Differences in oxygen consumption and critical oxygen levels of five stream fishes

by

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Abstract

Metabolic rate represents an integrated measure of a fish's physiology, particularly as it relates to stressors. A metric that is commonly used to determine the hypoxia tolerance of fishes is the determination of animal oxygen consumption rate (MO_2), which is thought to reflect the ability of an organism to extract oxygen from the environment to maintain routine metabolic rate as dissolved oxygen (DO) decreases. We used respirometry to quantify the influence of two abiotic factors as potential stressors on stream fishes: temperature and dissolved oxygen. We determined standard oxygen consumption rates and the critical oxygen level (DO_{crit}) of 5 fish species, one from the Tennessee River Basin (*Cottus carolinae*) and 4 from the Mobile River Basin (*Percina nigrofasciata, P. palmaris*, *Etheostoma jordani*, and *Cyprinella venusta)*. Combining intermittent flow with static respirometry allowed us to measure oxygen consumption as a function of dissolved oxygen concentration, as well as the DO_{crit}, at three different temperatures (20°, 24°, and 28°C for *E. jordani*, and *C. venusta,* 20°, 22°, and 24°C for *C. carolinae, P. nigrofasciata, and P. palmaris*). Linear and segmented regression analysis was used to determine if the oxygen curves had a DO_{crit}. Oxygen consumption patterns varied among species, *C. venusta* showing typical oxyregulating while the other species exhibited oxyconformation. Respiration increased with temperature for all species except *C. carolinae* which showed a declining trend. DO_{crit} did not change significantly with temperature among species, but generally remained constant with

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temperature. However, *C. carolinae* was again an exception, with the DO_{crit} showing a decline as temperature increased. The results of my study show that current management plans that maintain DO levels above 4 mg $O_2 \cdot L^{-1}$ likely not adequate to satisfy the oxygen demands of all the stream fishes tested. A DO level above 7 mg $O_2 \cdot L^{-1}$ would be required for *C. carolinae* which unlike the other species was not tolerant to low oxygen and temperature > 20°C. Although the minnow and darters were tolerant to low DO, downstream regulation of DO should also include periods of non-generation as well, as this could result in rapid declines of DO and increased temperatures well below the tolerances of these stream species.

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Introduction:

Metabolic rate represents an integrated measure of a fish's physiology, particularly as it relates to stressors. Abiotic factors such as water temperature and dissolved oxygen concentration are important factors affecting metabolic rates in teleost fishes (Fry 1971; Wooton 1991), and as a result, it is important to recognize how alterations of these environmental factors can alter the metabolic processes of the fishes. If metabolic rate falls below a minimum level, the fish will die. Alternately there exists a maximum sustainable rate of metabolism that the fish can only exceed by accruing an oxygen debt (Wootton 1999).

A metric that is commonly used to determine the hypoxia tolerance of fishes is the oxygen consumption (MO_2), which is thought to reflect the ability of an organism to extract oxygen from the environment to maintain routine metabolic rate as dissolved oxygen (DO) decreases (Tipathi et al. 2013). It is generally assumed that most fish fit into one of two categories of oxygen consumption: oxyregulators (Fry and Hart 1948; Beamish 1964c; Ultsch et al. 1978; Cech et al. 1979; Barnes and Carter 2011) or oxyconformers (Barnes and Carter 2011; Urbina et al. 2012; Tiffany et al. 2012; Tripathi et al. 2013). Fish that are able to maintain a constant MO_2 across a wide range of dissolved oxygen (DO) levels are called oxyregulators (Fry and Hart 1948; Beamish 1964c; Ultsch et al. 1978; Cech et al. 1979; Barnes and Carter 2011). Others reduce their oxygen consumption as DO decreases and are called oxyconformers (Pörtner et al. 1985; Tiffany et al. 2012; Urbina et al. 2012; Tripathi et al. 2013). Pörtner and Grieshaber (1993) divide oxyconformers into two separate types: 1) Oxyconforming with anaerobiosis (Figure 1a). This is defined by the failure of the oxygen transport system to

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the mitochondria (i.e., the transition from aerobic respiration to anaerobic respiration). The DO concentration at which the switch from aerobic to anaerobic respiration occurs is called the critical oxygen level (DO_{crit}). 2) Oxyconforming without anaerobiosis (Figure 1b). This is represented by the inability of an animal or tissue to maintain a constant rate of oxygen consumption. As a result oxygen consumption declines with declining DO, but the animals rely on anaerobiosis only at very low DO levels. Thus, only these aerobic oxyconformers should be considered as oxyconformers.

A low DO_{crit} is associated with greater hypoxia tolerance presumably because of improved oxygen uptake and transport to tissues at low water oxygen. Consequently, DO_{crit} has been employed routinely as an important measure of hypoxia tolerance in aquatic organisms including fishes (Scott et al. 2008; Speers-Roesch et al. 2012). In many studies, the presence/absence of a DO_{crit} is used to distinguish an oxyregulator from an oxyconformer (Tiffany et al. 2010; Barnes and Carter 2011; Urbina et al 2012). Thus, researchers often define DO_{crit} as the lowest DO level at which a fish can continue to maintain a constant MO_2. Below DO_{crit}, the fish ceases to oxyregulate and MO_2 decreases with decreasing DO (Fry and Hart 1948; Beamish 1964c; Ultsch et al. 1978; Cech et al. 1979; Urbina et al. 2012). However, Pörtner and Grieshaber (1993) demonstrated that a DO_{crit} is also exhibited by aerobic oxyconformers. As such, the DO_{crit} can also be used as an indicator of hypoxia tolerance in oxyconformers as it indicates the point at which aerobic oxyconformers transition to anaerobic oxyconformation.

Hypoxia tolerance is generally well studied in fishes that are recreationally (i.e., sport-fishes) and economically important (i.e., aquaculture), and as such provide a

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valuable source of information for management purposes (Cech et al. 1979; Redpath et al. 1983; Rice and Cochran 1984). For instance, the critical oxygen tensions and incipient lethal levels (ILL; the value of the factor beyond which the fish cannot survive) of Largemouth Bass, are used as a reference in the development and implimentation of hydroelectic dam operation protocols regarding the miminum dissolved oxygen reguirements (USFWS 2012). We know a great deal about the abiotic and biotic requirements of many sport fishes (e.g., Beamish 1964b; Redpath et al. 1983; Rice and Cochran 1984; Evans 1984), yet there is a lack of data relative to non-sport fishes. While many of the non-sport fishes do not have recreational or economic significance, they are ecologically important in many different ways. For example, many non-sport fishes are important hosts for freshwater mussel larvae. As obligate ectoparasites, mussel larvae (glochidia) development and dispersal often relies on a very specific and narrow range of host-fishes. Therefore, survival and upstream dispersal of mussels is dependent upon the presence of specific host-fishes (Barnhart et al. 2008). Appropriate fish hosts may not be present if DO levels fall below DO_{crit}. Additionally, many tachytictic mussels release conglutinates during warmer months (>25° C) when fish have higher respiratory demands and are likely more vulnerable to stress caused by variations in water temperature. Because most of these fishes are not well studied, relatively little is known about their habitat requirements, particularly as influenced by anthropogenic alterations, or their respective responses to temperature change and hypoxia. Alteration of environmental conditions, such as temperature and reduced DO, as might occur from the presence of impoundments, can greatly influence environmental stress on host fishes and is often the limiting factor determining survival of both mussels and fishes (Moss and Scott 1961;

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Fry 1971; Clarke and Johnston 1999). As such, the susceptibility of non-sport fishes to warming temperatures and decreasing dissolved oxygen needs to be characterized.

In this study I measure oxygen consumption rates and determine DO_{crit} of five common mussel host fishes across a range of temperatures and DO levels that are experienced during the summer and during periods of altered flow (hydroelectric generation vs. non generation periods). I use the relationship between oxygen consumption rate and ambient DO to categorize each species as either an oxyregulator or an aerobic oxyconformer, and to quantify the oxygen demand of each species above DO_{crit}. Furthermore, I utilize the observed DO_{crit} for each species to compare relative sensitivity to hypoxic conditions among species as temperature rises.

Materials and Methods:

Specimen Collection

Fish from the Tennessee River basin were collected from 2 sites during August 2012 and June 2013: the Little Paint Rock Creek and Yellow Branch Creek in Jackson County, AL. Fishes from the Mobile River basin were collected from 6 sites during January, March, and April of 2013: Choccolocco Creek in Talledega County, AL , Hatchet Creek in Coosa County, Hillabee Creek in Tallapoosa County, AL, Little Cahaba River and Oakmulgee Creek in Dallas County, AL, and Saugahatchee Creek in Lee County, AL. Collections were made by a 2 or 3 person crew and covered a distance of approximately 100 m downstream and 100 m upstream. To minimize the amount of disturbed sediment, each section was sampled while moving upstream. Fishes were collected with seines and nets in one pass along the combined 200 m using a backpack electroshocker (Electrofisher, Smith-Root, Inc., Vancouver WA) for 45-60 minutes.

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Fishes were held in aerated coolers and transported to a wet lab facility at Auburn University's E.W. Shell Fisheries Center. Additional fishes were provided by the Alabama Department of Conservation and Natural Resources Aquatic Biodiversity Center in Marion, Alabama in January and July of 2012 and May and August of 2013.

Experimental Design

Prior to experiments, fish were held at temperatures similar to those observed in creeks and rivers in Alabama during the summer (approximately 20°- 30° C) and that also represent the summer temperature range typically experienced by the host fishes in the Tennessee and Mobile River Basins during summer. At the project's initial start, an additional 10 Bronze Darters (not included in the eventual data set) were tested at 25 °C. All fish had acclimated to the temperature; however, when they were subjected to the respirometry measurement, respiration plummeted during the first cycle and did not recover for any of the following cycles. This indicated that the Bronze Darter had possibly reached an upper ILL and could not tolerate any additional stressor at this temperature. In addition, 12 Banded Sculpin (not included in the eventual data set) stopped eating when held at 25°C and thus could not be sufficiently acclimated prior to testing. As a result, Bronze Darter and Banded Sculpin were tested at 20°, 22°, and 24°C. Because of the small sample size and difficultly collecting Blackbanded Darter, these fish were also tested at 20°, 22°, and 24°C in an effort to reduce the likely hood of testing fish above a temperature that might be an upper ILL. Greenbreast Darter and Blacktail Shiner were tested at 20°, 24°, and 28°C. Greenbreast Darters were also acclimated at 25°C initially, and since they showed no signs of distress oxygen consumption data at this temperature and included in the eventual dataset. I conducted experiments on one species

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from the Tennessee River Basin: Banded Sculpin, *Cottus carolinae* (*N*=29). Four species from the Mobile River Basin were run: Blackbanded Darter, *Percina nigrofasciata* (*N*=16), Bronze Darter, *Percina palmaris* (*N*=19), Greenbreast Darter, *Etheostoma jordani* (*n*=22), and Blacktail Shiner, *Cyprinella venusta* (*n*=26). Fish were acclimated for at least one week to treatment temperatures in 113-L tanks. Water temperature was maintained ± 1°C with a combination of an aquarium heater and flow-through water chiller (1/3 hp Aqua Logic ® Delta Star® Research Chiller). During acclimation, fish were fed larval chironomids and *Chaoborus* until 24- 48 h prior to initiation of experiments, allowing for complete gut evacuation (Beamish 1964a). A 12L: 12D photoperiod was maintained throughout acclimation and testing.

After the acclimation period, individual fish were placed in one of four appropriately sized acrylic respirometry chambers submersed within a 400-L polyethylene tank. Chamber volume ranged from 20 – 100 ml with an additional tubing volume of 30 ml. Chambers were selected and fitted to the individual fish (1:10 ratio between wet weight of fish and volume of respirometer; Loligo Systems), limiting movement and reducing water volume without inducing additional stress on the fish. Air bubbles were removed and the chambers were sealed with threaded endcaps. A fishless control chamber was used to measure background oxygen change. All chambers were visually shielded from one another with black acrylic dividers to reduce visual stress. Fish were allowed to acclimate undisturbed in the chamber for 1 hour prior to measuring oxygen consumption. Oxygen electrodes (Strathkelvin Instruments, model 1302) were calibrated with fully aerated water (maximum DO) and a saturated sodium sulphite solution (DO = 0) at the treatment temperature before each experiment.

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Oxygen Consumption

To determine standard metabolism, I quantified oxygen consumption using an intermittent flow respirometry system. Chambers were plumbed so that water could be continually re-circulated through the chamber via tubing and a submersible pump (Elheim Universal, model 1046) as a closed system. Dissolved oxygen in each chamber was monitored via an oxygen meter fitted in a flow cell (Strathkelvin Instruments, model FC100) placed between the chamber and submersible pump. When the fish had reduced DO by1 to 2 $mg \cdot L^{-1}$ from the initial concentration, or 5-10 minutes had passed, the chamber was converted to an open chamber by opening flow valves and fresh oxygenated water flushed the O_2-depleted water from the chamber. This process was repeated 2 times to obtain two consecutive estimates of oxygen consumption rates for each individual fish. Measurements of O_2 consumption were calculated from the formula:

$$MO_2 = (CO_{2(a)} - CO_{2(b)})V \cdot T^{-1} \cdot B_w^{-1}$$

where MO_2 is the oxygen consumption rate ($mg\ O_2 \cdot g^{-1} \cdot hour^{-1}$), $CO_{2(a)}$ is oxygen concentration in water at the start of the measurement period ($mg \cdot L^{-1}$), $CO_{2(b)}$ is the O_2 concentration at the end of the measurement period ($mg \cdot L^{-1}$), V is the volume of the chamber (L), T is the time elapsed during the measurement period (hour), and B_w is the weight of the fish (Cech 1990).

