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Effects of Temperature and pO_2 on the Heart Rate of Juvenile and Adult Freshwater Mussels (Bivalvia: Unionidae)

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ABSTRACT. The heart rate of juvenile and adult *Utterbackia imbecillis* and *Pyganodon cataracta* was monitored during experimental manipulation of temperature and pO_2. Animals that had been acclimated to 15 and 25°C for 1 week were exposed to an ascending series of temperatures (10, 15, 20, 25 and 30°C). The effects of oxygen tension on heart rate were assessed by subjecting mussels to a descending series of oxygen tensions (100, 75, 50, 25, 5 and 0% air saturation). Results indicated that the heart rates of juvenile and adult mussels are markedly affected by experimental temperature, with Q_{10}'s approaching 4.5. Acclimation had no effect on adult mussels. However, juvenile *U. imbecillis* exhibited inverse acclimation; whereas, juvenile *P. cataracta* fit a typical pattern of acclimation (Prosser's Type IVA). When exposed to a descending series of oxygen tensions, adult *U. imbecillis* maintained a constant heart rate until a significant increase occurred at 25% air saturation, followed by a significant decrease at 5 and 0%. Juvenile *U. imbecillis* maintained a constant rate until bradycardia occurred at pO_2's of 5 and 0% air saturation. Juvenile and adult *P. cataracta* exhibited similar patterns, with sustained rates until a significant decrease occurred at 0% air saturation. The differences in acclimation patterns exhibited by juvenile and adult mussels may reflect differences in the thermal conditions experienced by these two life-history stages; however, there are no data available that characterize the microhabitat of juvenile freshwater mussels. *Utterbackia imbecillis* appears to be more sensitive to low oxygen levels than *P. cataracta*, and the heart rate of juveniles of both species is more responsive to hypoxia than that of adults. COMP BIOCHEM PHYSIOL 114A;2:135–141, 1996.

KEY WORDS. Unionidae, *Utterbackia*, *Pyganodon*, oxygen tension (pO_2), temperature, heart rate, juvenile mussels, acclimation

INTRODUCTION

Metabolic rate traditionally is measured by monitoring oxygen consumption ($\dot{V}O_2$) or heat production via calorimetry. However, difficulties arise when measuring metabolism in very small animals, and the requirements of aquatic respirometry are often logistically troublesome (32). Thus, an assessment of heart rate, a parameter that often reflects metabolic rate and is amenable to quantification, is commonly used in studies on molluscs (23,25). Pickens (26) found higher heart rates with increasing temperature in the mussels *Mytilus edulis* and *M. californianus*, while deFur and Mangum (11) showed that the heart rate for *Spisula solidissima* increases and decreases in direct response to increasing or decreasing temperature. Similarly, Lowe (21) determined that the heart rate of the bivalves *Mya arenaria* and *Crassostrea gigas* responded nearly immediately to rapid changes in temperature.

Other studies of bivalve molluscs have monitored heart rate in response to experimental manipulation of pO_2. In most adult species, there appears to be some degree of regulation of heart rate until exposure to some critical pO_2, which results in a pronounced bradycardia (5,11,14,25). An increase in heart rate precedes the ultimate bradycardia that occurs at exposure to hypoxic conditions in the bivalves *Mytilus edulis* (2), *Modiolus demissus* (3), *Pecten maximus* (4), and *Arctica islandica* (36). Occasionally the amplitude may be reduced at a higher pO_2 than that which induces the bradycardia, as for example in the freshwater mussel, *Anodonta cygnea* (25).

The metabolism of small, juvenile animals often exhibits an increased sensitivity to environmental stress when compared to larger adults (27). For freshwater mussels, the limited evidence available (13) suggests that juvenile unionids are especially sensitive to changes in the environment, and may have much narrower limits of physiological tolerance than adults. In the research reported herein, the physiological responses of juvenile and adult freshwater mussels, *Py-*

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Abbreviations–HF: Huynh-Feldt.

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ganodon cataracta (Say) and *Utterbackia imbecillis* (Say), to environmental stress have been investigated. The responses of heart rates of juvenile and adult mussels to a series of experimental temperatures for two acclimation groups have been characterized. Q_{10} values and the patterns elicited by acclimation were determined. The effects of a declining series of oxygen tensions on heart rate have been examined for juvenile and adult *P. cataracta* and *U. imbecillis*.

MATERIALS AND METHODS

Collection and Maintenance of Animals

Non-gravid adult mussels (*Utterbackia imbecillis*, 8.0–8.5 cm shell length; *Pyganodon cataracta*, 13.5–14.5 cm shell length) were collected from Davis Pond (Davidson, Mecklenberg County, North Carolina) and Meyer's Pond (Winston-Salem, Forsyth County, North Carolina), respectively. Mussels collected in June (*U. imbecillis*, water temperature = 29°C) and April (*P. cataracta*, water temperature = 17°C) were brought to the two acclimation temperatures (15 or 25°C) over a period of 3 days. They then were held at the acclimation temperature for 7 days prior to conducting experiments. For the pO_2 experiments, adult mussels of both species collected in August (water temperature = 29°C) were brought to the experimental temperature of 22°C over a period of 3 days. All pO_2 experiments were conducted between the fifth and seventh day following collection.

