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Respiratory response to temperature and hypoxia in the zebra mussel *Dreissena polymorpha*

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Abstract

The effects of temperature acclimation, acute temperature variation and progressive hypoxia on oxygen consumption rates ($\dot{V}_{O_2}$) were determined for the zebra mussel *Dreissena polymorpha*. In the first experiment, after acclimation to 5, 15 or 25 °C for at least 2 weeks, $\dot{V}_{O_2}$ was determined at 5 °C increments from 5 to 45 °C. V_{O_2} increased in all three acclimation groups from 5 to 30 °C, corresponding to the normal ambient temperature range for this species. Mussels displayed imperfect temperature compensation at temperatures above 15 °C, but exhibited little acclimatory ability below 15 °C. In the hypoxia experiment, V_{O_2} was determined over the course of progressive hypoxia, from full saturation (oxygen tension [P_{O_2}] = 160 Torr [21.3 kPa]) to a P_{O_2} at which oxygen uptake ceased (< 10 Torr [1.3 kPa]). Mussels were acclimated to either 5, 15 or 25 °C for at least 2 weeks and their respiratory response to progressive hypoxia was measured at three test temperatures (5, 15 and 25 °C). The degree of oxygen regulation increased with increasing test temperature, particularly from 5 to 15 °C, but decreased with increasing acclimation temperature. The decreased metabolic rate observed for warm-acclimated animals, particularly in the upper portion of the temperature range of the zebra mussel, may allow for conservation of organic energy stores during warm summer months. Compared to other freshwater bivalves, *D. polymorpha* is a relatively poor oxygen regulator, corresponding with its preference for well-oxygenated aquatic habitats. In addition, a new quantitative method for determining the degree of oxygen regulation is presented.

Keywords: *Dreissena polymorpha*; Hypoxia; Respiration; Temperature acclimation; Oxygen regulation; Oxygen conformity

1. Introduction

Recently, many studies have examined the physiological ecology of the zebra mussel *Dreissena polymorpha*, an important fouling exotic species that successfully colonized North America in the 1980s (Alexander et al., 1997). Several experiments have examined the respiratory responses of zebra mussels to environmental factors (for a review see McMahon, 1996). Studies to date include the examination of O_2 uptake rate (Mikheev, 1964; Alimov, 1975; Garton and Haag, 1991), seasonal changes in O_2 consumption rates (Mikheev, 1964; Lyashenko and Karchenko, 1989; Quigley et al., 1993), responses to acute temperature change (Dorgello and Smeenk, 1988), and responses to suspended inorganic sediments (Alexander et al., 1994; Summers et al., 1996). There have been no detailed studies of the effects of

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laboratory temperature acclimation or progressive hypoxia on O_2 uptake rates published for *D. polymorpha*. This paper presents the results of laboratory experiments examining the effects of temperature acclimation on the respiratory response of *D. polymorpha* to acute temperature change and progressive hypoxia. Our results are discussed in comparison to other bivalve mollusks, and in regard to this species' potential physiological and ecological adaptations for North American freshwater habitats.

2. Materials and methods

2.1. Maintenance and acclimation

Zebra mussels (*D. polymorpha* Pallas 1771) were collected near Monroe, Michigan on the southwestern shore of Lake Erie. They were shipped overnight to Texas by air freight wrapped in moist paper toweling in an insulated container containing coolant packs. On arrival, mussels were rinsed free of sediments and debris and held in continuously aerated, dechlorinated tap water at 5 °C in a refrigerated 284 l Living Stream® holding tank. The mussels were held without feeding for a short period (<60 days) at 5 °C prior to the experiments; zebra mussels have been shown to remain in excellent physiological condition prior to experimentation under these conditions (Chase and McMahon, 1995). Prior to the experiment, we acclimated samples of approximately 100 adult zebra mussels to one of three acclimation temperatures (5, 15, or 25 °C) for at least 14 days in 8 l of aerated, dechlorinated tap water held in 10 l plastic aquaria. The bottoms of the holding aquaria were covered with 2.5 mm square plastic microscope slide cover slips to which zebra mussels attached via their byssal threads. Attachment to cover slips allowed mussels to be removed from the aquarium and used in O_2 uptake experiments without cutting or disturbing their byssal attachments.

