Exploring relationships between oxygen consumption and biologger-derived estimates of heart rate in two warmwater piscivores

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ABSTRACT

Estimating metabolic rate in wild, free-swimming fish is inherently challenging. Here, we explored using surgically implanted heart rate biologgers to estimate metabolic rate in two warmwater piscivores, bowfin *Amia calva* (Linneaus 1766) and largemouth bass *Micropterus salmoides* (Lacepède 1802). Fish were surgically implanted with heart rate loggers, allowed to recover for 24 h, exposed to a netting and air exposure challenge, and then placed into respirometry chambers so that oxygen consumption rate ($\dot{M}_{O2}$) could be measured in parallel to heart rate (f_H) for a minimum of 20 h (*ca*. 20 estimates of $\dot{M}_{O2}$). Heart rate across the duration of the experiment (all at 19°C) was significantly higher in largemouth bass (mean ± s.d., 45 ± 14 beats min^{-1}; range 18 - 86) than in bowfin (27 ± 9 bpm; range 16 – 98). Standard metabolic rate was also higher in largemouth bass (1.06 ± 0.19 mg O_2 kg^{-1} min^{-1}; range 0.46 – 1.36) than in bowfin (0.89 ± 0.17 mg O_2 kg^{-1} min^{-1}; range 0.61 – 1.28). There were weak relationships between f_H and $\dot{M}_{O2}$, with heart rate predicting ~28% of the variation in oxygen consumption in bowfin and 23% in largemouth bass. The shape of the relationship differed somewhat between the two species, which is perhaps unsurprising given their profound differences in physiology and life history, illustrating the need to carry out species-specific validations. Both species showed some potential for a role of f_H in efforts to estimate field metabolic rates, although further validation experiments with a wider range of conditions (e.g., digestive states, swimming activity) would likely help improve the strength of the $\dot{M}_{O2} - f_H$ relationship for use in field applications.

Keywords: electrocardiogram, ECG, bioenergetics, biologger, cardiac output

Introduction

A large amount of research in biology is focused on examining how variation in metabolic rate shapes and responds to ecological and evolutionary processes (Tomlinson et al., 2014; Ricklefs & Wikelski, 2002; Réale et al., 2010). In fishes, bioenergetics modeling provides a holistic, first-principles framework for quantifying how fish respond to environmental dynamics (Jobling 1994; Deslauriers et al., 2017). Metabolic rate, a crucial parameter in the field of ecological energetics and in bioenergetics modeling (Winberg 1960), is typically estimated via the rate of oxygen consumption ($\dot{M}_{O2}$) by confining fish in respirometry chambers in the laboratory (Nelson 2016). However, our ability to understand metabolism in wild fish and to develop ecologically accurate bioenergetic models could be improved by being able to quantify natural variation in metabolic rate of fish *in situ* (Cooke et al. 2016).

There has long been an interest among fish biologists in using electronic sensor tags to remotely estimate metabolic rate in free swimming fish. Heart rate (f_H), which can be estimated via electrocardiogram (ECG) data, has the potential to act as a proxy for metabolic rate, because the heart is directly involved in the uptake and transport of oxygen (Butler et al., 2004). Heart rate tags have been the subject of experimentation with fish for decades, having been usually custom-made (e.g., Priede, 1974; Priede & Tytler, 1977; Armstrong, 1986; Kneis & Siegmund, 1976) and difficult to work with, often being restricted to a laboratory setting or including external battery pack (e.g., Cooke et al. 2004). In recent years, researchers have begun using commercially available heart rate loggers developed by Star Oddi (https://www.star-oddi.com/; e.g., Prystay et al. 2017, Brijs et al. 2019) which are much smaller than older models and measure electrocardiograms (ECGs) through electrodes on the side of the logger and calculate f_H using pre-programmed algorithms. These loggers can be surgically implanted in wild fish and

make intermittent recordings for weeks or months, overcoming limitations associated with laboratory experiments (see Cooke et al. 2016), although recapture of the study animals is required for logger download; a logistical challenge for many wild fishes. As well, f_H needs to be validated as a proxy for metabolic rate before use and validations likely need to be done on a genus- or species-specific basis (Butler et al., 2004). Even in the 'best-case scenario', f_H is only likely to provide a relative approximation of $\dot{M}_{O2}$ because $\dot{M}_{O2}$ is modulated by physiological functions other than f_H, such as the stroke volume of the heart and the rate of oxygen diffusion at the gills (e.g., changes in ventilation rate; Farrell 1991). However, in free-swimming fish, f_H currently represents one of our only options for attempting to indirectly estimate variation in $\dot{M}_{O2}$ because both $\dot{M}_{O2}$ and f_H typically respond similarly to swimming activity (Webber et al. 1998), recovery from exercise (Prystay et al. 2017), feeding and digestion (Armstrong 1986), or variation associated with temperature (Priede 1983).

