



Comparison of oxygen consumption in freshwater mussels (Unionidae) from different habitats during declining dissolved oxygen concentration

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Abstract

The rate of oxygen consumption (OC) of 9 species of freshwater mussels was measured under declining dissolved oxygen (DO) concentrations. The effects of temperature for some species also was investigated. The pattern of the OC vs. DO curve for each species was used in a hyperbolic model to compare abilities to regulate OC under low oxygen conditions. At 24.5 °C, *Pyganodon grandis* (from lakes), *Amblema plicata* and *Quadrula pustulosa* (from mud or sand in large rivers), *Elliptio complanata* (from pool areas in rivers), and *Elliptio fisheriana* and *Elliptio lanceolata* (from bank margins of rivers) were better able to maintain OC under low DO than were *Villosa iris* and *Villosa constricta* (which inhabit riffles) and *Pleurobema cordatum* (found in rivers with moderate flow). *Villosa iris* was especially sensitive to low oxygen conditions. The ability to maintain normal OC at low DO was improved considerably at 16.5 °C for *V. iris*, *P. grandis* and *E. complanata*. It is concluded that oxygen regulation ability appears to be related to the degree of hypoxia a species normally experiences in its habitat type, and it is enhanced at low temperature. The measurement of OC vs. DO may be a useful technique for estimating DO water quality criteria for endangered species because it is noninvasive.

Introduction

The ability to maintain a relatively constant uptake of oxygen under conditions of low oxygen availability is important for organisms that may encounter low dissolved oxygen (hypoxia) frequently in their environment (reviewed by Herreid, 1980). If the rate of oxygen consumption (OC) is not maintained at low levels of dissolved oxygen, anaerobic metabolism, which is inefficient with respect to energy production, may be activated in the tissues (Gade & Grieshaber, 1988). This ability to maintain OC (generally called metabolic regulation) in water of reduced DO has been widely investigated for marine species of bivalves from a range of habitats (e.g. Bayne, 1971; Taylor & Brand, 1975; Shumway & Koehn, 1982; Wang & Widdows 1993). These studies indicate that the extent of metabolic regulation varies with the environmental

conditions, body size, physiological state of the animals, and most especially, the habitat where the species is generally found.

The capacity for metabolic regulation in freshwater bivalve species has received relatively little attention. The fingernail clam appears to have a high capacity in that it retains a relatively constant rate of OC down to an oxygen tension of around 16 mm Hg (Wait and Neufield, 1977). Lewis (1984) found that *Elliptio complanata* and *Pyganodon grandis* were also exceptionally good oxygen regulators. In contrast, *Corbicula fluminea*, which inhabits only well-oxygenated habitats, exhibits an OC rate that is dependent on oxygen concentration at nearly all levels of DO (McMahon, 1983). Sheldon & Walker (1989) compared two Australian species of freshwater mussels and found they respond metabolically to low DO very differently from each other. McMahon (1991) in his review of the

ecology of freshwater molluscs suggested that species living in aquatic habitats periodically subjected to hypoxia are better able to regulate OC under declining DO conditions. However, the data are limited and no broad investigation of the Unionidae has been done, especially for species from riverine habitats.

The purpose of this study was to determine the ability of several freshwater unionid species from different habitats to regulate OC under declining DO. The resulting data might be used along with other information in establishing DO water quality criteria for these species. As this study involved freshwater mussels often of limited availability, and included a state-protected species (*Elliptio lanceolata*), the experiments were non-invasive and the mussels were released to their collection sites after the experimentation.