Critical Oxygen Level

Critical O_2 levels (DO_{crit}) were measured with a final O_2 depletion cycle following the routine MO_2 measurements as described above. The flow of O_2 saturated water was stopped, and dissolved oxygen was measured as described for the routine measurements; however, in this final run, each fish was allowed to deplete the O_2 in its

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chamber to < 1 $mg \cdot L^{-1}$ or to the point where fish began showing obvious signs of distress (e.g., loss of equilibrium). After each trial, all fish were measured (TL nearest mm), and weighed (nearest g). Ammonia levels within each chamber were measured to assess accumulation of metabolic wastes, which may have affected respiration.

Statistical Analysis

To determine DO_{crit}, individual data points for each fish's MO_2 at a given temperature were plotted against the DO level at which they were obtained. The individual data points were also averaged across all individual fish within bins of 0.25 $mg \cdot L^{-1}$and plotted against DO values. Both the values for each individual fish and the pooled averages were used to calculate the DO_{crit}. Individual data and pooled averages were tested with linear regression and segmented linear regression (2 and 3 segment) analysis (R Studio; R Core Team) to determine the best fit for the data. This analysis permitted assessment of whether the data were best fit with a linear relationship (i.e. oxyconforming) or whether a DO_{crit} could be determined at an intersection point of two distinct linear relationships. The best fit was determined by using the Akaike's Information Criterion (AIC). While models of the pooled averages and the population data points produced similar estimates to the models of individual fish, the models from the individual fish data were used to calculate respiration estimates for a species, in an effort to incorporate all individual variation. If a DO_{crit} could be calculated, the significance of the mean of the individual slopes above DO_{crit} was determined by a t test to indicate if the MO_2 was constant (insignificant from 0; i.e. oxyregulating) or declining (significantly different from 0; i.e. oxyconforming). The predicted respiration (MO_2) at DO = 5 $mg \cdot L^{-1}$ (P_{MO}) for each individual fish's model was used to determine the effect of

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temperature on respiration. This DO level was selected because it did not rely on fish having a standard metabolic rate (SMR), as is the case with oxyregulators and not with oxyconformers, and was generally between the DO_{crit} and DO at maximum respiration for each species. Banded Sculpin was the only exception where the effect of temperature on respiration was not compared using the predicted MO_2 at DO = 5 $mg \cdot L^{-1}$, due to the diverse patterns of oxygen consumption curves that occurred. Analysis of variance (ANOVA) was used to determine if intraspecific fish MO_2 and DO_{crit} were affected by temperature. All assumptions of normality and homogeneity of variance of residuals were tested and met. Tukey's honestly significant difference (HSD) post hoc test was used to determine differences among specific temperature treatments. Values were considered significant at $\alpha \leq 0.05$.

Results:

Fish generally remained stationary throughout testing and showed little signs of stress, such as loss of equilibrium or increased movement, until reaching the lowest DO levels tested. At the end of all experiments, ammonia concentrations were ≤ 0.5 ppm. Bronze Darter, Greenbreast Darter, Blackbanded Darter, and Banded Sculpin showed the least movement (little to none) during testing, while the Blacktail Shiner occasionally exhibited loss of equilibrium at normoxic DO levels. Individuals that moved continuously or demonstrated signs of stress throughout the experiment (e.g., entire range of DO) were removed from the data set.

The five species that I tested each had a uniquely differing respiration curves that fit both oxyregulation and oxyconformation. A comparison of the first two respirometry cycles with the third and final cycle showed a consistent trend of respiration at normoxia

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for each fish, thus indicating there was no stress response to the intermittent flushing cycles. The best fit regression for all species was either a two-segment or three-segment regression. The occurrence of a threshold (intersect of two regressions) indicated that each species exhibited a critical DO level (DO_{crit}).

Blacktail Shiner

Twenty-six individuals were tested (n =10 at 20°; n =9 at 24°; n =7 at 28°C) with mass ranging from 1.55 to 7.50 g. The best fitting model for both the pooled averages (i.e., means calculated across all individuals within 0.25 $mg \cdot L^{-1}$ DO bins) and the population data points (i.e., all data points across all individuals within a temperature) (Figure 2a) was a two-segment regression (Table 1; Figure 2b). The two-segment regression was also the best fitting model for each individual fish (Table 2; Figure 2c). The slopes above DO_{crit} for each model fitted to the pooled averages (Figure 2b) appeared similar to the means of the individual fish (Figure 2c), and the means of the individual fishes (including all variation) did not differ significantly from 0 (all $p > 0.08$). As such, the Blacktail Shiner would be considered an oxyregulator with a measurable standard metabolic rate at each temperature (Table 3).

The temperature x mass interaction was not significant for either respiration (MO_2) or the critical DO level (DO_{crit}). MO_2 significantly increased with temperature ($p < 0.001$) but was not affected by mass ($p > 0.08$). Among temperatures, the P_{MO} (Table 3) was significantly higher at 28°C than 20° and 24° (ANOVA: $F_{2, 23} = 6.776$, $p < 0.005$; Figure 3). There was no effect of temperature or mass for DO_{crit}. DO_{crit} at 20°, 24°, and 28°C was 2.44 ± 0.55, 2.14 ± 0.41, and 2.49 ± 0.56 mg $O_2 \cdot L^{-1}$, respectively.

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Bronze Darter

Nineteen individuals were tested (n =4 at 20°; n =10 at 22°; and n =5 at 24°C) with mass ranging from 0.54 to 5.01 g. The best fitting model for both the pooled averages and the population data points (Figure 4a) was the two-segment regression (Table 4; Figure 4b). The two-segment regression was also the best fitting model for the individual fish (Table 2; Figure 4c). The slopes above DO_{crit} for each model fitted to the pooled averages (Figure 4b) appeared similar to the mean of the slopes of the individual fish (Figure 4c). All slopes were significantly greater than 0. As such Bronze Darter would be considered an oxyconformer.

There was a significant temperature x mass interaction on respiration ($p < 0.002$) with P_{MO} increasing with mass at 20°C while at 24°C MO_2 decreased as mass increased (Figure 5). Individual differences between temperatures showed that P_{MO} (Table 3) was significantly higher at 24°C than at 20°C (ANOVA: $F_{2,16} = 4.083, p < 0.04$; Figure 6). The temperature x mass interaction for DO_{crit} was not significant, but DO_{crit} differed between 22° and 24°C (ANOVA: $F_{2,16} = 4.19, p < 0.04$; Figure 7). DO_{crit} at 20°, 22°, and 24°C was 2.26 ± 0.71, 3.39 ± 1.09, and 2.00 ± 0.88 mg $O_2 \cdot L^{-1}$, respectively. There was no effect of mass on DO_{crit}.

Greenbreast Darter

Twenty-two individuals were tested (n =5 at 20°; n =8 at 24°; n =5 at 25°; n =4 at 28°C) with mass ranging from 1.55 to 7.50 g. The best fitting model for both the pooled averages and the population data points (Figure 8a) was the three-segment regression (Table 5; Figure 8b). This indicated an upper threshold (DO_{UT}) that occurred at normoxic DO levels. The three-segment regression was also the most frequent best fit

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among individual fish (Table 2; Figure 8c). The slopes above DO_{crit} and below the upper threshold for each model fitted to the pooled averages (Figure 8b) appeared similar to the means of the slopes for the individual fish (Figure 8c). All slopes were significantly different from 0 except for the slope of the pooled averages model and the mean value of individual fish model at 28°C ($p > 0.05$). As such, including the increased respiration above the upper threshold, Greenbreast Darter would be considered an oxyconformer.

There was no significant interaction of temperature x mass for either respiration, DO_{crit}, or DO_{UT}. Temperature significantly affected MO_2 ($p < 0.003$) while mass did not ($p > 0.54$). P_{MO} (Table 3) increased significantly from 20° to 24°C and remained elevated for the treatments at 25° and 28°C (ANOVA: $F_{3,18} = 12.36$, $p < 0.001$; Figure 9). There was no effect of temperature or mass on DO_{crit} or DO_{UT}. DO_{crit} at 20°, 24°, 25°, and 28°C was 1.99 ± 0.71, 2.41 ± 0.95, 2.16 ± 0.20, and 2.59 ± 1.17 mg $O_2 \cdot L^{-1}$, respectively.

Blackbanded Darter

Sixteen individuals were tested (n =5 at 20°C; n =5 at 22°C; and n =6 at 24°C) with mass ranging from 0.54 to 5.01 g. The best fitting models for both the pooled averages and the population data points (Figure 10a) were the two-segment (at 24°C) and three-segment regressions (at 20° and 22°C) (Table 6; Figure 10b). The two and three-segment regressions were also the best fitting models for the individual fish (Table 2; Figure 10c). Although the two and three-segment models were almost evenly distributed among individuals at 20°C (2:3) and 22°C (3:2), the most prevalent model at 24°C (5:1) was the two-segment regression. The slopes above DO_{crit} and below the upper threshold (when present) for each model fitted to the pooled averages (Figure 10b) appeared similar

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to the means of the slopes for the individual fish (Figure 10c). All slopes were significantly different from 0 except the mean of the individual fish at 22°C (Figure 10c). As such, including the increased respiration above the upper threshold, Greenbreast Darter would be considered an oxyconformer.

The temperature x mass interaction was not significant for respiration, DO_{crit}, or DO_{UT}. Both temperature ($p < 0.02$) and mass ($p < 0.05$) significantly affected MO_2. The P_{MO} (Table 3) at 24°C was higher than 20° and 22°C (Figure 11) and decreased as mass increased (Figure 12). There was no effect of temperature or mass on DO_{crit} or the DO_{UT}. DO_{crit} at 20°, 22°, and 24°C was 2.63 ± 0.69, 2.78 ± 0.61, and 3.05 ± 0.74 mg $O_2 \cdot L^{-1}$, respectively.

Banded Sculpin

Twenty-nine individuals were tested (n =11 at 20°C; n =10 at 22°C; and n =8 at 24°C) with mass ranging from 0.89 to 3.01 g. For this species, oxygen consumption curves varied distinctively across temperatures. As a result, model selection for the pooled averages and population data points (Figure 13) also had a large degree of variation. The best fitting model at 20°C for the pooled averages was a two-segment regression (Table 7; Figure 13b) while a linear regression best fit the population data points (Table 7; Figure 13c). The best fit model for both the pooled averages and population data points at 22°C was a two-segment regression (Table 7, Figure 13b,c). The best fit model for both the pooled averages and population data points at 24°C was the three-segment regression (Table 7; Figure 13b,c). Model selection for the individual fish (Table 2) did not reflect the model selection for the pooled averages or the population data points (Table 7). Individual fish curves demonstrated two patterns within

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each temperature that were best fit with a two-segment regression (pattern 1[P1]; Figure 14a) and a three-segment regression (pattern 2 [P2]; Figure 14b). While Blackbanded Darter also exhibited model selection for both the two and three-segment regressions, it did not have significant variation between DO_{crit} and P_{MO} across temperature as exhibited by Banded Sculpin. Four fish were sexed under a dissecting microscope after the experiment at 20°C to determine if either pattern (P1 or P2) was specific to a sex. Of the four fish, a male and female fell under the P1 and two females fell under the P2. Thus sex was not clearly linked to either pattern. The respiration of P1 fish, typically fitted with a two-segment regression, increased rapidly as DO decreased from normoxic levels to an asymptote that was quickly followed by a linear decline (Figure 14b). Only at 24°C did the two-segment regression not show the elevated respiration at higher DO levels. The respiration of P2 fish, fitted with a three-segment regression, was similar to the P1 fish; however, the rapid increase in respiration with decreasing DO was delayed until DO decreased from normoxic levels to mid range DO levels (Figure 14c). Thus, the DO occurring at the asymptote of MO_2 (MO_{2A}), or the "hump", is the DO_{crit} in the P1 and P2 models. All but four fish had a calculable DO_{crit}. Data from these four fish were fitted with a linear regression. While comparisons and estimates of DO_{crit} could not be calculated for these four individuals, each model showed a similar rate of decline below DO_{crit} to that of the P1 models. Due to the varying occurrence of DO_{crit} across temperatures, the calculation of a predicted MO_2 occurring above and below any threshold at a given DO was not possible. Thus the MO_{2A} was used to compare respiration with temperature and mass instead of P_{MO} (Table 8). Analysis of P1 and P2 models was done separately to prevent the individual variation of fish from being masked

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as the population means were. In both patterns, the inability to maintain a constant rate of MO_2 would indicate Banded Sculpin is an oxyconformer at these temperatures.

In the P1 fish, all fish tested at 20°C were fit with the two-segment model, while 2 of 4 fish tested at 22°C and 2 of 5 fish tested at 24°C were fit best by a linear regression (i.e., oxyconforming). There was no temperature x mass interaction effect on respiration or DO_{crit}. Temperature significantly affected MO_{2A} ($p < 0.001$) and DO_{crit} ($p < 0.001$). There was a significant decline of MO_{2A} (Table 8) from 20° to 24°C (ANOVA: $F_{2,10} = 17.01$, $p < 0.001$; Figure 15). The DO_{crit} also declined significantly as temperature increased from 22° to 24°C (ANOVA: $F_{2,10} = 35.82$, $p < 0.001$; Figure 16). DO_{crit} at 20°, 22°, and 24°C was 6.56 ± 1.03, 6.93 ± 0.28, and 2.18 ± 0.67 mg $O_2 \cdot L^{-1}$, respectively. Neither MO_{2A} nor DO_{crit} were affected by mass as a main effect.