This study assessed the use of f_H as a proxy for metabolic rate in two freshwater fish, bowfin *Amia calva* and largemouth bass *Micropterus salmoides*. These species were selected due to their broad distribution in North American freshwaters and the fact that both grow to a size appropriate for use of electronic tags of the size we used here (13 mm diameter × 39.5 mm long). Cardiac physiology has been examined in these species a handful of times (bowfin – Porteus et al., 2014a, 2014b; largemouth bass – Cooke et al., 2004, 2002). Given the heart's direct role in modulating oxygen transport and uptake (Satchell 1991), we hypothesized that there would be a positive relationship between f_H and $\dot{M}_{O2}$ in both bowfin and largemouth bass. Because of the profound differences between bowfin and largemouth bass, we predicted that the heart rate-metabolic rate relationship would differ between these species. The largemouth bass is a warmwater piscivore with common teleost features and anatomy. Bowfin is the one of few extant

members of the infraclass Holostei; they differ from largemouth bass in several obvious ways, the most notable of which is that they use a gas bladder to gulp and breathe air, an adaptation that makes them well suited to hypoxic waters (Porteus et al. 2014). To facilitate the validation of the relationship between $\dot{M}_{O2}$ and f_H, we exposed every fish to a confinement + air exposure stressor immediately before entry into the respirometers. That step was designed to cause some level of exhaustion and physiological recovery to elicit a broader range of f_H and $\dot{M}_{O2}$ than would be expected in fish simply resting in respirometers. Given that relationships between f_H and $\dot{M}_{O2}$ are not always entirely clear (Thorarensen et al. 1996), laboratory experiments are an important first step in evaluating the utility of f_H loggers for studying ecological energetics of wild fish (Muller et al. 2020, Zrini & Gamperl 2021).

Methods

Fish Collection

Twenty-one (21) bowfin (mean mass = 1561 g, range = 467-2404 g; total length, TL, mean = 568 mm, 395-666 mm) and 30 largemouth bass (661 g mean, 358-1288 g range; mean TL 351 mm, 292-444 mm range) were collected by boat electrofishing from the Detroit River in the waters around Fighting Island in LaSalle, Ontario (42°13'14.1"N 83°06'53.2"W, 42°11'26.6"N 83°06'55.4"W) between May 15 and June 11, 2019. Once caught, fish were held in an onboard aerated tank transported to the nearby (total time of 15-180 min onboard) Freshwater Restoration Ecology Centre in LaSalle ON (42°14'10.4"N 83°06'17.9"W). Upon arrival, fish were given either an anchor tag or a PIT (passive integrative transponder) tag for identification and transferred into indoor 850L holding tanks set to either 14°C or 19°C depending on tank

availability as there were multiple experiments running parallel within the same facility that required the tank systems to be set to these two temperatures. All respirometry and f_H data were collected at 19°C, which was closer to the median river temperatures during the period when the experiment was taking place. Therefore, some fish were acutely acclimated from 14°C to 19°C after implantation of f_H loggers, meaning these fish were not 'fully acclimated' to the experimental temperature. However, both species live in shallow thermally dynamic environments and in the wild can freely move through thermally heterogeneous environments, so we considered these thermal changes to be within the scope of what is ecologically realistic. Tanks were on a recirculation system with UV sterilization, aeration, and filtration of dechlorinated municipal water, with temperature regulated by thermostat-controlled chillers. Fish were held in a seven-day quarantine, as per institutional animal care requirements (for biosecurity purposes), prior to use in experiments. No fish died, and all animals appeared to be in good condition following the quarantine period. All procedures for this study were approved by the University of Windsor Animal Care Committee following guidance set by the Canadian Council on Animal Care (Animal Utilization Project Proposal #19-08).

