Determination of Species-Specific Dissolved Oxygen and Temperature Requirements for Non-Game Riverine Fishes[1]

Final Report
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INTRODUCTION

The Southeastern United States possesses the richest aquatic biodiversity and the highest degree of endemism in North America (Jenkins et al. 2015), but that biological plenty unfortunately also comes with some of the highest imperilment rates on the continent (Warren et al. 1997; MRBMRC 2010). In fact, over 40% of the federally listed animals in the US Southeast Region are freshwater mussels, snails, and fishes that occur in Alabama. Protecting these imperiled animals at landscape scales requires knowledge regarding how multiple uses of waterways impact the risk of imperilment or extinction. Large and small dams are prevalent on the landscape (Graf 1999; Downing et al. 2006), many of which impact downstream water quality in terms of critical levels of temperature and dissolved oxygen (DO; Pringle et al. 2000; Santucci et al. 2005). According to a review of 300 Federal Energy Regulatory Commission (FERC) project records, more than 40% of projects had a specific requirement to maintain DO, 37% had DO monitoring requirements, and 38 and 35% had mitigation recommendations or changes in project operations, respectively (EPRI 1992). The U.S. Fish and Wildlife Service requires information to inform decision making relative to recovery actions, prioritization of stream and river reaches for restoration or reintroduction efforts, and for assistance during permitting where imperiled fishes and mussels are concerned (MRBMRC 2010).

Maintenance of minimum DO concentration is required for survival of most aquatic organisms. This can be particularly important when stream flows are interrupted by a dam causing DO and temperature fluctuations that sometimes exceed the tolerances of the organisms living downstream of the impoundment. Large instream hydropower dams are required to maintain a minimal level of dissolved oxygen in discharged water, typically determined based on the temperature and oxygen requirements of largemouth bass or other game fishes (U.S. EPA

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1986, as cited by FERC in their 2012 Biological Opinion for the Coosa River). However, while we know a great deal about the abiotic and biotic requirements of many game fishes (e.g., Beamish 1964a; Redpath et al. 2010; Rice and Cochran 1984; Evans 1984), there is a surprising lack of data regarding critical physiological limits of most non-game fishes. As such, threshold values that have been determined for game fishes may not adequately represent the temperature and dissolved oxygen requirements of other ecologically important species. In certain cases where flow from impoundments is reduced, temperature and/or DO may quickly exceed the tolerance limits of less-studied species while remaining within accepted bounds determined for largemouth bass. Given the potential importance of many non-game fishes as mussel hosts and as potentially important components of the food web, exceeding these limits could clearly have ecological implications for the entire aquatic ecosystem.

An approach that has been commonly used to evaluate hypoxia tolerance of fishes is to quantify oxygen (O_2) consumption (MO_2), which reflects the ability of an organism to extract oxygen from the environment required to maintain routine metabolic rate as DO changes (Tripathi et al. 2013). Historically, fish were assumed to fit into one of two categories of oxygen consumption response to declining DO: oxyregulators (Fry and Hart 1948; Beamish 1964b; Ultsch et al. 1978; Cech et al. 1979; Barnes et al. 2011) or oxyconformers (Barnes et al. 2011; Urbina et al. 2012; Tiffany et al. 2010; Tripathi et al. 2013) (Figure 1, from Hartline et al. in review). Fish that can maintain a constant MO_2 across a wide range of dissolved oxygen (DO) levels are considered oxyregulators (Figure 1a; Fry and Hart 1948; Beamish 1964b; Ultsch et al. 1978; Cech et al. 1979; Barnes et al. 2011), while those that reduce their oxygen consumption as DO decreases are considered oxyconformers (Figure 1b; Pörtner et al. 1985; Tiffany et al. 2010; Urbina et al. 2012; Tripathi et al. 2013). In both cases, there exists a critical DO concentration

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(DO_{crit}), below which the ability of an organism to obtain oxygen from its environment rapidly declines with declining oxygen availability (Jobling 1994; Rogers et al. 2016). Because a lower DO_{crit} is associated with a greater capacity to obtain oxygen in low DO environments, it is frequently used as an indicator of hypoxia tolerance in fishes (Speers-Roesch et al. 2012).

Another approach to reduce the confusion caused by attempts to categorize organisms into one of two distinct metabolic patterns is the concept of a regulation index (RI; Mueller and Seymour 2011) which recognizes that there is a continuum of metabolic patterns. The RI quantifies the relative ability of an organism to regulate oxygen uptake as DO declines to zero, rather than constraining an organism to be defined in a binary way as either a regulator or a conformer (Figure 1c).

In previous work (Hartline et al. in review), we quantified the influence of temperature and dissolved oxygen on oxygen consumption rate, critical oxygen level (DO_{crit}), and the regulation index of five non-game fish species- blacktail shiner (*Cyprinella venusta*), blackbanded darter (*Percina nigrofasciata),* bronze darter *(P. palmaris*), greenbreast darter (*Etheostoma jordani*), and banded sculpin (*Cottus carolinae*). Using closed respirometry, we were able to measure oxygen consumption as a function of dissolved oxygen concentration at three different temperatures. Oxygen uptake patterns for those species represented a continuum between regulation and conformation, and the ability to regulate was affected differently by temperature among species, declining with increasing temperature in blackbanded darter, not affected by temperature in bronze darter, greenbreast darter, and blacktail shiner, and increasing with temperature for banded sculpin. Critical oxygen levels increased with temperature for blacktail shiner, greenbreast darter, and blackbanded darter, but did not change with temperature for bronze darter or banded sculpin. This among-species variation further supports the need for

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information concerning effects of low DO across species and that protections based on data from a limited number of taxa may not effectively protect habitat for entire fish communities.

In the current study, we used respirometry to quantify dissolved oxygen uptake across temperatures for several additional species of non-game fishes in an effort to provide a broader database from which to generate predictions and ultimately help in determining suitable conditions in waters below impoundments. Species were all from the southeastern U.S. and it is our goal that our findings and predictions will assist in decision making for specific projects (i.e., SHC, conservation design, and Alabama's Strategic Habitat Unit-SHU Project), as well as for being applicable to delivery of conservation actions relative to impounded river systems and habitats across the region and beyond.

