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Metabolic, respiratory and cardiovascular responses to acute and chronic hypoxic exposure in tadpole shrimp *Triops longicaudatus*

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Summary

Hypoxic exposure experienced during sensitive developmental periods can shape adult physiological capabilities and define regulatory limits. Tadpole shrimp were reared under normoxic (19–21 kPa O_2), moderate (10–13 kPa O_2) or severe (1–3 kPa O_2) hypoxic conditions to investigate the influence of developmental oxygen partial pressure (P_{O_2}) on adult metabolic, respiratory and cardiovascular physiology. Developmental P_{O_2} had no effect on metabolic rate or metabolic response to hypoxic exposure in adults. All rearing groups decreased O_2 consumption as water P_{O_2} decreased. Heart rate, stroke volume and cardiac output were independent of P_{O_2} down to 5 kPa O_2 in all rearing groups. Below this, cardiac output was maintained only in tadpole shrimp reared under severe hypoxic conditions. The enhanced ability to maintain cardiac output was attributed to an increase in hemoglobin concentration and O_2-binding affinity in those animals. Oxygen-delivery potential was also significantly higher in the group reared under severe hypoxic conditions (1336 μl O_2 min^{-1}) when compared with the group reared under normoxic conditions (274 μl O_2 min^{-1}). Differences among the rearing groups that were dependent on hemoglobin were not considered developmental effects because hemoglobin concentration could be increased within seven days of hypoxic exposure independent of developmental P_{O_2}. Hypoxia-induced hemoglobin synthesis may be a compensatory mechanism that allows tadpole shrimp to regulate O_2 uptake and transport in euryoxic (O_2 variable) environments. The results of this study indicate that increased hemoglobin concentration, increased O_2-binding affinity and transient decreases in metabolic demand may account for tadpole shrimp hypoxic tolerance.

Key words: invertebrate, development, physiology, hypoxia, oxygen partial pressure.

Introduction

Hypoxic exposure can elicit a range of physiological and metabolic responses in aquatic crustaceans with ‘oxygen conformation’ and ‘oxygen regulation’ at the ends of a response continuum (Hochachka, 1988; McGaw et al., 1994; Reiber, 1995; Reiber and McMahon, 1998; Wilkens, 1999). Oxygen conformers are able to reduce metabolic demand so that O_2 consumption ($\dot{V}_{O_2}$) is coupled to environmental O_2 partial pressure (P_{O_2}) (Loudon, 1988; Hochachka et al., 1999; Boutilier, 2001). Oxygen regulators, by contrast, maintain $\dot{V}_{O_2}$ independent of environmental P_{O_2} down to a point where the O_2 required for aerobic processes becomes limiting (P_{CRIT}) (Herreid, 1980; Hochachka, 1988). Below P_{CRIT}, either tissue oxygen demand decreases or anaerobic processes become increasingly important in meeting energy requirements (Hochachka, 1988). Above P_{CRIT}, O_2 uptake and delivery may be enhanced through a variety of compensatory mechanisms including adjusting ventilatory parameters (rates and volumes), increasing internal convective processes such as heart rate and stroke volume and changing perfusion patterns (McMahon, 1988; Hervant et al., 1995; Willmer et al., 2000; Boutilier, 2001; Hochachka and Lutz, 2001; Hopkins and Powell, 2001; Hoppeler and Vogt, 2001). Additionally, some animals can modulate the O_2-binding affinity of their respiratory proteins, which enhances O_2 uptake at the respiratory surface and increases total O_2 capacitance (Wells and Wells, 1984; Kobayashi et al., 1988; Hochachka et al., 1999; Willmer et al., 2000).

Hypoxic exposure during development (embryonic or larval periods) may elicit a similar suite of compensatory responses or may result in long-term or permanent changes to the morphology and/or physiology of the animal and thus impact the adult hypoxic response (Bamber and Depledge, 1997; Bradley et al., 1999; Willmer et al., 2000; Gozal and Gozal, 2001; Peyronnet et al., 2002). The physiological consequences of hypoxia-induced developmental plasticity can be a reduced

metabolic rate (Hochachka et al., 1999; Boutilier, 2001), greater respiratory gas exchange surface area (Loudon, 1988; Loudon, 1989), increased respiratory capacities and/or enhanced convection and O_2 delivery mechanisms (Banchero, 1987; McMahon, 1988; Graham, 1990; Szewczak and Jackson, 1992; Childress and Seibel, 1998; Pelster, 1999; McMahon, 2001). Transcriptional regulation accounts for many of these long-term physiological and morphological changes associated with developmental exposure to hypoxia. A well-documented example of this type of developmental hypoxic response in crustaceans is the increase of existing O_2-binding proteins as well as the production of new higher-affinity proteins that result in long-term increases in O_2-uptake and capacitance (Wells and Wells, 1984; Snyder, 1987; Kobayashi et al., 1988; Hervant et al., 1995; Astall et al., 1997; Hou and Huang, 1999; Wiggins and Frappell, 2000; Barros et al., 2001).

The degree to which developmental P_{O_2} influences adult metabolic, respiratory and cardiovascular hypoxic responses was investigated using tadpole shrimp *Triops longicaudatus*. Several features of tadpole shrimp make them well suited to study the effects of acute and developmental hypoxic exposures. They are often faced with environmental P_{O_2}s below their P_{CRIT}, yet the mechanisms by which they tolerate such conditions are poorly understood (Horne and Beyenbach, 1971; Hillyard and Vinegar, 1972; Eriksen and Brown, 1980; Scholnick, 1995; Scholnick and Snyder, 1996). Tadpole shrimp have high metabolic rates and respiratory structures (epipodites) that are thought to be inadequate to maintain O_2 uptake in their euryoxic habitats (Fryer, 1988; Horne and Beyenbach, 1971; Hillyard and Vinegar, 1972; Scott and Grigarick, 1978; Eriksen and Brown, 1980; Scholnick, 1995; Scholnick and Snyder, 1996). A tubal, myogenic heart devoid of vasculature produces the only internal convective currents in tadpole shrimp (Yamagishi et al., 1997; Yamagishi et al., 2000). Large, extracellular hemoglobin (29 subunits) is produced in response to hypoxic exposure, but the mechanisms of this hypoxic induction are not known (Horne and Beyenbach, 1971; Scholnick and Snyder, 1996). Finally, tadpole shrimp have a comparatively rapid generation time and amenability to laboratory culture, which make them tractable organisms for developmental investigations (Fryer, 1988; Horne and Beyenbach, 1971; Scott and Grigarick, 1978).

Tadpole shrimp were reared under normoxic (19–21 kPa O_2), moderate (10–13 kPa O_2) or severe (1–3 kPa O_2) hypoxic conditions to determine whether differences in developmental P_{O_2} were sufficient to change adult metabolic, respiratory or cardiovascular system physiology and hypoxic sensitivity. Compensatory respiratory and cardiovascular processes that enhance O_2 uptake, internal convection and perfusion should increase in response to hypoxic exposure. We hypothesized that tadpole shrimp reared under hypoxic conditions would have decreased metabolic sensitivity to P_{O_2} change, increased physiological responses to hypoxic exposure and increased hemoglobin concentration and O_2-binding affinity relative to those reared under normoxic conditions (Wells and Wells, 1984; Snyder, 1987; Kobayashi et al., 1988; Hervant et al., 1995; Astall et al., 1997; Hochachka et al., 1999; Hou and Huang, 1999; Barros et al., 2001; Boutilier, 2001).