Heart rate logger implantation

Following the quarantine period, fish had Star-Oddi DST-Milli-HRT loggers (11.8 g in air, 13mm diameter × 39.5 mm length, Star-Oddi, Gardabaer, Iceland; https://www.star-oddi.com/) surgically implanted into the body cavity. Loggers were programmed using the software Mercury (Star-Oddi, Gardabaer, Iceland; https://www.star-oddi.com/) to turn on and record ECG for 7.5 seconds at 80 Hz every 10 min, from which f_H was calculated (beats min^{-1}) and given a quality index value using a pre-programmed algorithm. The loggers were programmed to save a

raw trace of the ECG data every six hours, which could be used to manually confirm the accuracy of the onboard f_H algorithm. In addition, the loggers recorded visceral temperature at each measurement period (data not presented here; all fish were held at a common temperature after logger implantation). After programming, loggers were fitted with a size two suture, and sterilized along with all tools by immersion in betadine solution prior to surgery. A 20 L anaesthetic bath was prepared with a concentration of 125 mg L^{-1} tricaine methanesulfonate (MS-222) buffered by 250 g L^{-1} sodium bicarbonate. Individual fish were placed in the bath and left until they lost equilibrium (typically 4-5 min) and then measured (length to nearest mm, weight to the nearest 0.5 g). Fish were continuously supplied with aerated water with a maintenance dose of 80 mg L^{-1} MS-22 (160 mg L^{-1} sodium bicarbonate) during surgery. Based on the differing position of the pelvic and pectoral fins (and associated cartilage) relative to the pericardium, an incision of two centimetres was made either between the pelvic fins just off the ventral midline (bowfin) or between the pelvic and pectoral fins on the left side (largemouth bass) of the fish. Loggers were inserted and oriented within the body cavity such that both electrodes were facing the pericardial cavity. Loggers were secured in place with an anchor suture and the incision closed with two sutures; all sutures were done using a 2-3-2 knot. Fish were placed in an 80 L aerated recovery tank until they had fully regained their righting reflex (*ca*. 5-10 minutes) and then transferred to a 19°C 850 L holding tank for 24 hours of recovery from surgery. Depending on the timing of the preceding trials, the respirometers were, in a few cases, available at the time fish came out of surgery (previous fish had been removed from respirometers) – in those cases, fish were transferred into respirometers immediately after surgery to begin their recovery (see below) to increase the amount of $\dot{M}_{O2}$ data we could compare

against f_H.

Respirometry

After the 24-hour recovery period, fish were exposed to a handling stressor to elicit an elevated metabolic rate (or AMR, active metabolic rate) to facilitate a broader range of both f_H and $\dot{M}_{O2}$ values for the purpose of validation. The handling stressor involved fish being netted using a knotless nylon dipnet and then held at the water-air interface for two minutes (at least one set of gills always submerged) and then completely air exposed for one minute. At the end of the air exposure, fish were sealed into respirometry chambers that were either 16 L (internal dimensions 42 cm long × 20.2 cm wide × 19 cm deep; 26 largemouth bass, two bowfin) or 74.2 L (internal dimensions 80.5 cm × 30.5 cm × 30.5 cm; five largemouth bass, 20 bowfin), with the size of the chamber used for a particular fish being driven by the size of the animal. Typically, 4 or 5 respirometers were running in parallel, and fish were all exposed to the stressor and transferred to respirometers within a five-minute period. Once all fish were in respirometers, the respirometer flush pumps were manually switched off for an initial sealed measurement period to estimate AMR. Flush pumps were then switched back on when DO reached ~7 mg/L (~80% air saturation) and thereafter controlled with timers set for 30-minute flush and 15-minute measurement (sealed) periods (74.2 L chambers), or with a 20-minute flush and 10-minute seal cycle (16 L). Respirometry trials then continued for a minimum of 20 hours, and up to 45 hours in some cases because of logistical constraints (availability of the research team). Mixing was maintained in each chamber through a recirculation line (Clark et al., 2013) which contained an optical oxygen probe connected to a Firesting system (PyroScience, Aachen, Germany) for DO measurements (at 0.5 Hz; e.g., as in Raby et al., 2020; Sundin et al., 2019). Each chamber was

also outfitted with a standpipe for overflow of which two had temperature probes placed in them (Clark et al., 2013). Background (microbial) measurements were made in each chamber before and after each trial (Sundin et al., 2019). In data analyses, background $\dot{M}_{O2}$ was always subtracted from total respiration to calculate fish $\dot{M}_{O2}$ and always remained below 11.5% of fish $\dot{M}_{O2}$ (median = 0.89%).