OBJECTIVES

Our overall objective for the proposed research was to quantify the oxygen uptake responses to declining dissolved oxygen for several non-game fish species and how those responses were affected by temperature.

METHODS

Fish were collected from Chewacla Creek (32.556174, -85.468419), Choctafaula Creek (32.466722, -85.641234; sometimes this site is mistakenly identified as Chewacla Creek on digital maps), and Big Wills Creek (34.571256, -85.617058). Stoneroller *Campostoma oligolepis*, blacktail shiner *Cyprinella venusta*, banded sculpin *Cottus carolinae*, rough shiner *Notropis baileyi*, and striped shiner *Luxilus crysocephalus* were collected via seining and backpack electrofishing (LR-24; SmithRoot, Vancouver, WA, USA), held in aerated coolers, and

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transported to the laboratory where they were placed into holding tanks and acclimated to lab conditions (21°C; 12:12 hr light/dark) for at least ten days before exposure to any temperature increase or decrease, or respirometry measurements. Temperatures were then gradually increased or decreased (1°C per day) until reaching the experimental temperature at which they were to be texted; fish were acclimated to that temperature for an additional ten days prior to respirometry runs. Individual fish were run at only one experimental temperature. Temperatures used during respirometry ranged from a baseline of 18°C to a maximum of 30°C at 3°C increments for all species except banded sculpin. Baseline temperature for banded sculpin was 14°C to a max of 26°C increasing in two-degree increments. Temperatures were chosen based on literature review estimates of sub-lethal temperatures (Smith and Fausch 1997; Beitinger et al. 2000).

Respirometry. A respirometry "run" was defined as the duration of time spent inside the respirometer chamber by an individual fish during which temperature, oxygen uptake, and dissolved oxygen concentration were measured, and oxygen uptake and routine metabolic rate were calculated. Fish feeding was suspended for 48 hours prior to a run to allow complete gut evacuation. Prior to placement in a respirometer chamber, individual fish were weighed (nearest 0.01 g). To correct for background respiration by bacteria, blank control runs were performed both before and after the experimental runs. Eight individual fish were randomly assigned to one of eight glass chambers that were submerged in a large reservoir of dechlorinated tap water. Temperature of the water housing the chambers matched the acclimation temperature of the fish holding tanks, and vision of individual fish was obscured by barriers around the chambers to prevent any unnecessary stimulation due to viewing other fish for the duration of a run. Once

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inside the chamber, an opaque covering was placed over the entire large water reservoir containing the respirometer chambers and fish were allowed to acclimate. Given that acclimation times can be variable both among species and among individuals within species, combined with variation in acclimation times that have been used previously in the literature, we allowed fish to acclimate in the chambers overnight for at least 12 hrs.

Each respirometer (Loligo® Systems, Tjele, Denmark) consisted of a flush pump, a recirculating pump, a fiber-optic oxygen probe, and the respirometer chamber (containing the species of interest). Oxygen concentration was measured via the fiber-optic probe. During the acclimation period, the respirometer was run as an intermittent system during which it alternated between a closed and an open system. While closed, the fish could reduce the oxygen concentration by a small amount (see below) after which the system switched to an open system with normoxic conditions. Fish were never exposed to a hypoxic level of oxygen concentration, and DO always remained at or above 80% saturation throughout the intermittent period. A diel light cycle was maintained which was light during 0700-1900 and dark from 1900-0700. Runs were only performed during daylight hours to minimize any diurnal effects. Fish were placed into the respirometry chamber no later than 1800 on the day prior to the trial. Individual fish were used only once.

Measures of hypoxia tolerance. Once the acclimation time had elapsed, the respirometer was set to closed so that water recirculated, oxygen was consumed, and oxygen uptake could be quantified. During this closed period, oxygen was consumed by the fish, and the (declining) oxygen concentration was measured via the fiber optic oxygen probe. As oxygen concentration declined, the fish was observed until a concentration of 1 mg/L of oxygen was reached or until

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fish movement became erratic. The fish was then removed from the chamber and humanely euthanized using a buffered solution of MS-222 (Auburn University Standard Operation Guideline AP-101). This technique was repeated for a minimum of 6 similarly-sized individuals of each species at each temperature. Three response variables were quantified-- oxygen uptake (MO_2), critical dissolved oxygen concentration (DO_{crit}), and the regulation index (RI). These three variables were compared across temperatures within species using one-way ANOVAs and Tukey's post hoc comparisons in RStudio. We used linear and quadratic regressions to compare response variable across temperatures; linear and quadratic models were compared for best model fit using AIC_C. The model with the best fit (lowest AIC_C) was used in the analysis. Finally, we compared normoxic MO_2 between species at a given temperature using ANOVA with Tukey's post hoc comparisons to quantify differences in metabolic rate among species.

RESULTS

Fish adjusted quickly to the lab, readily feeding on manufactured feed and displaying no behaviors that could be interpreted as induced by stress (e.g., listlessness, erratic swimming, piping, etc.) prior to a respirometry run. Once placed inside the respirometry chamber, fishes acclimated to the flow of the flush and recirculating pumps, displaying minimal movement within minutes and well before covering the basin with the opaque covering.

Stoneroller

During acclimation to 30°C, we observed that stonerollers fed less and over time their physical condition visibly declined. During the 2-week acclimation period, we also observed substantial mortality (9 out of 14 individuals died). Given this, we do not include any data for stonerollers at 30°C.

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Stonerollers had the highest overall metabolic rate of all species tested and the effect of increasing temperature on metabolic rate was significant (ANOVA, Tukey's, $p = 0.004$; Figures 2, 7). Metabolic rate significantly increased with increasing temperatures (linear regression, $p = 0.005$; Figure 2). Stoneroller was the only species we tested whose DO_{crit} increased significantly with increasing temperature (linear regression, $p = 0.01$; Figure 2). Ability of stonerollers to regulate oxygen consumption significantly decreased as temperature increased (linear regression, $p = 0.005$; Figure 2).

Blacktail shiner

Blacktail shiner metabolic rate decreased significantly as temperature increased (linear regression, $p = 0.002$; Figure 3). Increasing temperature had no effect on DO_{crit} or RI for blacktail shiner (linear regression, $p = 0.95$ and $p = 0.049$, respectively; Figure 3).