Materials and methods

Mussels

Adult specimens of 9 species of freshwater mussels were collected from various field locations (means wet body mass \pm 1 SE including shell are given for each species). *Pyganodon grandis* (137.5 \pm 11.5 g, $n=8$ for the 24.5 °C measurements; 105.2 \pm 9.1 g, $n=20$ for the 16.5 °C measurements) were collected from Claytor Lake, Virginia. *Elliptio complanata* (38.4 \pm 3.8 g, $n=17$ for the 24.5 °C measurements; 64.9 \pm 6.5 g, $n=12$ for the 16.5 °C measurements), *Elliptio fisheriana* (14.1 \pm 1.2 g, $n=10$), and *Elliptio lanceolata* (10.1 \pm 0.8 g, $n=20$) were collected from pools of the Nottoway River, and sandy substratum and clay bank of the Rappahannock River, Virginia, respectively. *Villosa iris* (12.5 \pm 0.9 g, $n=20$ for the 24.5 °C measurements; 14.6 \pm 1.1 g, $n=16$ for the 16.5 °C measurements) and *Villosa constricta* (10.0 \pm 2.9 g, $n=19$) were taken from riffles in the North Holston Fork River and Nottoway River, Virginia, respectively. *Amblema plicata* (192.4 \pm 8.2g, $n=15$), *Quadrula pustulosa* (156.7 \pm 2.4 g, $n=22$) and *Pleurobema cordatum* (210.0 \pm 8.2 g, $n=16$) were collected by diving in Kentucky Lake, lower Tennessee River, Tennessee. Animals to be tested at 24.5 °C were all collected during summer or autumn months, whereas those to be tested at 16.5 °C were collected from November through January.

All of the mussels were acclimated in aquaria with sand substratum and a flow-through, temperature-controlled (\pm 1 °C) dechlorinated water system for a

minimum of 1 week before OC measurements were begun. Acclimation temperatures were the same as test temperatures, and the water was maintained by aeration at near oxygen saturation. A photoperiod of 12 light: 12 dark was maintained at all times. The animals were fed a commercial algae diet (SUN Chlorella 'A' granules by YSK International Crop., Japan) each morning. Those animals to be used for measuring the OC rate were not fed in the morning of a test.

Oxygen consumption measurements

The rate of oxygen consumption was measured by placing single mussels in respirometer chambers equipped with an oxygen probe (YSI 5750) in the lid, a screen above the floor, and a magnetic stirring bar on the floor. Water temperature was maintained at 24.5 °C. Other specimens of *Villosa iris*, *E. complanata*, and *P. grandis* were acclimated for at least a week, and OC measured at a lower temperature of 16.5 °C, during the months of November through January. For the OC measurements, animals were placed in the chamber 3 h before a run began in the morning between 0900 and 1100 h. To reduce microbial OC, the chamber was disinfected with boiling water between each experiment, and UV sterilized water continuously flowed through the chamber before the measurements began. An experimental run started with shutting off the flow, and then as the mussel consumed the oxygen in the chamber, the DO was recorded on a strip-chart recorder. Different-sized chambers were used according to the size of the mussels, so that the duration of time to deplete the oxygen was more than 6 h but less than 10 h (except for the low temperature experiments.) as the background OC tended to increase slightly after 12 h. The OC rate was expressed as mg O₂ kg⁻¹ gross weight h⁻¹. The background oxygen consumed by the probe, chamber and water was also measured but usually ignored as it equaled less than 3% of the overall DO consumed by the mussels.

Results

Villosa iris and *Villosa constricta*

The OC rate of *V. iris* at 24.5 °C declined gradually as the DO declined (Fig. 1), which showed that they tend to be oxygen conformers. However, the ability to regulate the rate of OC of *V. iris* was much improved in those acclimated and run at 16.5 °C. The OC of

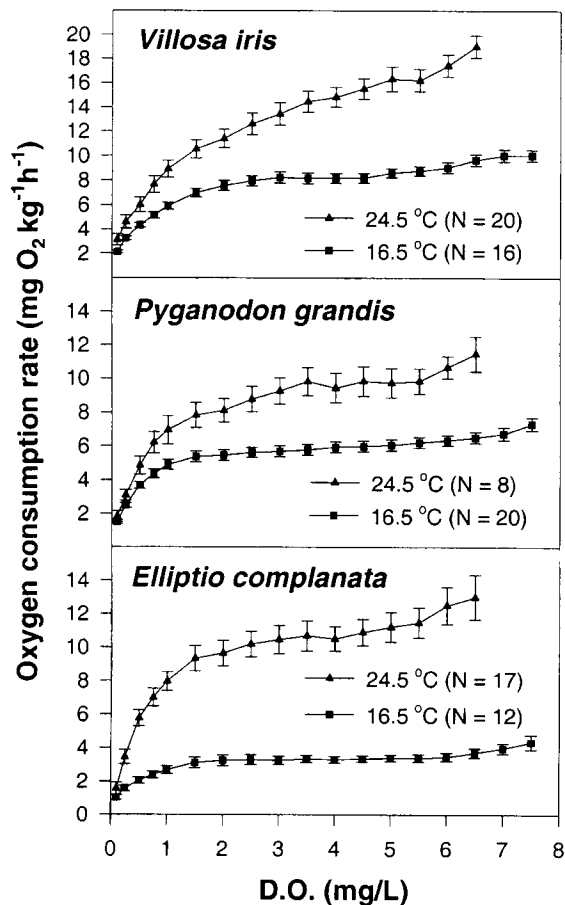


Figure 1. Oxygen consumption rate of *Villosa iris*, *Pyganodon grandis* and *Elliptio complanata* under declining dissolved oxygen concentration at 24.5 °C and at 16.5 °C. Points are means \pm SE.

