantly affected by the number of predators approaching the nest (Fig. 2A,B, Table 2). Fish across groups spent the majority of the time on nest tending behaviours, which was inversely related to the number of predators. The remaining percentage of time was spent on nest defending, which was positively correlated to the number of predators. A weak trend for a difference between branchial/coronary ligated and sham operated fish was observed on day 2 ($P = 0.149$, Fig. 2A), however, no such trend was observed on day 3 (Fig. 2B).

There were no statistically significant differences between treatment groups for average time to first attack during the predator interaction (i.e., pumpkinseed in a jar, 27.0 ± 8.8 s vs. 12.8 ± 4.6 s, respectively, Fig. 2C) nor average aggression score (22.9 ± 3.7 vs. 27.2 ± 3.6 aggression behaviours, respectively, Fig. 2D).

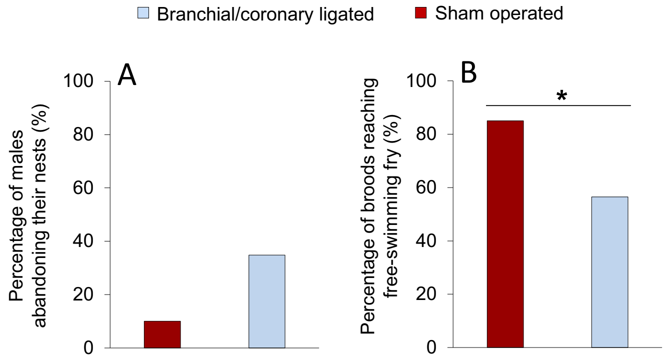

Fig. 1. Effects of branchial and coronary ligation on the percentage of nest abandonment and capacity of paternal smallmouth bass (*Micropterus dolomieu*) to rear their broods to free-swimming fry. Percentage of males abandoning their nests (A) and the percentage of broods reaching the free-swimming fry stage (B) in sham operated (red symbols, $n = 20$) and branchial and coronary ligated (blue symbols, $n = 23$) paternal smallmouth bass. Between groups differences were determined by a Fischers exact test (A) or the test of two proportions (B). Asterisk (*) denote statistically significant differences ($P \leq 0.05$) between the groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Effects of branchial/coronary ligation on parental care behaviours in paternal bass instrumented with heart rate loggers

Fish instrumented with heart rate loggers displayed similar patterns in the percentage of time spent on nest tending and defending behaviours as non-logger fish, i.e., a negative and positive relationship with the number of predators on both days 2 and 3, respectively (Fig. 3A,B, Table 2). Similarly, the linear relationships between behaviours and number of predators was also more pronounced on day 2 than day 3. Again, there were no differences between treatment groups for any of the behaviours.

There was a trend towards a difference between ligated and sham operated fish for the time to first attack (23.3 ± 5.4 s vs. 11.6 ± 2.4 s, respectively, $P = 0.11$, Fig. 3C), however, no differences were observed between groups for aggression score (31.2 ± 4.5 vs. 34.7 ± 5.0 aggression behaviours, respectively, Fig. 3D).

3.4. Effects of branchial/coronary ligation on heart rate performance in parental bass instrumented with heart rate loggers

There were no statistically significant differences between branchial/coronary ligated and sham operated fish for any of the heart rate variables (Fig. 4A-F). Resting heart rate averaged at 24.0 ± 1.4 and 25.8 ± 1.7 beats min^{-1}, and routine heart rate averaged at 30.9 ± 1.5 and 32.3 ± 1.8 beats min^{-1} in ligated and sham operated fish, respectively (Fig. 4A,B). The post-chase average maximum heart rate was 70.5 ± 3.7 and 67.5 ± 2.6 beats min^{-1} in ligated and sham operated fish, and the maximum heart rate during the predator exposure was 57.4 ± 3.0 and 64.7 ± 2.9 beats min^{-1}, respectively (Fig. 4C,D). The heart rate scope was 47.4 ± 3.0 and 41.7 ± 3.1 beats min^{-1} in branchial/coronary ligated and sham operated fish, respectively (Fig. 4E). The percent routine heart rate within the scope for heart rate scope ($R\%_{\text{Heart rate}}$) was 14.8 ± 3.0 and 15.9 ± 1.7% in branchial/coronary ligated and sham operated fish, respectively (Fig. 4F). There were no significant correlations between any of the heart rate variables with fish size nor any of the parental care behaviours.

