

Oxygen consumption by juvenile *Pyganodon cataracta* (Bivalvia: Unionidae) in response to declining oxygen tension

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ABSTRACT: The physiology of very young freshwater mussels is largely unstudied. Historically the near impossibility of acquiring sufficient numbers of juveniles from the field precluded experimental investigation. Advances with *in vitro* and *in vivo* techniques for inducing metamorphosis of glochidia larvae in the laboratory have been tempered by generally low survivorship and relatively poor growth of the resulting juveniles. As a result, except for work on nutrition, most physiological studies have been limited to using 1-2 wk old juveniles of only a few species in bioassays for toxicology. Success in rearing *Pyganodon cataracta* for 24 wks permitted the determination of the rate of oxygen consumption of individual juvenile mussels using micro-respirometry. Although the pattern of response to declining PO_2 varied among individuals, especially in respect to whether animals remained open and siphoning for the duration of an experiment, consistent data were acquired for nine animals. Juveniles exhibited partial oxyregulation from full saturation to a critical partial pressure (P_c) of about 115 mm Hg. This pattern contrasts somewhat with the oxyconformity previously reported for adults of this species. The rate of oxygen consumption in the regulatory phase of the response curve ($\dot{V}O_{2max}$) was 87.7 ml O_2 $g^{-1} h^{-1}$. These results constitute the first data on respiratory physiology of such young freshwater mussels.

Keywords: metabolism, respiration, freshwater, mussel, oxyconformity.

The complexity of the life cycle of unionoid mussels, with recently metamorphosed glochidia larvae entering the benthos as ≈ 200 -350 μm thin-shelled juveniles that are highly vulnerable to mortality (Young and Williams 1984), makes it unlikely that such young mussels can routinely be collected from the field. In an extensive field study, Neves and Widlak (1987) recovered only 27 <1 year-old juveniles (mean shell length = 2.7 mm; range = 0.8-5.0 mm) from 75 quantitative samples in a 100 m transect of a stream that harbored dense populations of adults of seven species of mussels. The abundance of juveniles in nature often must be inferred by extrapolation of data for relatively large (e.g., >25 mm) size classes of individuals (Negus 1966). The concept of juvenile may extend to mussels that are several years old (Sparks and Strayer 1998).

The use of *in vivo* procedures (infecting fish hosts in the laboratory with glochidia and awaiting their metamorphosis) or *in vitro* techniques (incubating glochidia in tissue culture) can yield sufficient numbers of very young mussels (days to months old) to facilitate their experimental study. However, the conclusion of Russell-Hunter (1964) that the biology of juvenile freshwater mussels is essentially unknown is nearly as true today as it was 35 years ago.

Limited understanding of this critical stage in mussel life histories is beginning to emerge, with nearly all the advances having been made within the past decade

(Table 1). However, only 12 species, of the nominal 300 or so in North America alone, have been subjects of experimental analyses, with the majority being used exclusively in toxicological bioassays (Table 1). Fundamental aspects of the physiology, ecology and behavior of juvenile freshwater mussels remain largely unknown.

The dearth of information about young freshwater mussels is in sharp contrast to the level of understanding of the biology of marine bivalves, especially commercially important species. For example, larvae and juveniles of the blue mussel, *Mytilus edulis*, have been studied extensively in respect to feeding biology, utilization of dissolved organic matter, aerobic and anaerobic components of metabolism, and factors affecting growth (Wang and Widdows 1991, Widdows 1991). The influence of environmental factors including food, temperature, salinity, dissolved oxygen, and toxicants on growth and survival of larvae and juveniles of this important species also has been thoroughly investigated (Widdows 1991). Comparable data, as well as evidence for complex interactions among environmental parameters and ontogenetic changes in functional capacities of larvae, are available for the American oyster, *Crassostrea virginica* (Widdows *et al.* 1989). Such studies would serve as instructive models for basic investigations of freshwater mussel biology.

Factors influencing the rate of oxygen consumption of adult unionids and other freshwater bivalves have been

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Table 1. Aspects of the biology of recently metamorphosed unionoid* mussels.