Data analysis and statistics

After respirometry trials, fish were euthanized using a 15 mL L^{-1} clove oil bath, loggers were retrieved, and their data downloaded. Loggers were re-programmed, sterilized and used in subsequent trails. The mean slope from each respirometry measurement period was calculated using LabChart Reader (ADInstruments, Sydney, Australia), leaving out the first and last minute of the slope to avoid any lag at the beginning of the cycle and any influence from the pumps turning back on at the end. This data was used to determine $\dot{M}_{O2}$ using the equation

$$\dot{M}_{O2} = V_{RE} \times M_W^{-1} \times \delta CO_2 \delta t^{-1}$$

in which V_{RE} is the effective chamber volume (in L), M_W is the mass of the fish (in kg), and $\delta CO_2 \delta t^{-1}$ is the linear slope from oxygen decrease during sealed cycles (mg O_2 kg^{-1} min^{-1}) (Clark et al., 2013). SMR was calculated as the 15^{th} percentile of all data points for individuals (Chabot et al. 2016), RMR (routine metabolic rate) was the 50^{th} percentile, AMR was the 100^{th} percentile ; each of these variables used $\dot{M}_{O2}$ data and is presented in mg O_2 kg^{-1} min^{-1}.

Heart rate data were automatically assigned a quality index ranking (0 – best, 3 – poorest) based on how 'clean' the ECG is using a proprietary Star-Oddi algorithm pre-programmed into loggers, meant to indicate the reliability of the f_H estimate as the ECG data can be noisy

depending on the position of the logger within the body cavity, the strength of the electric signal from the heart, and on surrounding electronic noise (Brijs et al. 2018; Muller et al. 2020; Zrini & Gamperl 2021). Heart rate data were filtered to include only points of quality index 0–1 (55% for bowfin, 56% for largemouth bass). Data were then inspected and filtered further. Specifically, we used a smoothing function whereby any data point that showed a greater difference than 10 bpm to the immediately preceding *and* proceeding points were removed. We also removed values >100 for bpm (ca. 0.03% of data points) because we considered these to be physiologically unrealistic at these temperatures. However, for model fitting only points within 20 – 80 bpm for largemouth bass and ≤50 bpm for bowfin were kept due to lack of sufficient data beyond these ranges to produce robust model estimates (these ranges were manually chosen based on visual inspection of the distribution of all data points). Of the 21 bowfin and 30 largemouth bass that received loggers, eight bowfin and 12 largemouth bass yielded sufficient high-quality f_H data for statistical analysis for a total of 17.4% and 20.8% of data points respectively after filtering.

Filtered f_H data were then matched to corresponding $\dot{M}_{O2}$ data points for the same animal that had a timestamp within eight (8) minutes. The timestamp for f_H data were exact times (the measurement periods were only 7.5 s); for the longer, $\dot{M}_{O2}$ measurement periods (10 or 15 min long, depending on respirometer size), the timestamp represented the time at the middle of the measurement period. If any f_H data point fell within 8 min of an $\dot{M}_{O2}$ timestamp, it was kept and matched with that $\dot{M}_{O2}$ data point. This meant that measurements of $\dot{M}_{O2}$ were typically assigned to one or two measurements of f_H. Note: we assessed the resulting model fits (R^2) based on 6, 7, 8, 9, and 10 min windows for matching $\dot{M}_{O2}$ and f_H data and found subtle differences, with 8 min providing the best model fit; smaller sampling windows meant a closer temporal match between

data points, but a smaller sample size for modeling. Linear mixed effects models (LME) and general additive mixed models (GAMM) were applied to the relationships between f_H and $\dot{M}_{O2}$ for both species to determine model of best fit for each. We modeled the effect of f_H (fixed effect) on $\dot{M}_{O2}$ (response variable) with animal ID as a random effect (n = 8 animals for the bowfin model, n = 12 animals for the largemouth bass model). We assessed whether the data met the assumptions of parametric statistics by plotting model residuals against fitted values and all predictor variables, by use of q-q plots, and by use of autocorrelation function plots. Models were fit using a temporal autocorrelation structure (using the 'corARMA' function in the package 'mgcv') to control for the lack of independence of data points from the same fish measured at proximate times. We compared model fits using AIC and log-likelihood tests. Model terms are reported as significant at $P < 0.05$. Data files and analysis code for this paper are publicly archived with figshare: https://doi.org/10.6084/m9.figshare.15090936.v2.