2.2. Oxygen consumption rates

We determined the whole animal O_2 consumption rates ($\dot{V}_{O_2}$, expressed as μl O_2 consumed h^{-1}) using Clark-type polarographic O_2 electrodes (YSI model 53 silver-platinum O_2 electrodes), whose output was monitored by a strip chart recorder (McMahon, 1985). We removed epibionts and debris from the mussel's shells, and placed the mussels inside the cylindrical respiration chambers. Chambers were filled with dechlorinated tap water (between 5 and 7 ml, depending on mussel size). Chamber water was passed through a 0.45 μm mesh filter to remove bacteria, and a small amount of the antibiotic streptomycin was added to the chamber water to inhibit bacterial growth during $\dot{V}_{O_2}$ determinations (McMahon, 1985). A plastic snap ring was inserted above the mussels within the chamber. The snap ring supported a magnetic stirrer above the mussels that allowed the continuous circulation of water past the electrode face during the experiment, thus replenishing the water immediately around the electrode membrane. The bottom side of the circular stirring magnet was flat, reducing the water movement in the lower portion of the chamber occupied by the mussels. We noted that circulation of chamber medium did not appear to adversely affect specimen behavior; most mussels gaped normally and extended their siphons within 5 min of being placed in the chambers. Chambers were held in a jacketed water bath, through which water from a Lauda K-2/R refrigerated circulator maintained chamber water temperatures within 0.05 °C.

2.3. Experiment 1: metabolic response to increasing temperature

Respiratory response to acute temperature change was recorded for replicate animals ($n=12$) from each of three acclimation groups (mussels were maintained at either 5, 15, or 25 °C for at least 2 weeks prior to the experiment). Replicates consisted either of a single large mussel with shell length (SL) >12 mm, or a group of two or three smaller mussels (SL<12 mm). Several small animals were used in some replicates to increase the respirable tissue mass of the sample. In replicates consisting of more than one individual, mussels were of similar size (±0.5 mm). Replicate size classes ranged from 9 to 17 mm SL in all three temperature acclimation groups, and the mean SL or dry tissue weight (DTW; see below) of an acclimation group was not significantly different (ANOVA, $P<0.5$) from the mean size or mass of any other group.

Specimens were first placed in the chambers at their acclimation temperature (AT). After a 20 min habituation period, the O_2 electrode was calibrated in a blank chamber that was fully air

saturated with O_2 (P_{O_2} 160 Torr or 21.3 kPa). The O_2 uptake of the blank medium was then determined, and subsequent sample V_{O_2} determinations of the experimental replicates were adjusted by subtracting the O_2 uptake of the water medium in the blank. The O_2 electrode was then placed sequentially in the experimental chambers, and the O_2 uptake of each replicate mussel sample was determined. The respiratory rate was first determined at the mussels' AT. The O_2 uptake of each replicate sample was monitored from near full air O_2 saturation (P_{O_2} = 21.3 kPa) for 10–30 min, during which the chamber P_{O_2} was generally reduced by approximately 5–10%. For each replicate, whole-animal oxygen consumption rates (V_{O_2}, in μl O_2 h^{-1} at STP) were estimated from the rate of the initial 5–10% decline in chamber water O_2 concentration (McMahon, 1985).

For the 15 and 25 °C acclimated animals, after determination of V_{O_2} at their AT, chamber water temperature was lowered approximately 1 °C 5 min^{-1} down to 5 °C, and blank V_{O_2} and sample V_{O_2} were determined as described above. The initial measurements of the 5 °C acclimation group started at 5 °C. After measurement of V_{O_2} at 5 °C, chamber temperature was raised by 5 °C at approximately 1 °C per minute and the V_{O_2} was recorded again. Respiratory rates were determined at successive 5 °C increments in chamber water temperature until the upper lethal temperature was reached. This upper lethal temperature was indicated by an extensive valve gape by the animals and by a marked decline in V_{O_2} (occurred at 40 °C for 5 °C acclimated specimens and approximately 45 °C for 15 and 25 °C acclimated mussels).

After completion of V_{O_2} determinations, the mineral components of the shell were dissolved in 11% nitric acid by volume (McMahon, 1985). Each replicate's shell-free DTW was determined by removing the shell periostracum and byssal threads, and drying the remaining tissue mass at 90 °C to constant dry weight (±0.1 mg). We divided the DTW and V_{O_2} measurements for replicate samples containing more than one mussel by the number of individuals in the sample (n = 2 or 3). The whole animal oxygen consumption rates (V_{O_2}, in μl O_2 h^{-1} at STP) were then divided by DTW to produce mass-specific oxygen consumption rates (V_{O_2}, in μl O_2 h^{-1} mg^{-1}).