Materials and methods

Study organism and rearing conditions

Sediments containing tadpole shrimp (*Triops longicaudatus* LeConte) cysts were collected from an ephemeral pool in Brownstone Basin (12.7 km west of Las Vegas, NV, USA; 1425 m elevation; 36.2500° N, –115.3750° W). Water P_{O_2} and temperature in Brownstone Basin pool ranged from 2 to 32 kPa O_2 and 17.6 to 31.7°C, respectively (Harper, 2003). Three 150-liter insulated aquaria (normoxic=19–21 kPa O_2, moderate hypoxic=10–13 kPa O_2 and severe hypoxic=1–3 kPa O_2) were set up in the laboratory using deionized water and sediments taken from the pool. A model GF-3/MP gas flowmeter (Cameron Instruments Company, Ontario, Canada) was used to regulate a mixture of N_2 and room air. Oxygen partial pressure in each aquaria was monitored for a 48-h period each week using data-logging dissolved O_2 meters (Model 810 Orion Dissolved Oxygen Meter; Orion Research, Boston, MA, USA).

Each aquarium was equipped with ultraviolet (3% UVB and 7% UVA) enhanced lights (Super UV Reptile Daylight Lamp; 20 W; Energy Savers Unlimited Inc., Chicago, IL, USA) that established a 13 h:11 h L:D cycle. A timed circulating water bath (Model VT513; Radiometer, Copenhagen, Denmark) and heat exchange coil were used to cycle temperature with the lights. The aquaria started to warm two hours after the lights were turned on and continued for five hours each day to establish a 23–28°C temperature cycle. Dry sediments (100 g) containing tadpole shrimp cysts were added to each aquarium weekly. Animals ate algae, detritus and small invertebrates that hatched from the sediment. Tadpole shrimp were identified as *Triops longicaudatus* (Sassaman, 1991). Aquaria were drained and refilled monthly.

Metabolism

Standard metabolic rate was measured when animals were active because they were infrequently quiescent. To assess the confounding effect of P_{O_2} on activity, individual animals (N=10) were placed in a marked cylindrical 10-ml flow-through chamber and videotaped during progressive hypoxic exposure. Animals were acclimated (30 min) to the chamber under normoxic conditions. A gas flowmeter (Model GF-3/MP) was used to regulate the mixture of N_2 and air. Chamber P_{O_2} was decreased from 20 to 2 kPa O_2 at a rate of 5 kPa O_2 h^{-1}. The number of times the animal crossed defined marks on the chamber was averaged over one-minute intervals to produce an index of activity in response to P_{O_2}.

Individual animals from each rearing group (N=7 per rearing group) were placed in a 125-ml closed-system darkened respirometry chamber at 28°C. The chamber had a plastic grate on the bottom under which a magnetic stirring rod was placed to ensure thorough mixing of the chamber water. Animals were acclimated for 30 min and then the chamber was sealed.

Oxygen content of the chamber was monitored using a model 781 Strathkelvin O_2 meter (Strathkelvin Instruments, Glasgow, UK). Oxygen consumption was calculated based on the following equation:

$$\dot{V}_{O_2} = (V_r \, \Delta P_{WO_2} \, \beta_{WO_2}) / \Delta t M_d \, , \quad (1)$$

where $\dot{V}_{O_2}$ is oxygen consumption, V_r is the volume of water in the respirometer, ΔP_{WO_2} is the change in oxygen concentration of the water, β_{WO_2} is the capacitance of oxygen in water, Δt is duration in minutes, and M_d is the dry mass of the animal measured in grams (Piiper et al., 1971). Individual tadpole shrimp dry mass was determined by drying the animal at 60°C until three constant mass measurements were obtained. Oxygen consumption rates were calculated for successive 5-min intervals and expressed as mass-specific O_2 uptake (μl O_2 g^{-1} h^{-1}). The experiment was performed without animals in the chamber, and the $\dot{V}_{O_2}$ rates obtained from three replicates were used to calculate a mean correction factor for microbial respiration.

Animals reared under normoxic (N=10) and severe hypoxic (N=10) conditions were used to assess anaerobic lactate metabolism in tadpole shrimp. Lactate concentration was measured for five animals pre-treatment and five animals after exposure to severe hypoxic conditions (2 kPa O_2) for 12 h at 23°C. Lactate concentrations in the experimental chamber water were also determined. Hypoxic conditions were maintained using a gas flowmeter (Model GF-3/MP) to control the mixture of N_2 and room air. Hemolymph was collected in glass capillary tubes by dorsal heart puncture. Lactate concentrations were determined for 10 μl water samples and 10 μl hemolymph samples mixed with 1.0 ml lactate reagent solution (#735-10; Trinity Biotech, St Louis, MO, USA). Hemolymph lactate concentrations were measured enzymatically (Sigma Diagnostics, St Louis, MO, USA; Sigma Lactate Kit #735) at 540 nm.

Ventilation

Ventilatory rate and amplitude were measured in response to hypoxic exposure in order to assess the effects of developmental P_{O_2} on adult ventilatory hypoxic response. Adult tadpole shrimp from each rearing group (N=13 per rearing group) were held in a 30-ml flow-through chamber (temperature, 25°C). Tadpole shrimp were secured in the chamber with an applicator stick and cyanoacrylate glue on the lateral carapace. They were inverted in the chamber to allow the ventral surface to be viewed. Movements of the respiratory appendages were videotaped (60 Hz sampling speed) under a dissecting microscope (Leica Stereozoom 6 Photo) using a video camera (Oscar Color Camera Vidcam), super VHS video recording system (Panasonic PV-54566) and Horita time code generator (VG 50; Horita Co., Inc., Mission Viejo, CA, USA). Tadpole shrimp were acclimated for 30 min and then exposed to four environmental P_{O_2}s (20, 13.3, 10 and 1 kPa O_2) in random order. Thirty minutes was allowed for acclimation once a new P_{O_2} was achieved. Animals were not returned to normoxia between exposures. Time-encoded video was analyzed frame-by-frame on an editing tape player (Panasonic AG-DS550) to determine ventilation rate and amplitude. Ventilation rate (frequency) was measured as number of appendage beats per minute. The amplitude of appendage beats was determined as the mean distance that the 4th and 5th appendages separate during five subsequent ventilatory strokes (Harper, 2003).

Cardiac physiology

Heart rate, stroke volume and cardiac output were measured in response to hypoxic exposure in order to assess the effects of developmental P_{O_2} on adult cardiac hypoxic responses. Adult tadpole shrimp from each rearing group (N=13 per rearing group) were secured in a 30-ml flow-through experimental chamber as previously described. Animals were acclimated for 30 min and then exposed to five environmental P_{O_2}s (26.7, 20, 13.3, 10 and 4 kPa O_2) in random order. Thirty minutes was allowed for acclimation at each P_{O_2}. Cardiac contractions were videotaped as previously described. Heart rate (f_H; beats min^{-1}) was measured when the time-encoded video was advanced frame-by-frame on an editing tape player (Panasonic AG-DS550, Cypress, CA). The tadpole shrimp heart was modeled as a cylinder with a volume of $\pi r^2 h$, where r is half the width of the heart and h is length. Images of the heart were collected during maximal [end diastolic volume (EDV)] and minimal [end systolic volume (ESV)] distention. Those images were dimensionally analyzed using Scion Imaging software (National Institutes of Health, Bethesda, MD, USA). Stroke volume (V_S; μl $beat^{-1}$) was calculated as the difference in heart volume between EDV and ESV. Cardiac output ($\dot{Q}$; μl min^{-1}) was calculated as the product of f_H and V_S.