Results

There were clear differences in f_H and $\dot{M}_{O2}$ patterns between bowfin and largemouth bass. While bowfin demonstrated a much narrower range of f_H when looking at the 5th and 95th percentiles (bowfin – 19 – 41 beats min^{-1}; largemouth bass – 27 – 69 beats min^{-1}), the full range of f_H in bowfin (16 – 98 beats min^{-1}) was comparable to largemouth bass (18 – 86 beats min^{-1}) (Fig. 1a). However, largemouth bass exhibited greater increases in f_H in response to the acute stressor (median increase of 32 beats min^{-1}) than did bowfin (median of 15 beats min^{-1}) (Fig. 2). Mean f_H across the entirety of the trial was higher in largemouth bass (45 ± 14 beats min^{-1}; mean ± SD) than in bowfin (27 ± 9 beats min^{-1}; Welch's t-test, pooling data for both species $t_{3728.5} = -43.4$, P

< 0.001). A total of 986 measurements of $\dot{M}_{O2}$ were collected among 21 bowfin and 1872 among 30 largemouth bass that could be used to estimate SMR, RMR, and AMR. SMR and AMR were higher in largemouth bass than in bowfin while RMR was lower (t-tests, $F_{46.4}$ = -3.26 [SMR], $F_{48.9}$ = -1.42 [AMR], $F_{28.1}$ = 3.01 [RMR], all $P < 0.001$).

The strength of the relationship between f_H and $\dot{M}_{O2}$ was weak in both species, with differences in the shape of the relationship. A linear mixed-effects model was the best fit for the bowfin data (marginal R^2 [fixed effects only] = 0.28, conditional R^2 [fixed + random effects] = 0.49;), whereby $\dot{M}_{O2}$ increased by 0.058 mg O_2 kg^{-1} min^{-1} for each 1-bpm increase in f_H (P < 0.001). For largemouth bass, a GAMM (non-linear model) was the best fit for the data (Adjusted R^2 = 0.23; $P < 0.001$ for the main effect of f_H). A non-linear GAMM smoothing function fit best to the largemouth bass data because there appeared to be a linear relationship up to a f_H of *ca*. 66, at which point there was an inflection whereafter $\dot{M}_{O2}$ increased at a higher rate with further increases in f_H . However, there were few data above that 66 bpm inflection point leading to wider confidence intervals (Fig. 3).

Discussion

In this experimental study using wild-caught fishes, we found weak relationships between heart rate (f_H) and rate of oxygen consumption ($\dot{M}_{O2}$) for bowfin and largemouth bass using heart rate loggers and respirometry. These experiments were carried out under a constrained range of conditions, whereby all fish were in the laboratory, fasted, recovering from a moderate handling stressor, and confined in static respirometry chambers at a constant temperature. We had expected to see some variation in f_H and $\dot{M}_{O2}$ from spontaneous activity within the respirometry

chambers, though both species mostly appeared to remain relatively 'calm' throughout. Perhaps partly because of that lack of activity-driven variation, f_H predicted only ~28% and 23% of the variation in $\dot{M}_{O2}$ for bowfin and largemouth bass, respectively. One possibility is that our measurements of both parameters were too infrequent to detect brief bouts of spontaneous activity that were not accompanied by excess post-exercise oxygen consumption (the latter would mean a sustained increase in f_H and $\dot{M}_{O2}$ even after activity ceases). Regardless, heart rate has long been thought of as an imperfect proxy for $\dot{M}_{O2}$ in fish – as it was in the present study – for a variety of reasons, including the fact that f_H is one of several ways fish modulate their oxygen uptake and transport (stroke volume plays an important role in cardiac output; Thorarensen et al., 1996; Priede & Tytler, 1977). However, there is growing evidence (relative to early syntheses by Farrell 1991) that many fish are frequency modulators (including largemouth bass; Cooke et al. 2004) and thus heart rate may indeed be a useful proxy. This proof-of-concept experiment suggests that while heart rate loggers may have some role in the 'toolbox' to help quantify metabolic rate in wild fishes (Cooke et al. 2016), validation is needed and their utility may be limited in some species. While f_H on its own appears to be a poor choice for precisely estimating energy expenditure in these species, it could be useful for probabilistically assigning different physiological states (e.g., stressed *vs*. unstressed, resting *vs*. active, digesting *vs*. fasted). In any case, our findings underline the need for species-specific validation experiments before f_H can be of use for studying the ecophysiology of fish in the wild.