We ran a repeated measures multifactor analysis of variance to examine the effect of temperature on the mass-specific oxygen consumption rates (V_{O_2}), with DTW as a covariate, AT as a treatment variable, and test temperature (TT) as a repeated measure variable. In addition, we calculated Q_{10} values for each 5 °C change in TT, and we also produced an overall Q_{10} value for each acclimation group for a 20 °C range, from 5 to 25 °C. We also calculated acclimation Q_{10} values [$Q_{10(acc)}$] (see McMahon, 1985, 1991 and the discussion for further details) comparing the response of the 5 °C acclimated animals (tested at 5 °C) to 15 °C acclimated animals and to the 25 °C acclimated animals (each tested at their AT), and also compared the 15 °C acclimated mussels to the 25 °C acclimated mussels.

2.4. Experiment 2: metabolic response to progressive hypoxia

We determined the oxygen uptake rate in response to progressive hypoxia for mussels (n = 8, SL = 9–18 mm) from the 5, 15 and 25 °C acclimation groups at each of three test temperatures (5, 15 and 25 °C). Mean animal size (expressed as either SL or as DTW) in each acclimation group was not significantly different from each other (ANOVA, $P < 0.5$). The calculation of V_{O_2} was with O_2 electrodes as described above, except that monitoring of O_2 uptake occurred continuously from 100% O_2 saturation (P_{O_2} = 160 Torr [= 21.3 kPa]), until a chamber P_{O_2} (< 10 Torr) was reached at which sample O_2 uptake ceased. For each replicate, V_{O_2} was computed over sequential 5% decreases in P_{O_2} from 100% air saturation, to the P_{O_2} at which O_2 uptake ceased. The respiratory response to progressive hypoxia was determined initially at each replicate samples AT (5, 15 or 25 °C) and then sequentially to the other two test temperatures. We observed that the behavior of the mussels was similar at all three test temperatures (the mussels opened their valves and began siphoning within a few minutes of handling), suggesting that they were not stressed by exposure to the range of temperatures used.

2.5. Data analysis of the hypoxia experiment

We used a new method for analysis of O_2 regulatory capacity, which we call the 'Regulation Value' or R (Alexander et al., unpublished). This method involves expressing each $\dot{V}_{O_2}$ value record-

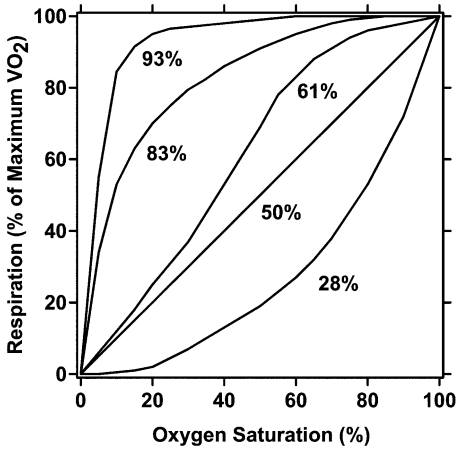

Fig. 1. Hypothetical curves of standardized O_2 uptake rates (V_{O_2}, vertical axis) vs. oxygen tension (P_{O_2} in Torr) ranging from 0 to 160 Torr at full air O_2 saturation (horizontal axis). The curves represent varying degrees of O_2 regulatory ability as indicated by the $\geq R$ values labeling each curve. An R value of 50% represents oxygen conformity, where O_2 uptake declines directly with P_{O_2}. R values above 50% are indicative of progressive increase in O_2 regulatory ability, while R values progressively falling below 50% represent animals that are hypoxia sensitive, where the metabolic rate drops dramatically with a slight decline in P_{O_2}.

ed for each replicate sample at each 5% decrease in O_2 concentration as a percentage of the maximum V_{O_2} recorded across all tested P_{O_2} values. The maximum standard $\dot{V}_{O_2}$ value thus is assigned a value of 100% and the minimum $\dot{V}_{O_2}$ value is 0%. The R value for an individual is calculated as the integrated sum of standard $\dot{V}_{O_2}$ values across 100–0% of full air O_2 saturation in 5% increments and the integrated sum expressed as a percentage of that which would be recorded for a theoretically perfect O_2 regulator. For example, an individual displaying perfect oxygen regulation would have an R of 100%, and an animal exhibiting strict oxygen conformity (where oxygen uptake declines in direct proportion to the declines in P_{O_2}) would have an R of 50% (Fig. 1). R values between 50 and 100% are indicative of oxygen regulation, with higher values indicating greater regulatory ability. R values below 50% suggest that the organism is extremely hypoxia-sensitive, where the metabolic rate of hypoxia-sensitive animals drops rapidly in response to a slight decline in P_{O_2} (Fig. 1). For the replicate samples used from each acclimation group, the standard $\dot{V}_{O_2}$ values were computed at each 5% progressive decrease in P_{O_2} for each test temperature (5, 15 and 25 °C). Standard V_{O_2} values were then used to compute R values for each individual replicate as described above. We used a multiple factor analysis of variance, with R values as the dependent variable, DTW as a covariant, AT as a treatment variable, and test temperature as a repeated measure variable.