Area of Investigation	Species	Reference	
Toxicology metals, pesticides, industrial effluent	<i>Utterbackia</i> (=Anodonta) <i>imbecillis</i>	Keller and Zam 1991 Johnson <i>et al.</i> 1993 Warren <i>et al.</i> 1995 Warren 1966 McKinney and Wade 1996 Keller and Ruessler 1997	
	<i>Villosa lienosa</i> , <i>V. villosa</i>	"	
	<i>Lampsilis straminea claibornensis</i>	"	
	<i>L. subangulata</i>	"	
	<i>Elliptio icterina</i>	"	
	<i>Pyganodon</i> (=Anodonta) <i>grandis</i>	"	
	<i>L. cardium</i> (=ventricosa)	Lasee 1991	
	<i>V. iris</i>	Jacobson <i>et al.</i> 1993	
	Growth and Survival diets, maintenance	<i>U. imbecillis</i>	Hudson and Isom 1984 Starkey <i>et al.</i> 1999
		<i>V. iris</i>	Gatenby <i>et al.</i> 1996, 1997 O'Beirn <i>et al.</i> 1998
<i>L. fasciola</i>		"	
<i>P. grandis</i>		Gatenby <i>et al.</i> 1996, 1997	
<i>Margaritifera margaritifera</i>		Buddensiek 1995	
Physiological Effects temperature, oxygen		<i>U. imbecillis</i> , <i>P. cataracta</i>	Dimock and Wright 1993 Polhill and Dimock 1996
	Behavior feeding, burrowing	<i>V. iris</i>	Yeager <i>et al.</i> 1994
<i>U. imbecillis</i>		Wright 1995	

*Families Margaritiferidae, Hyriidae and Unionidae

studied in some detail (McMahon 1991). A few studies have specifically addressed the metabolic response of adult mussels to decreasing oxygen tension (Hiestand 1938, Lewis 1984, Sheldon and Walker 1989, Massabuau *et al.* 1991, Tankersley and Dimock 1993). For many freshwater bivalves, exposure to hypoxia rather than to complete anoxia may be a recurring and physiologically stressful condition (Johnson and McMahon 1998). The dual but opposite effects of temperature on oxygen solubility and bivalve metabolic rate, together with the hydro-dynamics especially of lentic habitats and the burrowing habit of adult (Amyot and Downing 1991) or juvenile mussels (Neves and Widlak 1987, Yeager *et al.* 1994) can result in mussels encountering oxygen stress. Juvenile mussels may be especially vulnerable (Dimock and Wright 1993) to the oxygen profile of subsurface interstitial water, within which oxygen tension and the degree of saturation can decrease 60-80 % within the first 1-2 cm (Buddensiek *et al.* 1993).

The present study examines the rate of oxygen consumption by 5-month old *Pyganodon cataracta* in response to decreasing oxygen tension. The results indicate that the rate of consumption is dependent upon ambient oxygen level except at relatively high oxygen tensions. The work provides the first data on respiratory physiology of any juvenile unionid mussel.

Methods

Juvenile *Pyganodon cataracta* were reared from glochidia larvae excised from the gill of an adult female from Meyers' Pond, Forsyth County, NC, (January, water temp = 9 °C). The mussel was held in a refrigerated aquarium that was raised 1 °C d⁻¹ to 16 °C, at which *in vitro* culturing of glochidia was initiated. Glochidia were cultured using techniques of Isom and Hudson (1982) as modified by Hudson and Shelbourne (1990) and Dimock and

Wright (1993). Once begun, the cultures were raised 1 °C d⁻¹ to 20 °C in a temperature-controlled CO₂ incubator. The glochidia metamorphosed to juvenile mussels on the 11th day of incubation.

Since the juveniles were part of a growth study, they were not fed for 5 d post-metamorphosis and then received approximately 1.5 x 10⁶ cells ml⁻¹ d⁻¹ of *Chlorella vulgaris* (Carolina Biological Supply Co., Burlington, NC). At 2-wk post-metamorphosis they were placed on a diet of live *C. vulgaris* and spray-dried *Schizochytrium* (Algamac-2000, Bio-Marine Inc., Hawthorne, CA) in a 1:1 mixture equivalent to a total of 0.02 mg dry wt ml⁻¹ d⁻¹. The diet was prepared fresh every 2-3 wks and kept refrigerated.