Heart rate and its relationship with metabolic rate in untethered animals (i.e., using loggers or transmitters) has been studied in several fish species that have produced comparable results to the species used here. Armstrong (1986) found a positive linear relationship between these variables up to 55 beats min^{-1} in northern pike (*Esox lucius*), after which the linear

relationship dissolved due to larger increases in metabolic rate relative to f_H, similar to what we found in largemouth bass. Atlantic salmon (*Salmo salar*) also showed a strong linear relationship between f_H and metabolic rate on a logarithmic scale, one that extends past the point at which both pike (Lucas, 1994) and largemouth bass appear to dissolve. Other species, however, show weaker relationships between f_H and metabolic rate, such as cod (*Gadus morhua*), which demonstrated a large range of $\dot{M}_{O2}$ values per f_H value (Priede & Tytler, 1997),. Strong intraspecific variation may also explain weak relationships between f_H and $\dot{M}_{O2}$ in fish (Lucas, 1994; Scharold & Gruber, 1991; Farrell 1991). Indeed, in our study the random effect term significantly improved model fits for both species (different intercepts for each individual animal), confirming that there are individual differences in the precise relationship between $\dot{M}_{O2}$ and f_H. In addition, longer trials than the 24-48 h timespan used here could facilitate recovery from surgery and better f_H-$\dot{M}_{O2}$ relationships; Weber et al. (1998) found that the relationship between these variables improved over seven days in Atlantic cod.

Aspects of the 'lifestyle' of the two species studied here may be relevant to consider when interpreting the weak relationships between f_H and $\dot{M}_{O2}$ we observed. Bowfin and largemouth bass are both ambush predators that live primarily in shallow, warm habitats with submerged vegetation. Our test of the f_H-$\dot{M}_{O2}$ relationship was quite conservative in the sense that we exposed fish to a narrow set of conditions: one temperature, confinement in a respirometer in the lab, with all fish being post-absorptive – considering this context casts a more positive light on the somewhat weak relationship we did observe in the two species. Given that both species are sit-and-wait predators, there would likely be a benefit to including post-prandial metabolic responses in future f_H validation experiments. For example, in the invasive lionfish, another sit-and-wait predator, the rise in metabolic rate during digestion eclipses that from

exhaustive exercise (Steell et al. 2019). Indeed, the relative advantage of f_H over accelerometry as a means of remotely quantifying energy use is that it can detect the postprandial metabolic response (i.e., specific dynamic action; Secor 2009). In contrast, for more 'active' species like salmonids, it could be that f_H has stronger relationships with other variables (Clark et al. 2010) than in sit-and-wait predators like those used here.

Despite the loggers used here being commercially available and 'user-friendly', there are technical challenges and limitations in their use, not unlike previously used custom-made heart rate loggers (e.g., Kojima et al., 2003; Campbell et al., 2005). For example, only 17.4% of the data points from 30 largemouth bass and 20.8% of data from 21 bowfin were of reliable quality to be used in analysis. That shortcoming highlights the need for careful pilot experiments to determine optimal logger settings and placement in the body cavity (i.e., surgical methods; steps we were unable to take with the present study). For example, in this study loggers were placed perpendicular to the incision with the electrodes facing the pericardial cavity; another option to explore would be to place them parallel to the incision with the electrodes facing the abdominal muscles (Brijs et al., 2018). Placement of the logger relative to the heart also likely plays a role in its efficiency, the further away the weaker the ECG signal, and to optimize results, the loggers should be kept within 20 mm of the heart (Brijs et al., 2018). Exact logger placement depends on the anatomy of the species (Muller et al. 2020). For bowfin and largemouth bass, the position of the pelvic and pectoral fins and surrounding cartilage dictated where the incision could be made safely (without damaging important structures like cartilage). Loggers in our study were approximately 5-20 millimetres from the pericardium, with some variation related to body size, although it was difficult to verify the exact positioning of the tag once it was inside the animal (in part because it could have moved slightly, despite being sutured to the body wall).