Rough shiner

Increasing temperature had a significant effect on the metabolic rate of rough shiners (quadratic regression, $p = 0.038$; Figure 4). However, DO_{crit} did not differ across tested temperatures (linear regression, $p = 0.22$; Figure 4). Rough shiner RI had a curvilinear response with temperature, with RI being highest at 18°C, decreasing at 21°C and 24°C, but increasing at 27°C (ANOVA, Tukey's, $p < 0.001$; Figure 4).

Striped shiners

Metabolic rate was significantly related to temperature in a curvilinear fashion (quadratic regression, $p = 0.003$; Figure 5). There was no response of DO_{crit} to temperature ($p = 0.52$; Figure 5). And striped shiner RI declined significantly (albeit in a curvilinear fashion) as temperature increased (quadratic regression, $p < 0.023$; Figure 5).

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Banded sculpin

Similar to stonerollers, substantial mortality of banded sculpins was observed at temperatures above 26°C. Given this, no data were recorded for banded sculpin above this temperature.

Banded sculpin metabolic rate increased, and RI decreased as temperature increased (quadratic regression, $p < 0.001$ and linear regression, $p = 0.01$, respectively; Figure 6); however, increasing temperature had no effect on DO_{crit} (linear regression, $p = 0.50$; Figure 6).

At 14°C, the uptake of oxygen by banded sculpin reduced oxygen concentration to a very low level (< 1 mg/L O_2), with the data recording software indicating that oxygen consumption ceased, and visually fish did not appear to be respiring prior to removal from the chamber. However, once the chamber was flushed with freshly oxygenated water, banded sculpin individuals recovered and began to respire (as noted by opercular movement).

Across species

We were able to compare metabolic rates across species at four temperatures: 18, 21, 24, and 27°C. At 18°C, no significant differences were quantified among the four species compared (banded sculpin was not included in the analysis at 18 or 27°C). At 21°C, banded sculpin and stoneroller metabolic rates differed significantly (ANOVA, $p < 0.001$; Figure 8). At 24°C, stoneroller and striped shiner metabolic rates did not differ (ANOVA, $p = 0.99$; Figure 8) but were significantly higher than those of blacktail shiner, rough shiner, and banded sculpin (ANOVA, $p < 0.001$; Figure 8). At 27°C, stoneroller and striped shiner metabolic rate did not differ (ANOVA, $p = 0.13$, Figure 8) but were significantly higher than those of blacktail shiner and rough shiner, which did not differ from each other (ANOVA, $p = 0.85$; Figure 8).

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DISCUSSION

Consistent with the work of Hartline et al. (in review), our results clearly demonstrate the variation that exists both among species and among individuals within a species relative to metrics quantifying effects of reduced DO and temperature. We found that metabolic rate increased with temperature for four species (stoneroller, rough shiner, striped shiner, banded sculpin) and decreased with temperature for one species (blacktail shiner). Similarly DO_{crit} was positively related to temperature for one species (stoneroller), while being unrelated for the others. And the regulation index was negatively related to temperature for two species (stoneroller, banded sculpin), curvilinearly related for two species (rough shiners, striped shiner), and unrelated for one species (blacktail shiner). And given the range of responses that we found for the four cyprinid species, there was clearly no overarching taxonomic consistency among species within a family.

We chose the species that we studied for several reasons. Stoneroller and striped shiner were chosen based on their ubiquitous distribution. Blacktail shiner and banded sculpin were chosen to expand on some of the measures that Hartline et al. (in review) had made previously with a different experimental setup; in addition, banded sculpin had provided some unique results previously and we wanted to further study this species across a broader range of temperatures. We chose to study several members of the Cyprinidae to evaluate whether there was any consistency in responses among species within a taxonomic group (i.e., family).

Stoneroller was the only species that we found to have significant relationships for all three metabolic parameters (MO_2, DO_{crit}, RI) with increasing temperatures. Metabolic rates varied by 3-4 times across species, and stoneroller and striped shiner had the highest measured metabolic rates of our five study species. Anecdotally, it appeared that stoneroller body

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condition declined during the two-week acclimation to 30°C. At 30°C individuals began to feed less frequently than they fed at 27°C and below, they appeared to lose weight, and swimming/schooling appeared to be a strenuous activity. With oxygen uptake, the typical or expected relationship with increasing temperature is an increase in metabolic rate to a maximum temperature, followed by a decline at which point the individual fish's metabolism is reduced to compensate for suboptimal temperatures (Pörtner 2010). We did not observe any such decline in stoneroller due to the inability to acclimate them to 30°C. It is possible that such a reduction in metabolic rate would be observed between our upper test temperature (27°C) and 30°C, but that was not tested in our work. However, we do expect that a critical point for stonerollers to maintain physiological function lies in that temperature range between 27-30°C. Stoneroller metabolism at these higher temperatures could be sufficiently high to cause a decline in body condition. While we did observe a more typical pattern in striped shiner metabolic rate (i.e., increasing with temperature to a point above which metabolic rate declines), there did appear to be a maximum metabolic rate at 24-27°C above which it declined, suggesting that a critical point for striped shiner to maintain physiologic function lies somewhere between 27-30°C. Relative to physical condition, if striped shiners exhibit observable declines in body condition at high temperatures, theses symptoms did not present within the two week acclimation period of this study.

Interestingly, we found that blacktail shiner metabolic rate decreased with increasing temperatures, which was in contrast to results from Hartline et al. (in review). These data would suggest that blacktail shiners either favored higher temperatures or were less able to regulate metabolic rate as temperature increased; however, temperature had no effect on their regulation ability or critical oxygen level, which is similar to Hartline et al. (in review). The actual values

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of metabolic rate were similar between our study and Hartline et al. (in review), and the overall appearance of no effects of temperature on other metrics of their ability to regulate are similar between studies, so the reason for the different relationship of metabolic rate with temperature remains unclear.