4. Discussion

In this study, we assessed the effects of branchial ligation of the pair of second gill arches on parental care behaviours, heart rate performance metrics and reproductive fitness in paternal smallmouth bass. In accordance with our hypothesis, occluding ¼ of the branchial blood flow, as well as the blood flow to the heart via the coronary artery, negatively affected reproductive fitness, where fewer broods reared by ligated males reached the free-swimming fry stage. In turn, this was associated with a tendency for a higher degree of nest abandonment in ligated males relative to sham operated fish (see methods section for full disclosure regarding the statistical analytical approach for these variables). There were no differences between groups in terms of the capacity of males that did not abandon their nests, to rear their brood to free-swimming fry. Moreover, the reduced fitness could not be related to any significant impacts of the ligation on the parental care behaviours assessed here, thus contrary to our hypothesis. Worth noting, however, is the trend (i.e., $P = 0.149$, see Table 2) towards a difference between the ligated and sham operated fish that was observed during the first assessment of the behaviours (i.e., day 2 of the experimental protocol) in non-logger fish, which indicated that ligated bass displayed less aggressiveness towards predators and spent more time on nest tending behaviours relative to sham operated fish. It is possible that the tendency for lower aggressiveness in some of the ligated fish may have led to

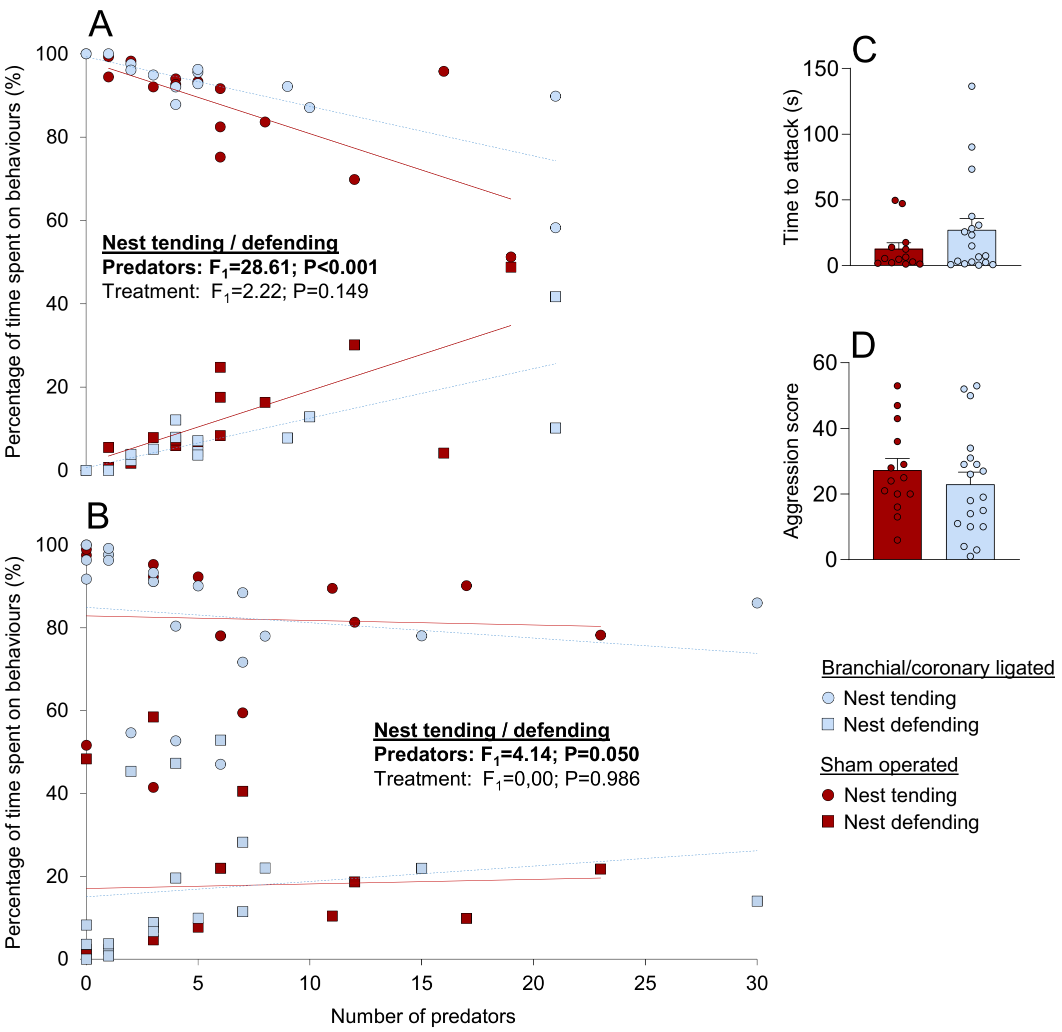

Fig. 2. Effects of branchial/coronary ligation on parental care behaviours in paternal smallmouth bass (*Micropterus dolomieu*). Nest tending and defending behaviours during a 20 min time window (A, on day 2; B, on day 3), as well as the time to first attack (C) and the aggression score (D) during a 5 min exposure to a nest predator (Pumkinseed, *Lepomis gibbosus*, on day 3), in sham operated (red symbols, solid lines, $n_{day\ 2} = 14$, $n_{day\ 3} = 16$) and branchial/coronary ligated (blue symbols, dashed lines, $n_{day\ 2} = 15$, $n_{day\ 3} = 18$) paternal smallmouth bass. Statistical details from one-way ANCOVAs (covariate: number of predators) are displayed in panels A and B, and significant results ($P \leq 0.05$) are highlighted in bold text. There were no statistically significant differences between treatment groups in regards to the variables in panels C and D, as assessed by independent two tailed *t*-tests. The linear equations for the depicted relationships are detailed in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increased brood predation during day 2, which subsequently reduced the brood size (i.e., egg score). Indeed, parental care investment decisions are known to be affected