V. constricta at 24.5 °C (Fig. 2), on the other hand, behaved more like a regulator when $DO \geq 4$.

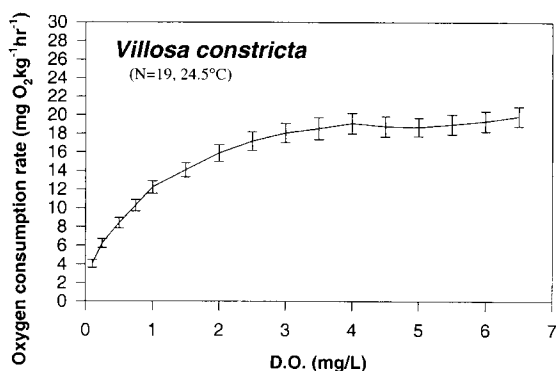


Figure 2. Oxygen consumption rate of *Villosa constricta* under declining dissolved oxygen concentration at 24.5 °C. Points are means \pm SE.

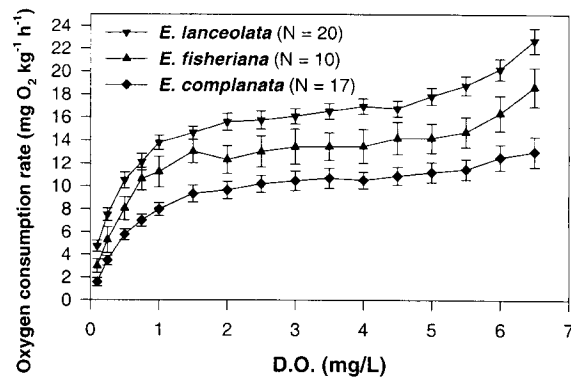


Figure 3. Comparison of oxygen consumption rate of three *Elliptio* species under declining dissolved oxygen concentration at 24.5 °C. Points are means \pm SE.

Elliptio complanata, *Elliptio fisheriana* and *Elliptio lanceolata*

The OC curves of the three *Elliptio* species at 24.5 °C (Fig. 3) exhibited regulation when $DO \geq 1-2$ mg l^{-1} , indicating that they were able to regulate to a lower DO than *V. iris* and *V. constricta*. As with *V. iris* at the lower temperature, *E. complanata* at 16.5 °C (Fig. 1) also behaved like a regulator.

Pyganodon grandis

The OC curve of *P. grandis* at 24.5 °C (Fig. 1) is similar to that of *E. complanata* (Fig. 1). They both responded like regulators when the DO was ≥ 1.5 mg l^{-1} . The overall rate of metabolism of *P. grandis* was reduced at 16.5 °C, and the ability to maintain OC in low DO was improved at this lower temperature (Fig. 1).

Amblema plicata, *Quadrula pustulosa* and *Pleurobema cordatum*

The OC of *A. plicata* and *Q. pustulosa* at 24.5 °C (Fig. 4) were similar to that of *P. grandis*, in that they responded like regulators when $DO \geq 2.5$ mg l^{-1} . *P. cordatum*, however, exhibited less ability to regulate OC than the other two species (Fig. 4).