3. Results

3.1. Experiment 1: metabolic response to increasing temperature

For both the 15 and 25 °C acclimated replicate samples, no significant differences were recorded between the initial $\dot{V}_{O_2}$ measured at a replicates AT and the $\dot{V}_{O_2}$ later recorded at that same temperature as $\dot{V}_{O_2}$ was measured at progressive 5 °C increases from 5 °C (paired t-tests, $n=12$, 15 °C acclimated group, $P=0.9$; 25 °C acclimated group, $P=0.5$). We argue from this result that the reduction of chamber water temperature to 5 °C from the initial AT did not affect the mussels subsequent respiratory response for the mussels acclimated to warmer temperatures (15 and 25 °C). Therefore, only the second $\dot{V}_{O_2}$ value recorded at the AT was used in the subsequent data analysis.

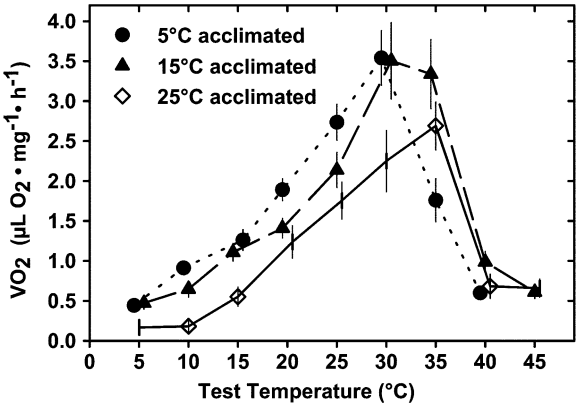

Fig. 2. Oxygen consumption rates ($\dot{V}_{O_2}$) in the zebra mussel (*D. polymorpha*). Animals were acclimated either to 5 (●), 15 (▲) or 25 °C (◇). The metabolic response of each replicate ($n=12$ in each acclimation temperature treatment) was monitored at 5 °C increments in test temperature from an initial test temperature of 5 °C, until a lethal temperature was attained (40–45 °C). Some points are slightly offset for clarity. Bars represent standard errors of the mean.

Table 1
Q_{10} values indicating response of O_2 consumption rate to temperature increase over a 5–45 °C in 5 °C intervals for *D. polymorpha* acclimated to 5, 15 or 25 °C

Test temperature	Acclimation temperature		
range (°C)	5 °C	15 °C	25 °C
5–10	4.831	1.596	0.771
10–15	1.768	4.504	18.323
15–20	2.444	1.655	6.790
20–25	2.055	2.277	2.282
25–30	1.632	2.337	1.329
30–35	0.211	0.861	1.867
35–40	0.00010	0.098	0.052
40–45	–	0.387	0.0086

Test temperature has a strong effect on the metabolic rate of each acclimation group (Fig. 2). The $\dot{V}_{O_2}$ values progressively increased in all three acclimation groups over a range of TTs (from 5 to 30 °C). Both AT (Repeated Measured ANOVA, tests of hypotheses for between-subject effects, $F=15.13$, $P<0.0005$) and test temperature (Wilks=Lambda, $F=19.83$, $P<0.0001$) significantly affected $\dot{V}_{O_2}$, but in different ways. As the TT increased, from 5 to 30 °C in all AT groups, metabolic rates increased. As AT increased, the metabolic rate decreased at any given test temperature. A significant AT*TT interaction was found (Wilks=Lambda, $F=4.0$, $P<0.007$), but the rate-temperature curves of each acclimation group did not intersect over the 5–30 °C range (Fig. 2), showing that at a given test temperature, the respiratory rates of warmer acclimated animals were lower than that of cooler acclimated animals. The cause of the AT*TT interaction is observed at the lower test temperatures of 5 and 10 °C (Fig. 2), where the metabolic rates of 25 °C acclimated animals were considerably depressed compared to those of colder acclimated animals. This interaction between AT and test temperature is also indicated in the Q_{10} results, as discussed below.

The temperature of maximal $\dot{V}_{O_2}$ was 30 °C for the 5 and 15 °C acclimated mussels and 35 °C for those acclimated to 25 °C (Fig. 2). Above these temperatures, $\dot{V}_{O_2}$ declined dramatically, suggesting that individuals were dying from temperature stress. Death at these elevated temperatures was also indicated by widely gaping shell valves.