by the changes in brood size in teleost fish exhibiting parental care of their young (Goldberg et al., 2020; Hanson et al., 2007; Zuckerman et al., 2014). For example, experimental reductions of brood size led to reduced brood defence in smallmouth bass (Ridgway, 1989), and increased the likelihood of nest abandonment in smallmouth and largemouth bass (Hanson et al., 2007; Steinhart et al., 2008; Zuckerman et al., 2014). While there were no clear links between the initial brood size (i.e., on day 1) and nest abandonment for the ligated and sham operated fish that did abandon their nests (egg scores of 4.1 ± 0.8 and 3.4 ± 0.5 (average ± SD), respectively), it is also possible that changes in brood size occurred at some other time point during or after the current experimental protocol, which may have triggered some of the males to abandon their nests.

The causal mechanisms behind the tendency for reduced aggressiveness on day 2 (a finding which should be interpreted with caution as it is not statistically significant) remains unknown, but may reflect that the branchial/coronary ligation reduced branchial oxygen uptake and thus aerobic capacity, which may have compromised the capacity to chase away predators. Indeed, occluding blood flow (via ligation) through one or several gill arches reduced the oxygen tension of the arterial blood in rainbow trout (Davis, 1971). Moreover, Duthie and Hughes (1987) found that while gill damage to the first and/or second gill arch did not significantly affect standard oxygen consumption rate in rainbow trout during resting conditions, it did impair maximum oxygen consumption rate as well the critical swim speed (U_{crit}), indicating that gill damage may be detrimental during periods of high aerobic demand. The tendency for a reduced aggressiveness in ligated fish on day 2 may also relate to other factors not assessed here, e.g., a heightened stress response following the ligation (i.e., by the ligation procedure per se or by the physiological effects of the ligation). For example, Algera et al. (2017a) showed that experimental elevations in the stress hormone cortisol (via intracoelomic implants) reduced the swimming activity (burst swimming, e.g., when chasing away predators) of paternal smallmouth bass, and suggested that this reduction in parental care investment could have negative fitness implications for this species. Indeed, experimental elevations in cortisol levels have also been shown to increase the likelihood of nest abandonment in largemouth bass (O'Connor et al., 2009) and to reduce the nest success in paternal

Table 2

Linear equations from the relationships between parental care behaviours and number of brood predators (Figs. 1 and 2) in branchial/coronary ligated and sham operated paternal smallmouth bass (*Micropterus dolomieu*).