In the P2 fish, the temperature x mass interaction was not significant for MO_{2A}, DO_{crit}, or DO_{UT}. Neither temperature nor mass significantly affect MO_{2A} (ANOVA: $F_{2,9} = 3.919$, $p = 0.059$); however, the decrease of MO_{2A} from 20° to 22°C was significant (adjusted $p = 0.05$; Figure 17). Neither DO_{crit} nor DO_{UT} were affected by temperature or mass as main effects. DO_{crit} at 20°, 22°, and 24°C was 4.99 ± 1.81, 4.05 ± 0.95, and 4.27 ± 1.49 mg $O_2 \cdot L^{-1}$, respectively.

Discussion

Standard Oxygen Consumption

Because the standard metabolic rate (SMR) is defined as the lowest rate of oxygen consumption for oxyregulators (i.e., MO_2 is constant) (Krogh 1914; Fry and Hart 1948; Beamish and Mookherjii 1964), only Blacktail Shinerhad a measurable SMR predicated on the basis it was the only fish that exhibited a constant MO_2. Furthermore,

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while Blacktail Shiner remained an oxyregulator as temperature increased, there was a significant increase in SMR as temperature increased.

Relative to the darter species, the most obvious pattern they shared was the conformation above DO_{crit}. This was consistent across temperatures, and in most cases the degree of conformation (i.e., the slope of MO_2 versus DO) tended to increase with temperature. This was true for species best fit with a two-segment model (Bronze Darter) and the species best fit with a three-segment model (Greenbreast and Blackbanded Darter). Of the darters fitted with a three-segment model, respiration rate increased significantly at normoxic levels above the upper threshold. Studies of invertebrates such as the lugworm, *Arenicola marina,* (Schöttler et al. 1983) and bristle worm, *Scolopos armiger,* (Schöttler et al. 1988) have shown these two oxyconformers have two critical PO_2 values. However, for these "true" oxyconformers, the lower critical PO_2 indicated the shift to anaerobiosis while the upper critical PO_2 indicated a transition from oxyregulation to oxyconformation. In the case of Greenbreast and Blackbanded Darter, the MO_2 above the upper threshold was not constant, and therefore neither species was oxyregulating above the threshold.

With regards to the oxygen consumption curves of the Banded Sculpin, there existed both interspecies and intraspecies variation at all temperatures tested. The P1 and P2 fish illustrated a significant increase of MO_2 as the DO_{crit} was reached, followed by a rapid decline of MO_2 below the DO_{crit}. The difference between the two patterns was distinguished by the delayed response to lowering DO levels. While this phenomenon was significantly affected by temperature, neither MO_2 nor DO_{crit} increased as temperature increased. Instead, there was a steady decline in all parameters as

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temperature increased suggesting the fish had reached (and possibly surpassed) the plateau of their metabolic ILL, resulting in a significant decline in MO_2. One likely explanation is the P1 fish experience a transition to anaerobiosis at a normoxic DO_{crit} while the P2 fish experience the transition to anaerobiosis at a DO_{crit} within approximately 50 to 80% of normoxic levels. Because the P1 fish experienced a more rapid transition to anaerobiosis at a higher DO_{crit}, they would also be more likely to shift to a completely linear or "true" oxyconformation for the entire range of DO levels as the upper ILL of temperature is approached. While it would be impossible to test this hypothesis without the use of calorimetry or metabolite testing, we can observe this trend as temperature increases in the P1 fish. As DO declined from normoxia at 20°C, 6 of 7 fish had an immediate increase in MO_2 leading to a DO_{crit} at relatively high DO levels. The remaining fish had an elevated MO_2 at normoxia that was equivalent to the peak MO_2 of the other six fish; yet it was not a "true" linear conformer. As DO declined at 22° and 24°C, 50% (2 of 4) and 40% (2 of 5), respectively, of fish were linearly oxyconforming at normoxic levels. Clearly there is an increased occurrence of linear or "true" oxyconformation at normoxic levels at these higher two temperatures. Furthermore, of the three fish tested at 24°C that were not "true" conformers at normoxia, the MO_2 no longer increased, or peaked, exhibiting a DO_{crit} at higher DO levels, but instead exhibited a DO_{crit} that was significantly lower than fish ran at 20° and 22°C, shifting from 6.93 + 0.28 to 2.18 ± 0.67 mg $O_2 \cdot L^{-1}$ indicating an overall decline in metabolism.

While there are many factors that affect SMR and the DO_{crit} (e.g., temperature acclimation, state of nutrition, state of development, stress, and activity; Herreid 1980) I

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careful controlled each of these factors to reduce the potential for these effects from influencing my measurements. There is an additional effect of pre- and post-spawning condition that could have also caused an increase in metabolism resulting in oxyconformation. Spawning temperatures for the darter species range from 13° to 29°C and last from mid-March to late June, depending on latitude, (Boschung and Mayden 2004) during which many of our data were collected. It is possible that oxyconformation in these darters was due to elevated metabolic requirements of reproduction at the temperatures tested. However, with the Banded Sculpin, spawning occurs at a much cooler temperature, around 14°C, during mid-January to mid-March (Boschung and Mayden 2004). As such, it is unlikely the oxygen consumption curves of this fish were related to pre or post-spawning metabolism. Because the objective of this study was to quantify oxygen consumption and effects of temperatures occurring during summer, it is nonetheless important to recognize that the increased oxygen requirement at these temperatures is leading to oxyconformation, regardless of whether it is influenced by reproductive response to temperature.

Another possible influence that is debated within the literature is the use of closed versus open respirometry (Steffensen 1989). In closed respirometry an excess of metabolic wastes and CO_2 could potentially affect respiration (Cech 1990). However, a comprehensive review of literature studying the effects of acidification on aquatic organisms by Ishimatsu et al. (2008) showed there was no effect of CO_2 accumulation on fish respiration. Furthermore, Urbina et al. (2012) showed that measurements of respiration of the Inanga, *Galaxis maculatus,* taken by closed respirometry and intermittent respirometry were not significantly different. In this experiment, the use of

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intermittent respirometry in the first two cycles, prior to the third closed cycle, allowed for the flushing and removal of metabolic wastes accumulated while the fish acclimated to the chamber. The measured ammonia at the end of each run in this study was ≤ 0.5 ppm, which is below total ammonia levels generally considered to be safe for coldwater species such as salmonids (Stickney and Hohler 1990). In this regard it would be unlikely that the oxygen consumption rates were affected by the procedures of this experiment.

Critical DO Level

Many studies define DO_{crit} as the lowest DO level that a fish can maintain its MO_2, which is the point where the fish ceases to oxyregulate (Fry and Hart 1948; Beamish 1964c; Ultsch et al. 1978; Cech et al. 1979; Urbina et al. 2012). Typically, the presence of a DO_{crit} is a strict condition that distinguishes an oxyregulator from an oxyconformer (Tiffany et al. 2010; Barnes and Carter 2011; Urbina et al 2012). However, Portner and Grieshaber (1993) demonstrated that a DO_{crit} occurs in both oxyregulators and oxyconformers. Furthermore DO_{crit} is not only represented by a decrease of MO_2 with decreasing DO but can also be represented by an increase in MO_2 (Castleberry and Cech 1986; Pörtner and Grieshaber 1993). Here, we have shown that out of five fish species living in similar habitats, there was a typical oxyregulator (Blacktail Shiner) and four intermediate oxyconformers (Bronze Darter, Blackbanded Darter, Greenbreast Darter, and Banded Sculpin) that exibit a DO_{crit}.

While there were differences of oxygen curves among species, there was a general increase of DO_{crit} with temperature in most fish; however, temperature did not

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have an overall significant affect on DO_{crit}. This was not expected, as some literature suggests that any factor leading to an increased demand in tissue oxygen (e.g., increased temperature) would also affect the DO_{crit} (Jobling 1995). Cech et al. (1979) found in their study of Largemouth Bass that the DO_{crit} increased significantly as temperature increased. In my study, excluding Banded Sculpin, all other fish species had a low DO_{crit} ranging from 1.99 ± 0.71 to 3.39 ± 1.09 mg $O_2 \cdot L^{-1}$ regardless of temperature tested. The DO_{crit} of Banded Sculpin was significantly higher than all the other species, ranging from 2.18 ± 0.67 to 6.93 ± 0.28 mg $O_2 \cdot L^{-1}$ (P1) and 4.05 ± 0.95 to 4.99 ± 1.81 mg $O_2 \cdot L^{-1}$ (P2). Only at what appears to be the upper ILL of Banded Sculpin did the P1 fish have an equivalent DO_{crit} to the other species.

Mass Relationship

The relationship between mass and respiration was differed among all the species. In general, mass did not show a strong effect on respiration. Only Bronze Darter had a significant effect on respiration, but from the interaction of mass and temperature. While there was an additive effect of mass and temperature on respiration in the remaining species, generally mass was not significantly related to MO_2. Again, this was not expected, as it is typically thought that mass is directly related to metabolism and respiration. Small fish will consume more oxygen than larger conspecifics on a unit-weight basis (Jobling 1995). This allometric relationship is described by Winberg (1956) as

$$VO_2 = 0.307 \cdot W^{0.81}$$

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where VO_2 is the rate of oxygen consumption, W is the weight, and 0.81 is the weight exponent. Thus this equation results in an exponential decline in oxygen consumption per-gram as mass increases. While the species tested in this study generally had a decline of mass specific respiration as mass increased, it was not significant. Furthermore there were instances where the opposite relationship was observed. Such was the case of Bronze Darter which at 20°C the respiration increased as mass increased, but at 24°C respiration decreased as mass increased. Similar to the relationship of mass and MO_2, the relationship between mass and DO_{crit} was not significant in all species; however, there was an additive effect of mass and temperature on the DO_{crit} of the P1 fish of the Banded Sculpin, but this was the only occurrence of a relationship and the mass effect was not significant. Thus there was a large amount of interspecies and intraspecies variation in the relationship between respiration and fish mass.

Management and Research Implications

This study has shown that five stream fish species, often coexisting in similar habitats, have very different oxygen consumption and temperature requirements. Based on a review of the literature, no previous studies have illustrated these types of intermediate oxygen consumption curves in other fishes. These findings have important implications for both management and future research.

Management considerations of the temperature and oxygen requirements of these stream species must be inclusive of all species found within these stream habitats as opposed to only a few well studied species. While the DO_{crit} was generally found at low DO levels (1.99 ± 0.71 - 3.39 ± 1.09 mg $O_2 \cdot L^{-1}$) for the darters and minnow, it was significantly higher for the sculpin. Additionally, the effect of temperature differed

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across species. The Greenbreast Darter, Bronze Darter, Blackbanded Darter, and Banded Sculpin all showed a significant increase in respiration with a 4°C (20°- 24°C) temperature increase while Blacktail Shiner only showed a significant increase of respiration after an increase of 8°C (20°- 28°C). The fish least tolerant to temperature increase was Banded Sculpin which showed a significant decline in respiration when temperatures exceeded only 20°C. However, Bronze Darter was also unable to tolerate temperatures above 24°C. The Greenbreast Darter was an exception in that even after significantly increasing its respiration from 20°- 24°C, it did not show respiratory distress at 25° and 28°C. Concomitantly, Greenbreast Darter also demonstrated an upper respiration threshold at normoxic levels, above which respiration was significantly increased. Whether this increased temperature tolerance is linked with the increased respiration above the upper threshold requires additional research into their physiology, possibly incorporating calorimetry and metabolite analysis to define the point at which the transition from aerobiosis to anaerobiosis occurs. Blackbanded Darter also had an upper threshold at normoxic levels, but fewer individuals exhibited the upper respiratory threshold as temperature increased to 24°C, making it is difficult to compare to Greenbreast Darter, where most individuals exhibited an upper respiratory threshold as temperature increased. It is this variation between species, and even among individuals within species that illuminates the difficulty in management of our aquatic resources. While the precedent for most regulations involving the minimum dissolved oxygen requirement of water released downstream of dams is based on the temperature and oxygen requirements of the Largemouth Bass or other sport fishes, it may not adequately cover the requirements of other ecologically important species. In certain cases where

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the flow from impoundments is reduced, temperatures could quickly exceed the tolerances of these stream fishes while remaining within the limits of Largemouth Bass.

In terms of research implications, the variation of oxygen consumption curves among these similar species and among individuals within species presents a multifaceted problem. First, in dealing with the interspecies differences of oxygen consumption, the traditional placement of organisms into either oxyregulating or oxyconforming categories leaves little room for fish that fall between the groups. Careau et al. (2008) points out that most of the error associated with movement during SMR measurement is actually the individual responses based on coping styles or "personality". Therefore, by removing one coping style, associated with more active individuals, the researcher has biased their data toward the less active individuals. The oxyconforming fish in this study did not fit the completely linear decline as is often represented by a "true" oxyconformers, yet they undoubtedly did not maintain a constant respiration and were thus not oxyregulating. Had the requirements of this study maintained the conditions of strict regulation and conformation, the data set would have been reduced drastically, and would ultimately be biased toward whatever regulators or conformers remained. In the case of Greenbreast and Blackbanded Darters, the rapid increase in MO_2 as the DO approached and surpassed the upper threshold was consistent in all three respirometry cycles indicating MO_2 rose each time DO increased. Had the data points been considered to be outliers, the oxygen consumption curves would look much different, and a different interpretation would be applied. It is possible that this rapid change in respiration at normoxic levels has occurred in other studies but was assumed to be machine error or an artifact of stress due

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to a lack of acclimation, and as such removed from analysis as a presumed outlier (Ultsch et al. 1978).