Banded sculpin metabolic rates were low, potentially reflecting its role as a benthic species or general description of being a coolwater species (Baltz et al. 1982; Brown 1989). Interestingly, the results reported here for banded sculpin differ from those reported in Hartline et al. (in review). Inclusion of the lower 14°C temperature allowed us to quantify the increase in MO_2 that occurred between 14°C and 17°C. This may help to explain the unique results found by Hartline et al (in review), where banded sculpin respiration rate decreased and RI increased with increasing temperature (albeit only for temperatures between 20-24°C versus the range from 14-26°C used here). Acute thermal maximum for mottled sculpin, *Cottus bairdi*, after acclimation at 15 °C was documented in the review paper by Beitinger et al. (2000), where the endpoint of sculpin thermal tolerance was determined by the onset of muscle spasms at 30 °C (Kowalski et al. 1978). At the same acclimation temperature, the slimy sculpin, *Cottus cognatus*, exhibited loss of equilibrium at 26.3 and 27.3 °C (Otto and O'Hara Rice 1977). More recently, studies have compared thermal maxima for a variety of southeastern sculpin species and found similar values for banded sculpin acclimated to 15 °C (Walsh et al. 1997). Long-term exposure and acclimation to temperatures higher than 26°C may be difficult to study based on the findings of these studies.

Another interesting result for banded sculpin was the observed cessation of respiration at 14°C. In some trials at 14°C, as dissolved oxygen levels decreased to between 1 and 2 mg/L, banded sculpin were observed to have stopped respiring; however, prior to this, individuals did

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not exhibit any visible distress behavior. Instead, sculpin remained at the bottom of the chamber in a manner that was not distinguishable from that at normoxia. Cutaneous respiration has been observed in marine and intertidal cottids (Martin 1991), but even that would not explain the complete lack of oxygen decline inside the closed respirometry chamber. Our immediate conclusion was that the non-respiring individual had died without exhibiting distress that we observed to be typical for shiners exposed to oxygen levels $\leq$1 mg/L. However, when the chamber was flushed with oxygenated water and the individual removed, the apparently "dead" fish resumed respiration with visible opercular beats. Prior to the flushing, no movement was observed. The cool temperature and low oxygen environment may have induced a torpor-like state in some individuals, allowing them to reduce metabolic activity to survive unfavorable conditions (as seen in *Notothenia coriiceps*; Campbell et al. 2008). These conditions lasted no longer than twelve hours so this could be a response to acute stress of low dissolved oxygen at low water temperature. The behavior was not observed at higher temperatures.

MANAGEMENT IMPLICATIONS

The results presented here stress the importance of including multiple metrics or endpoints when using physiological data in support of management decisions. If we were to rely only on DO_{crit} to support management plans, we could potentially misidentify temperatures or dissolved oxygen levels that are more broadly required for species survival. A significant change in metabolic rate with temperature did not necessarily equate to significant responses of RI or DO_{crit} to temperature. For example, blacktail shiner metabolic rate decreased significantly with temperature while no effects were observed relative to either DO_{crit} or RI versus temperature. Rough shiner and striped shiner DO_{crit} were not significantly affected by increasing

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temperature but metabolic rate and their ability to regulate were significantly affected. Clearly, the response of species to temperature and DO is complex and we are only now beginning to piece together the larger picture. Considering that some of these species are also hosts for glochidia larvae for freshwater mussels (which have their own responses to fluctuations in water temperature and dissolved oxygen), the situation becomes even more complex. Additional data are always needed, but in this case it is particularly important. Only with additional data can we start to generate the comprehensive and broad-scale view of the dissolved oxygen concentrations that would be required for the long-term survival and success of diverse fishes and the rest of their aquatic communities.

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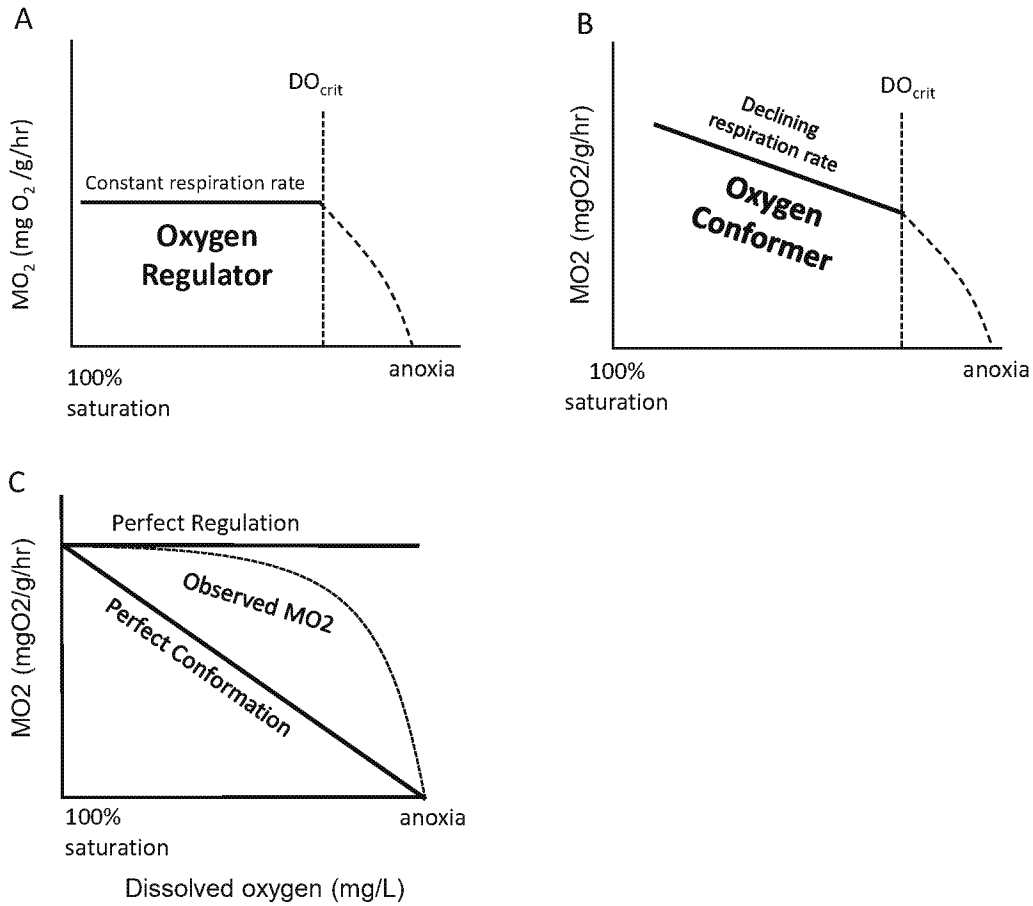

Figure 1. Oxygen uptake patterns ($\dot{M}O_2$) graphed as a function of DO of (A) an oxyregulator and (B) an oxyconformer. DO_{crit} is defined as the DO threshold below which respiration rates show a marked change. (C) Oxygen uptake patterns ($\dot{M}O_2$) graphed as a function of DO indicating the range of values of the Regulation Index (RI; Mueller and Seymour 2011), with solid lines indicating either perfect regulation (RI = 1) or perfect conformation (RI = 0) and the dashed line indicating an intermediate RI value.