Behaviour	Day	Sham operated	Branchial/coronary ligated
		Non logger fish (Fig. 2)	
Nest tending	2	$y = -1.7424\times + 98.291; R^2 = 0.506$	$y = -1.1889\times + 99.333; R^2 = 0.602$
	3	$y = -0.1097\times + 82.907; R^2 = 0.002$	$y = -0.3713\times + 84.941; R^2 = 0.026$
Nest defending	2	$y = 1.7424\times + 1.7085; R^2 = 0.506$	$y = 1.1889\times + 0.6669; R^2 = 0.602$
	3	$y = 0.1097\times + 17.093; R^2 = 0.002$	$y = 0.3713\times + 15.059; R^2 = 0.026$
		Heart rate logger fish (Fig. 3)	
Nest tending	2	$y = -0.7525\times + 96.67; R^2 = 0.833$	$y = -0.6232\times + 95.248; R^2 = 0.871$
	3	$y = -0.4766\times + 94.37; R^2 = 0.437$	$y = -0.2767\times + 87.296; R^2 = 0.123$
Nest defending	2	$y = 0.7525\times + 3.3299; R^2 = 0.833$	$y = 0.6232\times + 4.7516; R^2 = 0.871$
	3	$y = 0.4766\times + 5.6302; R^2 = 0.437$	$y = 0.2767\times + 12.704; R^2 = 0.123$

smallmouth bass (Algera et al., 2017b; Dey et al., 2010). Thus, it is possible that the tendency for a reduced aggressiveness observed in ligated fish relates to elevated cortisol levels on day 2, and/or that this may have contributed to the reduced nest success in this group. However, the tendency for reduced aggressiveness was absent on day 3, and there were no differences between the treatment groups in non-logger fish in terms of the interaction with the introduced nest predator in a jar. Thus, any potential effects of the ligation on inducing a stress response, or in relation to compromising branchial oxygen uptake or circulatory oxygen transport capacity, had apparently subsided by day 3. In the latter case, this could reflect that some measure had been elicited to compensate for a declining oxygen uptake and/or transport, e.g., by increasing oxygen transport capacity of the blood by increasing the amount of circulatory red blood cells (e.g., via erythropoesis or splenic release of red blood cells) and thus hemoglobin content which can occur in fish, e.g., during/following stress (see review by Harter and Brauner, 2017).

There were no differences between groups regarding the parental behaviours in fish instrumented with heart rate loggers on either day of the behavioural assessments. This contrasts with the tendency for a reduced aggressiveness on non-logger fish on day 2, the reasons for which are unknown. There was, however, a trend towards a slower reaction time (i.e., time to attack) in ligated fish when confronted with a nest predator in a jar. While this may be interpreted as reduced aggression in ligated relative to sham operated fish, this was not reflected in the subsequent behaviour during the predator interaction. For example, aggression score did not differ between groups. Whether the lowered reaction time reflects compromised oxygen uptake or transport capacity remains unknown. Nonetheless, that is unlikely, as we did not detect any significant correlations between the heart rate performance metrics and predator interaction variables. Again, it is possible that the slower reaction time relates to an elevated stress response in ligated fish. However, Algera et al. (2017b) and Zolderdo et al. (2016) evaluated the effects of elevated cortisol levels on these behavioural parameters in paternal smallmouth bass by a similar methodological approach as in the current study, but concluded that elevated cortisol did not significantly impact the aggression score during the predator interaction. These authors did not, however, assess the time to first attack.

We did not observe any correlations between heart rate performance metrics and the assessed parental care behaviours in the paternal bass in either experimental treatment group. This is largely consistent with similar findings of Prystay et al. (2019), except for one contrasting observation in that Prystay and colleagues did find that the aggression score positively correlated with $R\%_{\text{Heart rate}}$, which we did not observe here in either group. The cause for this discrepancy between our studies remain unknown, but may relate to the slight differences in methodological approaches between studies, e.g., the sham/ligation treatment per se may have affected the aggression score of the fish in the current study.