This exclusion of individual variation that does not fit strict definitions can lead to the second problem of a biased interpretation of data. When measuring SMR and critical DO levels, researchers typically group individuals to quantify shared trends, or population means, in an effort to reduce the noise obscuring the underlying effect. While this may be appropriate much of the time, it is not always the case. In the majority of fish within this study, the population means and the models fitted to the means were similar to the individual fish models, and thus could be used to accurately describe the trends in respiration. However, when the population means of Banded Sculpin were considered it is obvious there are little similarities between comparisons of the individual models and the pooled averages. In fact, if using only the population means and models as descriptive analysis of respiration, the individual trends are completely masked. Certainly, a consistent trend as temperature increased would be impossible to discern from analysis of the population means, much less discernment of two separate patterns of respiration within each treatment.

Furthermore, measuring the SMR at a given DO level, without first confirming the oxygen consumption pattern of a fish can lead to a false estimation of metabolism. Open or flow-through respirometers are often used to measure SMR, of oxyregulators, while maintaining oxygen concentration at a constant level, generally with a difference of 0.5 – 1.0 mg $O_2 \cdot L^{-1}$ between inflowing and outflowing water (Cech 1990). Walsh et al. (1997) measured SMR on several sculpin species, including Banded Sculpin, across temperatures ranging from 15° to 25°C, using flow-through respirometry while carefully

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maintaining DO at normoxic conditions. My results contradict the assumption that Banded Sculpin are oxyregulators at temperatures ≥ 20°C, and thus the measured SMR values given by Walsh et al. (1997) do not appear to accurately describe metabolic rates of this species. Again, it is likely that the assumption of oxyregulation has remained unverified for other studies that used respirometry to calculate SMR.

Conclusion

The results of my study show that current management plans that maintain DO levels above 4 mg $O_2 \cdot L^{-1}$ are likely adequate to satisfy the oxygen demands of Blacktail Shiner, Bronze Darter, Greenbreast Darter, and Blackbanded Darter based on the critical DO level of each species. However, this is not the case with Banded Sculpin, which had a much higher critical DO level than the other four species and thus might require the DO level be maintained above 7 mg $O_2 \cdot L^{-1}$. Temperature should also be carefully considered when setting future oxygen requirements. More precisely, as temperature increased to 24°C there was a significant effect on respiration of each darter and minnow species. It is likely that temperatures at or above 20°C are stressful for Banded Sculpin. Thus, stagnant conditions with higher water temperatures should be avoided by maintaining some flow of water downstream from impoundments. With regards to future research, assumptions of oxyregulation must still be verified before measuring standard metabolic rate, and individual variation within species should be included into interpretation of SMR to avoid masking overall trends.

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ED_005603A_00000028-00040

Table 1. Blacktail Shiner model selection for the pooled averages and population data points based on difference of AIC (AIC_{Δ}) from the best fitting model and the R^2 values.

Pooled averages

	Linear		2-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.67	64.48	0.96	0.00
24	0.69	71.14	0.97	0.00
28	0.64	54.08	0.96	0.00

Population data points

	Linear		2-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.57	171.10	0.74	0.00
24	0.61	198.82	0.82	0.00
28	0.51	96.24	0.72	0.00

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Table 2. The model selection results showing the proportion of individual fish (*n*) from each species, at each temperature, that best fit under the linear, two-segment, and three-segment regressions, as determined by AIC. NA denotes where fish did not fit the model.

Species	temp	*n*	Linear	2-segment	3-segment
Blacktail Shiner	20	10		10	NA
	24	9		9	NA
	28	7		7	NA
Bronze Darter	20	4	1	2	1
	22	10	1	5	4
	24	5	1	4	
Greenbreast Darter	20	5			5
	24	8		2	6
	25	5		2	3
	28	4		1	3
Blackbanded Darter	20	5		3	2
	22	5		2	3
	24	6		5	1
Banded Sculpin	20	11		7	4
	22	10	2	1	7
	24	8	2	3	3

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Table 3. Respiration data (means ± SD) for four fish species at 3-4 temperatures including the standard metabolic rate (SMR), predicted respiration rate (P_{MO}), critical oxygen level (DO_{crit}), and the DO level above the critical oxygen level (DO_{UT}).

Species	Temp (C)	SMR mg $O_2 \cdot g^{-1} \cdot hour^{-1}$	P_{MO} mg $O_2 \cdot g^{-1} \cdot hour^{-1}$	DO_{crit} mg $O_2 \cdot L^{-1}$	DO_{UT} mg $O_2 \cdot L^{-1}$
Blacktail Shiner	20	0.177 ± 0.039	0.176 ± 0.038	2.44 ± 0.55	
	24	0.182 ± 0.021	0.184 ± 0.021	2.14 ± 0.41	
	28	0.226 ± 0.032	0.231 ± 0.033	2.49 ± 0.56	
Bronze Darter	20		0.140 ± 0.027	2.26 ± 0.71	
	22		0.161 ± 0.025	3.39 ± 1.09	
	24		0.193 ± 0.036	2.00 ± 0.88	
Greenbreast Darter	20		0.078 ± 0.016	1.99 ± 0.71	6.97 ± 0.64
	24		0.140 ± 0.024	2.41 ± 0.95	6.55 ± 0.77
	25		0.151 ± 0.025	2.16 ± 0.20	6.96 ± 0.76
	28		0.143 ± 0.016	2.59 ± 1.17	5.87 ± 0.42
Blackbanded Darter	20		0.128 ± 0.023	2.63 ± 0.69	8.09 ± 0.33
	22		0.114 ± 0.016	2.78 ± 0.61	7.24 ± 0.25
	24		0.162 ± 0.023	3.05 ± 0.74	7.48

ED_005603A_00000028-00043

Table 4. Bronze Darter model selections for the pooled averages and population data points based on difference of AIC (AIC_{Δ}) from the best fitting model and the R^2 values. NA denotes where model would not statistically fit.

Pooled averages

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.79	7.85	0.86	0.00	0.86	4.06
22	0.88	24.08	0.95	0.00	NA	NA
24	0.89	19.68	0.95	0.00	NA	NA

Population data points

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.53	16.51	0.58	0.00	NA	NA
22	0.70	116.11	0.75	0.00	NA	NA
24	0.69	27.34	0.74	0.00	NA	NA

ED_005603A_00000028-00044

Table 5. Greenbreast Darter model selection for the pooled averages and population data points based on difference of AIC (AIC_{Δ}) from the best fitting model and the R^2 values.

Pooled averages

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.815	35.73	0.866	29.35	0.949	0.00
24	0.954	10.83	0.969	3.11	0.976	0.00
25	0.899	61.56	0.967	29.48	0.988	0.00
28	0.961	23.59	0.965	23.29	0.987	0.00

Population Data points

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.581	56.59	0.637	24.57	0.676	0.00
24	0.660	10.11	0.671	2.13	0.677	0.00
25	0.737	104.96	0.799	21.39	0.814	0.00
28	0.862	16.72	0.877	5.87	0.886	0.00

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Table 6. Blackbanded Darter model selection for the pooled averages and population data points based on difference of AIC (AIC_{Δ}) from the best fitting model and the R^2 values.

Pooled averages

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.72	55.12	0.91	20.50	0.96	0.00
22	0.82	72.03	0.90	57.77	0.99	0.00
24	0.88	43.94	0.98	0.00	0.98	2.71

Population data points

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.67	121.51	0.84	10.11	0.85	0.00
22	0.72	70.62	0.80	20.58	0.83	0.00
24	0.80	67.43	0.86	0.00	NA	NA

ED_005603A_00000028-00046

Table 7. Banded Sculpin model selections for the pooled averages and population data points based on difference of AIC (AIC_{Δ}) from the best fitting model and the R^2 values. NA denotes where model would not statistically fit.

Pooled averages

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.42	65.17	0.92	0.00	NA	NA
22	0.57	28.03	0.83	0.00	NA	NA
24	0.83	47.70	0.95	10.93	0.97	0.00

Population data points

	Linear		2-segment		3-segment	
temp	R^2	AIC_{Δ}	R^2	AIC_{Δ}	R^2	AIC_{Δ}
20	0.42	0.00	NA	NA	NA	NA
22	0.33	48.20	0.41	0.00	NA	NA
24	0.59	60.13	0.67	2.10	0.68	0.00

ED_005603A_00000028-00047

Table 8. Banded Sculpin respiration data (means ± SD) at each of the temperatures, for the pattern 1 (P1) and pattern 2 (P2) fish, including the respiration rate at the asymptote of the critical oxygen level (MO_{2A}), critical oxygen level (DO_{crit}), and the DO level of the threshold above the critical oxygen level (DO_{UT}).

Temp (C)	MO_{2A} mg $O_2 \cdot g^{-1} \cdot hour^{-1}$		DO_{crit} mg $O_2 \cdot L^{-1}$		DO_{UT} mg $O_2 \cdot L^{-1}$	
	(P1)	(P2)	(P1)	(P2)	(P1)	(P2)
20	0.358 ± 0.063	0.247 ± 0.079	6.56 ± 1.03	4.99 ± 1.81		5.98 ± 2.21
22	0.222 ± 0.149	0.156 ± 0.031	6.93 ± 0.28	4.05 ± 0.95		5.64 ± 1.38
24	0.100 ± 0.040	0.205 ± 0.006	2.18 ± 0.67	4.27 ± 1.49		4.80 ± 1.61

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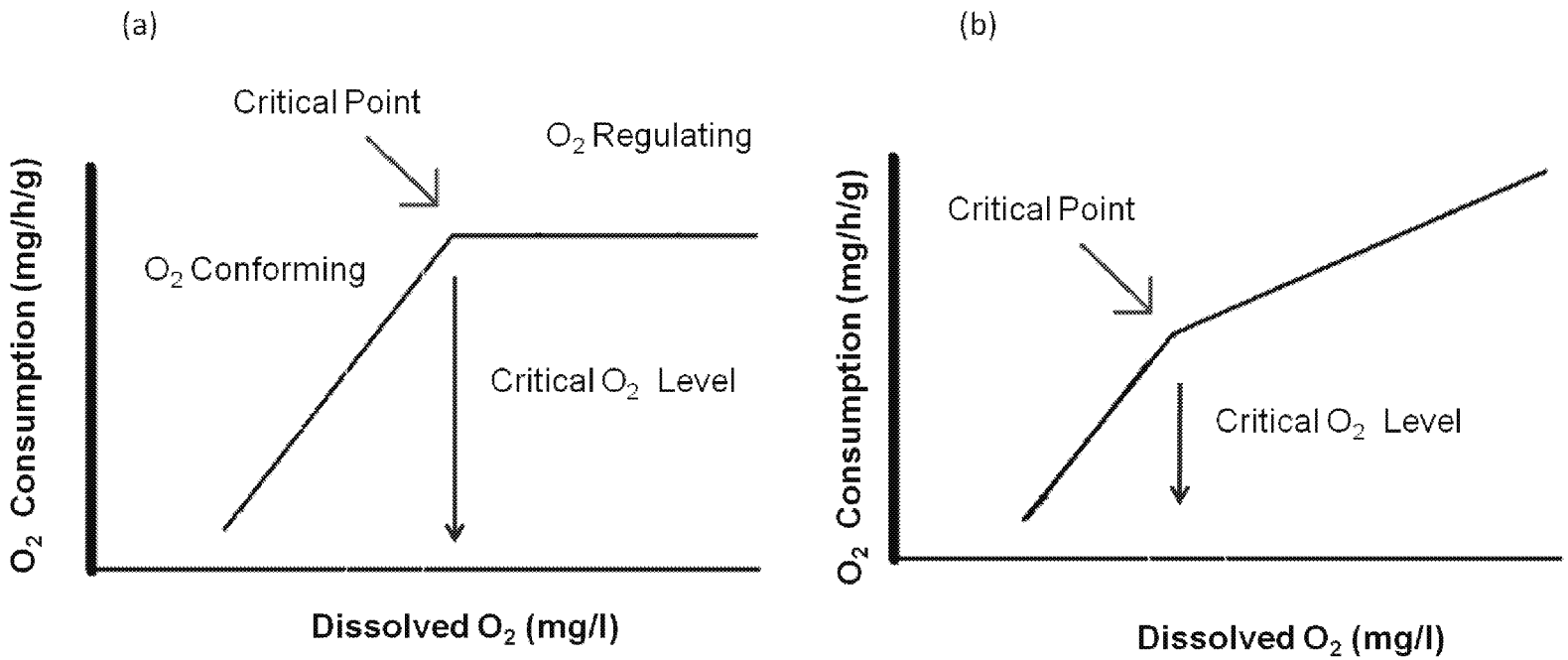
(a)
Critical Point
O_2 Regulating
O_2 Conforming
Critical O_2 Level
O_2 Consumption (mg/h/g)
Dissolved O_2 (mg/l)
(b)
Critical Point
Critical O_2 Level
O_2 Consumption (mg/h/g)
Dissolved O_2 (mg/l)