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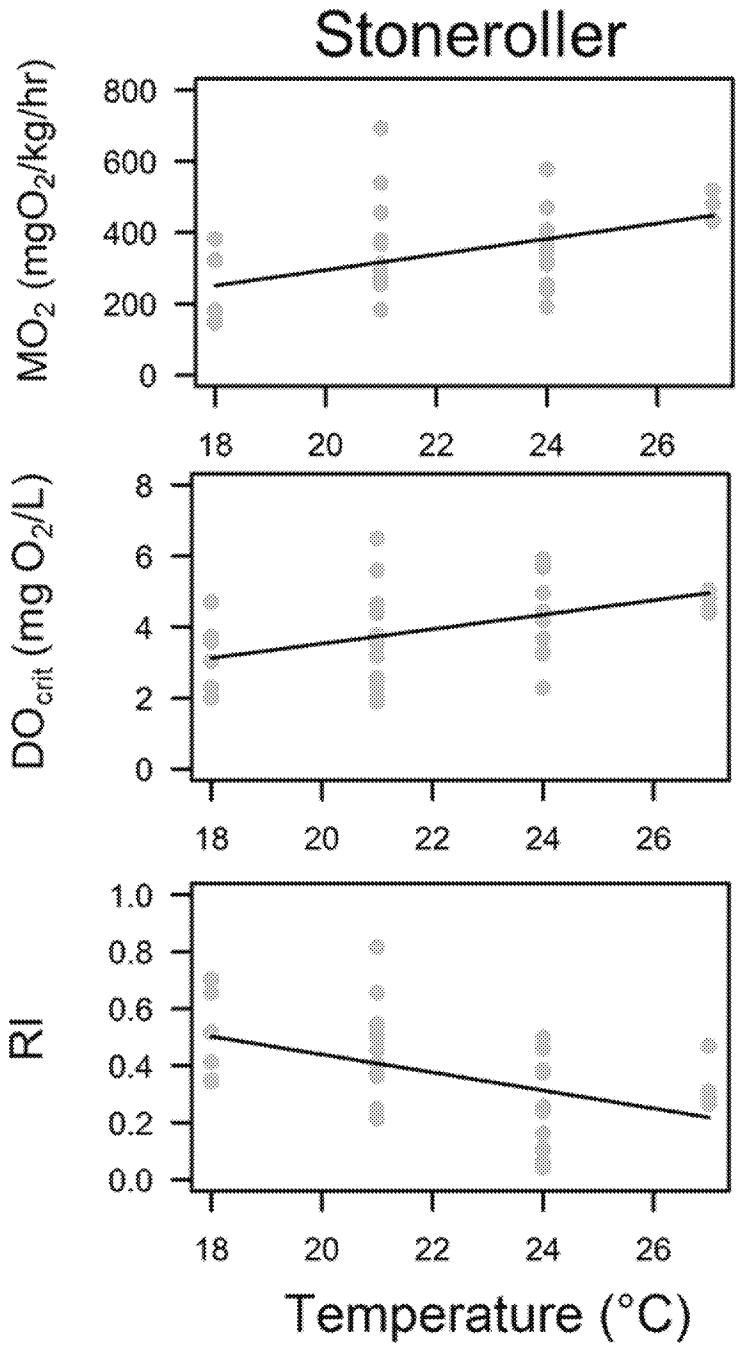

Figure 2. Metabolic rate, critical dissolved oxygen, and regulation index of stonerollers across a temperature range from 18 to 27°C. Fitted lines denote significant relationships.