Juveniles (50 chamber⁻¹) were reared in 10 cm i.d. ABS-plastic cylinders with 204 µm Nitex mesh as the bottom. Each chamber was placed in a 14 cm x 14 cm polyethylene container with enough calcium-enriched artificial pond water (Ca-APW) [25 mg CaCO₃ L⁻¹ added to the recipe of Dietz and Alvarado (1970)] to yield ≈200 ml inside the chamber. Animals were fed daily and the water was changed every other day, at which time approximately 700 mg dry wt L⁻¹ of <63 µm autoclaved pond silt was added to each chamber. Mussels were transferred to clean chambers every 10 d to 2 wks. Cultures were held at 21 °C under a 10L:14D photoperiod.

The rate of oxygen consumption was determined for 9 juveniles that were 22-23 wks old [length = 6.2 ± 0.4 mm (±SE), range = 4.9-8.3 mm; wet wt (including shell) = 27.5 ± 4.8 mg (± SE), range = 16.1-56.8 mg]. Rates were measured at 20 °C on individual mussels in water-jacketed RC 300 micro-respirometry chambers (operational volume 0.3-1.0 ml) with Model 1302 micro-cathode oxygen sensors (Strathkelvin Instruments, Glasgow, Scotland) and a Cameron Instrument Company (Port Aransas, TX) dual channel oxygen meter. The output from the meter was fed into an MP100 data acquisition system (BioPac Systems, Santa Barbara, CA) and recorded and displayed with AckKnowledge Version 3.25 software (BioPac Systems) running on an IBM 380D ThinkPad computer. An identical oxygen sensor and chamber served as a control.

An individual mussel that had been held in clean Ca-APW for 24 h without food was placed in the respirometer to which had been added autoclaved sand (particle size 250-500 µm) to a depth of about 2 mm, together with enough Ca-APW (750-1000 µl) to accommodate the mussel. The mussel was allowed to burrow until it became quiescent, with the siphons extended. During this period the chamber was gently aerated through a 27 G syringe needle to maintain the PO₂ near air saturation (PO₂ = 152-155 mm Hg). Control chambers received identical treat-

ment. When the mussel appeared to be quiescent, the chamber was sealed by insertion of the oxygen sensor. The software continuously (30 samples s⁻¹) recorded the PO₂ (mm Hg) from both the experimental and control chambers. The animals were allowed to deplete the PO₂ to ≈10 mm Hg (about 7% of air saturation), which required from 2-4 h depending upon the size of the animal and the volume of water in the respirometer.

Oxygen consumption was determined at target PO₂'s of 135, 125, 100, 75, 50, 25 and 15 mm Hg, (approximately 90, 80, 65, 50, 16 and 10% of air saturation, respectively): Weight-specific rates ($\dot{V}O_2$, µl O₂ g⁻¹ h⁻¹) were calculated from the slope (±10 min around the target value) of the time vs PO₂ curves, based on the oxygen capacitance (ml O₂ l⁻¹ mm Hg⁻¹, Cameron solubility tables) and corrected for any change in the control chamber. Since the mussels were small, few in number, and intended to be used in subsequent studies, no animal was sacrificed. The data are based on the total wet wt of each mussel (including the shell) as determined after each experiment for animals that were gently wiped dry with absorbent tissue and weighed with a Cahn C-31 microbalance (Cahn Instruments, Inc., Cerritos, CA). Shell lengths (maximum dimension parallel to the hinge) were taken with image analysis software and a video camera on a Nikon SMZ-2T dissecting microscope. Regression analysis (Microsoft Excel™) was used to determine whether there was a significant effect of shell length or animal mass on $\dot{V}O_2$ over the range of sizes of mussels employed.