Q_{10} values, indicating the factor by which $\dot{V}_{O_2}$ would increase for a 10 °C increase in test temperature, were computed from the adjusted mean $\dot{V}_{O_2}$ values recorded across each successive 5 °C increase in test temperature for each acclimation group (Table 1). Above 30° for 5 °C acclimated mussels, and 35 °C for 15 °C acclimated mussels, the Q_{10} values fell well below 1.0, indicative of marked reduction in V_{O_2} with increasing temperature (Table 1, Fig. 2) as mussels succumbed to lethal temperatures. In contrast, Q_{10} values for 25 °C acclimated individuals between test temperatures of 10 and 15 °C, and between 15 and 20 °C, were rather elevated (18.3 and 6.8, respectively), compared to typical values for ectothermic animals (Table 1). The elevated Q_{10} values for the 25 °C acclimated animals across the 10–20 °C TT range collaborate the significant TT*AT interaction observed in the ANOVA, suggesting that $\dot{V}_{O_2}$ in 25 °C acclimated individuals was substantially depressed at lower temperatures ($\leq$15 °C), with a relatively large increase in metabolic rate occurring with slight increases in temperature above this range (Table 1, Fig. 2). Between 20 and 35 °C, Q_{10} values of 25 °C acclimated individuals fell to 1.33 and 2.28, indicative of a more typical respiratory response to temperature, while between 35 and 40 °C, a very low Q_{10} value (0.052) indicated a marked reduction in V_{O_2}, associated with exposure to lethal temperatures (Table 1, Fig. 1).

Within each acclimation group, the overall Q_{10} values were calculated for a 20 °C range (from 5 to 25 °C) that brackets the temperature range many mussels would experience in the wild. The overall Q_{10} values were 2.34, 2.29 and 3.11 for the 5 °C, 15 °C and 25 °C acclimation groups, respectively.

The acclimation Q_{10} value ($Q_{10(acc)}$) obtained by comparing the response of 5 °C acclimated mussels tested at 5 °C to the respiratory rates of 15 °C acclimated mussels tested at 15 °C was 2.55. The $Q_{10(acc)}$ comparison between 15 °C acclimated and 25 °C acclimated animals was considerably lower (1.25). Overall, $Q_{10(acc)}$ between 5 and 25 °C acclimated mussels was 1.78 (Fig. 1).

3.2. Experiment 2: metabolic response to hypoxia

The results of the progressive hypoxia experiment indicated that both AT and TT significantly affected oxygen regulation (Figs. 3 and 4). Mean R values ranged from a low of 51.5% ($\pm$2.9 SE) for 25 °C acclimated mussels tested at 5 °C, to 81.4% ($\pm$1.4 S.E.) for 5 °C acclimated individuals

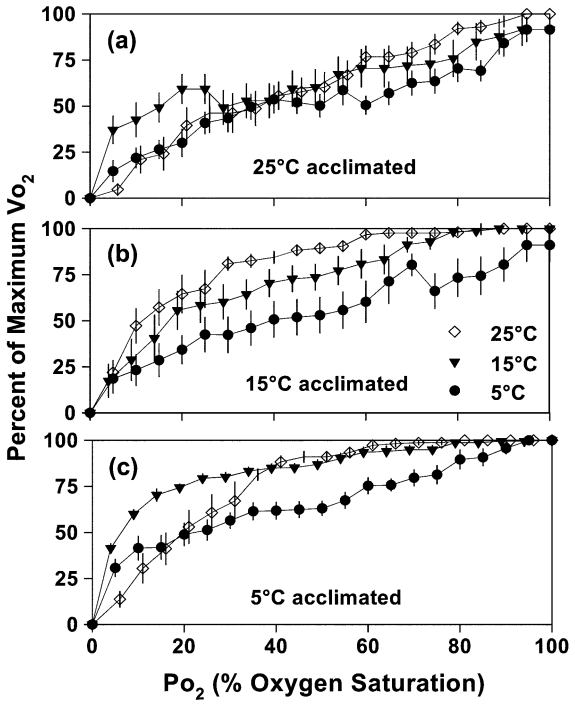

Fig. 3. Metabolic response to progressive hypoxia in the zebra mussel (*D. polymorpha*). Oxygen consumption rate values (V_{O_2}, expressed as fraction of the maximum rate recorded for a given animal over the range of oxygen tensions) in response to progressive hypoxia, from full air O_2 saturation ($P_{O_2}=160$ Torr) to the P_{O_2} at which O_2 uptake ceased (<10 Torr). At each P_{O_2} increment (5% oxygen saturation increments, or 8 Torr), the average responses of the replicate animals ($n=8$ in each acclimation temperature treatment) are plotted. Animals were acclimated either to 25 °C (a), 15 °C (b), or 5 °C (c). The response to hypoxia was measured in replicate animals from each acclimation group to all three test temperatures: 5 (●), 15 (▲) and 25 °C (◇). Some points are slightly offset for clarity. Bars represent standard errors of the mean.