Figure 1

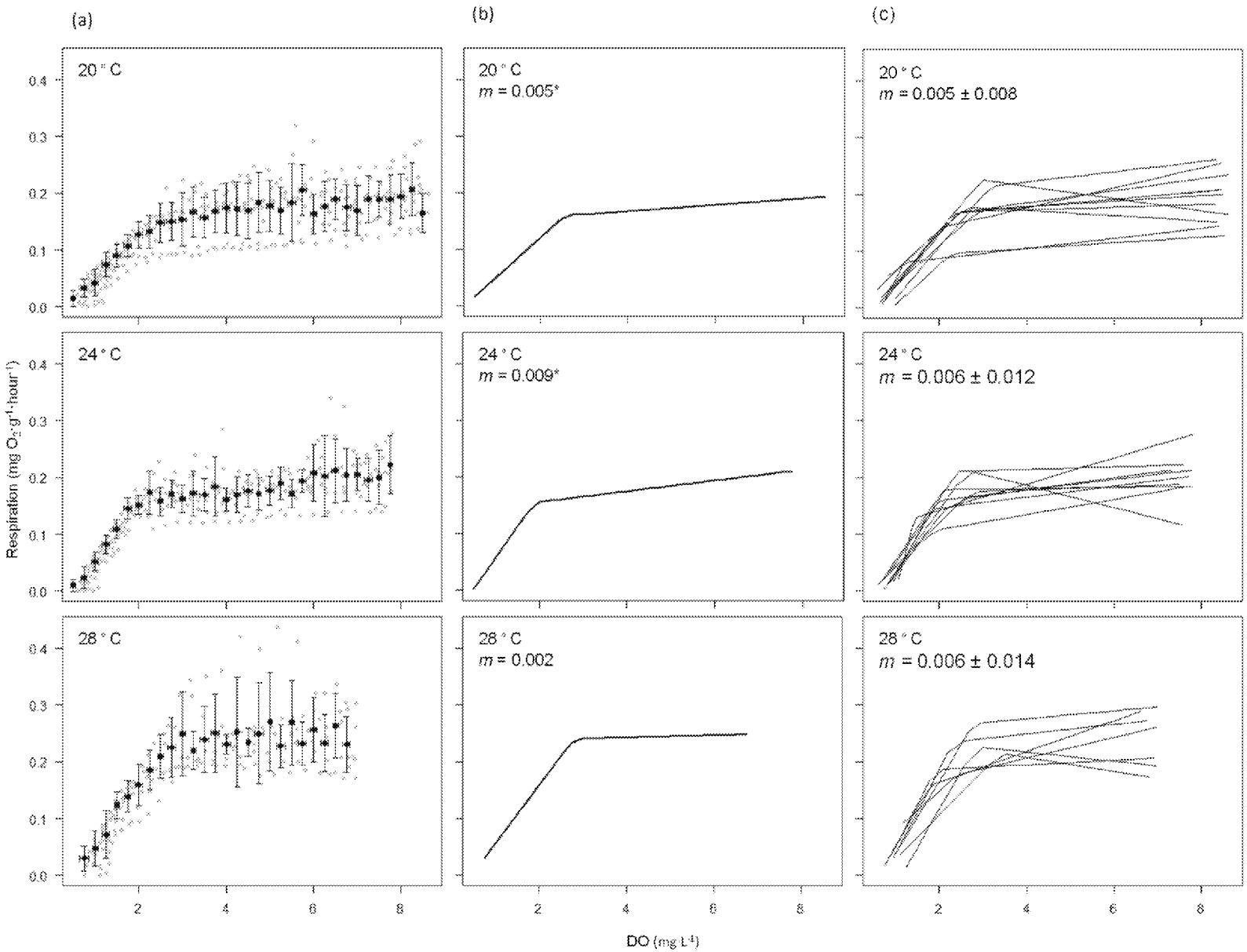
(a)
(b)
(c)
20 ° C
20 ° C
m = 0.005*
20 ° C
m = 0.005 ± 0.008
24 ° C
24 ° C
m = 0.009*
24 ° C
m = 0.006 ± 0.012
28 ° C
28 ° C
m = 0.002
28 ° C
m = 0.006 ± 0.014
Respiration (mg O2·g-1·hour-1)
DO (mg L-1)
0.0
0.1
0.2
0.3
0.4
2
4
6
8

Figure 2

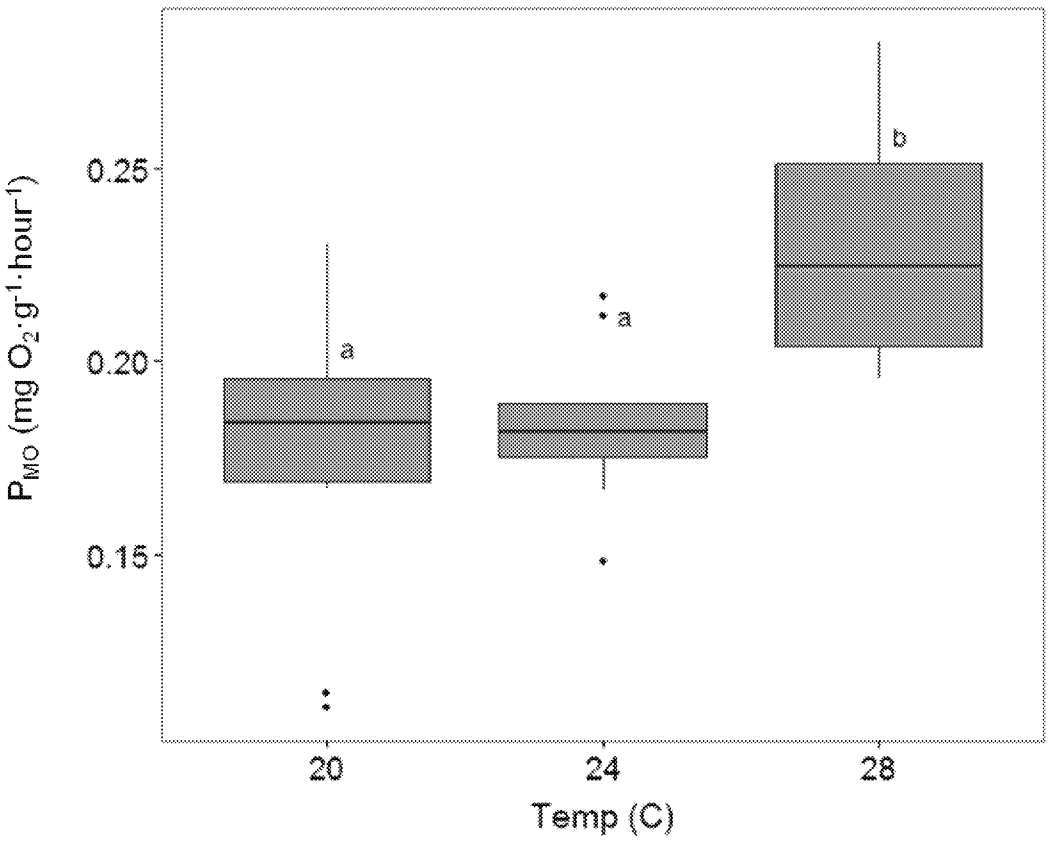

Figure 3

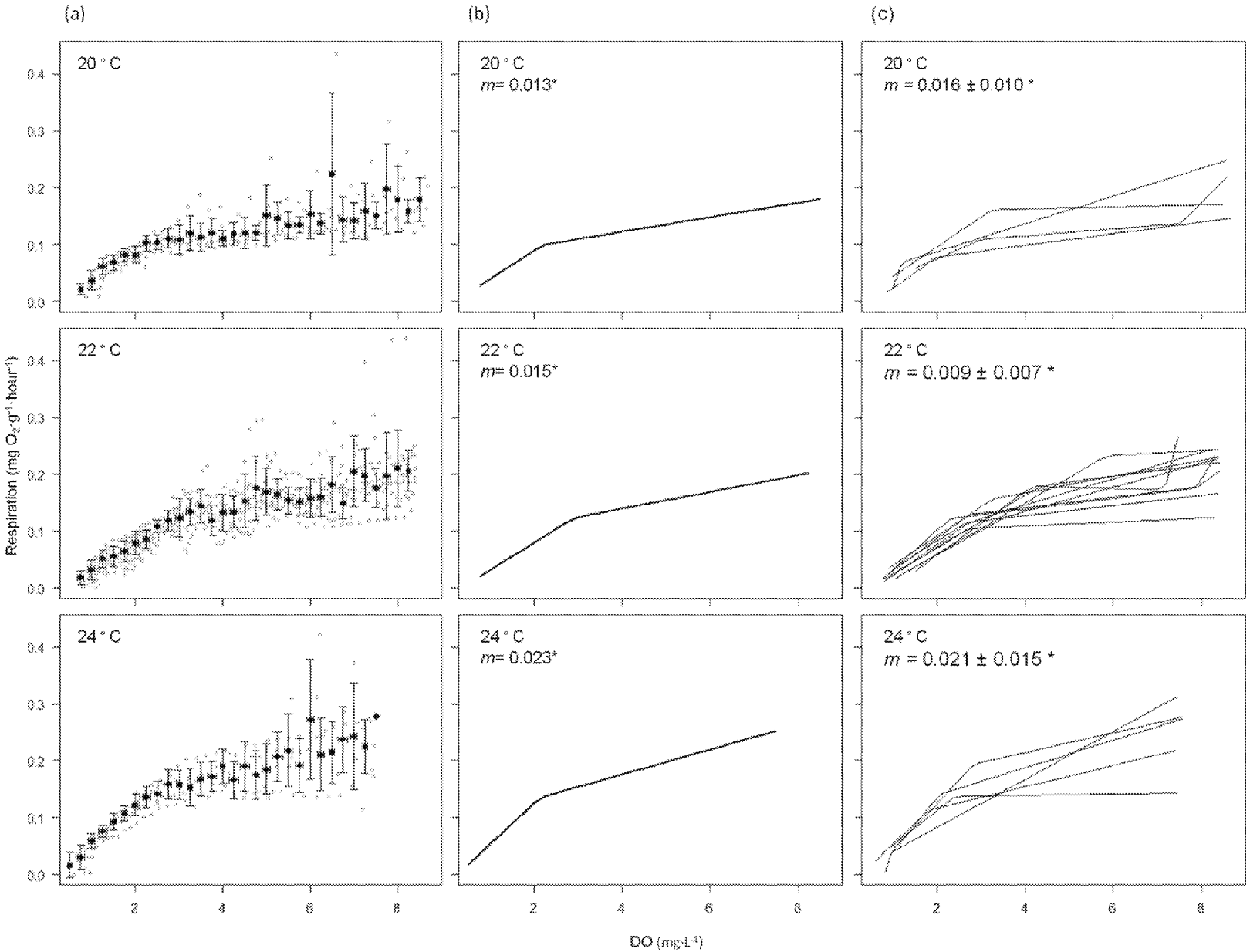
(a)
(b)
(c)
20 ° C
20 ° C
m= 0.013*
20 ° C
m = 0.016 ± 0.010 *
22 ° C
22 ° C
m= 0.015*
22 ° C
m = 0.009 ± 0.007 *
24 ° C
24 ° C
m= 0.023*
24 ° C
m = 0.021 ± 0.015 *
Respiration (mg O2·g-1·hour-1)
DO (mg·L-1)
0.0
0.1
0.2
0.3
0.4
2
4
6
8

Figure 4

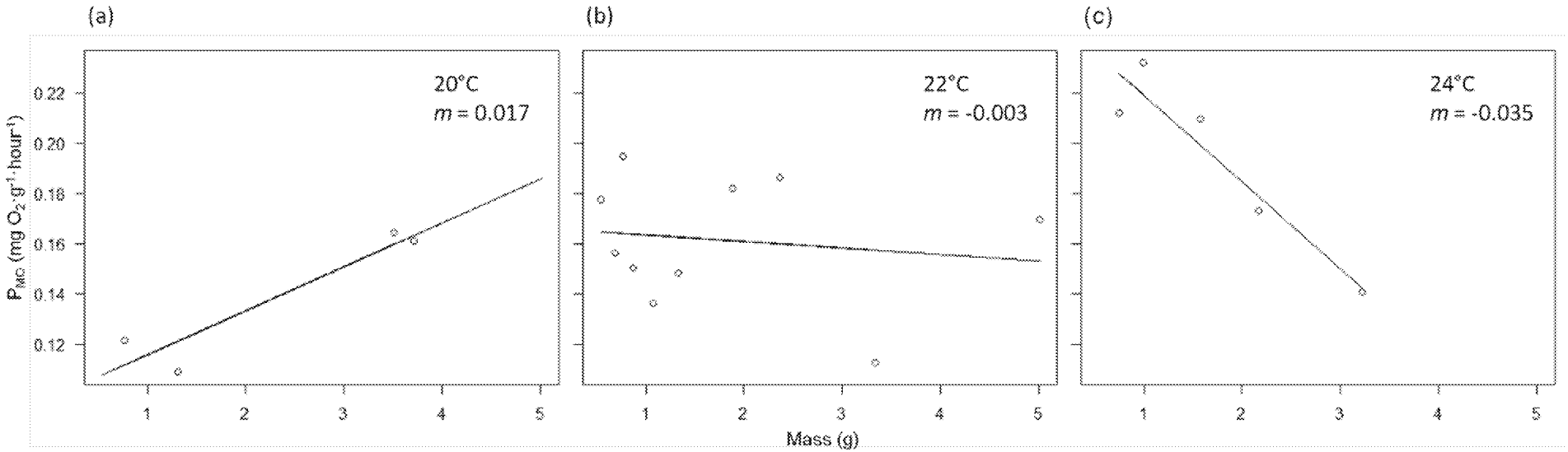
(a)
(b)
(c)
20°C
m = 0.017
22°C
m = -0.003
24°C
m = -0.035
P_MO (mg O2·g-1·hour-1)
0.12
0.14
0.16
0.18
0.20
0.22
1
2
3
4
5
Mass (g)