Juvenile mussels were reared from glochidia larvae removed from gravid adult *U. imbecillis* in May and June (water temperature = 25°C) and from gravid adult *P. cataracta* collected in December (water temperature = 7°C). Glochidia of *U. imbecillis* were cultured at 22°C. Gravid adult *P. cataracta* were brought to 15°C over a period of 7 days before glochidia of *P. cataracta* were cultured such that the temperature of the incubator was increased 1°C/day to 22°C. Juvenile mussels (400–600 μm in length) used in these studies were obtained by transformation of glochidia using *in vitro* culturing techniques outlined by Isom and Hudson (20), and modified by Hudson and Shelbourne (18) and Dimock and Wright (13).

Temperature Effects

The effect of acute changes in temperature on the heart rate of adult mussels (N = 10 for each species) acclimated to 15 and 25°C was determined by impedance conversion of cardiac contractions using stainless steel electrodes (A-M Systems, Inc.). To minimize trauma to a mussel, a dental drill was used to drill about ¾ of the way through the valves, and a small hole then was completed by careful rotation of a steel needle. The electrodes were carefully positioned on either side of the pericardial cavity of the mussel and were held in place by modeling clay. Electrodes were connected to an impedance converter (UFI Instruments, Model 2991), which detected changes in impedance accompanying each contraction and relayed the signals to an A/D converter (MacPaq Model MP100, BIOPAC Systems, Inc.) connected to a Macintosh Quadra 650 computer. Data acquisition software (Acqknowledge III for the MP100WS, BIOPAC Systems, Inc.), running on the Macintosh computer, provided recordings of the heart rate of the adult mussels.

After the placement of electrodes, a mussel was mounted to a Plexiglas stand by gluing one of the valves to a 1-cm diameter plastic bolt. The mussel was then placed in 3.5 liters of aerated artificial pond water (APW: 0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl, 0.25 mM $CaCO_3$; pH 7.8; total Ca = 25 mg/liter) in a Lucite chamber (23 cm L × 15 cm W × 16 cm H) that was held at the appropriate acclimation temperature (15 or 25°C). The mussel was considered adjusted to the chamber when it opened its valves and began to siphon (30–60 min). A measurement of the animal's acclimated heart rate was then recorded for 2 min, following which the animal was exposed to an ascending series of temperatures (10, 15, 20, 25 and 30°C). Preliminary experiments showed that a hypodermic thermister probe inserted into the viscera near the pericardial cavity required ≈15–20 min to reach the new target temperature; thus, a 30-min exposure allowed for the tissues of the animal to reach each new target temperature. The appropriate experimental temperatures were achieved by mixing 6 and 40°C APW from temperature-controlled reservoirs. Because sex could affect the animal's response, immediately following experiments with *P. cataracta*, the animals were sexed by examining the structure of the water-tubes of the outer demibranchs (34) (five males and five females were used). *U. imbecillis* is a hermaphroditic species.

Because of their small size and relatively transparent shells, the heart rate of juvenile *U. imbecillis* and *P. cataracta* (N = 20 for each species) was measured by direct visual observation of the heart through the shell. Juvenile mussels (7 days post-transformation) were acclimated for 1 week at 15 or 25°C. Individual acclimated juveniles were then placed in 20-μl capillary tubes that partially restricted normal locomotion but allowed for gaping of the shells and irrigation of the mantle cavity. The capillary tubes were immediately placed in 430 ml of APW in a plastic chamber (13.5 cm L × 11 cm W × 6 cm H) held at the animal's acclimation temperature (15 or 25°C). The capillary tubes were immobilized by two rows of 5 × 5-mm rubber blocks that were affixed to the bottom of the chamber. The capillary tubes could be rotated in place, which facilitated positioning the juveniles for the direct observation of the heart using an inverted microscope (130×) (American Optical Corporation, Buffalo, NY). Three juveniles could be processed sequentially with this system within 4–5 min.

The heart rate of juveniles was determined initially at their acclimation temperature, and then each was exposed

to an ascending series of experimental temperatures (10, 15, 20, 25 and 30°C). Exposure to a new experimental temperature was accomplished by drawing water of the appropriate temperature into the capillary tubes via a 50-μl syringe (Hamilton Co., Reno, NV) attached to the capillary tube via polyethylene tubing. Sufficient water was flushed through the capillary tube to displace the original volume of water in the tube 2½ times. The capillary tube then was placed back into the plastic chamber, which had been changed to the desired next experimental temperature (±0.1°C) by a constant temperature circulator (Fisher Model 90, Pittsburgh, PA). The heart rate was determined after a 15-min exposure to each new temperature. Since preliminary trials demonstrated no change in the heart rate of juveniles after 1 hr of maintenance within the capillary tubes, any observed changes were attributed to the experimental treatments.