Discussion

Oxygen consumption of bivalves is often calculated on the basis of the dry flesh weight, however this requires sacrificing the specimen, which we wanted to avoid in this study. In a pilot group of measurements

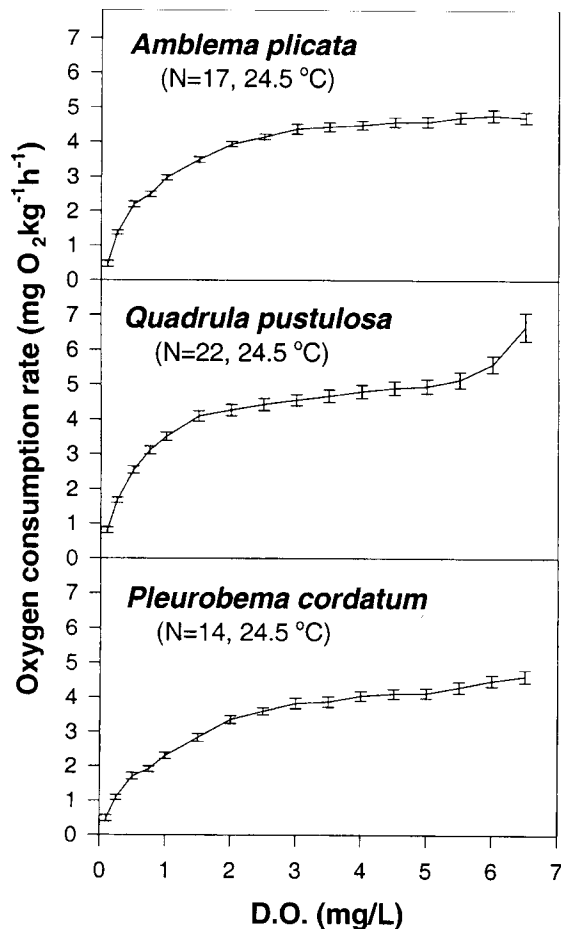


Figure 4. Oxygen consumption rate of *Amblema plicata*, *Quadrula pustulosa* and *Pleurobema cordatum* under declining dissolved oxygen concentration at 24.5 °C. Points are means \pm SE.

made in our laboratory, a linear relationship was observed between gross body weight (i.e. including the shells) and wet weight and dry weight of the flesh ($r^2 = 0.93$ and 0.88 , respectively, $n=22$) for *E. complanata*. Hence, the specific OC calculated by gross body weight reflected the relative values of that calculated by wet flesh weight or dry weight. In this study, we focused on the pattern of OC changes under low DO rather than comparing the absolute values of specific OC among different species. Because the weight of the shell is included in these calculations, the absolute values of OC cannot be compared with most other values in the literature.

In order to quantify the ability of an animal to maintain OC in low DO, some (e.g. Hornbach, 1991) have utilized the hyperbolic model $OC = DO/K1 + K2 \times DO$, based on Bayne (1971). In this model, K1 and

K2 are obtained from the regression of the weight-specific OC/DO to DO, where K1 is the intercept and K2 is the slope. The ratio K1/K2 can be used as an index of respiratory independence from oxygen concentration. The lower the K1/K2 value, the greater the capacity to maintain OC (i.e. act as a regulator), since OC is more like a constant under varying dissolved oxygen levels. Conversely, the higher the K1/K2 value, the smaller the capacity to regulate OC, since OC is more dependent on DO.

In Table 1, we relate the K1/K2 values of the species we tested with their respective typical habitat type. *Villosa iris* and *V. constricta*, which generally live in well oxygenated stream and river riffles, and *P. cordatum*, which occurs in areas of moderate flow and adequate oxygenation, exhibited the poorest ability to regulate OC under conditions of low oxygen availability (i.e. they have the highest K1/K2). *Pyganodon grandis*, *A. plicata*, *Q. pustulosa* and *E. complanata*, which live in lentic habitats and lotic areas where DO typically declines in summer from algal blooms or organic decomposition, tend to generally have a greater ability to regulate OC than the *Villosa* species. Byrne et al. (1995) also demonstrated that *E. complanata* tolerates a range of hypoxic conditions, even with zebra mussels (*Dreissena polymorpha*) attached to its valves. The species *E. fisheriana*, which lives in sand and is possibly exposed to hypoxia below the benthic surface, exhibited the greatest ability to regulate OC. Of course, these are only trends with some overlap in habitat and physiological ability of the species, but they do seem to reflect differences that may be important biologically.

A possibly confounding variable is body size. Hornbach (1991) noted that larger specimens of the same species had slightly lower K1/K2 ratios (i.e. they regulated OC better) than smaller ones. The *Villosa* spp. that we used were the smallest of the species tested and had the highest K1/K2 ratios. However, it should be noted that the three *Elliptio* species we tested had the lowest K1/K2 ratios and were only slightly larger than the *Villosa* spp.