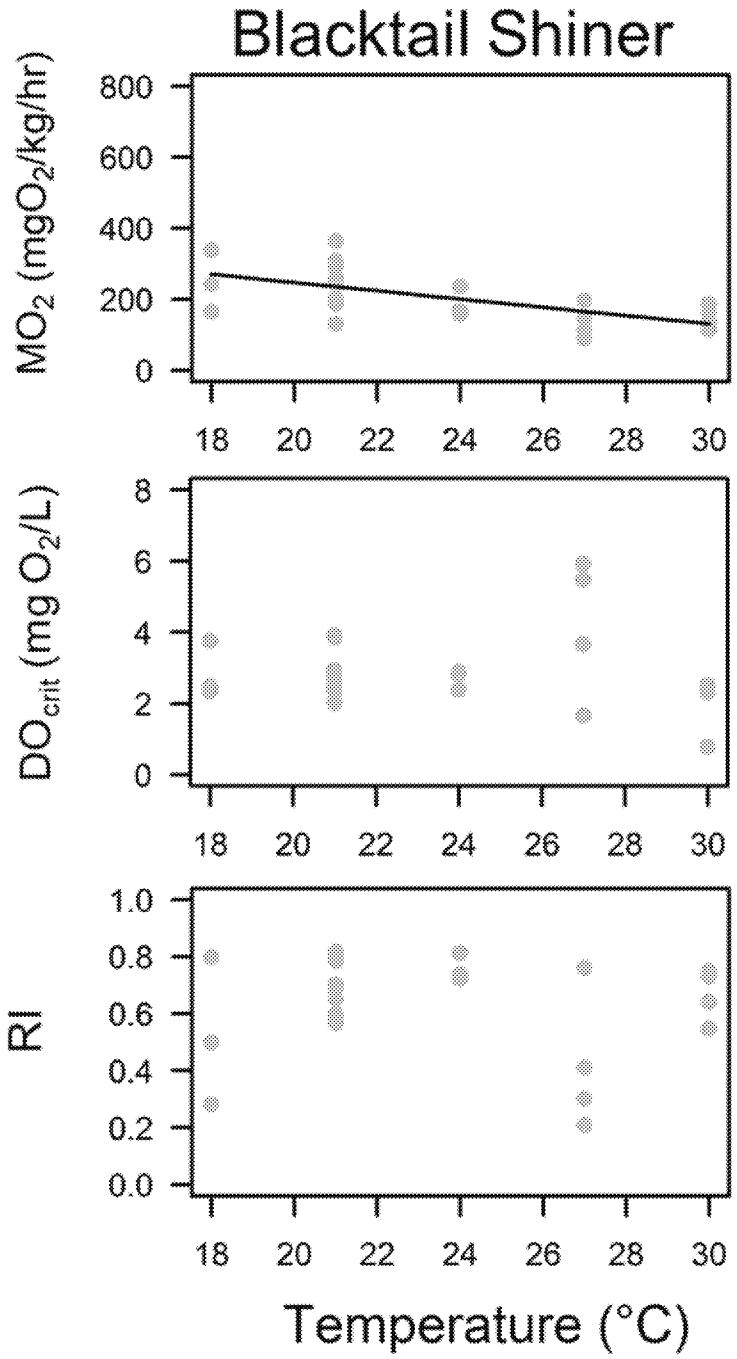

Figure 3. Metabolic rate, critical dissolved oxygen, and regulation index of blacktail shiner across a temperature range from 18 to 30°C. Fitted lines denote significant relationships.

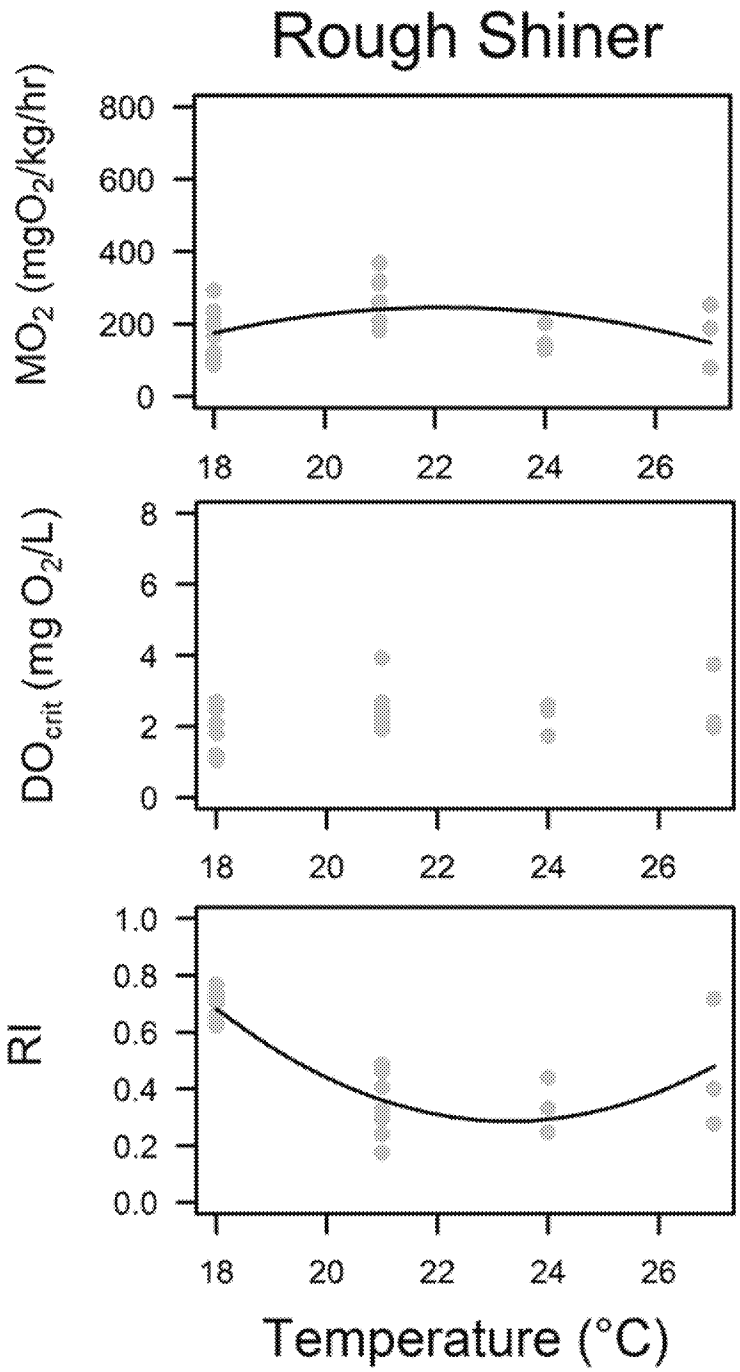

Figure 4. Metabolic rate, critical dissolved oxygen, and regulation index of rough shiners across a temperature range from 14 to 27°C. Fitted lines denote significant relationships.