Effects of pO_2

The effect of pO_2 on the heart rate of adult mussels (N = 10 for each species) was determined by measuring changes in heart rate when animals were exposed to a descending series of pO_2's (152, 114, 76, 38, 7.6 and 0 mm Hg). Heart rate was determined by impedance changes as described above. Following the experimental preparation of the mussel, each individual was placed in 16 liters of APW (152 mm Hg = 100% air saturation = 20.3 kPa) in a 40-liter aquarium (51 cm L × 26 cm W × 31 cm H) maintained at 22°C by a constant temperature circulator (Haake Instrument, Inc.). Two magnetic stir bars were positioned to provide adequate mixing of the water. Preliminary experiments were conducted on both species to determine the duration of exposure to a new pO_2, which induced a cardiac response. These results indicated that an exposure of 1 hr ensured that the heart had been affected by a change in pO_2 and had stabilized. Thus, heart rate was recorded following a 1-hr exposure to each new pO_2. The pO_2 was regulated by two flow meters that provided the proper mixing of N_2 and compressed air to obtain each target pO_2. Control animals of both species were exposed continuously to 100% air saturation (152 mm Hg; 20.3 kPa) and their heart rates were recorded every hr for 6 hr, which was the duration of an experimental series. Following each experiment, adult *P. cataracta* was sexed as described above (six male and four female mussels were used).

The effects of pO_2 on the heart rate of juveniles (N = 20 for each species) were examined for 14-day-old animals that had been reared at 22°C and 100% air saturation. Individual juveniles were drawn into 20-μl capillary tubes that had been filled with fully saturated water (152 mm Hg; 20.3 kPa). Three capillary tubes, each with one juvenile, were positioned in the chamber as described above except that the chamber was filled with 430 ml of APW at 100% air saturation held at 22°C. After a 15-min exposure, heart rate was determined by direct observation, as above. Following measurements at 100% air saturation (152 mm Hg), juveniles were exposed sequentially to a pO_2 of 114, 76, 38, 7.6 and 0 mm Hg. Water of the appropriate pO_2 was drawn into the capillary tubes using a 50-μl syringe, which displaced the volume of water in the tubes 2½ times. Heart rate was counted after a 15-min exposure to each target pO_2. To control for effects of experimental manipulation and changes in condition of the animals over time, control animals in capillary tubes were exposed to similar experimental manipulations but were flushed with 100% air saturated APW for all six of the 15-min exposure periods.

Statistical Analysis

The heart rates for juvenile *P. cataracta* and adults of both species were transformed (log X) to normalize the data and remove heteroscedasticity. Values were back-transformed prior to plotting in the figures. In order to assess the effects of acclimation and sex on the response of *P. cataracta* to the experimental temperatures, heart rates were analyzed using an analysis of variance with repeated measures (ANOVAR) run from SAS procedure GLM (The SAS (r) System, Release 6.07, SAS Institute Inc.). Similarly, the effect of acclimation on the response of adult *U. imbecillis* and juvenile mussels of both species was analyzed using an ANOVAR. Separate ANOVAR's were run on the control and the experimental groups of juveniles and adults of both species to determine if there were changes in heart rate as a function of pO_2. Because the hypothesis of the compound symmetry of the covariance matrix (Mauchley's criterion) should be tested prior to the examination of ANOVAR results, Huynh-Feldt (HF) corrected significance values are presented when Mauchley's criterion is rejected (19). Statistical significance is considered as $P < 0.05$. When significance was indicated by ANOVAR, a posteriori comparisons were made using Tukey's Studentized Range (HSD) Test (38).

RESULTS

Temperature Effects

Stepwise increases in temperature resulted in subsequent increases in heart rate for both acclimation groups (15 and 25°C) of adult and juvenile *P. cataracta* (Figs 1A, 1C). Neither acclimation ($F = 0.35$; df = 1, 16; $P = 0.56$) nor sex ($F = 0.81$; df = 1, 16; $P = 0.38$) had significant effects on the response of adult mussels; however, the response curve of cold acclimated juvenile *P. cataracta* was significantly different ($F = 34.23$; df = 1, 38; $P < 0.001$) from that of warm acclimated animals (Fig. 1C), with significant within-subject effects of temperature ($F = 1412$; df = 4, 152; (HF) $P < 0.001$) and the temperature × acclimation interaction ($F = 46.77$; df = 4, 152; (HF) $P < 0.001$). A comparison of the responses of juvenile and