Figure 5

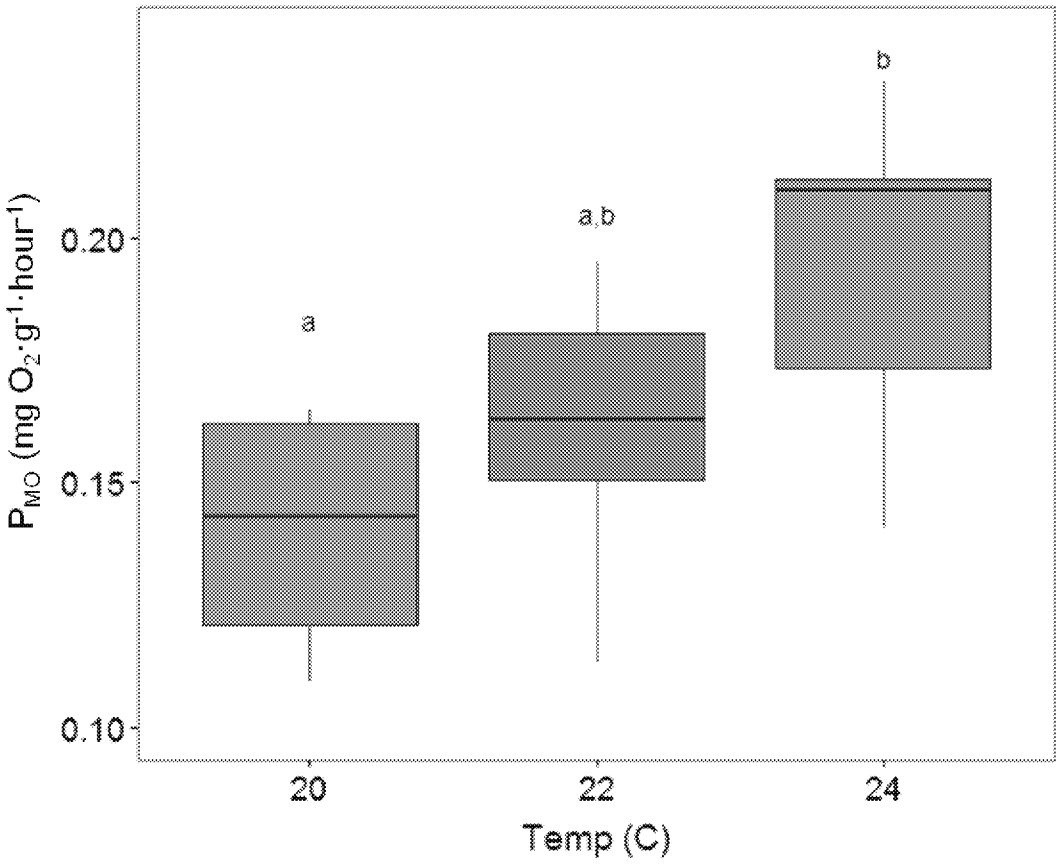

Figure 6

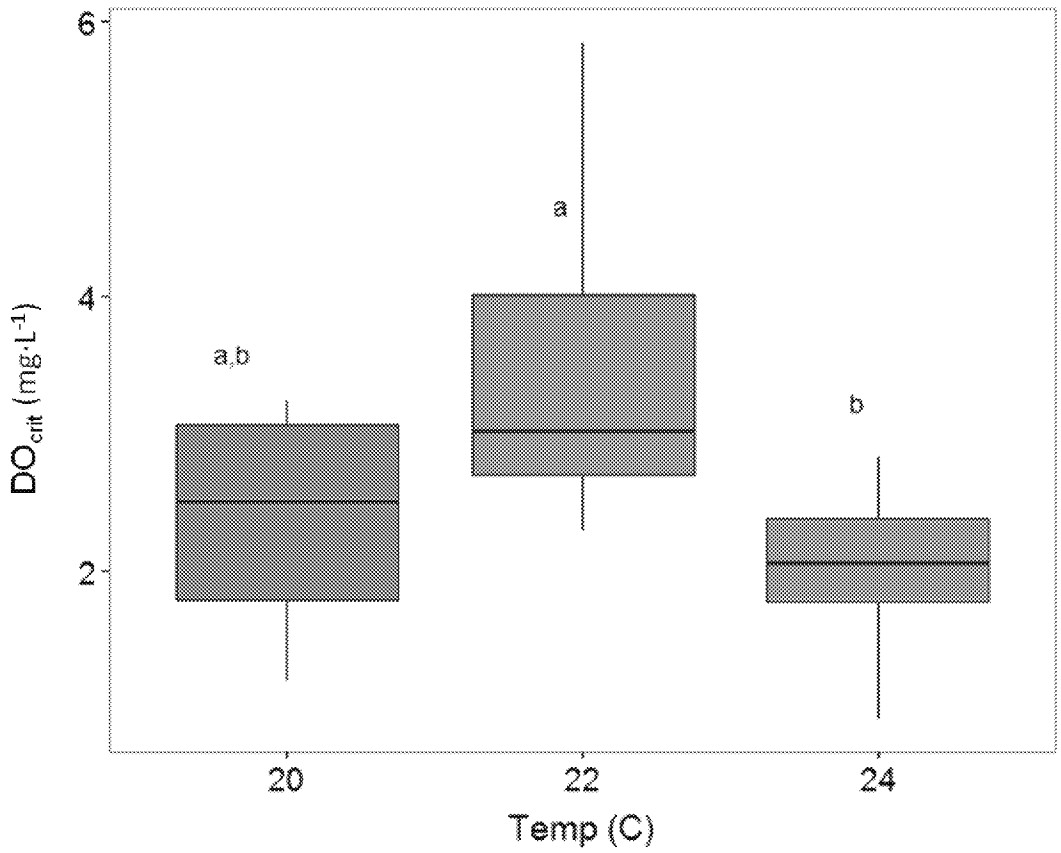

Figure 7

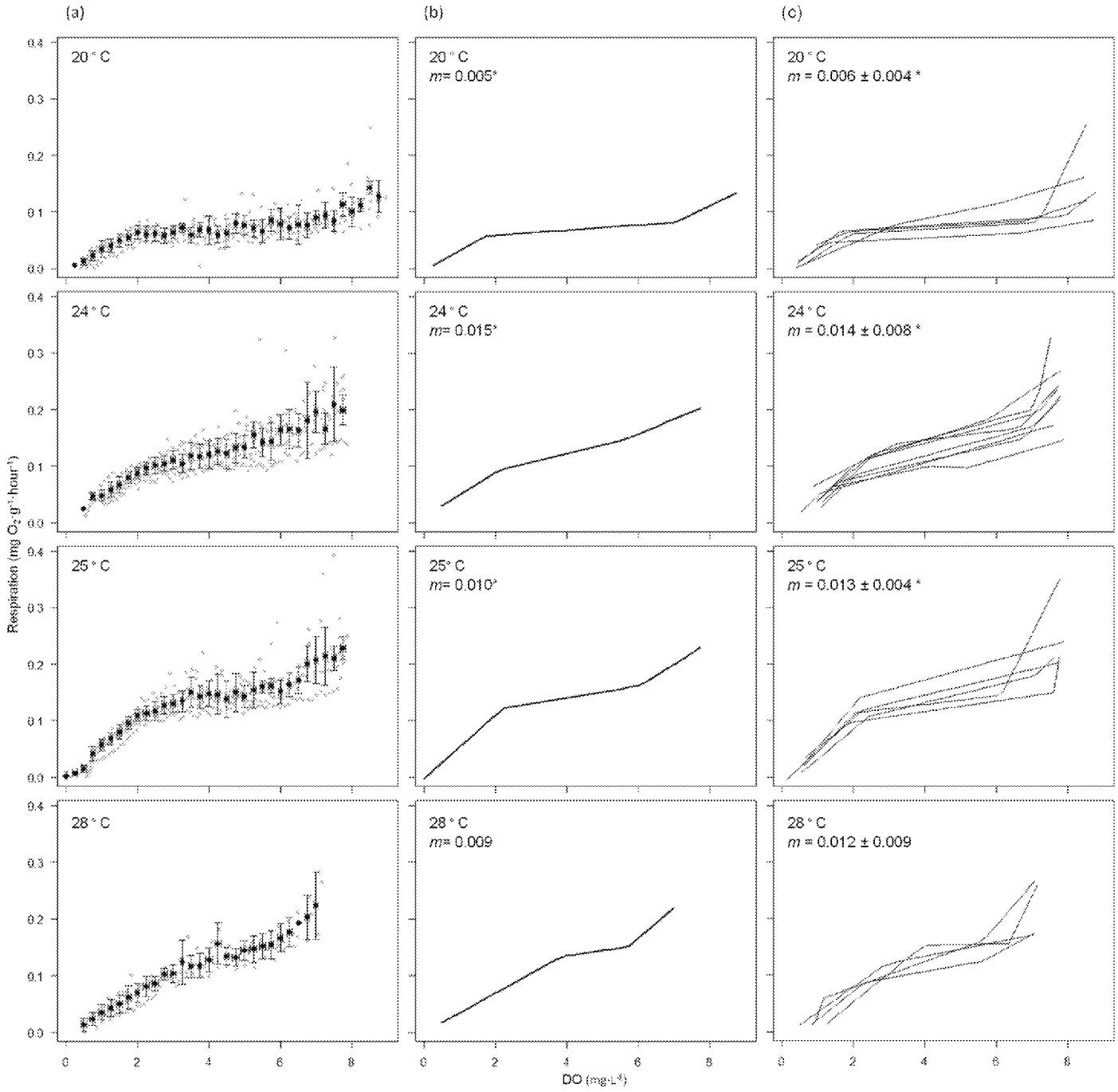
(a)
(b)
(c)
20 ° C
20 ° C
m= 0.005*
20 ° C
m = 0.006 ± 0.004 *
24 ° C
24 ° C
m= 0.015*
24 ° C
m = 0.014 ± 0.008 *
25 ° C
25° C
m= 0.010*
25 ° C
m = 0.013 ± 0.004 *
28 ° C
28 ° C
m= 0.009
28 ° C
m = 0.012 ± 0.009
Respiration (mg O2 g-1 hour-1)
DO (mg L-1)

Figure 8

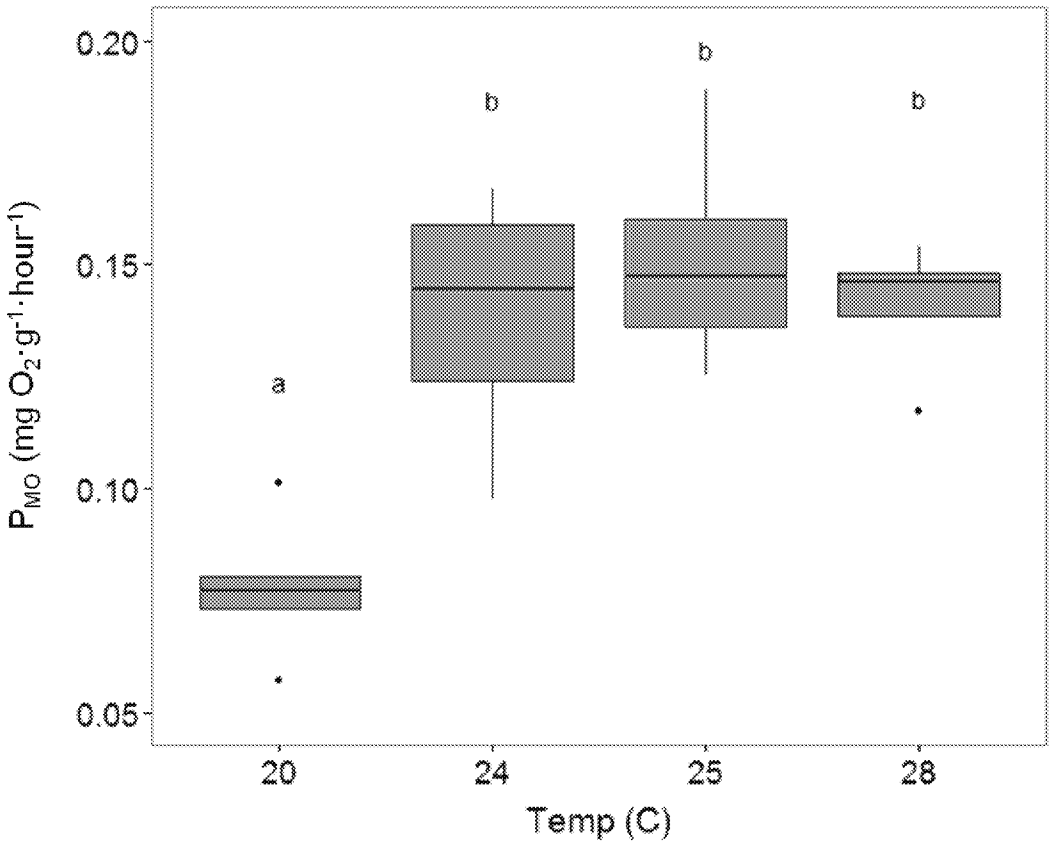
0.20
0.15
0.10
0.05
P_{MO} (mg $O_2 \cdot g^{-1} \cdot hour^{-1}$)
a
b
b
b
20
24
25
28
Temp (C)