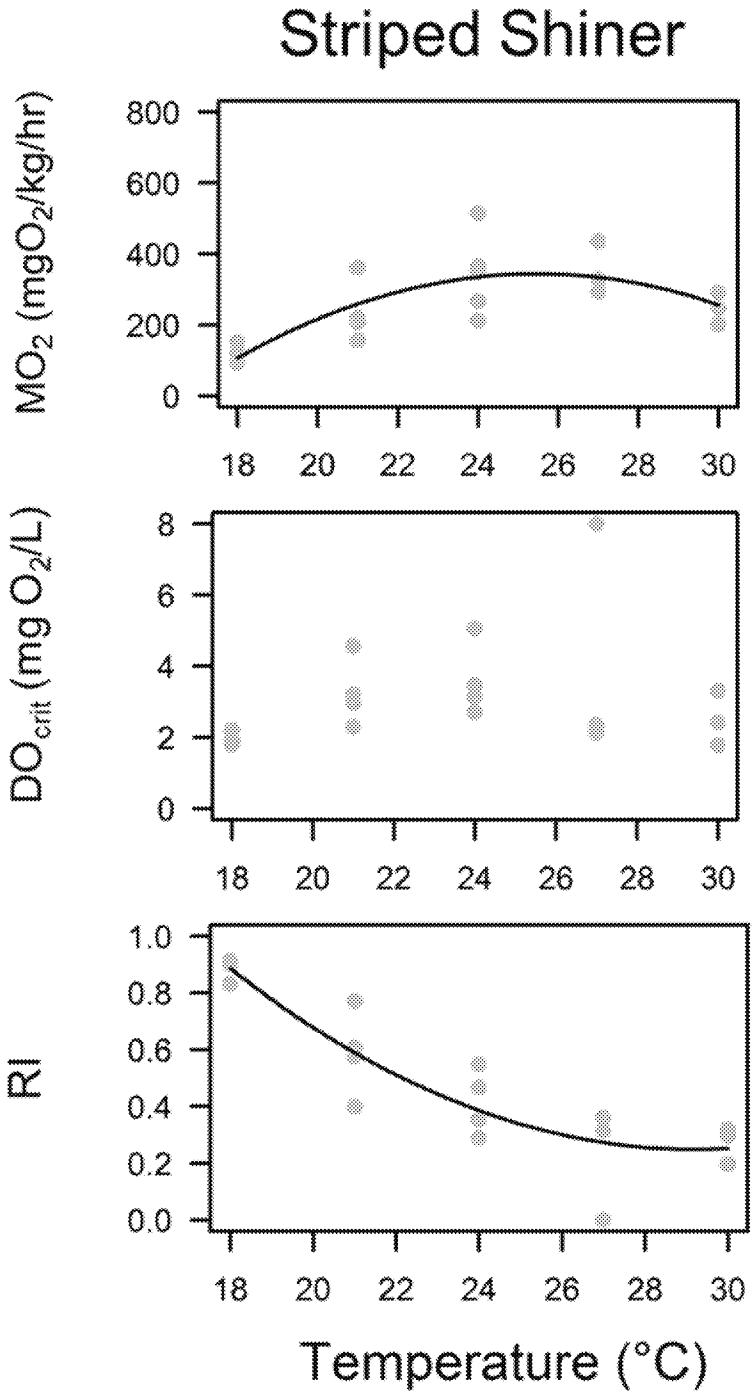

Figure 5. Metabolic rate, critical dissolved oxygen, and regulation index of striped shiner across a temperature range from 14 to 26°C. Fitted lines denote significant relationships.

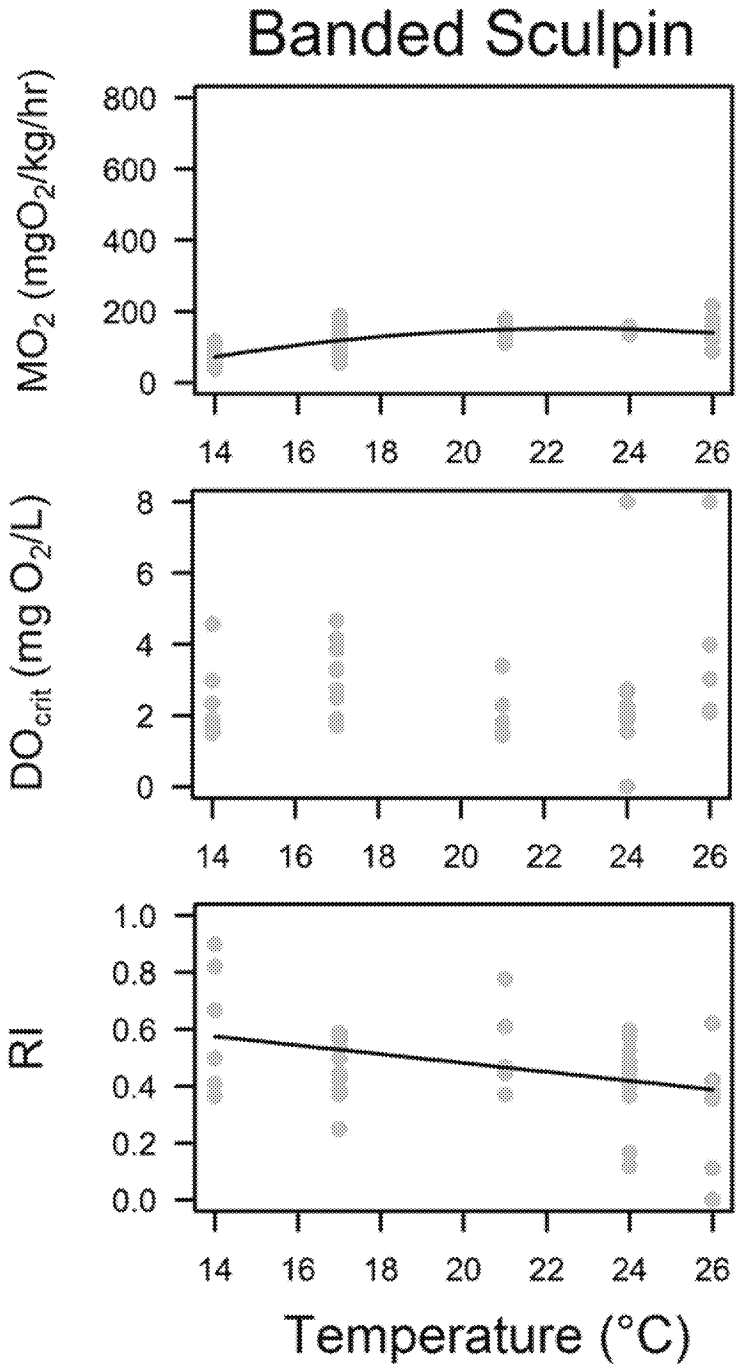

Figure 6. Metabolic rate, critical dissolved oxygen, and regulation index of banded sculpin across a temperature range from 14 to 26°C. Fitted lines denote significant relationships.