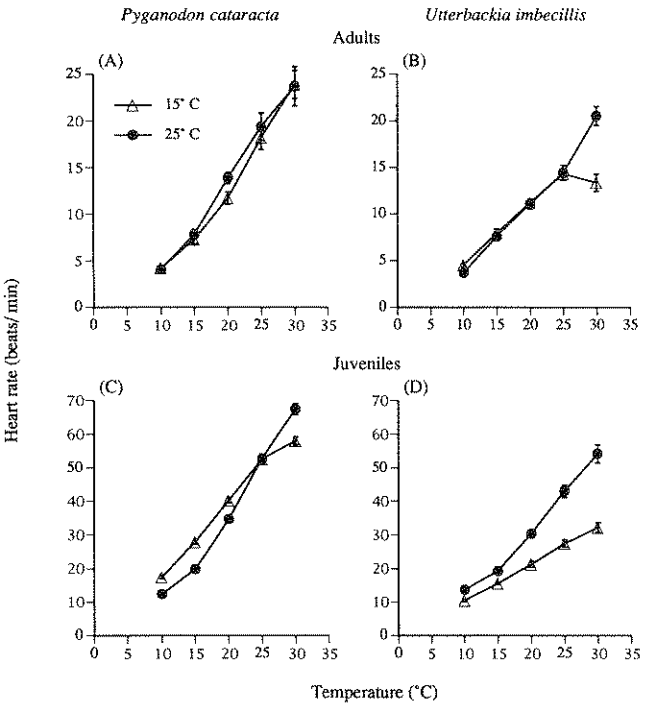

FIG. 1. Heart rates ($\bar{x}$ ± SE) of adult (n = 10) and juvenile (n = 20) mussels, *P. cataracta* and *U. imbecillis*, acclimated for 1 week to 15 and 25°C and exposed to an ascending series of temperatures (10, 15, 20, 25 and 30°C).

adult *P. cataracta* revealed that the heart rate of juvenile mussels was higher than that of adults at all exposure temperatures in the ascending series when animals were similarly acclimated (Figs 1A, 1C). Not surprisingly, Q_{10}'s for both warm and cold acclimation groups were higher at lower temperature intervals and decreased as temperature increased (Table 1). Although a trend of higher Q_{10}'s existed for warm-acclimated adults at lower temperature intervals (10–15°C and 15–20°C) and higher Q_{10}'s for cold-acclimated adults at the upper temperature intervals (20–25°C and 25–30°C), the mean Q_{10}'s at each 5-degree interval were not significantly different between acclimation groups (Table 1). Mean Q_{10}'s of juveniles at 10–15°C were not significantly different between the two acclimation groups, but juveniles acclimated to 25°C had significantly higher Q_{10}'s than those of 15°C-acclimated animals at the remaining three temperature intervals (Table 1).

The responses of comparably acclimated adult *U. imbecillis* (Fig. 1B) resembled those of *P. cataracta*, with no overall significant difference in response due to acclimation ($P = 0.14$; df = 1, 18; $P = 0.72$); however, cold-acclimated animals did have lower heart rates than warm acclimated animals when exposed to 30°C (*t*-test: $P < 0.05$). The response curve of juvenile *U. imbecillis* acclimated to 15°C was significantly different ($F = 63.71$; df = 1, 38; $P < 0.001$) from that of animals acclimated to 25°C (Fig. 1D), with significant within-subject effects of temperature ($F = 360.8$; df = 4, 152; (HF) $P = 0.001$) and the temperature × acclimation interaction ($F = 36.15$; df = 4, 152; (HF) $P = 0.001$). The heart rate of juvenile *U. imbecillis* was substantially higher than that of adults at all exposure temperatures for similarly acclimated animals (Figs 1B, 1D). Q_{10}'s of warm-acclimated adult mussels were significantly higher than those of cold-acclimated adults at the temperature intervals 10–15°C and 25–30°C, with no significant differences at the other intervals (Table 1). For juvenile *U. imbecillis*, mean Q_{10}'s at 10–15°C were not significantly different, but warm acclimated juveniles had significantly higher Q_{10}'s than those of cold acclimated animals at the other temperature intervals (Table 1).