Both the routine heart rate and the maximum heart rate recorded in the sham operated group related well to the previous assessment of these metrics in paternal smallmouth bass by Prystay et al. (2019), whom also used Star-Oddi heart loggers and an almost identical experimental protocol except for the sham/ligation intervention. It is worth mentioning, however, that our values for the heart rate metrics could have been even lower than reported here, considering that post-surgical recovery from the implantation with bio-loggers may take substantially longer (up to several weeks) than could be allowed for in the current experiment due to the limited time-frame of the brooding period (Hjelmstedt et al., 2020; Zrini and Gamperl, 2021). Contrary to our hypotheses, there were no effects of the branchial/coronary ligation on resting or routine heart rate, maximum heart rate nor heart rate scope in this species. This contrasts with previous observations of a considerable elevation in resting or routine heart rate in rainbow trout in which the coronary supply to heart had been abolished (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021), which led to a reduction in heart rate scope as maximum post-chase heart rate remains unaltered relative to sham operated trout (Ekström et al., 2018). Moreover, the elevated routine/resting heart rate likely aided to sustain cardiac output during routine/resting conditions as coronary ligation negatively impacts the maintenance of cardiac stroke volume in rainbow trout (Ekström et al., 2019; Morgenroth et al., 2021). The lack of an effect of the ligation on the heart rate metrics assessed here may not be surprising considering the fact that the compact myocardium only comprised ~10% of total cardiac mass, and thus, abolishing the blood supply via the coronaries only impacted a minor proportion of cardiac tissues in this species. Thus, the coronary ligation may only have had minor effects on stroke volume and the ability to maintain cardiac output in bass. While no apparent additional routes of a cranial (i.e., stemming from the gills) coronary supply could be observed during the assessments of the vascular anatomy conducted here (i.e., via injections of Microfil casting material into the hypobranchial artery), it is possible, yet unknown, that the compact myocardium receives oxygen from an alternative (e.g., caudal) route in smallmouth bass.

4.1. Conclusions and future perspectives

Based on our findings, we conclude that an abolishment of branchial and thus coronary blood flow (e.g., as may occur via gill damage following catch release fishing), negatively impacts reproductive fitness in smallmouth bass. Factors such as hook type and angler experience influence the likelihood of gill injuries (reviewed in Bartholomew and Bohnsack (2005) and Brownscombe et al. (2017b)) so we suggest that efforts to reduce gill injuries during angling will reduce sub-lethal consequences for released fish. While the causal factors behind our observations remains largely elusive, our findings did reveal tendencies towards reduced aggressiveness in ligated fish, which may have affected the brood status and/or parental care decisions to increase the likelihood of nest abandonment and failed broods. However, the reduced fitness was unrelated to an impairment of heart rate performance metrics in paternal bass. Indeed, abolishing the coronary blood flow to the heart did not significantly impact the heart rate metrics assessed here, which likely relates to a low dependence on the coronary circulation to maintain heart rate performances in this species during the conditions examined here. This begs the question how the coronary circulation benefits physiological performance traits in bass, and why it has been retained over evolutionary time in this species? It is often argued that the coronaries are relevant in very active, “athletic” teleost fish species able to sustain high levels of cardiac performance and which face periods of strenuous persistent aerobic exercise during their life history