Figure 9

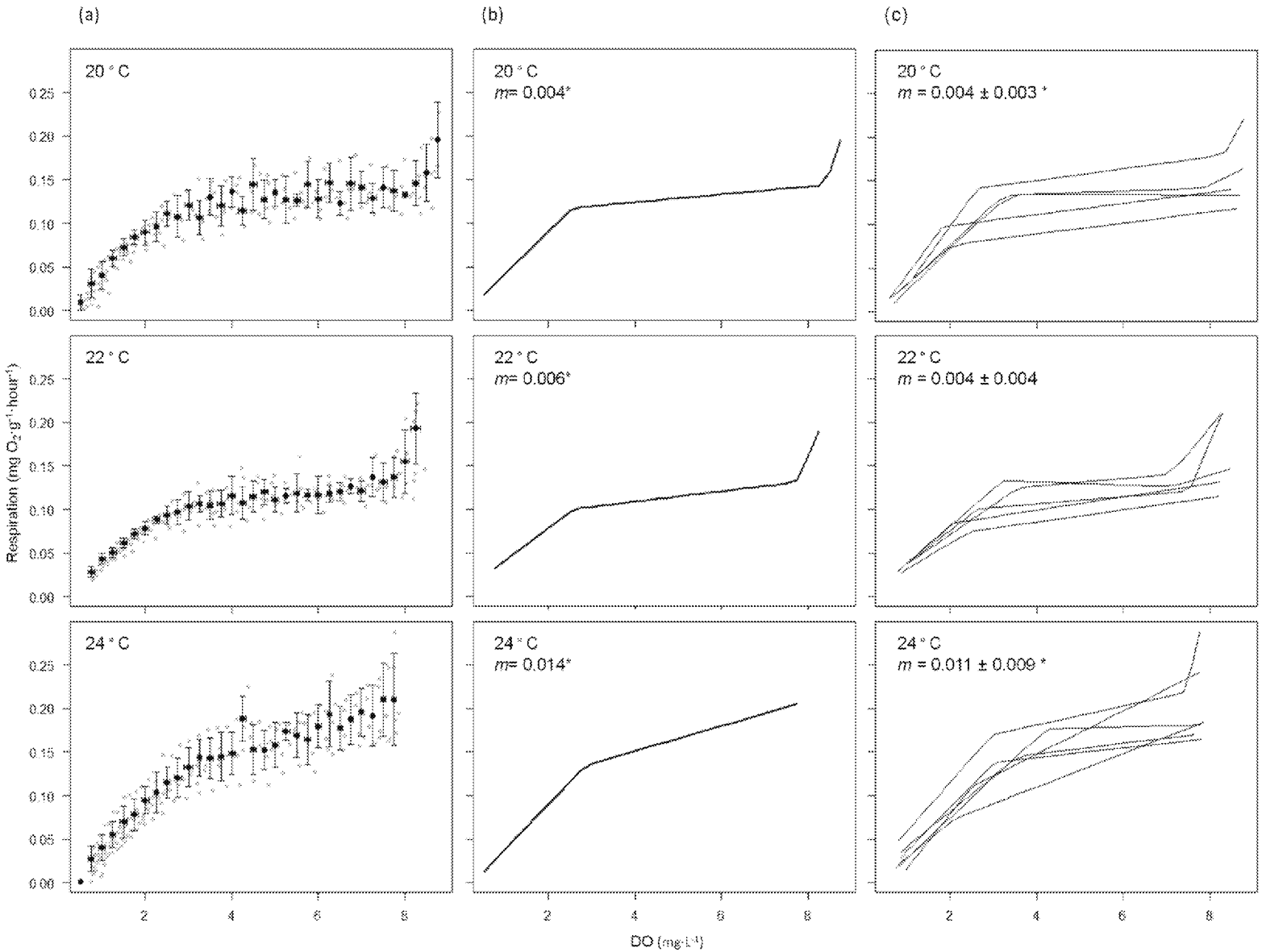
(a)
(b)
(c)
20 ° C
20 ° C
m= 0.004*
20 ° C
m = 0.004 ± 0.003 *
22 ° C
22 ° C
m= 0.006*
22 ° C
m = 0.004 ± 0.004
24 ° C
24 ° C
m= 0.014*
24 ° C
m = 0.011 ± 0.009 *
Respiration (mg O2·g-1·hour-1)
DO (mg·L-1)
0.00
0.05
0.10
0.15
0.20
0.25
2
4
6
8

Figure 10

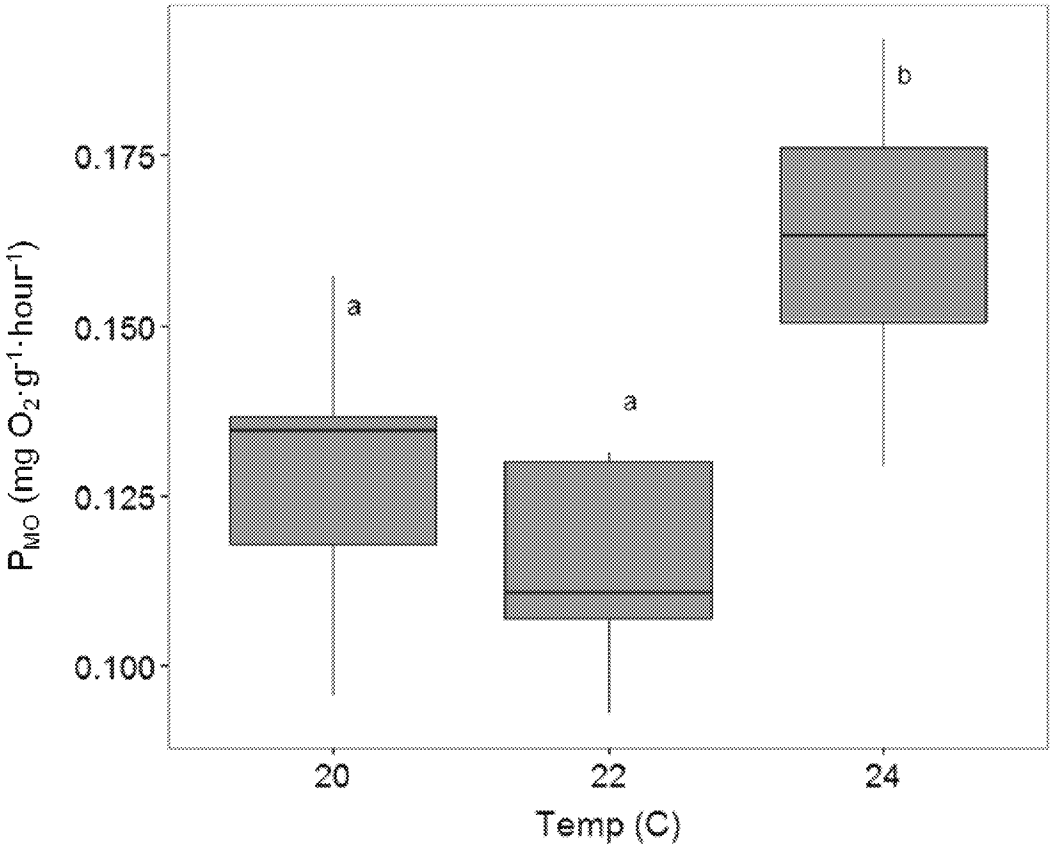
b
a
a
0.175
0.150
0.125
0.100
P_{MO} (mg $O_2 \cdot g^{-1} \cdot hour^{-1}$)
20
22
24
Temp (C)

Figure 11

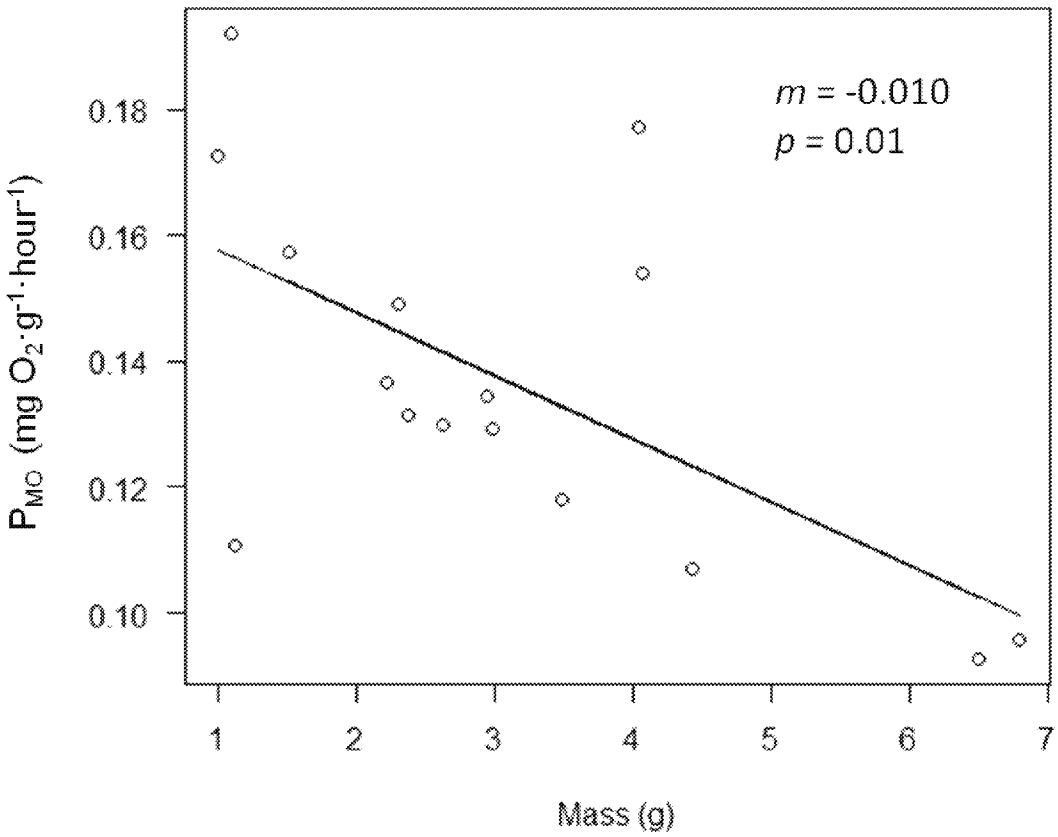
m = -0.010
p = 0.01
0.18
0.16
0.14
0.12
0.10
P_MO (mg O2·g-1·hour-1)
1
2
3
4
5
6
7
Mass (g)