Hemoglobin

Hemoglobin is the major protein in tadpole shrimp hemolymph (Horne and Beyenbach, 1971). Protein concentrations of animals from each rearing group (N=10 per rearing group) were determined to assess the influence of developmental P_{O_2} on hemoglobin production. Protein concentrations were determined for tadpole shrimp reared under normoxic conditions and exposed to severe hypoxic conditions for 5, 7 and 10 days (N=7 per day). Likewise, protein concentrations were determined for tadpole shrimp reared under severe hypoxic conditions and exposed to normoxic conditions for 5, 7 and 10 days (N=7 per day). Protein concentrations were determined using a Micro BCA Protein Assay Reagent Kit (#23235; Pierce, Rockford, IL, USA). Protein standards (2.0 mg ml^{-1} BSA in a solution of 0.9% saline and 0.05% sodium azide) were diluted with tadpole shrimp saline (5.84 mg NaCl, 7.45 mg KCl, 11.09 mg $CaCl_2$, 9.52 mg $MgCl_2$, 3.65 mg HCl and 1 ml H_2O) (Yamagishi et al., 2000) to form solutions with final BSA concentrations of 200, 40, 20, 10, 5, 2.5, 1 and 0.5 μg ml^{-1}. Working reagent was prepared by mixing 12.5 ml Micro BCA Reagent MA (sodium carbonate, sodium bicarbonate and sodium tartrate in 0.2 mol l^{-1} NaOH) and 12 ml Micro BCA

Reagent MB [bicinchoninic acid (4.0%) in water] with 0.5 ml Micro BCA Reagent MC (4.0% cupric sulfate, pentahydrate in water). One milliliter of each standard was added to appropriately labeled test tubes. A water blank and tadpole shrimp saline were used as controls. In each test tube, 1.0 ml working reagent was added and mixed. The tubes were covered with Parafilm™ and placed in a 60°C water bath for 60 min and then cooled to room temperature (23°C). Absorbance was measured at 562 nm, with corrections made for reference. A standard curve was produced to obtain hemoglobin concentrations of hemolymph samples.

Hemoglobin O_2-binding affinities were measured for animals from each rearing group to determine differences dependent on developmental P_{O_2}. Hemolymph (60 μl) was collected in glass capillary tubes from large tadpole shrimp (N=7 per rearing group) by dorsal puncture of the heart. Hemolymph was added into a small flow-through tonometer that opened into a narrow chamber (1 mm inner diameter) inside a cuvette (1 cm×1 cm×5 cm). The tonometer was placed on its side when hemolymph was added and during each equilibration step. This allowed the hemolymph to flow into the bulbous region of the tonometer. A stirring flea powered by a magnetic stirrer was placed into the tonometer to ensure thorough mixing of the hemolymph with inflowing gas mixtures. During spectrophotometric measurements, the tonometer was held upright so that the hemolymph flowed into the narrow chamber. The P_{O_2} of humidified inflowing gas (1000 sccm) was adjusted using a gas flowmeter (Model GF-3/MP; Cameron Instruments, Guelph, ON, Canada). Hemolymph was equilibrated for 20 min with normoxic air (20 kPa) and analyzed spectrophotometrically using a Turner spectrophotometer (Model 340; Mountain View, CA, USA) at a wavelength of 570 nm. This is the wavelength of maximal absorbance for oxy- and deoxy-hemoglobin for *Triops* (Horne and Beyenbach, 1974). Water was used as a reference. The absorbance of hemoglobin was determined after equilibration with air of 30, 4.0, 2.7, 1.3, 1.1, 0.8, 0.5 and 0 kPa O_2. Standard curves were constructed from the absorbance of hemoglobin at 0% and 100% saturation. Percent saturation for hemoglobin at each P_{O_2} was calculated using standard curves. Curve fitting of O_2 binding was calculated using SigmaStat 2.03 (SPSS Inc., Chicago, IL, USA). The P_{50} for each rearing group was determined as the P_{O_2} at which 50% saturation occurred (Bruno et al., 2001). Cooperativity (n_H) was calculated as the maximal slope of log[saturation/(1–saturation)] against log[P_{O_2}] (Bruno et al., 2001).

Oxygen-dependent changes in hemolymph pH were used to determine the significance of a Bohr shift in altering hemoglobin O_2-binding affinity. Hemolymph pH was measured using a PHR-146 Micro Combination pH Electrode (Lazar Research Laboratories, Inc., Los Angeles, CA, USA) inserted into the base of a flow-through chamber (20 μl). The P_{O_2} of inflowing humidified gas (1000 sccm) was adjusted using a gas flowmeter (Model GF-3/MP) to control a mixture of N_2 and room air. Hemolymph pH was determined after 10 min equilibration at 30, 4.0, 2.7, 1.3, 1.1, 0.8, 0.5 and 0 kPa O_2.

Oxygen-carrying capacity and delivery potential

The O_2-carrying capacity of 20 μl of O_2-saturated hemolymph was determined for animals from each rearing group (N=7 per rearing group) using methods described previously (Tucker, 1967). Hemolymph was saturated by equilibration with 30 kPa O_2. A potassium ferricyanide solution {6 g potassium ferricyanide [$K_3Fe(CN)_6$], 3 g saponin (Sigma) and 1 kg water} was added to a 10 ml glass syringe and degassed by plugging the syringe needle with a rubber stopper, pulling back gently on the plunger to create a vacuum and shaking. Extracted gas was expelled and the process was repeated a minimum of five times to ensure complete degassing. A microrespirometry chamber (400 μl) with O_2 electrode (Model 781 Strathkelvin oxygen meter) was filled with degassed potassium ferricyanide solution, plugged and stirred. After five minutes equilibration, the P_{O_2} in the chamber was measured. The stopper was removed from the chamber and 20 μl of hemolymph was injected into the chamber with the degassed potassium ferricyanide. After five minutes equilibration, the P_{O_2} in the chamber was determined. Hemolymph and degassed potassium ferricyanide solution were removed. Aerated potassium ferricyanide solution was added to the chamber, removed and added again. The chamber was left unplugged and the P_{O_2} determined after 20 min equilibration. The potassium ferricyanide solution was removed from the chamber and a solution of sodium sulfite and sodium borate [1 mg sodium sulfite (Na_2SO_3) and 5 ml 0.01 mol l^{-1} sodium borate ($Na_2B_4O_7$) (Sigma)] was added before the chamber was plugged again. After five minutes equilibration, the P_{O_2} of the chamber was determined. Hemolymph O_2 content (ml O_2 100 ml^{-1} hemolymph or Vol%) was calculated using the equation:

$$O_2 \text{ content} = (\Delta P_{O_2}/760)\alpha V(100/V_s) , \quad (2)$$

where ΔP_{O_2} is the change in P_{O_2} after injecting the hemolymph into the degassed potassium ferricyanide solution, α is the solubility coefficient of O_2 in the potassium ferricyanide solution, V is the chamber volume and V_s is the sample volume (Tucker, 1967).

Oxygen-delivery potential was calculated as the product of O_2 content and cardiac output and reported in μl O_2 min^{-1} (Ronco et al., 1991). Calculations for the determination of O_2 content and cardiac output were described above.