tested at 15 °C (Fig. 4). The capacity for oxygen regulation of $\dot{V}_{O_2}$ in *D. polymorpha* increased with decreasing AT (Repeated Measures ANOVA, $F=16.92$, $P<0.0005$). However, the capacity for oxygen regulation increased with increasing TT (Wilks = Lambda, $F=5.15$, $P<0.016$, Fig. 3). There were no significant DTW*TT or TT*AT interactions.

4. Discussion

4.1. Metabolic response to increasing temperature

The lack of a significant difference between the $\dot{V}_{O_2}$ initially measured at each replicate's AT and that subsequently measured at that AT during the incremental increase in test temperature indicated that the exposure of all experimental individuals to 5 °C at the beginning of the experiment did not affect their subsequent respiratory responses, regardless of the temperature to which they were acclimated. Our results suggest that short-term acute exposure to low temperatures is not stressful to *D. polymorpha*.

The observed decline in $\dot{V}_{O_2}$ with increasing AT is a metabolic temperature compensation response typical of most ectothermic species (Precht et al., 1973), including freshwater bivalves (McMahon, 1991). There was little difference in the $\dot{V}_{O_2}$ of 5 °C acclimated animals and 15 °C acclimated animals, but the $\dot{V}_{O_2}$ of the 25 °C acclimated animals was considerably lower at all test temperatures. *D. polymorpha* exhibits imperfect temperature compensation, with acclimatory responses best developed at temperatures above 15 °C. Quigley et al. (1993) also reported a similar pattern of compensation in *D. polymorpha*. Acclimation to higher temperatures resulted in a decline in $\dot{V}_{O_2}$ at test temperatures within the acutely tolerated temperature range of this species (McMahon et al., 1993).

The Q_{10} values for *D. polymorpha* fall between 2 and 3, a range considered normal for ectothermic species in general (Precht et al., 1973) and for

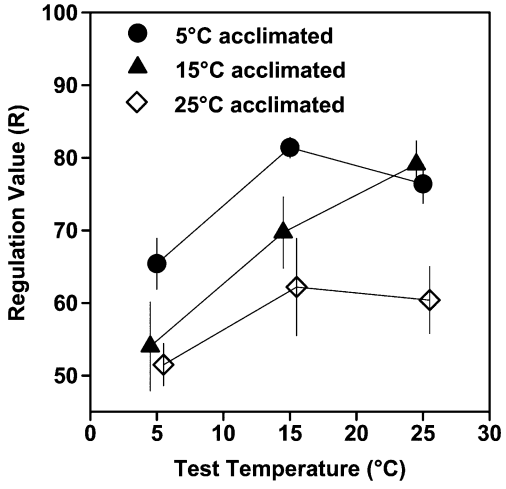

Fig. 4. Mean oxygen regulation values (*R* values) for the zebra mussel (*D. polymorpha*). Animals were acclimated to 5 (●), 15 (▲) and 25 °C (◇) and all animals were tested at all three test temperatures: 5, 15 and 25 °C. Higher *R* values indicate higher degrees of oxygen regulation. Bars represent standard errors of the mean.

freshwater bivalves in particular (McMahon, 1996). Such values indicate that metabolic rate in this species responds in a simple thermokinetic pattern to temperature increase and thus the zebra mussel's metabolic rate is not thermally regulated in response to acute temperature change (McMahon, 1991).