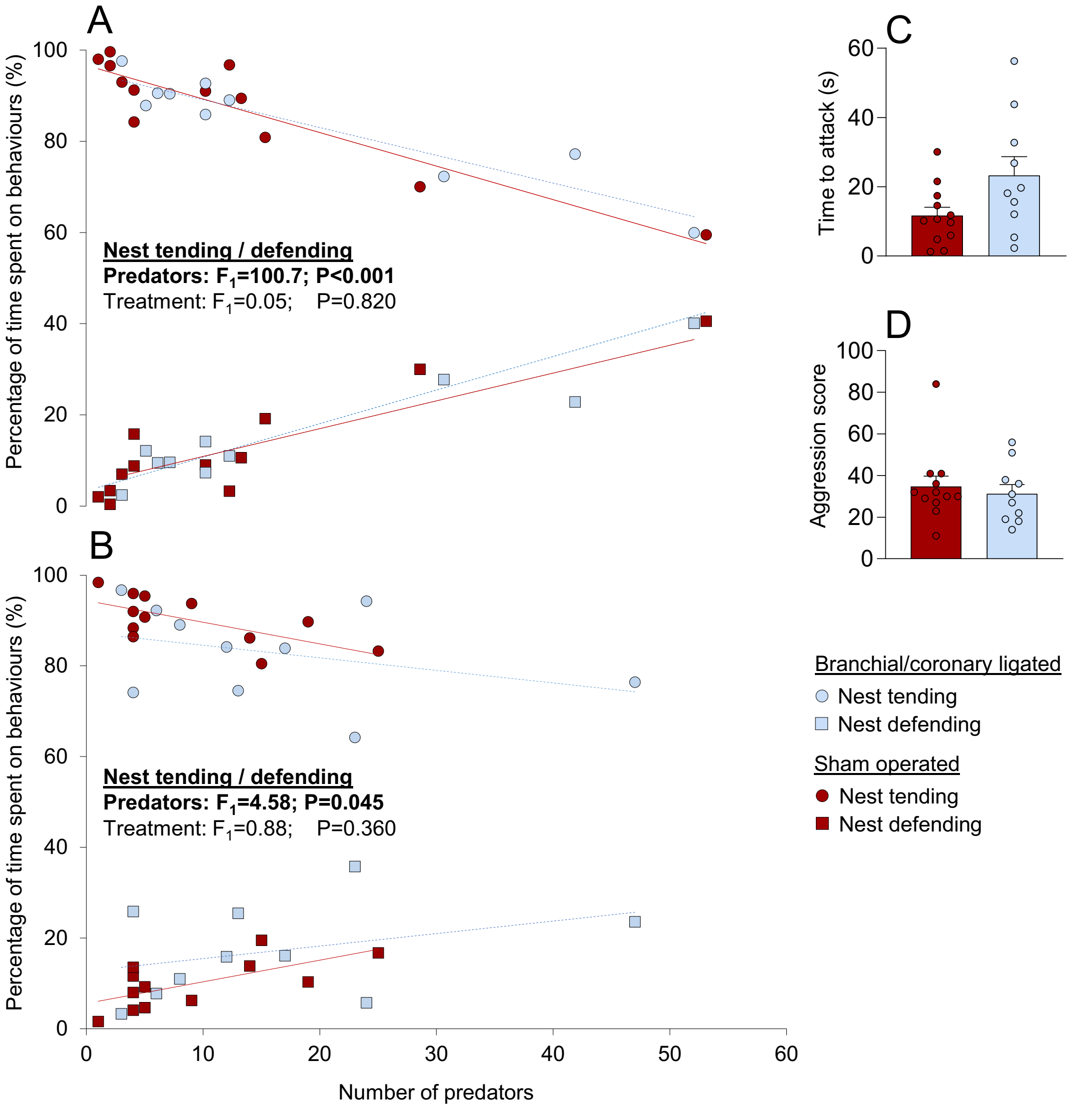

Fig. 3. Effects of branchial/coronary ligation on parental care behaviours in paternal smallmouth bass (*Micropterus dolomieu*) instrumented with heart rate loggers. Nest tending and defending behaviours during a 20 min time window (A, on day 2; B, on day 3), as well as the time to first attack (C) and the aggression score (D) during a 5 min exposure to a nest predator (Pumkinseed, *Lepomis gibbosus*, on day 3), in sham operated (red symbols, solid lines, $n_{day\ 2} = 12$; $n_{day\ 3} = 12$) and branchial/coronary ligated (blue symbols, dashed lines, $n_{day\ 2} = 10$, $n_{day\ 3} = 10$) paternal smallmouth bass instrumented with heart rate loggers (Star-Oddi). Statistical details from one-way ANCOVAs (covariate: number of predators) are displayed in panels A and B, and significant results ($P \leq 0.05$) are highlighted in bold text. There were no statistically significant differences between treatment groups in regards to the variables in panels C and D, as assessed by independent two tailed t-tests. The linear equations for the depicted relationships are detailed in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Farrell et al., 2012; Farrell and Smith, 2017). Smallmouth bass may not be considered as a particularly athletic fish species, at least according to this definition. It is therefore possible that the coronary circulation becomes more influential for governing cardiac and whole animal performance during exposure to environmental stressors, especially during their already energetically demanding spawning period. For example, acute warming and hypoxia elevates cardiac workload and/or reduces venous (luminal) oxygen supply to the heart (Clark et al., 2008; Ekström et al., 2016; Steffensen and Farrell, 1998), and constitutes circumstances believed to be important drivers for the evolution of coronary circulations in fish (Farrell et al., 2012). Another possibility, that currently remains unexplored (but see Roberts et al., 2020), is that chronic exposure