Effects of pO_2

Heart rates did not differ at any pO_2 until adult *P. cataracta* were exposed to anoxic conditions, which resulted in a significant decrease in heart rate to 6.5 ± 0.96 ($\bar{x}$ ± SE) beats/min ($F = 4.61$; df = 5, 45; $P < 0.001$) (Fig. 2A). The mean heart rate for control animals (held continuously

TABLE 1. Q_{10} ($\bar{x}$ ± SE) at each 5-degree temperature interval for the heart rates of cold (15°C) and warm (25°C) acclimated adult and juvenile *P. cataracta* and *U. imbecillis*

Species	Temperature interval °C	Adults		Juveniles	
		15°C	25°C	15°C	25°C
P. cataracta					
	10–15	3.26 ± 0.43	3.79 ± 0.40	2.67 ± 0.19	2.82 ± 0.29
	15–20	2.76 ± 0.37	3.55 ± 0.49	2.10 ± 0.06*	3.16 ± 0.18
	20–25	2.50 ± 0.29	2.20 ± 0.42	1.74 ± 0.08*	2.33 ± 0.07
	25–30	1.93 ± 0.27	1.56 ± 0.16	1.25 ± 0.06*	1.66 ± 0.06
U. imbecillis					
	10–15	3.14 ± 0.31*	4.50 ± 0.37	2.34 ± 0.16	2.06 ± 0.10
	15–20	2.08 ± 0.15	2.22 ± 0.18	1.87 ± 0.08*	2.59 ± 0.16
	20–25	1.66 ± 0.09	1.69 ± 0.10	1.69 ± 0.06*	2.01 ± 0.09
	25–30	0.95 ± 0.17*	2.07 ± 0.11	1.38 ± 0.04*	1.61 ± 0.09

*Significantly different between acclimation groups ($P < 0.05$) using Tukey's Studentized Range (HSD) Test.

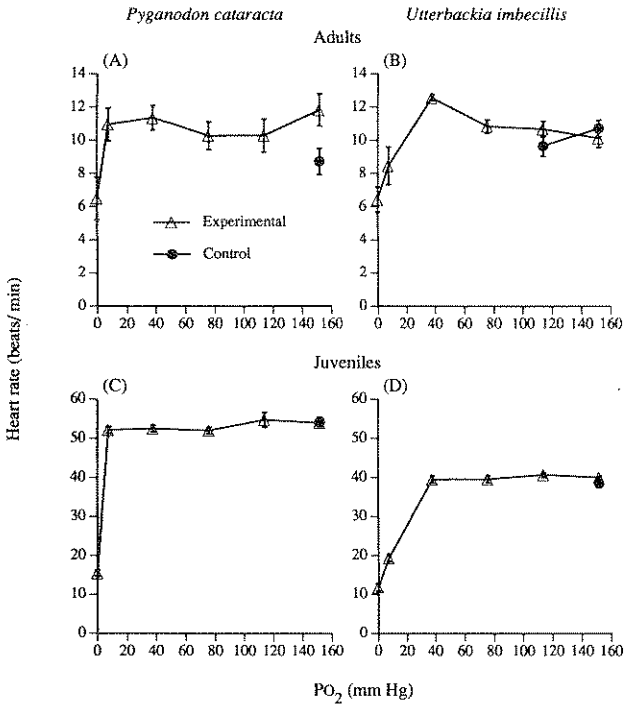

FIG. 2. The heart rate ($\bar{x} \pm SE$) of adult (n = 10) and juvenile (n = 20) mussels, *P. cataracta* and *U. imbecillis*, plotted as a function of oxygen tension (pO_2 = 152 mm Hg = 100% air saturation). Experimental animals were exposed to a descending series of oxygen tensions (100, 75, 50, 25, 5 and 0% air saturation), while control animals were held at pO_2 = 152 mm Hg for the entire 6-hr exposure.

at 100% air saturation) was not significantly different at any time throughout the entire 6 hr ($F = 2.51$; df = 5, 45; $P = 0.69$). Juvenile *P. cataracta* had a constant heart rate to 5% saturation (7.6 mm Hg), and then experienced a significant reduction to 15.5 ± 0.77 ($\bar{x} \pm SE$) beats/min at 0% saturation ($F = 201.46$; df = 5, 95; $P < 0.001$) (Fig. 2C). Rates for control juveniles were not significantly different throughout the entire 6 hr at 152 mm Hg ($F = 0.59$; df = 5, 95; $P = 0.70$).

Adult *U. imbecillis* responded to the declining pO_2's somewhat differently from adult *P. cataracta* (Fig. 2B). This species maintained a constant rate until 25% saturation (38 mm Hg), at which the rate increased significantly to 12.6 ± 0.17 ($\bar{x} \pm SE$) beats/min ($F = 45.56$; df = 5, 45; $P < 0.001$). This increase was followed by a significant decrease at 5% and a further decrease at 0% air saturation to 6.4 ± 0.76 ($\bar{x} \pm SE$) beats/min (Fig. 2B). The heart rate of control animals after the first hr was significantly higher ($F = 6.39$; df = 5, 45; $P < 0.001$) than rates for the remaining 2–6 hr of exposure to 152 mm Hg.