Oxygen consumption/oxygen transport coupling

The amount of coordination between O_2 demand and delivery was determined by comparing the ratio of $\dot{V}_{O_2}$ to cardiovascular transport. The degree of coupling was compared among rearing groups to determine the effects of developmental P_{O_2} on respiratory and cardiovascular system coordination. Indices of the relationship between $\dot{V}_{O_2}$ and hemolymph O_2 transport ($\dot{Q}_{O_2}$) were calculated using the equation:

$$\dot{V}_{O_2} / \dot{Q}_{O_2} = \dot{V}_{O_2} / C_{O_2} , \quad (3)$$

where $\dot{V}_{O_2}$ is oxygen consumption in $\mu l\ O_2\ g^{-1}\ h^{-1}$, C_{O_2} is oxygen content of fully saturated hemolymph ($\mu l\ O_2\ \mu l^{-1}$ hemolymph) and $\dot{Q}$ is cardiac output ($\mu l\ g^{-1}\ h^{-1}$) (Territo and Altimiras, 1998; Territo and Burggren, 1998). The ratio is unitless because $\dot{V}_{O_2}$ and $\dot{Q}_{O_2}$ are both expressed in $\mu l\ O_2\ g^{-1}\ h^{-1}$. A value of one suggests a strong coupling between O_2 demand and convective transport. Values below one indicate that circulatory O_2 transport capacity exceeds total $\dot{V}_{O_2}$. Values above one indicate that convective transport may limit O_2 supply.

Statistical analyses

All statistical analyses were run with SigmaStat 2.03 unless otherwise specified. Results are presented as means ± s.e.m. with statistical significance accepted at the level of $P<0.05$. Multiple pairwise comparisons were made using Bonferroni *t*-tests when rearing effects were significant, unless otherwise specified.

Metabolism

The strength of the relationship between activity level and P_{O_2} was measured using Pearson Product Moment Correlation. Comparisons of metabolic response to graded hypoxia were made among rearing groups using one-way repeated-measures analysis of variance (ANOVA). Comparison of lactate concentrations was made between normoxic and severe hypoxic rearing groups using a Student's *t*-test.

Ventilation

Comparisons of ventilatory amplitude and frequency among rearing groups were made using Kruskal–Wallis one-way ANOVA on ranks because data had unequal variance. Multiple pairwise comparisons were made using a Tukey test.

Cardiac physiology

Comparisons of heart rate, stroke volume and cardiac output to graded hypoxia were made among rearing groups using one-way repeated-measures ANOVA.

Hemoglobin

Comparisons of hemoglobin concentration were made among rearing groups using one-way ANOVA. The hemoglobin concentration of animals that had been switched from normoxic to severe hypoxic, and from severe hypoxic to normoxic conditions (after 5, 7 and 10 days), was analyzed using one-way repeated-measures ANOVA. Comparison of the O_2-binding affinity of hemoglobin from each rearing group was made using Friedman repeated-measures ANOVA on ranks because of unequal variance. Hemoglobin P_{50} was compared among rearing groups using Kruskal–Wallis one-way ANOVA on ranks because data were not normally distributed. Multiple pairwise comparisons were made using a Tukey test. Hemoglobin n_H was compared among rearing groups using analysis of covariance (StatView, 5.0.1; StatView Software, Cary, NC, USA). Multiple pairwise comparisons were performed using a Scheffe test and StatView. Comparisons of hemolymph pH with P_{O_2} were made among rearing groups using one-way repeated-measures ANOVA.

Oxygen-carrying capacity and delivery potential

Comparisons of the O_2-carrying capacity of hemolymph were made among rearing groups using Kruskal–Wallis one-way ANOVA on ranks because data did not have equal variance. Multiple pairwise comparisons were made using a Tukey test. Comparisons of the O_2-delivery potential of hemolymph were made among rearing groups using Kruskal–Wallis one-way ANOVA on ranks because data had unequal variance. Multiple pairwise comparisons were made using a Tukey test.

Oxygen consumption/oxygen transport coupling

Comparisons of the O_2 consumption/oxygen transport coupling were made among rearing groups using one-way repeated-measures ANOVA.

Results

Metabolism

Animal activity was not correlated with P_{O_2} and therefore did not confound $\dot{V}_{O_2}$ measurements. Developmental P_{O_2} had no effect on metabolic rate or metabolic response to hypoxic exposure (Fig. 1). All groups gradually decreased $\dot{V}_{O_2}$ down to a P_{CRIT} of 2 kPa O_2 (691±20 $\mu l\ O_2\ g^{-1}\ h^{-1}$ at normoxia to 274±9 $\mu l\ O_2\ g^{-1}\ h^{-1}$ at 2 kPa O_2 in normoxic animals; 714±14 $\mu l\ O_2\ g^{-1}\ h^{-1}$ at normoxia to 277±14 $\mu l\ O_2\ g^{-1}\ h^{-1}$ at 2 kPa O_2 in moderate hypoxic animals; and 728±3 6 $\mu l\ O_2\ g^{-1}\ h^{-1}$ at normoxia to 253±12 $\mu l\ O_2\ g^{-1}\ h^{-1}$ at 2 kPa O_2 in severe hypoxic animals). Baseline lactate levels for animals reared under normoxic (2.971±0.29 mmol l^{-1}) and severe hypoxic (3.142±0.33 mmol l^{-1}) conditions were not significantly different after 12 h of severe hypoxic

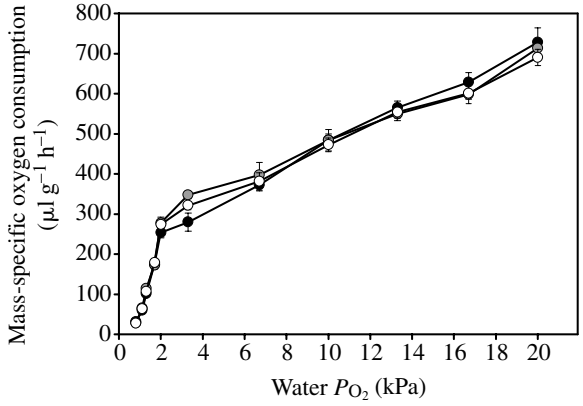

Fig. 1. Mass-specific O_2 consumption for tadpole shrimp reared under normoxic (19–21 kPa O_2; open circles), moderate (10–13 kPa O_2; gray circles) or severe hypoxic (1–3 kPa O_2; black circles) conditions exposed to progressive hypoxia. Values are means ± s.e.m. ($N \geq 7$). In some cases, the error bars were smaller than the symbols.

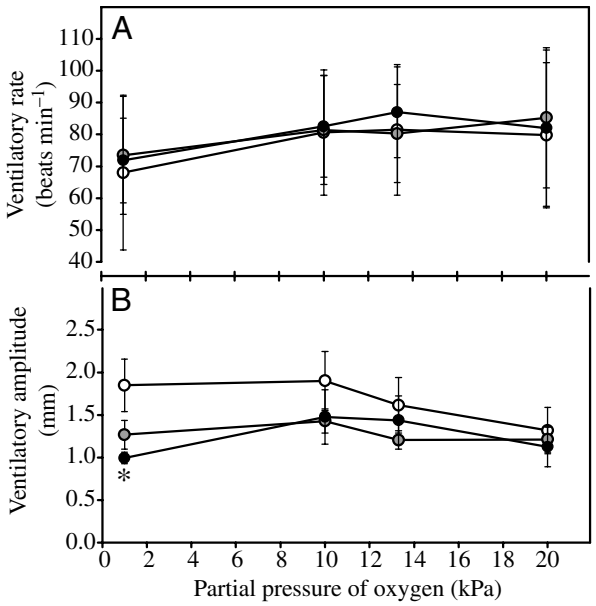

Fig. 2. (A) Ventilatory rate measured as the beats per minute of respiratory appendages and (B) ventilatory amplitude measured as the mean maximal distance between the 4th and 5th appendages during five consecutive ventilatory strokes. Tadpole shrimp reared under normoxic (19–21 kPa O_2; open circles), moderately hypoxic (10–13 kPa O_2; gray circles) or severe hypoxic (1–3 kPa O_2; black circles) conditions were exposed to four environmental P_{O_2}s (20, 13.3, 10 and 1 kPa O_2). Values are means ± s.e.m. ($N \geqslant 13$). *Significant difference from control animals at same P_{O_2} ($P<0.05$).