The relative degree of oxyconformity ($\dot{V}O_2$ declining linearly with decreasing PO₂) or oxyregulation ($\dot{V}O_2$ remaining constant over a range of PO₂'s) was assessed by fitting a quadratic polynomial model to the data using the procedures of Van Winkle and Mangum (1975). The coefficient for the quadratic term (β_2) is an index of the shape of the curve relating $\dot{V}O_2$ to PO₂. Oxyregulation is indicated if β_2 is significantly less than 0, while coefficients not significantly different from 0 are indicative of oxyconformity (Van Winkle and Mangum 1975).

The partial pressure of oxygen below which juvenile *P. cataracta* failed to maintain a constant rate of oxygen consumption, [i.e., the critical oxygen tension (P_c) (Herreid 1980)], was determined by fitting a segmented quadratic model (with plateau) to the response curve using nonlinear (least squares) regression. The procedure estimates P_c by fitting two models to the data that meet at P_c . At values above P_c , the equation yields a horizontal line or plateau (where $\dot{V}O_2 = \dot{V}O_{2max}$). Below P_c the quadratic model [$\dot{V}O_2 = \beta_0 + \beta_1 PO_2 + \beta_2 (PO_2)^2$] is fit to the data. All the parameters of the model including $\dot{V}O_{2max}$ and P_c were estimated iteratively using the SAS NLIN Procedure (SAS Institute 1990).

Results

The rates of oxygen consumption at each experimental PO_2 , together with the effects of declining oxygen tension on $\dot{V}O_2$ are depicted in Fig. 1. The variance among animals at a given oxygen tension (depicted by standard error bars on the figure) was relatively small at most points of measurement. This observation reflects the fact that the mussels burrowed into the shallow substratum and remained in place, with the siphons open, for the duration of most trials.

The $\dot{V}O_2$ declined in response to decreasing PO_2 at all oxygen tensions below about 115 mm Hg (the P_c , about 75 % of air saturation) (Fig. 1). $\dot{V}O_{2max}$, that is the rate at the calculated plateau from the P_c to $PO_2 = 135$ mm Hg was $87.7 \mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$. The $\dot{V}O_2$ for juvenile *P. cataracta*, as measured between 80 % and 100 % of air saturation (*i.e.*, on the plateau of the response curve), did not vary significantly either with shell length ($F_{1,7} = 1.9, P = 0.21, R^2 = 0.21$) or with the mass of the mussels ($F_{1,7} = 3.5, P = 0.11, R^2 = 0.32$).

The quadratic model provided a reasonably good fit to the data (Fig. 1, $R^2 = 0.72$). Although the β_2 value (-6.61×10^{-5}) was not significantly different from 0 ($t = -1.19, df = 4, P = 0.3$), it places the $\dot{V}O_2$ vs. PO_2 curve between the theoretical response curves D and E of Mangum and Van Winkle (their Fig. 1, 1973), underscoring the limited oxyregulatory capacity of these mussels (at $PO_2 > 75\%$ of air saturation).

Discussion

An historical approach to the study of aerobic metabolism of aquatic organisms often has included the assignment of experimental animals to a dichotomous designation of metabolic conformers or metabolic regulators (Herreid 1980). Assessment of the response to reduced oxygen tension is not always objective, with regulation or conformity or declaration of the critical partial pressure sometimes being determined by inspection of the response curves (Brand and Roberts 1973, Taylor and Brand 1975, Lewis 1984). Statistical