Figure 12

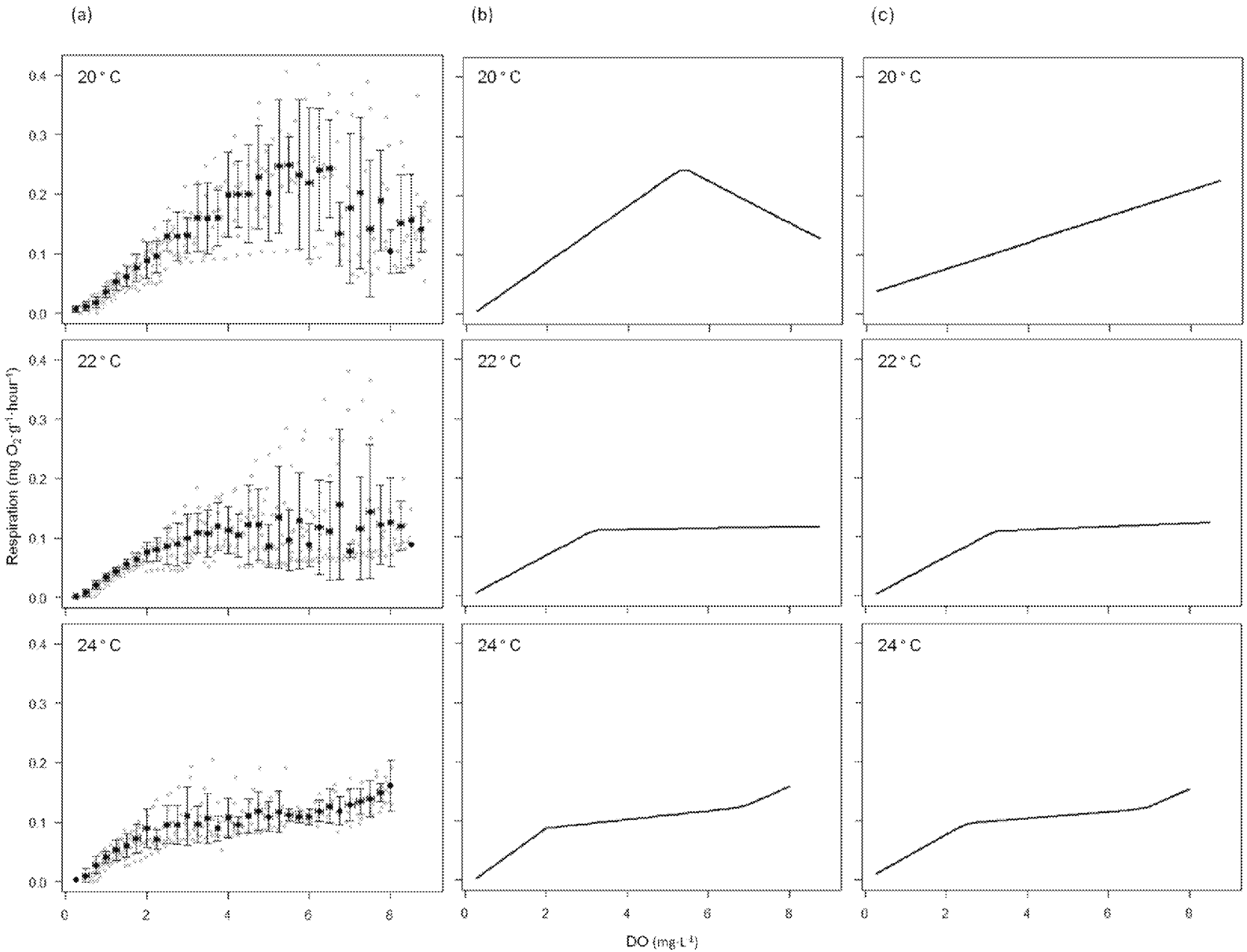

Figure 13

ED_005603A_00000028-00061

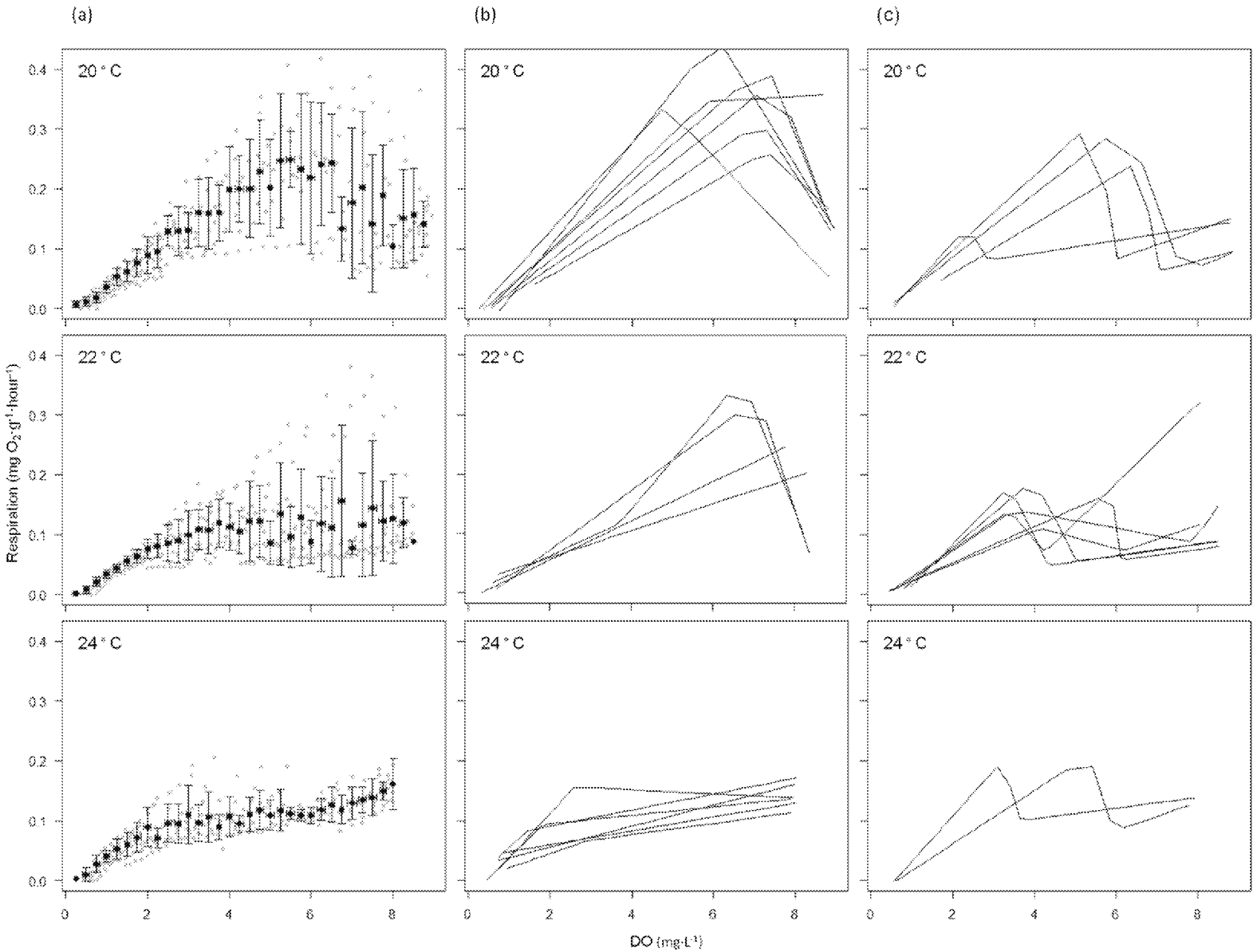

Figure 14

ED_005603A_00000028-00062

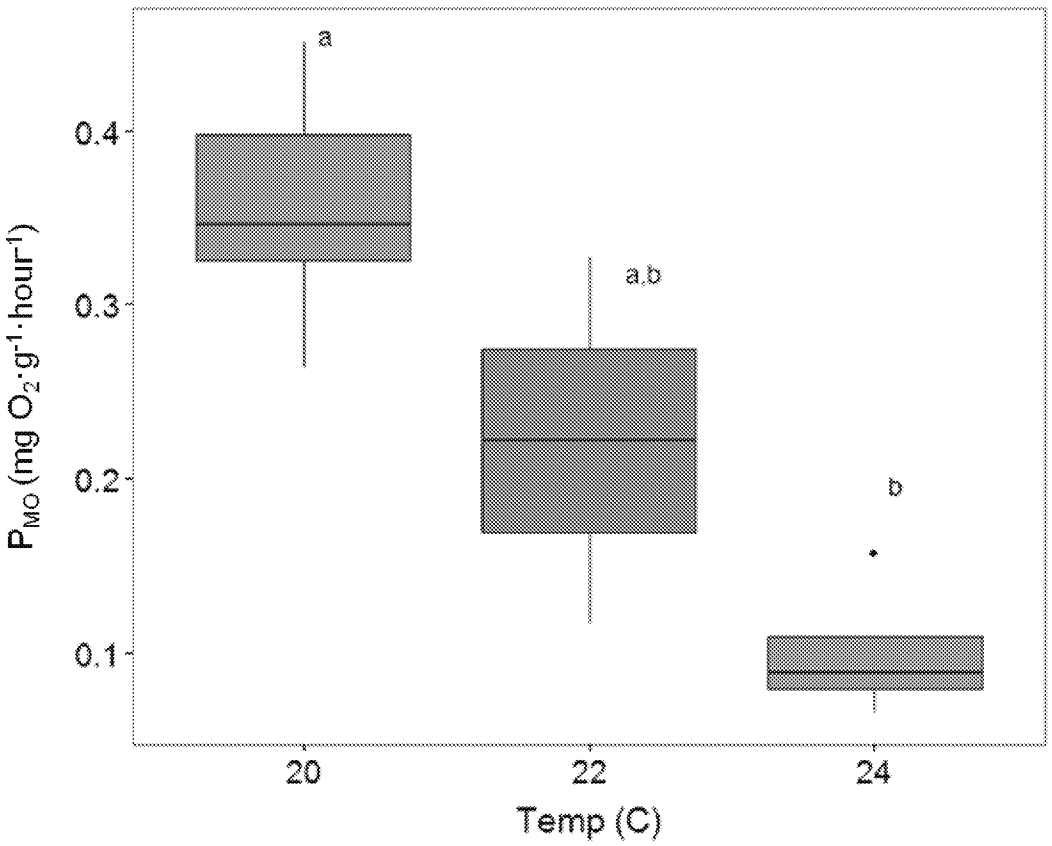

Figure 15

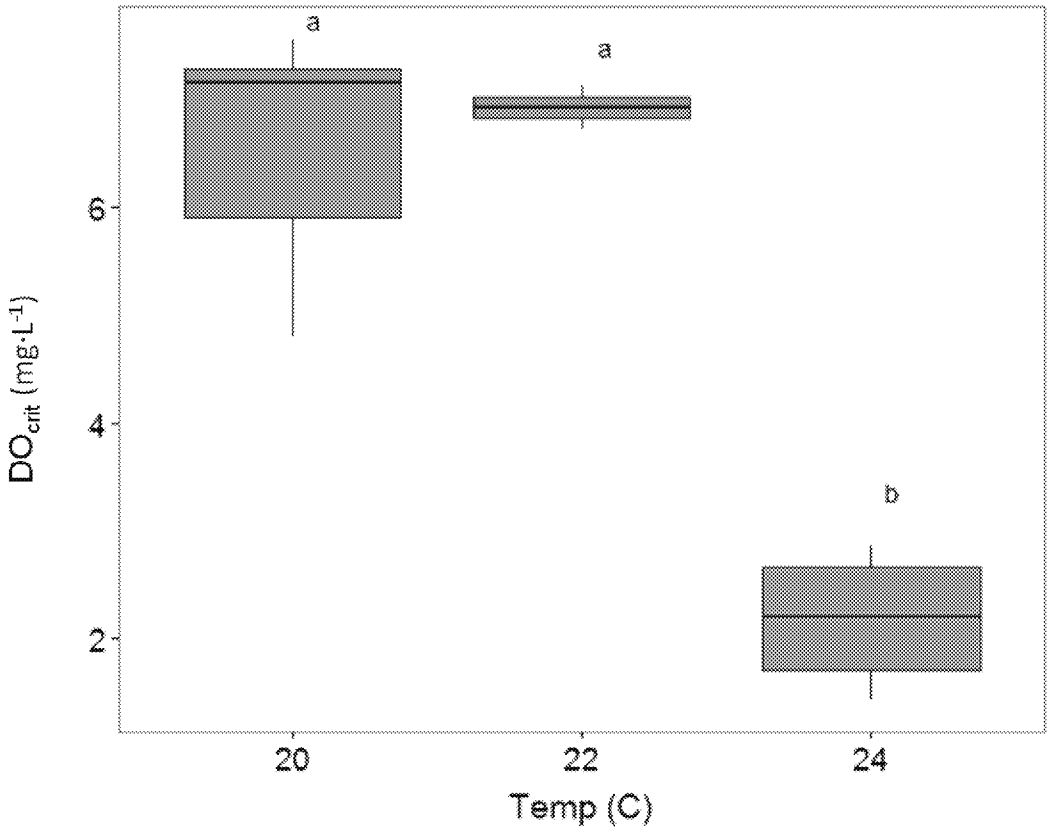

Figure 16

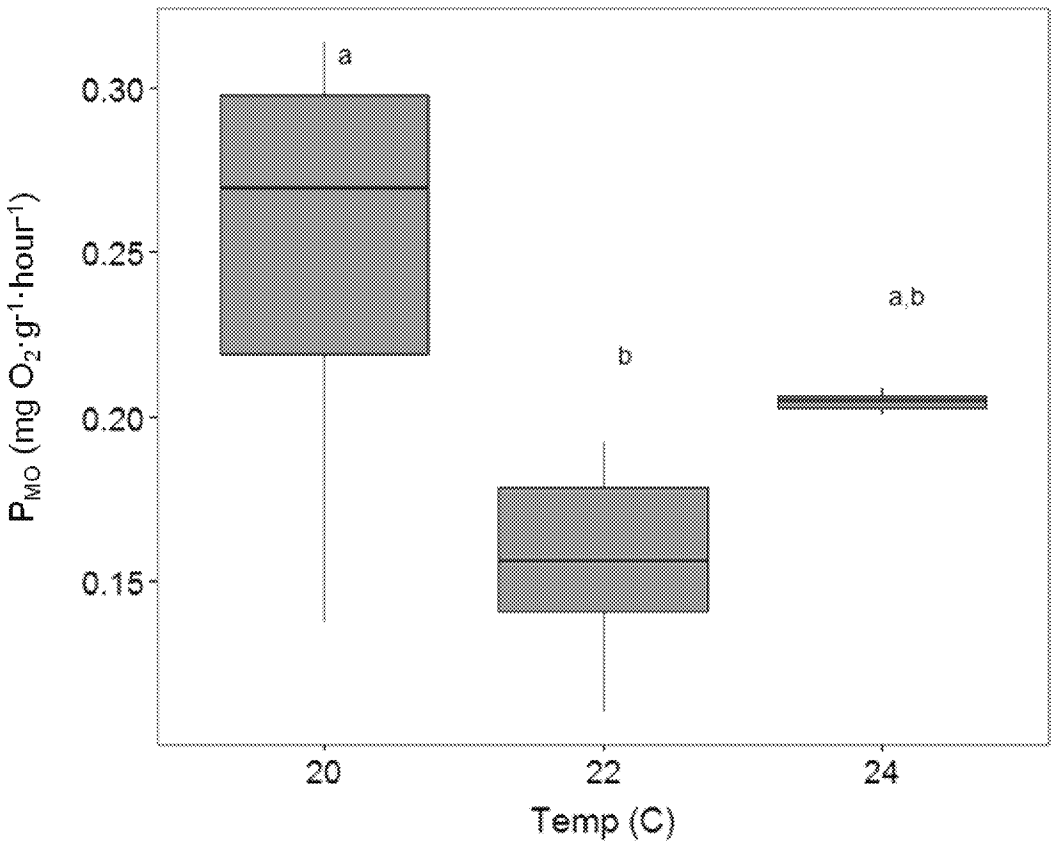

Figure 17

Appendix a. Anecdotal description of temperature observations and mortality of species acclimated during experiment.

Banded Sculpin

Narrative: Twelve fish held at 25°C for one week were observed to no longer feed. In the following two weeks, fish began to die until temperature was reduced.

Bronze Darter

Narrative: Ten fish were held at 25°C for one week. All fish appeared to acclimate and did not stop eating. Once the fish were placed inside the respirometry chambers and the experiment began, the oxygen consumption of each fish dropped unusually low on the first measurement cycle. Subsequent cycles showed that oxygen consumption did not increase after flushing the chamber with fresh oxygen saturated water, but instead remained low. Mortality occurred in 3 fish at DO levels ~ 5 to 6 mg·L.

Greenside Darter

Narrative: In three separate attempts, at least 12 or more fish were held at 20°C for a week in an effort to acclimate prior to testing. In each attempt, fish showed no signs of distress until after one week at which point fish began to show symptoms of disease. Within two to three weeks all fish died.

Redeye Bass

Narrative: Six fish acclimated to 20°C and tested well without signs of distress. However, in two separate attempts to acclimate fish at 22° and 24°C fish showed signs of disease and increased mortality.

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Tennessee Logperch

Narrative: Two fish were held in acclimation tanks for over 18 months at temperatures ranging from 20° to 28°C without showing any signs of stress.

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