Juvenile *U. imbecillis* maintained a constant heart rate from full saturation to 25% saturation, but their rate decreased significantly to 19.4 ± 0.59 ($\bar{x} \pm SE$) beats/min at 5% ($F = 380.53$; df = 5, 95; $P < 0.001$) (Fig. 2D). The rate declined further at 0% saturation (11.9 ± 0.39 ($\bar{x} \pm SE$) beats/min). The overall effect was a 75% reduction in heart rate at 0% saturation as compared to the normoxic rate. No significant differences occurred among control animals throughout the 6 hr at 100% saturation ($F = 1.66$; df = 5, 95; $P = 0.162$).

Heart rates of juvenile *P. cataracta* and *U. imbecillis* were substantially higher than those of adult mussels at each pO_2 (Fig. 2). Significant bradycardia only occurred at anoxia in both juvenile and adult *P. cataracta*, with juveniles having a 70% decrease (≈54 to 15 beats/min) from their initial rate compared to the 55% decrease (≈10.5 to 8.5 beats/min) observed in adults (Figs 2A, 2C). In contrast, a significant decrease in rate occurred at 5% air saturation in both juvenile and adult *U. imbecillis*, with juveniles experiencing a 50% decrease (≈40 to 20 beats/min) and adults about a 20% decrease (≈10.5 to 8.5 beats/min) as compared to the rates at 100% saturation. A further 25% decrease in rate occurred in both juvenile and adult *U. imbecillis* upon exposure to 0% saturation. As shown in Figs 2B and 2D, the significant increase in heart rate of adult *U. imbecillis* exposed to 25% saturation was not observed in juveniles of that species.

DISCUSSION

Sensitivity of heart rate to temperature is typical of many adult bivalves (9,11,12), and is underscored by results from this study that indicate that both adult and juvenile unionid mussels are markedly affected by temperature. Furthermore, the results reveal an inverse relationship between heart rate and body mass for these two size classes of freshwater mussels (juveniles = 400–600 μm; adults = 8–15 cm). Such scaling effects are common in the physiology of most animals (30).

Numerous ectotherms have been shown to exhibit a pattern of thermal acclimation referred to as normal compensation (Prosser's Type IIA or IVA acclimation) (28) in which animals acclimated at low temperature have higher rate functions at a series of experimental temperatures than those acclimated to a higher temperature (31). In contrast to this typical pattern, adult *P. cataracta* (Fig. 1A) and adult *U. imbecillis* (Fig. 1B) show no acclimation of their heart rate in response to maintenance at 15 and 25°C. The intertidal pulmonate limpet *Siphonaria oculus* also shows no acclimation of its heart rate, a response which Marshall and McQuaid (23) suggest is an energy-conserving metabolic adaptation that could counteract food shortages and would allow the metabolic rate to decline with a seasonal decrease in temperature. Since both adult *P. cataracta* and *U. imbecillis* experience seasonal fluctuations in environmental temperature, the absence of acclimation may be an energy-conserving adaptation at low environmental temperatures. However, the lack of acclimation of heart rate does not preclude compensatory changes in other rate functions

such as oxygen consumption or enzyme activity. For example, the heart rate of *Mytilus edulis* shows no acclimation to temperature from 5 to 25°C, while oxygen consumption and ventilation rate acclimate to 10, 15 and 20°C (37).

Juvenile *P. cataracta* exhibit normal acclimation (Prosser's Type IVA; Fig. 1C). This compensatory ability could be related to the timing of the life cycle of this mussel, since glochidia larvae are released from adult mussels in late winter/early spring to begin their parasitic phase on a fish host (33). This ectoparasitic phase probably lasts several weeks and results in the release of newly metamorphosed juveniles into the aquatic environment as temperatures approach 15°C in the Piedmont of North Carolina (33). This seasonal maturation of juvenile *P. cataracta* may be related to their ability to acclimate to 15°C and maintain their metabolism at a relatively constant level during their early development.

In contrast to *P. cataracta*, juvenile *U. imbecillis* exhibit inverse acclimation (Prosser's Type IIB), with cold-acclimated animals having lower heart rates than warm-acclimated animals at all experimental temperatures (Fig. 1D). Inverse acclimation has been reported for several pulmonate limpets (7,10,24), and for a few sphaeriid bivalves (16,17). It has been proposed that inverse acclimation functions to conserve energy stores during the overwintering period, when primary productivity is at a minimum and the animals may not be actively feeding (1,22). *U. imbecillis* has several reproductive cycles per year in North Carolina (personal observation). For juveniles that detach from their fish host in early winter, inverse acclimation might serve to conserve energy as animals undergo a metabolic "shut-down." In contrast, juveniles that metamorphose during warmer periods could exploit available resources for growth and maintenance because of their increased metabolic rate.