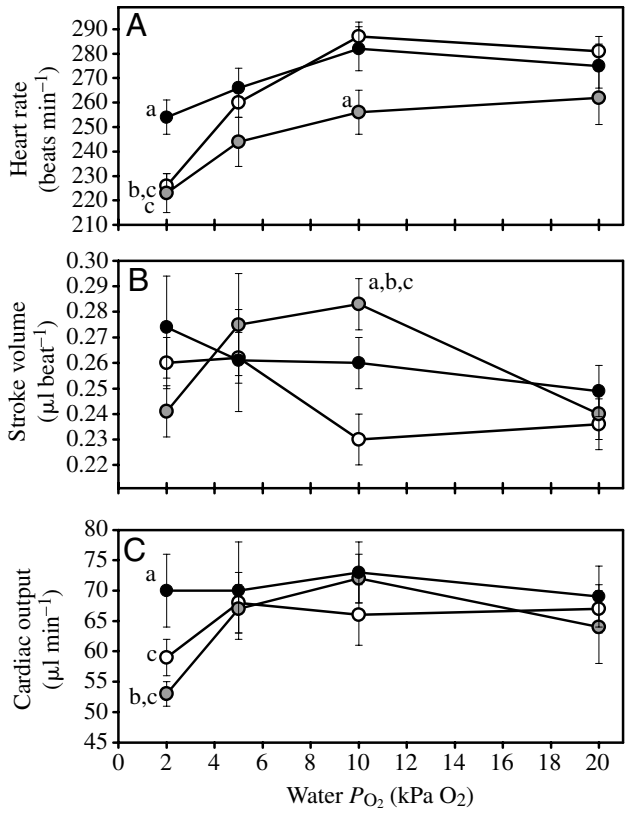

Fig. 3. (A) Heart rate, (B) stroke volume and (C) cardiac output of tadpole shrimp reared under normoxic (19–21 kPa O_2; open circles), moderately hypoxic (10–13 kPa O_2; gray circles) or severe hypoxic (1–3 kPa O_2; black circles) conditions exposed to normoxic (20 kPa O_2) and hypoxic (10, 5 and 2 kPa O_2) conditions. Values are means ± s.e.m. ($N \geqslant 13$). Significance is assumed at the level of $P<0.05$: [a]significant difference from control animals at same P_{O_2}; [b]significant difference from the same animal at preceding P_{O_2}; [c]significant difference from the same animals at 20 kPa O_2.

exposure (3.810±0.45 mmol l^{-1} and 3.304±0.59 mmol l^{-1}, respectively). No lactate was observed in the any of the water samples.

Ventilation

Ventilation rates were highly variable in all rearing groups. Tadpole shrimp ventilation rates were not different among rearing groups and did not differ within groups in response to hypoxic exposure (13.3, 10 and 1 kPa O_2) (Fig. 2A). Ventilatory amplitude did not differ within groups in response to hypoxic exposure (Fig. 2B). However, ventilatory amplitudes at 1 kPa O_2 were significantly different among the groups. Ventilatory amplitude was less in animals reared under severe hypoxic conditions when compared with animals reared under normoxic conditions but only at 1 kPa O_2.

Cardiac physiology

Heart rate response to hypoxic exposure was significantly different among the rearing groups. At low P_{O_2}, f_H decreased in tadpole shrimp reared under normoxic conditions from 281±6 beats min^{-1} at 20 kPa O_2 to 226±5 beats min^{-1} at 2 kPa O_2 (Fig. 3A). Animals reared under moderate hypoxia also showed a significant decrease in f_H, from 262±11 beats min^{-1} at 20 kPa O_2 to 223±8 beats min^{-1} at 2 kPa O_2. However, animals reared under severe hypoxic conditions did not exhibit a change in f_H with decreased P_{O_2} (275±9 beats min^{-1} at 20 kPa O_2 to 238±10 beats min^{-1} at 2 kPa O_2). Tadpole shrimp reared under normoxic (0.23±0.01 μl beat^{-1}) and moderate hypoxic conditions (0.28±0.01 μl beat^{-1}) had significant differences in Vs at 10 kPa O_2 (Fig. 3B). Those reared under normoxic conditions decreased Vs, whereas those reared under moderate hypoxic conditions increased Vs in response to severe hypoxic exposure. Yet neither hypoxic response was statistically significant due to the high variability of Vs; mean standard deviation was greater than the hypoxic response. Cardiac output was maintained in animals reared under severe hypoxic conditions down to 2 kPa O_2 (Fig. 3C). However, animals reared under moderate hypoxic conditions decreased $\dot{Q}$ from 64±6 μl min^{-1} at 20 kPa O_2 to 53±2 μl min^{-1} at 2 kPa O_2, and animals reared under normoxic conditions decreased $\dot{Q}$ from 67±4 μl min^{-1} at 20 kPa O_2 to 59±3 μl min^{-1} at 2 kPa O_2.

Hemoglobin

Hemoglobin concentrations measured as protein concentration were dependent on rearing P_{O_2} and were

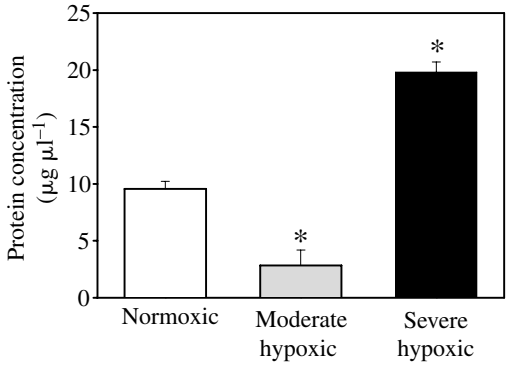

Fig. 4. Hemoglobin concentrations of tadpole shrimp reared under normoxic (19–21 kPa O_2), moderate (10–13 kPa O_2) or severe hypoxic (1–3 kPa O_2) conditions. Values are means ± s.e.m. ($N \geqslant 10$). *Significant difference from control (normoxic) animals ($P<0.05$).

significantly altered by chronic hypoxic exposure. Animals reared under normoxic (9.6±0.7 μg μl^{-1}) and moderate hypoxic (2.8±1.4 μg μl^{-1}) conditions had significantly less protein (hemoglobin) than those reared under severe hypoxic (19.8±0.9 μg μl^{-1}) conditions (Fig. 4). Concentrations in tadpole shrimp reared under normoxic conditions and transferred to severe hypoxic conditions increased significantly after 7 days (Fig. 5). After 10 days of severe hypoxic exposure, concentrations were not significantly different from animals that had been reared under severe hypoxic conditions. Adult tadpole shrimp reared under severe hypoxic conditions and switched to normoxic conditions did not decrease the amount of hemoglobin in their hemolymph for the 10 days investigated.