Heart rate loggers offer the potential of being able to estimate metabolic rate in free-swimming fish in the wild. In this study, we piloted the use of heart rate loggers as a proxy for metabolic rate in laboratory-held, wild-caught largemouth bass and bowfin. While the relationships between f_H and $\dot{M}_{O2}$ were not strong, our experiment involved a narrow range of conditions. Therefore, we suggest that the relationships we found represent sufficient evidence to consider further experimentation that includes a broader range of conditions in order to elicit more within-individual variability, including dynamic changes in temperature, oxygen level, activity levels (e.g., using a swim tunnel respirometer), and digestive states. Nevertheless, it may simply be that in these species, f_H on its own is not particularly useful for estimating metabolic rate. Accelerometry has also been shown to provide a good estimate of metabolic rate when used in conjunction with f_H (Clark et al., 2010); this combination is something to consider for future efforts to remotely measure metabolic rate in free swimming fish (a logger that measures acceleration, f_H, and temperature is available from the same manufacturer; /www.star-oddi.com). The differences between the two species illustrates the need for species specific validation of this relationship and the need for pilot studies to experiment with logger placement in relation to the heart.

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Contributions

G. D. R. and A. T. F. conceived and designed the experiment. T. E. P. and S. J. C. provided critical equipment and logistical support. C. L. J. D. and G. D. R. collected and analyzed the data. C. L. J. D. wrote the manuscript with input from all co-authors.

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Figure captions – Doherty et al. ms

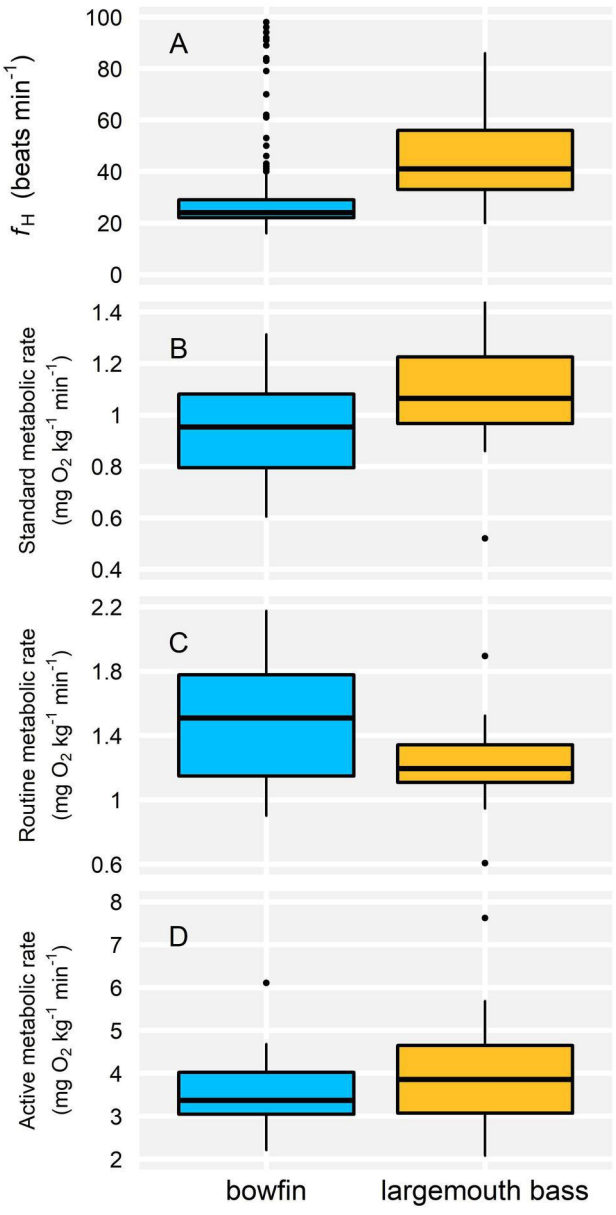
Figure 1: Physiological states exhibited by bowfin and largemouth bass held under controlled laboratory conditions at *ca*. 19°C. Top panel (a) is heart rate (f_H) with the following sample sizes: n = 1626 data points from 8 bowfin, 2092 data points from 12 largemouth bass. For the three oxygen consumption parameters: (b) standard metabolic rate (SMR,), (c) routine metabolic rate (RMR), there were 30 largemouth bass and 21 bowfin, (d) active metabolic rate (AMR)(one estimate per fish). For boxplots, the thick middle line is the median, the boxes extend to the 25th and 75th percentiles of the data, and the whiskers extend to the most extreme data points or 1.5× the interquartile range, whichever is less.