The Q_{10}'s of adult *P. cataracta* and *U. imbecillis* were generally unaffected by acclimation (Table 1) except for cold acclimated adult *U. imbecillis* exposed to 30°C (Fig. 1B; Q_{10} = 0.95 at 25–30°C, Table 1). The pronounced decrease in heart rate under these conditions may have foreshadowed lethality for this species. For juvenile mussels of both species, warm-acclimated animals had significantly higher Q_{10}'s at the three higher temperature intervals (Table 1). This increased temperature sensitivity with increasing acclimation or habitat temperature has been reported for various ectotherms including bivalve molluscs (29). Burky and Burky (8) suggest that the high Q_{10}'s for warm-acclimated pea clams may facilitate exploitation of "ideal" feeding conditions in increased temperatures conducive to maximal growth.

When adult and juvenile *P. cataracta* are exposed to declining oxygen tensions, heart rates do not change until animals are exposed to anoxic conditions (Figs 2A, 2C), which results in the distinctive bradycardia observed in other molluscs (5,14,25). However, juvenile *P. cataracta* appear to be much more sensitive to anoxia than adults (≈70% decrease *vs.* 55% decrease). This observation is consistent with previous work that demonstrated that juvenile *P. cataracta* succumb to anoxia within 24 hr (13), while adult bivalves often have extreme tolerance to anoxic conditions (6). The absence of tachycardia at intermediate oxygen levels in this species suggests that juveniles as well as adults are unable to regulate their oxygen consumption. For example, Tankersley and Dimock (35) showed that adult *P. cataracta* are oxyconformers from 100% to ≈40% air saturation.

Adult and juvenile *U. imbecillis* respond to declining oxygen tensions somewhat differently from *P. cataracta*, with bradycardia beginning at 5% air saturation and becoming more pronounced at 0% (Figs 2B, 2D). The occurrence of bradycardia at higher oxygen tensions suggests that *U. imbecillis* is more sensitive to low oxygen levels than *P. cataracta*. As with *P. cataracta*, the overall decrease in heart rate after exposure to anoxia is greater in juvenile *U. imbecillis* than adults (≈70% decrease *vs.* 45% decrease), which also is consistent with the observations of Dimock and Wright (13). The significant increase in heart rate at 25% air saturation observed in adult *U. imbecillis* (Fig. 2B), and not in juveniles (Fig. 2D) could result in increased perfusion of the gills and mantle, which might facilitate oxygen uptake. This is consistent with the ability of adult *U. imbecillis* to oxyregulate down to very low oxygen tensions (15). No comparable analyses of $\dot{V}O_2$ have been accomplished with any juvenile unionids.

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References

1. Bailey, S.E.R.; Lazaridou-Dimitriadou, M. Inverse temperature acclimation of heart rate in hibernating land snails. J. Comp. Physiol. 160:677–681;1991.
2. Bayne, B.L. Ventilation, the heart beat and oxygen uptake by *Mytilus edulis* L. in declining oxygen tension. Comp. Biochem. Physiol. 40A:1065–1085;1971.
3. Booth, C.E.; Mangum, C.P. Oxygen uptake and transport in the lamellibranch mollusc *Modiolus demissus*. Physiol. Zool. 51:17–32;1978.
4. Brand, A.R.; Roberts, D. The cardiac responses of the scallop *Pecten maximus* (L) to respiratory stress. J. Exp. Mar. Biol. Ecol. 13:29–43;1973.
5. Brand, A.R.; Morris, D.J. The respiratory responses of the dog cockle *Glycymeris glycymeris* (L.) to declining environmental oxygen tension. J. Exp. Mar. Biol. Ecol. 83:89–106; 1984.
6. Brooks, S.P.J.; de Zwaan, A.; van den Thillart, G.; Cattani, O.; Cortesi, P.; Storey, K.B. Differential survival of *Venus gallina* and *Scapharca inaequivalvis* during anoxic stress: covalent modification of phosphofructokinase and glycogen phosphorylase during anoxia. J. Comp. Physiol. 161:207–212; 1991.
7. Burky, A.J. Reverse acclimation at low temperature in the stream limpet, *Ferrissia rivularis* (Say). Acta Cient. Venez. 21:28;1970.