The trends we report here are in agreement with the limited published data in the literature on freshwater bivalves. Sheldon & Walker (1989) compared a species of mussel typical of flowing rivers with one found in impoundments that experience periods of low DO. The species from the river habitats (*Alathyria jacksoni*) was essentially a metabolic conformer, whereas the one from the impoundments (*Vesunio ambiguus*) exhibited metabolic regulation down to a partial pres-

Table 1. K1/K2 values calculated from the data in Figures 1–4 for the different species tested. K1 and K2 were obtained from the regression of the OC/DO to DO where K1 is the intercept and K2 is the slope. Twelve OC observations from a DO of 5.5–0.5 mg l⁻¹ were used for the regression of each species. *r*² for K2 was generally above 0.9

Species	Temp. (°C)	Typical Habitat	K1/K2
<i>E. fisheriana</i>	24.5	Along and under bank of river ^a	0.407
<i>E. lanceolata</i>	24.5	Sand bottom of river ^a	0.497
<i>E. complanata</i>	24.5	Pools of large rivers ^a	0.568
	16.5		0.307
<i>P. grandis</i>	24.5	Ponds and lakes ^a	0.608
	16.5		0.377
<i>Q. pustulosa</i>	24.5	Mud, sand or gravel of medium to large river ^b	0.611
<i>A. plicata</i>	24.5	Mud, sand or gravel of river ^b	0.711
<i>V. constricta</i>	24.5	Riffle area of river ^a	0.786
<i>P. cordatum</i>	24.5	Medium to large rivers with moderate flow ^b	1.152
<i>V. iris</i>	24.5	Riffle area of river ^a	1.30
	16.5		0.587

^aJohnson (1970).

^bCummings & Mayer (1992).

sure of 65 mm Hg. Hornbach (1991) observed that the freshwater clam *Musculium partumeium* shows excellent metabolic regulation down to a partial pressure of approximately 30 mm Hg. This species is commonly found in ephemeral ponds that experience frequent periods of low oxygen. These studies along with our results agree with the suggestions of McMahon (1991), that species living in aquatic habitats periodically subjected to prolonged hypoxia may have a greater ability to regulate OC under declining DO.

Temperature may be an important factor controlling tolerance of mussels to low oxygen, not only because saturation DO declines as temperature rises, but since they are poikilotherms, the rate of mussel metabolism is dependent on temperature. *Villosa iris* had the poorest ability to regulate OC among the species tested in our study. However, its ability to regulate increased greatly when the temperature was lowered to 16.5 °C. A similar effect of temperature was seen with *P. grandis* and *E. complanata* (Fig. 2, Table 1), as also occurred in the response of the fingernail clam *Sphaerium simile* (Waite & Neufeld, 1977). There the K1/K2 ratio increased markedly with a rise in temperature.

The mechanisms of metabolic regulation have received little attention in freshwater bivalves. Massabuau et al. (1991) reported that *Anodonta cygnea* (a freshwater bivalve) maintains OC independ-

ent of ambient oxygen down to a low level primarily by maintaining arterial blood Po₂ at low values, independent of oxygen partial pressure in the water. This is facilitated by the animals having a blood respiratory pigment with a high oxygen-binding affinity. In a pilot study in our laboratory (unpublished), we simultaneously measured the heart rate and OC in *P. grandis* and found that these mussels increased the heart rate when the DO was low, presumably to help maintain the OC. Undoubtedly, other species may use these along with other mechanisms to facilitate metabolic regulation, which is an interesting topic for further research.

The data presented in this paper may be used to suggest water quality criteria for minimum DO at temperatures of around 24 °C. To be sure, mortality data would be even better to use for such purposes, but when working with species that may be available in limited quantities (or be federally endangered species), this technique has the advantage of being noninvasive. If we assume that the DO level where OC vs. the DO curve begins to show a sizeable decline represents a transition below which the animals are not maintaining 'normal' OC, then at a DO below this, the animals may be under some degree of stress if the condition persisted for many hours or days (Davis, 1975). For *A. plicata*, *Q. pustulosa* and *E. complanata*, this transition is around 2–3 mg l⁻¹. For *P. cordatum*,

DO should be above 3.5 – 4.0 mg/L; and for *V. iris*, DO should probably be higher than 6 mg l⁻¹ to ensure that aerobic metabolism remains relatively unchanged.

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