Figure 2: Examples of (a) largemouth bass and (b) bowfin heart rate (f_H) data from two fish, highlighting the between-species differences in cardiac response to stressors applied in a controlled environment. The blue vertical lines indicates the time when surgery took place; the yellow vertical line is the handling and air exposure stressor. The matching oxygen consumption (= metabolic rate) data for the same individuals is shown in blue triangles (right axis).

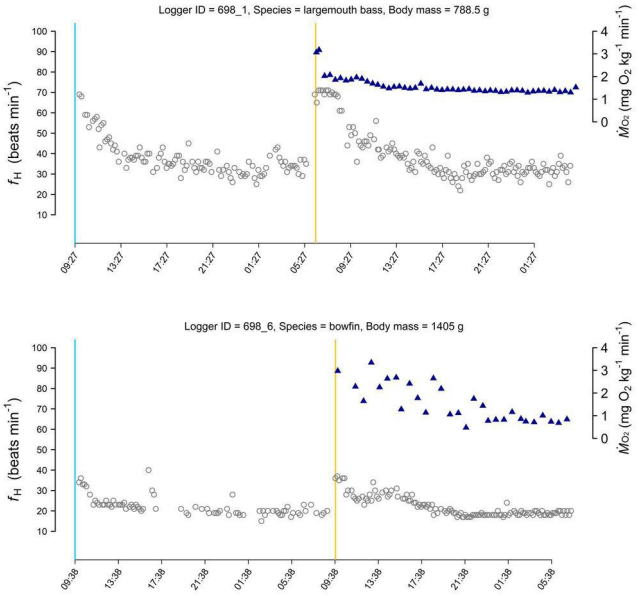
Figure 3: Scatterplot comparison of heart rate (f_H) and oxygen consumption rate ($\dot{M}_{O2}$) in a controlled environment overlaid by a general additive mixed model (largemouth bass, right panel) and a linear mixed effects model (bowfin, left panel), yellow data points (translucent) represent quality index zero (best) and blue quality index one (good) that passed our filtering steps and were used for statistical modeling (n = 599 data points from eight bowfin, 1068 data points from 12 largemouth bass).

Significance statement

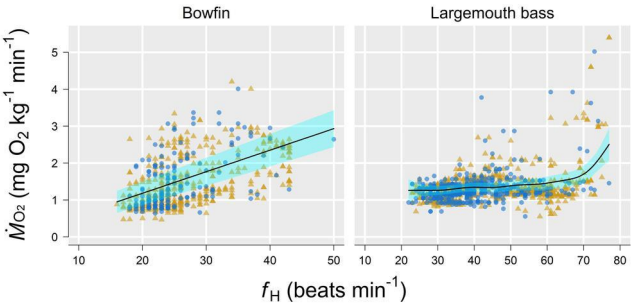
There is growing interest in using electronic tags to be able to remotely estimate bioenergetic paramteres in wild fishes. In this study, we used respirometry in the laboratory to assess the performance of heart rate loggers for estimating metabolic rate in bowfin *Amia calva* and largemouth bass *Micropterus salmoides*. The relationships between heart rate and metabolic rate were modest for both species (model R^2 of 0.23-0.28), suggesting heart rate loggers may have some (limited) utility in estimating field metabolic rate, perhaps when combined with other techniques like accelerometry.



JFB_14923_Fig 1 - stacked boxplot 20210630.jpg



JFB_14923_Fig 2 - bowfin-bass HR example plot 20210715.jpg



JFB_14923_Fig 3 - mo2_bpm_20210715v3.jpg