8. Burky, A.J.; Burky, K.A. Seasonal respiratory variation and acclimation in the pea clam, *Pisidium walkeri* Sterki. Comp. Biochem. Physiol. 55A:109–114;1976.
9. Davenport, J.; Carrion-Cotrina, M. Responses of the mussel *Mytilus edulis* L. to simulated subarctic tide pool conditions. J. Therm. Biol. 6:257–265;1981.
10. Davies, P.S. Environmental acclimation in the limpet *Patella vulgata* L. Nature 205:924;1965.
11. De Fur, P.L.; Mangum, C.P. The effects of environmental variables on the heart rates of invertebrates. Comp. Biochem. Physiol. 62A:283–294;1979.
12. Dietz, T.H.; Tomkins, R.U. The effect of temperature on heart rate of the freshwater mussel, *Ligumia subrostrata.* Comp. Biochem. Physiol. 67A:269–271;1980.
13. Dimock, R.V., Jr.; Wright, A.H. Sensitivity of juvenile freshwater mussels to hypoxic, thermal and acid stress. J. Elisha Mitch. Sci. Soc. 109(4):183–192;1993.
14. Gade, G.; Ellington, W.R. The anaerobic molluscan heart: adaptation to environmental anoxia. Comparison with energy metabolism in vertebrate hearts. Comp. Biochem. Physiol. 76A:615–620;1983.
15. Hiestand, W.A. Respiration studies with fresh-water molluscs: I. Oxygen consumption in relation to oxygen tension. Proc. Ind. Acad. Sci. 47:287–292;1938.
16. Hornbach, D.J. A review of metabolism in the Pisidiidae with new data on its relationship with life history traits in *Pisidium casertanum.* Am. Malacol. Bull. 3:187–200;1985.
17. Hornbach, D.J. The influence of acclimatization, temperature and size on the oxygen consumption of the freshwater clam, *Musculium partumeium* (Say). Comp. Biochem. Physiol. 101A:345–349;1992.
18. Hudson, R.G.; Shelbourne, C.W. Improved in vitro culture of parasitic freshwater mussel glochidia. Rep. Tenn. Valley Auth. 1990: 26 pp.
19. Huynh, H.; Feldt, L.S. Conditions under which mean square ratios in repeated measurements design have exact F-distributions. J. Am. Stat. Assoc. 65:1582–1589;1970.
20. Isom, B.G.; Hudson, R.G. In vitro culture of parasitic freshwater mussel glochidia. Nautilus 96:147–151;1982.
21. Lowe, G.A. Effect of temperature change on the heart rate of *Crassostrea gigas* and *Mya arenaria* (Bivalvia). Proc. Malac. Soc. Lond. 41:29–36;1974.
22. Marshall, D.J.; McQuaid, C.D. Relationship between heart rate and oxygen consumption in the intertidal limpets *Patella granularis* and *Siphonaria oculus.* Comp. Biochem. Physiol. 103A:297–300;1992.
23. Marshall, D.J.; McQuaid, C.D. Seasonal and diel variations of in situ heart rate of the intertidal limpet *Siphonaria oculus* Kr. (Pulmonata). J. Exp. Mar. Biol. Ecol. 179:1–9; 1994.
24. McMahon, R.F. Respiratory variation and acclimation in the freshwater limpet, *Laerapex fuscus.* Biol. Bull. 145:492–508; 1973.
25. Michealidis, B.; Athanasiadou, P. Effect of reduced oxygen tension on the heart rate and the kinetic properties of glycolytic key enzymes PFK, PK and glycogen phosphorylase from the freshwater mussel *Anodonta cygnea* (L.). Comp. Biochem. Physiol. 108B:165–172;1994.
26. Pickens, P.E. Heart rate of mussels as a function of latitude, intertidal height and acclimation temperature. Physiol. Zool. 38:390–405;1965.
27. Precht, H.; Christophersen, J.; Hensel, H.; Larcher, W. Temperature and Life. New York: Springer-Verlag; 1973: 779 pp.
28. Prosser, C.L. General Summary: The nature of physiological adaptation. In: Prosser, C.L., ed. Physiological Adaptation. Washington, D.C.: Am. Physiol. Soc.; 1958: 167–180.
29. Rao, K.P.; Bullock, T.H. Q_{10} as a function of size and habitat temperature in poikilotherms. Am. Nat. 88:33–43;1954.
30. Schmidt-Nielson, K. Scaling: why is animal size so important? Cambridge: Cambridge University Press; 1984: 1–241.
31. Segal, E. Acclimation in molluscs. Am. Zoologist 1:235–244;1961.
32. Steffensen, J.F. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiol. Biochem. 6:49–59;1989.
33. Tankersley, R.A. Larval brooding by the freshwater unionid mussel *Anodonta cataracta*: its effect on filtration, ventilation, and respiration. Ph.D. Dissertation, Wake Forest University, Winston-Salem, North Carolina.
34. Tankersley, R.A.; Dimock, R.V., Jr. Quantitative analysis of the structure and function of the marsupial gills of the freshwater mussel *Anodonta cataracta.* Biol. Bull. 182:145–154;1992.
35. Tankersley, R.A.; Dimock, R.V., Jr. The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel *Pyganodon cataracta.* Am. Midland Nat. 130: 146–163;1993.
36. Taylor, A.C.; Brand, A.R. Effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica* (L.). J. Exp. Mar. Biol. Ecol. 19:187–196;1975.
37. Widdows, J. Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis.* Mar. Biol. 20:269–276;1973.
38. Zar, J.H. Biostatistical Analysis. Englewood Cliffs, New Jersey: Prentice-Hall; 1984: 718 p.