to environmental warming and/or hypoxia leads to an increased reliance on the coronaries in fish, and so bass cardiac function may be more dependent on coronary oxygen supply during more challenging environmental conditions. Finally, as the proportion of compact myocardium relative to heart size is positively correlated with body size in various fish species (Farrell et al., 2012), as well as within species e.g., rainbow trout (Brijs et al., 2016; Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021), the coronary circulation may also become more important for sustaining physiological performance as bass grow larger. These possibilities deserve further scrutiny in the future.

Author contributions

S.J.C., T.S.P., and A.E. designed the study and A.E., T.S.P., A.E.I.A., A.C.B., P.E.H., A.J.Z., S.J.C. and E.M. conducted the experiments. S.J.C: and E.S. financed the study. A.E. performed data and statistical analyses and drafted the manuscript. All authors provided feedback and approved the final version of the manuscript.

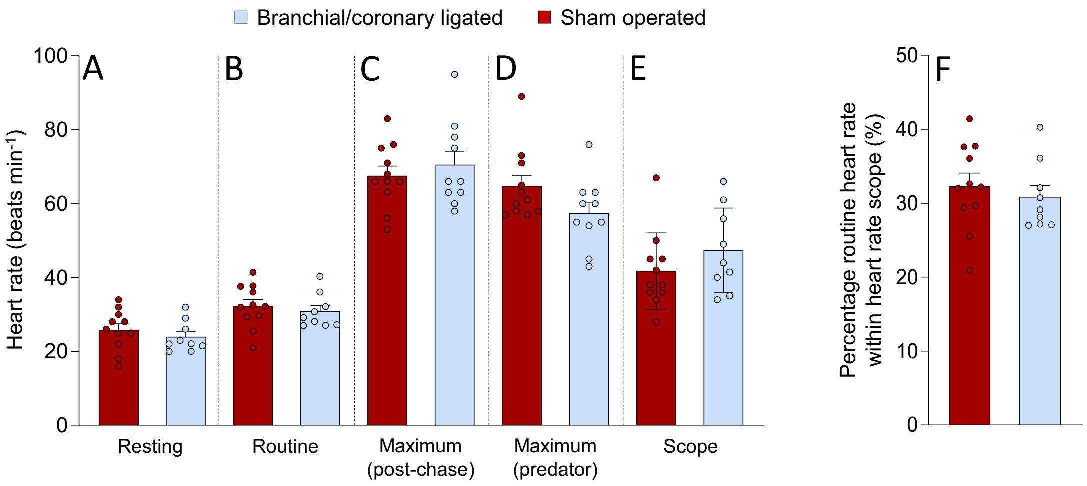

Fig. 4. Effects of branchial/coronary ligation on heart rate responses in paternal smallmouth bass (*Micropterus dolomieu*). Resting heart rate (A), routine heart rate (B), post-chase maximum (C) heart rate, the maximum heart rate during a 5 min exposure to a nest predator (Pumkinseed, *Lepomis gibbosus*) in a glass jar (D), heart rate scope (E, post-chase maximum heart rate – resting heart rate), as well as the percentage of routine heart rate within the scope for heart rate (F) in sham operated (red symbols, $n = 11$) and branchial and coronary ligated (blue symbols, $n = 10$) paternal smallmouth bass instrumented with heart rate and temperature loggers. There were no statistically significant differences ($P \leq 0.05$) between treatment groups in regards to any of the variables, as assessed by independent two tailed t-tests. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2022.111165.

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