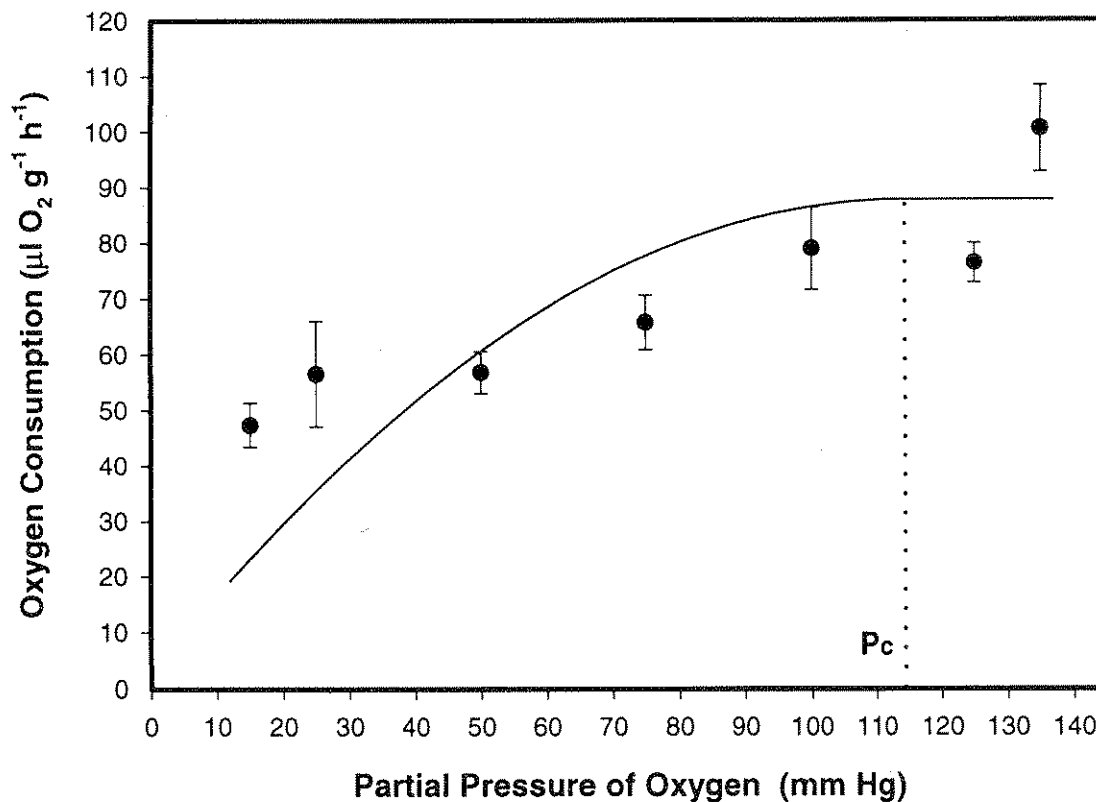


Figure 1. The rate of oxygen consumption of juvenile *Pyganodon cataracta* as a function of ambient oxygen tension. Points are the \pm SE, $N = 9$. The solid line depicts the fit of the quadratic polynomial model to the data. The vertical dashed line indicates the position of the critical oxygen tension (P_c).

evaluation of parameters indicative of regulation or conformity, and characterization of P_c , is preferable to subjective conclusions, and can readily be effected with the techniques of Van Winkle and Mangum (1975) or Yeager and Ultsch (1989). Fitting the data to a hyperbolic equation (Tang 1933), while occasionally employed (Bayne 1971, Taylor and Brand 1975, Dimock 1977), is probably less satisfactory (Mangum and Van Winkle 1973).

While the distinction between regulation and conformity may have heuristic value, especially when coupled with efforts to determine underlying mechanisms of regulation (e.g., Massabuau *et al.* 1991), the categorization may be a simplification of a complex suite of interactions that ultimately determine the magnitude and pattern of oxygen consumption. Mangum and Van Winkle (1973) cogently illustrated that regulation and conformity are neither species nor ecological absolutes, but rather are two ends of the spectrum of responses of species to declining PO_2 . The comprehensive summary of the effects of size, age, physiological condition and environmental factors on the interaction between $\dot{V}O_2$ and PO_2 by Herreid (1980) further illustrates the risk in making generalizations.

Tankersley and Dimock (1993) characterized the metabolic response of adult *P. cataracta* as oxyconformity, although their experimental design limited the exposure of adults to PO_2 's $>50\%$ of air saturation (about 75 mm Hg). The results with juvenile *P. cataracta* (Fig. 1) indicate that these animals have limited oxyregulatory capability above about 75 % air saturation. Below that oxygen tension, the $\dot{V}O_2$ of juveniles decreases in response to declining PO_2 .