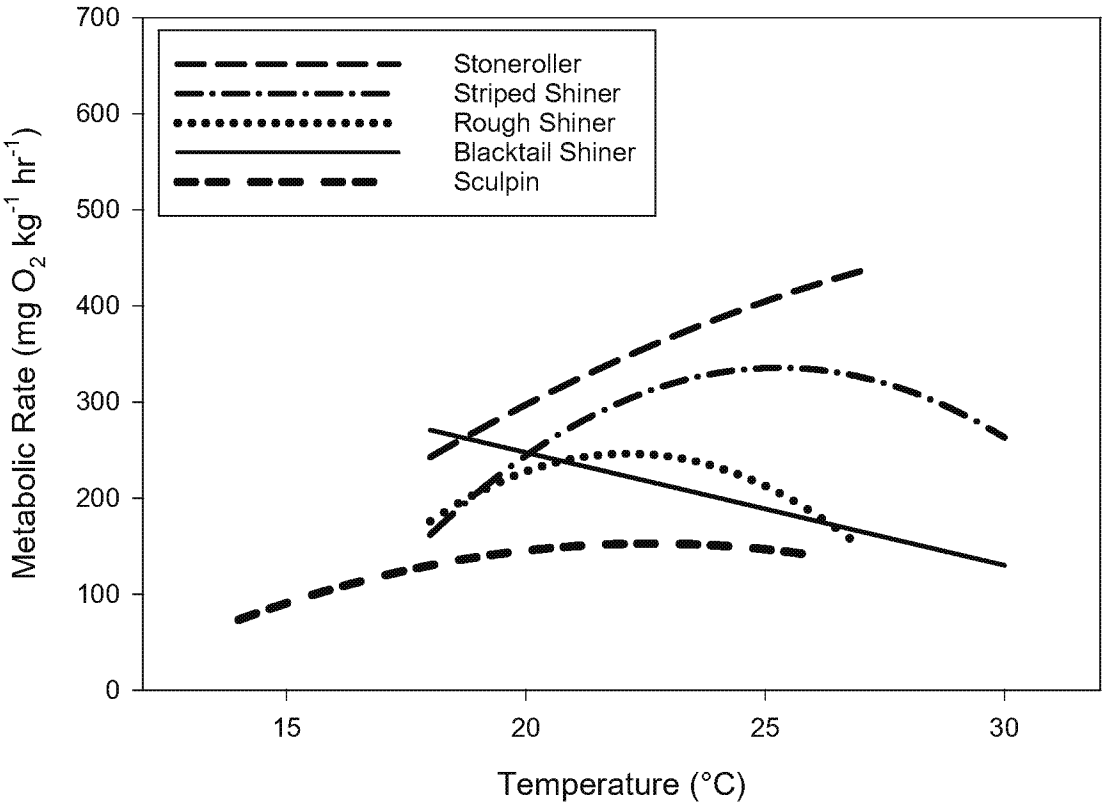

Figure 7. Predicted relationships between metabolic rate and temperature (14-30 °C) for the species tested here.

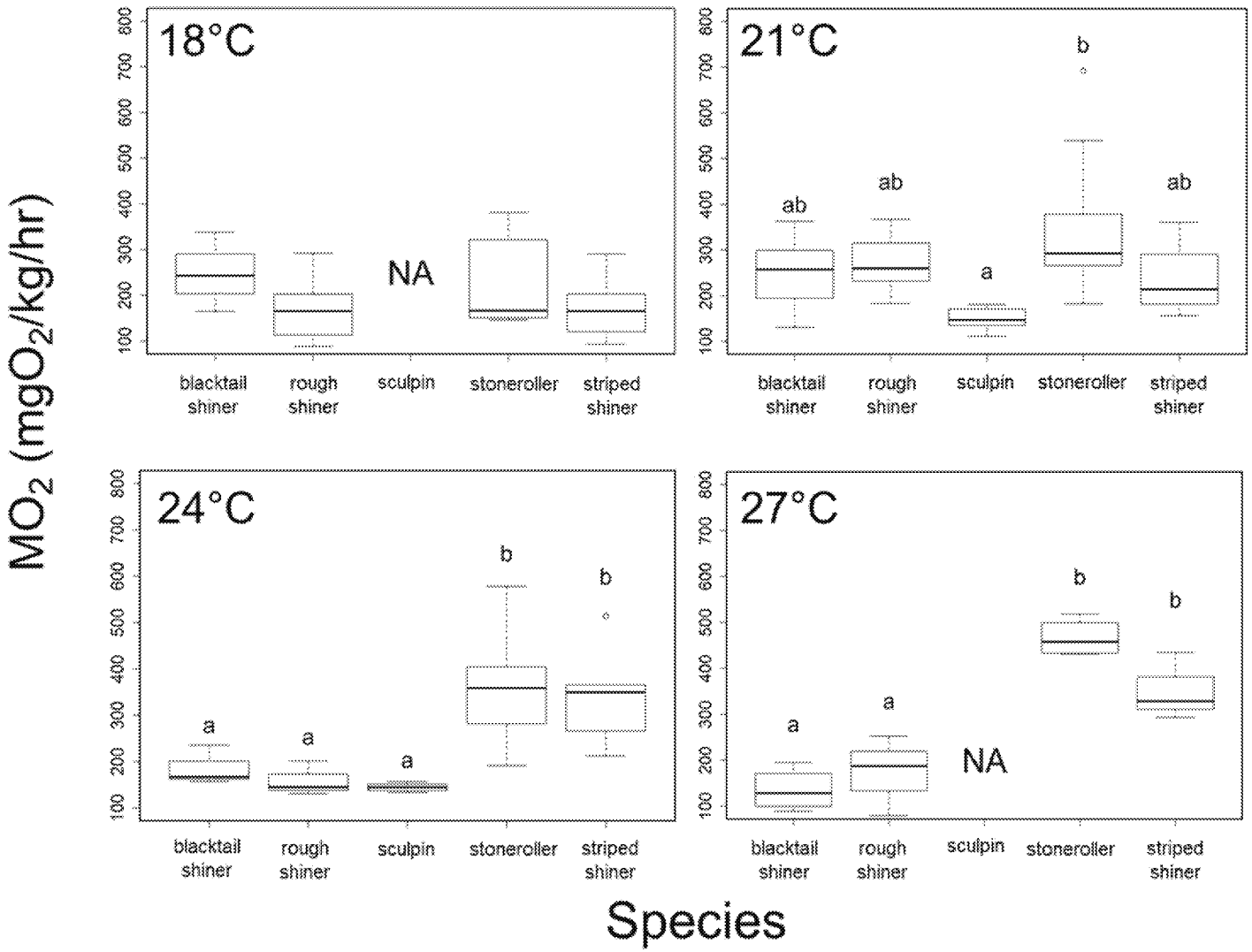

Figure 8. Metabolic rate of five study species compared at temperatures. "NA" denotes data for a species at a temperature was unavailable. Different letters within a panel indicate significantly different metabolic rates between species at that temperature.