The imperfect compensation pattern, as seen in *D. polymorpha*, is considered to reduce seasonal variation in metabolic rate relative to species incapable of metabolic temperature acclimation (Precht et al., 1973). The degree to which ectothermic animals are able to adjust their metabolic rates in response to changes in seasonal temperatures can be estimated by acclimatory Q_{10}=values [$Q_{10(acc)}$] (McMahon, 1991). Values of $Q_{10(acc)}$ near 1 indicate that metabolic temperature compensation is nearly perfect (V_{O_2} remains constant at different ATs after acclimation across a wide temperature range), while a $Q_{10(acc)}$ between 2 and 3 suggests that the species does not show any metabolic temperature compensation (McMahon, 1991). Zebra mussels show little capacity for respiratory temperature compensation in the lower portion of their tolerated temperature range (from 5 to 15 °C), but they are relatively good temperature compensators of $\dot{V}_{O_2}$ in the upper portion of their temperature range (from 15 to 25 °C). The ability to maintain a constant metabolic rate at high ATs might prevent wasteful catabolism of energy stores during high summer water temperatures. This ability to compensate at elevated summer temperatures may be particularly important for *D. polymorpha*, because catabolism approaches or surpasses assimilation rates above 20 °C in *Dreissena*, leaving little or no energy for allocation to growth or reproduction (Waltz, 1978). In contrast, the lack of metabolic temperature compensation below 15 °C means that metabolism declines considerably during the cold winter months when low food availability may occur. This metabolic decline during the winter would also conserve energy stores. Individual *D. polymorpha* lost only 9% of their dry tissue mass when starved over a 90 day period at 5 °C (Chase and McMahon, 1995), indicative of maintenance of extremely reduced metabolic rates at low, wintertime temperatures.

We consider the temperatures of maximal $\dot{V}_{O_2}$ observed in our study to represent the acute upper lethal limits for this species (approximately 30 °C for 5 °C and 15 °C acclimated individuals, and 35 °C for 25 °C acclimated individuals). These temperatures are similar to values obtained in other studies of *D. polymorpha* in the laboratory, which range between 33 and 41 °C (McMahon et al. 1993), and with the chronically tolerated upper thermal limit of approximately 30 °C suggested by Iwanyzki and McCauley (1993). Our results also generally agree with those obtained in field experiments conducted in outdoor mesocosms (Thorp et al., 1998, 2002); mortality increased sharply for zebra mussels collected from both Ohio River and Lake Erie at temperatures approximately 30–32 °C. Growth rates also were highest between 15 and 25 °C, and declined at higher temperatures (Thorp et al., 1998, 2002).

4.2. Metabolic response to hypoxia

Because of its low oxygen regulatory capacity, *D. polymorpha* appears to be poorly adapted to hypoxic conditions, compared to other bivalves (Burky, 1983; McMahon, 1991). Oxygen-regulating bivalves generally inhabit environments that are periodically hypoxic, such as hypolimnetic waters or hypoxic sediments (McMahon, 1991). Most freshwater unionid mussels are oxygen regulators, including *Elliptio complanata* (Lewis, 1984), *Anodonta grandis* (Lewis, 1984), and *A. cygnea* (Massabuau et al., 1991), and all can tolerate relatively extreme hypoxic conditions for many weeks (McMahon, 1991). Among North American freshwater bivalves, only the Asian clam *Corbicula fluminea* appears to be a poorer oxygen regulator than *D. polymorpha* (McMahon, 1979). The generally poor O_2 regulatory ability of *C. fluminea* is associated with this species' poor hypoxia tolerance and resulting restriction to well-oxygenated waters, and its preference for well-oxygenated substrata (McMahon, 1983). *D. polymorpha* likewise appears to be restricted to well-oxygenated lotic habitats or the oxygenated epilimnetic waters of large lakes (McMahon, 1996; Alexander et al., 1997).

Interestingly, temperature acclimation and test temperature affected the oxygen regulatory ability in *D. polymorpha* in different ways. In our study, acclimation to high temperatures decreased the capacity for O_2 regulation with progressive hypoxia regardless of TT, while increasing TT increased the capacity for regulation in all three acclimation groups. A similar increase in O_2 regulation with increasing TT has been reported for *Sphaerium*

simile (Waite and Neufeld, 1977). The increase in regulatory ability at higher test temperatures presumably is associated with increased metabolic capacity for ciliary ventilation and/or hemolymph perfusion of the gills. In the freshwater mussel, *A. cygnea*, the P_{O_2} of hemolymph leaving the gills is maintained at a constant, low value independent of ambient P_{O_2} (Massabuau et al., 1991), suggesting an ability to regulate the gill ventilation/perfusion ratio.

Zebra mussels are poor O_2 regulators when warm-acclimated, but their regulatory ability increases when cold-acclimated. This increased regulatory ability at low temperatures may be an adaptation to the hypoxic conditions generated by freezing over of its habitat. Winter ice cover prevents gas exchange at a lake's surface, leading to hypoxic conditions as the metabolic demands of bacteria remove oxygen from the water column. Increased O_2 regulatory ability at low winter temperatures could allow overwintering zebra mussels to remain aerobic, thus maintaining a higher production of ATP energy while preventing wasteful anaerobic metabolism of organic energy stores. A similar shift occurs in the freshwater pulmonate snail *Laevapex fuscus*, from conformity by warm-acclimated individuals to extreme oxygen regulation in cold-acclimated individuals experiencing hypoxia in ice covered habitats (McMahon, 1973).