The O_2-binding affinity of hemoglobin was dependent on developmental P_{O_2}. Hemoglobin O_2-binding affinity in

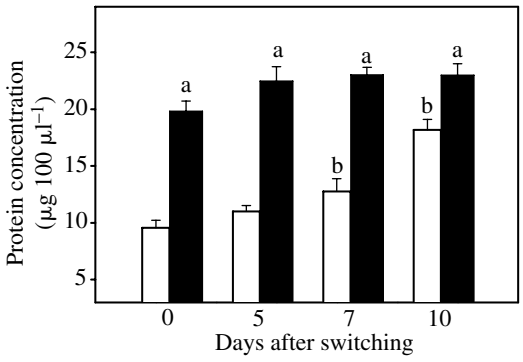

Fig. 5. Hemoglobin concentrations of tadpole shrimp reared under normoxic (19–21 kPa O_2) (open bars) conditions and then switched as adults to severe hypoxic (1–3 kPa O_2) conditions for 5, 7 or 10 days. Black bars indicate animals reared under severe hypoxic conditions and switched as adults to normoxic conditions for 5, 7 or 10 days. Day 0 is prior to switch. Values are means ± s.e.m. ($N \geqslant 7$). Significance is assumed at the level of $P<0.05$: [a]significant difference from normoxic animals at the same day; [b]significant difference within rearing groups from the values at day 0.

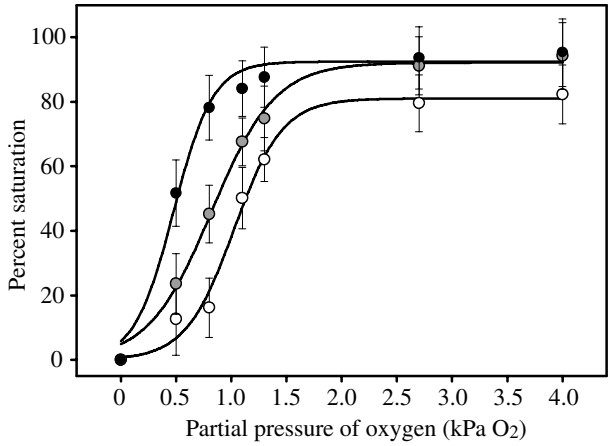

Fig. 6. Oxygen binding of hemoglobin from tadpole shrimp reared under normoxic (19–21 kPa O_2; open circles), moderately hypoxic (10–13 kPa O_2; gray circles) or severe hypoxic (1–3 kPa O_2; black circles) conditions at 25°C. The partial pressures of O_2 required to half-saturate hemoglobin (P_{50}) are given in Table 1. Values are means ± s.e.m. ($N \geqslant 7$).

animals reared under normoxic conditions was less than those reared under severe hypoxic conditions but not different from animals reared under moderate hypoxic conditions (Fig. 6). Hemoglobin cooperativity was significantly different among rearing groups (Fig. 7). Animals reared under normoxic conditions exhibited more cooperativity among hemoglobin subunits than those reared under moderate and severe hypoxic conditions. Oxygen-binding affinities and cooperativity values for hemoglobin from each rearing group are given in Table 1.

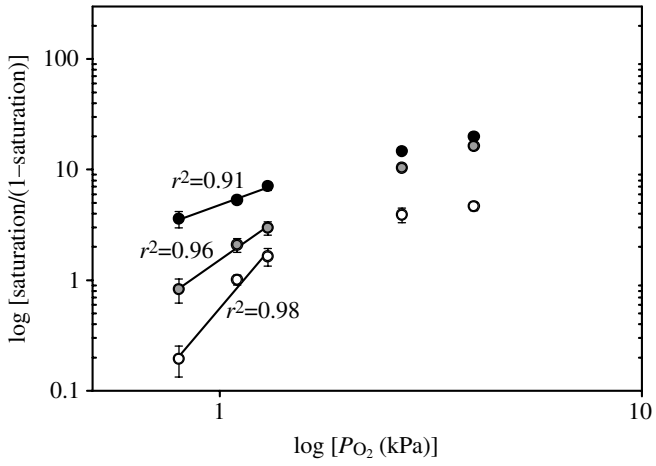

Fig. 7. Hill plot of hemoglobin for tadpole shrimp reared under normoxic (19–21 kPa O_2; open circles), moderately hypoxic (10–13 kPa O_2; gray circles) or severe hypoxic (1–3 kPa O_2; black circles) conditions. Maximal slope for each rearing group represented by regression line. Cooperativities (n_H) for each group are given in Table 1. Values are means ± s.e.m. ($N \geqslant 7$). In some cases, the error bars were smaller than the symbols.

Table 1. *Partial pressures of O_2 required to half-saturate hemoglobin (Hb P_{50}), cooperativities of hemoglobin subunits (Hb n_H), hemolymph O_2-carrying capacities and delivery potentials for tadpole shrimp reared under different P_{O_2}*

Developmental P_{O_2} (kPa O_2)	Hb P_{50} (kPa O_2)	Hb n_H	Oxygen carrying capacity (ml O_2 100 ml^{-1})	Delivery potential (μl O_2 min^{-1})
Normoxic (19–21)	1.14	4.5	4.1±0.6	274
Moderate hypoxic (10–13)	0.83	2.7*	8.3±1.0	529
Severe hypoxic (1–3)	0.5*	1.4*	19.4±1.6*	1336*

*Significant difference (P<0.05) from animals reared under normoxic conditions.

Oxygen-carrying capacity and delivery potential

Tadpole shrimp O_2-carrying capacity was dependent on developmental P_{O_2} (Table 1). Animals reared under severe hypoxic conditions had 5.2× the O_2-carrying capacity of those reared under normoxic conditions. Maximal O_2-delivery potential for tadpole shrimp was dependent on developmental P_{O_2}. Oxygen-delivery potential was significantly lower in animals reared under normoxic conditions (274 μl O_2 min^{-1}) relative to animals reared under severe hypoxia (1336 μl O_2 min^{-1}) (Table 1).

Oxygen consumption/oxygen transport coupling

The ratio of $\dot{V}_{O_2}/\dot{Q}_{O_2}$ was dependent on developmental P_{O_2}. Oxygen consumption/transport ratio was significantly higher (consistently above 1) in animals reared under normoxic conditions relative to those reared under moderate or severe hypoxic conditions (Fig. 8). Ambient P_{O_2} significantly affected $\dot{V}_{O_2}/\dot{Q}_{O_2}$ in animals reared under normoxic and moderate hypoxic conditions. Animals reared under moderate hypoxic conditions had a near coupling (1.18) of O_2 demand to cardiovascular supply at 20 kPa O_2, which decreased significantly with severe hypoxic exposure (2 kPa O_2). Animals reared under severe hypoxic conditions maintained $\dot{V}_{O_2}/\dot{Q}_{O_2}$ below 1 at each P_{O_2} investigated.

Discussion

Metabolism

The ability to regulate $\dot{V}_{O_2}$ may be related to the frequency and duration of hypoxic exposure that an animal typically encounters in its habitat (Chen et al., 2001). Tadpole shrimp inhabit euryoxic environments that can fluctuate daily by more than 26.7 kPa O_2 or remain almost anoxic (<1.3 kPa O_2) for months (Horne and Beyenbach, 1971; Scholnick, 1995). In addition, they vertically migrate from severely hypoxic (2 kPa O_2) sediments to hyperoxic (32 kPa O_2) surface water in a relatively short period of time (Scholnick and Snyder, 1996). Oxygen conformation to environmental P_{O_2} should be energetically favorable for organisms frequently exposed to hypoxic conditions on many temporal and spatial scales (Chen et al., 2001). Tadpole shrimp would be considered oxygen conformers as they did not tightly regulate $\dot{V}_{O_2}$ when P_{O_2} was decreased (Fig. 1). Instead, tadpole shrimp reduced O_2 demand to match O_2 supply.