Very young (1-wk old) *P. cataracta* die within 24 h when subjected to $<\approx 3$ mm Hg (<0.1 mg O_2 L^{-1} at 20 °C) (Dimock and Wright 1993). Although most of the juvenile mussels employed in the present study were not exposed to such a low PO_2 except very briefly, all mussels were subjected to ≈ 10 mm Hg for at least several minutes. One was exposed to <1 mm Hg ($\approx 0.6\%$ of air saturation) for about 2 h. Since none of the mussels died, and in fact survived for several weeks following these experiments, it is possible that 5-mo old *P. cataracta* are more resistant to hypoxia than are very young mussels.

The suite of changes in functional capacities as juvenile mussels mature is as yet unknown. Clearly, significant modifications in respiration and circulation accompany increasing size and ontogenetic changes in structure. Recently metamorphosed unionids begin benthic life with three pairs of poorly developed, posteriorly positioned gill filaments (Lasee 1991, Wright 1995). The mantle cavity is irrigated by an anterior to posterior ciliary-driven

current (Wright 1995) that is typical of very small adult or juvenile bivalves (Reid *et al.* 1992), and occurs at least for a time in all post-metamorphous bivalve molluscs (Ockelmann and Muus 1978). While a posterior tissue bridge between the two folds of mantle defines an exhalant aperture in 1-2 wk old juvenile unionids (Wright 1995), the development of functional inhalant and exhalant siphons awaits further growth and differentiation (Dimock and Tankersley, personal observation). The 'siphons' of a 1-hr old *Utterbackia imbecillis* are incorrectly identified in Fig. 1 of Johnson *et al.* (1993).

The 22-24 wk old juvenile *P. cataracta* of the present study had well developed inhalant and exhalant siphons, with the conventional ventral to dorsal posterior irrigation current of adult unionids. The anatomy of the gills was not examined. However, video recording of the hydrodynamics of particle entrainment in the flow generated by ciliation of the gill filaments together with successful capture of 10 μ m latex beads by ctenidial filtration of these juveniles (R. Dimock, unpublished observations), attest to the functional advances gained relative to those of very young mussels. It is likely that this enhanced functional capacity includes more effective gaseous exchange.

The microhabitats of juvenile unionoid mussels immediately following their metamorphosis and excystment from a host fish are unknown. For a bradytictic, winter brooder such as *P. cataracta*, juvenile mussels would be entering the benthic community in late spring or early summer. Their early development would occur in conditions of increasing water temperature accompanied by reduced oxygen solubility, which could be especially stressful in the interstitial milieu of lentic habitats (Buddensiek *et al.* 1993). These juveniles may be adapted to this seasonal temperature regime as indicated by the relatively low Q_{10} of the heart rate for animals acclimated to 15 °C (Polhill and Dimock, 1996). However, an ability to cope with hypoxia may well be augmented by even limited oxyregulatory capability. The hyriid mussel *Vesunio ambiguus* is especially resistant to hypoxia and also regulates at $PO_2 > 65$ mm Hg (Sheldon and Walker 1989).

There are no relevant data with which to directly compare these observations on rates of oxygen consumption of juvenile *P. cataracta*. However, in addition to the effects of oxygen availability on oxygen consumption by freshwater mussels described in the introduction of this paper, a variety of factors have been shown to influence the aerobic metabolism of unionacean mussels (Ornatowski 1967, Salanki and Lukacsovics 1967, Lomte and Nagabhushanam 1971, Badman and Chin 1973, Dietz 1974, Alimov 1975, McCorkle *et al.* 1979, Huebner 1982, Paterson 1983, Paterson and Cameron 1985, Kasprzak

1986a,b, Mane and Kulkarni 1988, Holwerda and Veenhof 1984, Bauer *et al.* 1991, Pandey and Datta-Munshi 1991, Naimo *et al.* 1992, Byrne and McMahon 1994, Roper and Hickey 1995). Previous studies have of necessity been confined to adult mussels. The only option for examining respiratory characteristics of very small freshwater bivalves has been to focus on the biology of the sphaeriid (pisidiid) clams (*e.g.*, Hombach 1985, 1991, Holopainen 1987). Perhaps now with the increased availability of juvenile freshwater mussels, it will be possible to address fundamental physiological questions associated with their ecology and ontogeny.

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