4.3. The utility of R in comparative physiology

One problem that has perplexed environmental physiologists for years is that no clear ecological or phylogenetic pattern appears to exist in the response to hypoxia (Tang, 1934; Bayne, 1973; Mangum and van Winkle, 1973; van Winkle and Mangum, 1975; Taylor and Brand, 1975; Herreid, 1980; Willmer et al., 2000; Randall et al., 2002). Animals described as either regulators or conformers are widely scattered among both ecological habitats and taxonomic groups, making broad generalizations difficult. Part of the problem is that the strict dichotomy of regulation or conformity is false (Mangum and van Winkle, 1973; Taylor and Brand, 1975), and a continuum of responses exists, with conformers on one end of the continuum and strong regulators on the other. Categorizing species simply as either conformers or regulators may be a vague and imprecise tool; however, other complications also exist. The degree to which regulation occurs varies not only between species, but among individuals in the same species as well (Herreid, 1980). In several studies, some individuals act as conformers, but others were described as regulators under similar conditions (e.g. Bayne, 1971; Duke and Ultsch, 1990). Different environmental conditions and the physiological state of the animals also influence the degree of regulation (Herreid, 1980).

Several quantitative methods currently exist for describing the degree of $\dot{V}_{O_2}$ regulation in organisms subjected to progressive hypoxia. All of these methods involve fitting $\dot{V}_{O_2}$ (often in standardized form) as the dependent variable to a regression equation, with O_2 concentration (usually as P_{O_2}) as the independent variable. Linear, semi-logarithmic, exponential, hyperbolic and quadratic regression models have all been used, from which a regression coefficient is obtained and used as the measure of the degree of O_2 regulation (Tang, 1934; Bayne, 1973; Mangum and van Winkle, 1973; van Winkle and Mangum, 1975; Yeager and Ultsch, 1989).

We believe that these regression methods have not been completely satisfactory in describing the degree of O_2 regulation of $\dot{V}_{O_2}$, for several reasons. First, the regressions cannot always be readily fit to V_{O_2}–P_{O_2} curves, particularly those of species which are extremely adept at oxygen regulation. A second reason is that $\dot{V}_{O_2}$ varies in an unpredictable manner over the course of progressive hypoxia in many species. $\dot{V}_{O_2}$ may initially increase, not decrease, with progressive hypoxia. Metabolic demands at lower oxygen concentrations may increase due to increased ventilation rates and/or perfusion rates. However, in other species, metabolic demands may become suppressed with even small reductions in P_{O_2}. A third reason that regression models may be imperfect tools is because regression coefficients do not relate in an easily comprehensible way to the degree of oxygen regulation. For example, one common method of reporting the degree of regulation is the use of the quadratic coefficient of a quadratic equation fitted to the data, multiplied by 1000 ($b_2 \times 10^3$ value, Mangum and van Winkle, 1973). The resulting $b_2 \times 10^3$ values are usually small and negative, making species comparisons or ecological comparisons difficult.

One analytical tool currently in use is the determination of the critical oxygen tension, or P_c, for animals under specific environmental conditions.

Many regulators maintain a constant metabolic rate over declining oxygen levels down to a point at which they switch to acting as conformers; this point is defined as P_c. The P_c value is useful in defining what is meant by a strong regulator, in that strong regulators should have low P_c values, and weaker regulators have higher P_c values. However, clearly defined P_c points do not exist in many cases (Herreid, 1980), and authors may vary considerably in what value of P_c they consider to be indicative of regulation (Hill, 1976).

We suggest that our new descriptive statistic, *R*, has many advantages over previously used regression statistics. First, *R* can accurately assess the degree of regulation. Second, *R* is much less affected by the degree of irregularity of the V_{O_2}–P_{O_2} response curve. Third, *R*'s relation to the degree of oxygen regulation is easily understood: progressively higher *R* values above 50% indicate a greater degree of oxygen regulation, and *R* near 50% indicate oxygen conformity. One additional advantage to *R* is that it is very amenable to statistical analysis and subsequent interpretation, as shown by our study. We believe that in addition to various descriptive statistics currently in use (such as P_c), *R* can be an important analytical tool for comparing the regulatory responses of different animals to hypoxic conditions.

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