Sensitivity to hypoxic exposure may be altered in animals exposed to hypoxic conditions throughout development due to ontogenetic changes in physiological capabilities and metabolic demand (Schulte, 2001). Adult animals that develop under hypoxic conditions often have decreased metabolic rates and decreased hypoxic sensitivity relative to animals that develop under normoxic conditions (Pichavant et al., 2001; Sokolova and Portner, 2001; Cech and Crocker, 2002; Chapman et al., 2000; Hammond et al., 2002). Yet developmental P_{O_2} did not affect adult tadpole shrimp metabolic rate or metabolic response to acute hypoxic exposure. Tadpole shrimp that develop under hypoxic conditions must balance immediate O_2 demand with long-term requirements for completing their life cycle within 30–40 days (Horne and Beyenbach, 1971). Therefore, transient decreases in $\dot{V}_{O_2}$ in response to ambient changes in P_{O_2} may be more beneficial than permanent decreases in $\dot{V}_{O_2}$ that could ultimately limit growth and development.

Anaerobic metabolism can be used to supplement aerobic metabolism and decrease sensitivity to hypoxic exposure in many aquatic organisms (Truchot, 1980; Childress and Seibel, 1998). Tadpole shrimp do not appear to utilize anaerobic metabolic pathways that end in lactic acid production;

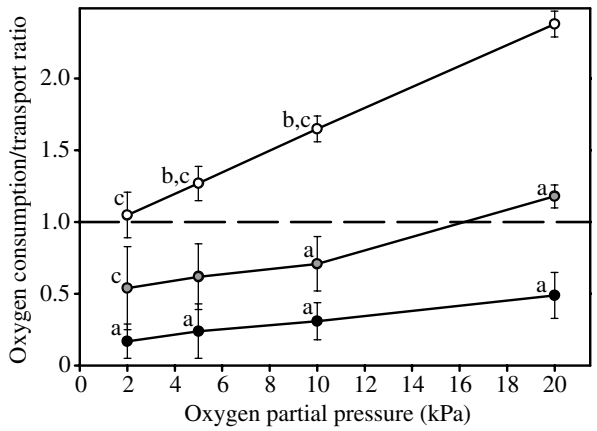

Fig. 8. Changes in O_2 consumption/transport ratio with P_{O_2} for tadpole shrimp reared under normoxic (19–21 kPa O_2; open circles), moderately hypoxic (10–13 kPa O_2; gray circles) or severe hypoxic (1–3 kPa O_2; black circles) conditions. Values are means ± s.e.m. (N⩾7). Significance is assumed at the level of P<0.05: [a]significant difference from control animals at same P_{O_2}; [b]significant difference from the same animal at preceding P_{O_2}; [c]significant difference from the same animals at 20 kPa O_2.

hemolymph lactate levels did not increase after 12 h of severe hypoxic exposure (1.3 kPa O_2). However, tadpole shrimp may employ other anaerobic pathways that were not investigated by the current study (i.e. pathways ending in pyruvate, valeric acid or alanine).

Ventilation

Organisms from a variety of phyla, including crustaceans (Hervant et al., 1995), birds (Faraci, 1991; Maina, 2000), bats (Maina, 2000), humans (Gozal and Gozal, 2001; Hoppeler and Vogt, 2001), mussels (Chen et al., 2001), fish (Galis and Barel, 1980; Cech and Crocker, 2002) and toads (Hou and Huang, 1999) increase ventilation rate and/or volume in response to acute hypoxic exposure. Tadpole shrimp apparently lack this typical ventilatory response or perhaps the response was not observed due to the length of acclimation prior to data collection (Fig. 2). Ventilation rates did not increase in response to hypoxic exposure down to 1 kPa O_2. Further, developmental P_{O_2} did not affect ventilation rates under normoxic conditions or in response to hypoxic exposure. Tadpole shrimp appendages are used for locomotion and the generation of feeding currents, in addition to respiratory gas exchange (Fryer, 1988). If ventilatory rates were increased in response to hypoxic exposure, it could cause the tadpole shrimp to swim faster and/or may alter the currents used for food collection. Typical ventilatory hypoxic responses may be lacking because those alterations may have adverse effects on locomotion and feeding.

Ventilatory amplitude was used in this study as an index of ventilation volume. Ventilatory amplitude did not increase in response to hypoxic exposure in any of the rearing groups. Below P_{CRIT}, amplitude was lowest in tadpole shrimp reared under severe hypoxic conditions but was not significantly different from amplitude under normoxic conditions. Lower ventilatory amplitude in animals reared under severe hypoxic conditions may result from direct effects of O_2 limitation on the respiratory appendage movement or may be a strategy to minimize metabolic demand under severe hypoxic conditions (Maina, 2000). Additionally, alterations in the angle of the appendages could account for ventilatory volume changes without observed changes in amplitude.

Cardiac physiology

Tadpole shrimp cardiovascular responses to hypoxic exposure follow a unique pattern unlike that documented for other crustaceans (McMahon, 2001). In most crustaceans, acute hypoxic exposure results in a decrease in f_H (bradycardia) and concomitant increase in V_S to maintain or increase $\dot{Q}$ (Reiber, 1995; McMahon, 2001). Smaller crustaceans, such as water fleas (*Daphnia magna*) (Paul et al., 1998) and grass shrimp (*Palaemonetes pugio*) (Harper and Reiber, 1999), increase f_H (tachycardia) to maintain $\dot{Q}$ in response to hypoxic exposure. In tadpole shrimp, however, cardiac function appears to be highly insensitive to hypoxic exposure (Fig. 3). Again, it should be acknowledged that this may be an artifact of the experimental protocol in that an acute hypoxic response may have been present but not observed because it took place during the acclimation period. Those animals reared under normoxic and moderate hypoxic conditions did not change f_H or V_S when exposed to 5 kPa O_2. Below this, a bradycardia was observed and resulted in decreased $\dot{Q}$. All cardiac parameters were maintained down to 1 kPa O_2 in tadpole shrimp reared under severe hypoxic conditions.

The difference between the cardiac response of tadpole shrimp and those of other crustaceans may result from differences in mechanisms of cardiac regulation. The heartbeat of many crustaceans is regulated through periodic bursting activity of cardiac ganglion, excitatory neurons that innervate the myocardium (Yamagishi et al., 2000). Hypoxia-induced bradycardia in those crustaceans may be mediated by a direct effect of lack of O_2 to the cardiac ganglion (Wilkens, 1999). Tadpole shrimp hearts have a myogenic mechanism of regulation whereby the cardiac muscle has endogenous rhythmic properties and does not rely on neural impulses to contract (Yamagishi et al., 1997; Yamagishi et al., 2000). The heart of tadpole shrimp does not respond to hypoxic exposure in the typical compensatory manner observed in other crustaceans. Myogenic regulation apparently supports cardiac function over a wide range of P_{O_2}s, including exposure to severe hypoxic conditions.

Hemoglobin

Hemoglobin concentrations obtained in this study were comparable to previously reported concentrations for *Triops* (Horne and Beyenbach, 1974; Scholnick and Snyder, 1996; Guadagnoli et al., 2005). Hemoglobin concentration was higher in animals that developed under severe hypoxic conditions, yet was not proportional to developmental P_{O_2} (Fig. 4). Animals reared under moderate hypoxic conditions produced less hemoglobin than animals reared under either normoxic or severe hypoxic conditions. Moderate hypoxic conditions may represent the level of P_{O_2} in which O_2 uptake is sufficient to meet metabolic demand. Alternatively, it may represent a trigger for genetic up- or down-regulation of hypoxia-inducible genes prior to the translation of hemoglobin protein subunits.

Hypoxia-induced hemoglobin synthesis appears to be a compensatory response that allows *T. longicaudatus* and several other branchiopods to regulate O_2 uptake and transport in their euryoxic habitats. Hypoxic exposure (4–5 kPa O_2) induced more than a 10-fold increase in hemoglobin concentration within 10 days in adult *D. magna* (Goldmann et al., 1999) and a threefold increase within three weeks in adult brine shrimp (*Artemia salina*) (Gilchrist, 1954). Hypoxic exposure (1–3 kPa O_2) induced a significant increase in protein concentrations within seven days in adult *T. longicaudatus* (Fig. 5). Since hemoglobin is the only major protein in tadpole shrimp hemolymph, protein concentration was accepted to be representative of hemoglobin concentration (Horne and Beyenbach, 1971). Within 10 days,

hemoglobin concentrations increased to the levels observed in tadpole shrimp reared under severe hypoxic conditions. The induction of hemoglobin synthesis observed in *T. longicaudatus* was less than the induction observed in *A. salina* or *D. magna*. These results indicate that tadpole shrimp exposed to hypoxic conditions acclimate by increasing hemoglobin concentration. Once the Hb is produced, though, it remains even when animals are returned to normoxic water (Fig. 5) (Guadagnoli et al., 2005).

Hemoglobin O_2-binding affinity was enhanced (decreased P_{50}) in tadpole shrimp reared under severe hypoxic conditions relative to those reared under normoxic conditions (Fig. 6). Differences in O_2-binding affinity are thought to have resulted from changes in subunit assembly of the functional hemoglobin molecule (Guadagnoli et al., 2005). This has been observed in other branchiopods such as *D. magna* and *A. salina* (Bowen et al., 1969; Waring et al., 1970; Sugano and Hoshi, 1971; Kobayashi et al., 1988; Goldmann et al., 1999). Specific assembly of hemoglobin subunits could be dependent on environmental P_{O_2}, internal chemistry or protein concentration (Sugano and Hoshi, 1971; Kobayashi et al., 1988; Fago and Weber, 1995; Goldmann et al., 1999). Since each subunit differs in O_2-binding affinity and cooperativity, changes in assembly directly affect O_2-binding affinity of the functional hemoglobin molecule (Kobayashi et al., 1988). The observed differences in hemoglobin O_2-binding affinity and cooperativity support the hypothesis that different P_{O_2} levels induce different hemoglobin isoforms or differential subunit assembly in *T. longicaudatus* (Figs 6, 7).

Oxygen-carrying capacity and delivery potential

Tadpole shrimp reared under severe hypoxic conditions appear to have an enhanced ability to transport and possibly store O_2 obtained from the environment due to their increased O_2-carrying capacity (Table 1). Tadpole shrimp reared under severe hypoxic conditions had a fivefold increase in hemolymph O_2-carrying capacity compared with those reared under normoxic conditions. Increased hemolymph O_2-carrying capacity resulted from increased hemoglobin concentration and O_2-binding affinity observed in those animals. Hemoglobin with high O_2-carrying capacity can often serve a storage function (Fago and Weber, 1995). Tadpole shrimp frequently surface and expose their respiratory appendages to the air–water interface. Tadpole shrimp may obtain and store O_2 from the higher P_{O_2} surface water for use during feeding and hunting in severely hypoxic regions of the pool. Surfacing behavior has been shown to increase with decreased P_{O_2}, further supporting a storage function of tadpole shrimp hemoglobin (Scholnick and Snyder, 1996).

Oxygen-delivery potential, which takes into account hemolymph O_2 content and $\dot{Q}$, appeared to be dependent on developmental P_{O_2}. Maximal O_2-delivery potential for tadpole shrimp was lowest in animals reared under normoxic conditions and highest in those reared under severe hypoxic conditions (Table 1). However, this was not a direct effect of developmental environment since hemoglobin synthesis could be increased throughout the life of the animal. Adult tadpole shrimp reared under normoxic conditions significantly increased hemoglobin concentrations in response to severe hypoxic exposure.

Oxygen consumption/oxygen transport coupling

The coupling of $\dot{V}_{O_2}$ with cardiovascular transport reveals the level of coordination in tissue O_2 demand relative to O_2 delivery (Territo and Burggren, 1998). In tadpole shrimp, differences in cardiovascular contribution observed among the rearing groups were due to increased hemoglobin concentration and O_2-binding affinity since there was no observed cardiovascular hypoxic response. Oxygen consumption of tadpole shrimp reared under normoxic conditions was not as dependent on cardiovascular transport as it was in tadpole shrimp reared under hypoxic conditions (Fig. 8). The coupling of $\dot{V}_{O_2}$ with transport was consistently above 1 in tadpole shrimp reared under normoxic conditions; however, $\dot{V}_{O_2}/\dot{Q}_{O_2}$ decreased in response to hypoxic exposure. This reduction suggests that $\dot{V}_{O_2}$ was reduced relative to cardiac output since (1) cardiac output did not increase in response to hypoxic exposure and (2) hemoglobin levels remain constant during acute hypoxic exposure. Animals reared under normoxic conditions increased convective processes to supply O_2 demands or relied on diffusional processes when exposed to severe hypoxic conditions.

Cardiovascular contribution to O_2 delivery was greatest in animals reared under severe hypoxic conditions. Oxygen delivery was enhanced in those animals *via* increased hemoglobin concentration and O_2-binding affinity. The increased O_2 delivery to the aerobic, metabolically active heart muscle may have supported cardiac contraction at normoxic rates under hypoxic conditions. Tadpole shrimp reared under severe hypoxic conditions maintained f_H and $\dot{Q}$ when exposed to severe hypoxic conditions while the other rearing groups did not. It was concluded that as tadpole shrimp produce more hemoglobin, they increase O_2 delivery relative to O_2 supply and enhance cardiac function during hypoxic exposure.

Conclusions

Developmental P_{O_2} may not be sufficient to induce permanent changes in adult tadpole shrimp physiological capabilities. Metabolic rate, ventilatory rate and metabolic response to acute hypoxic exposure were independent of developmental P_{O_2}. Differences in cardiac response to hypoxic exposure among the rearing groups were probably due to compensatory increases in hemoglobin concentration and O_2-binding affinity rather than to developmental differences. Based on the results of this study, hypoxia-induced hemoglobin synthesis represents an effective compensatory mechanism that allows tadpole shrimp to flexibly regulate O_2 uptake and transport under euryoxic conditions. Differences that result from increased hemoglobin concentration should not

be considered developmental effects until it is shown that (1) hemoglobin type or subunit arrangement is dependent on developmental P_{O_2} and not directed by ambient P_{O_2} during hemoglobin synthesis or (2) hypoxia-inducible genes were switched on during development and are continually expressed in the adult even if the animal is exposed to normoxic conditions.

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