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Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small?

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Three physiological variables, haematocrit, haemoglobin concentration and ventilation frequency, were measured to test how fathead minnows *Pimephales promelas* and small and large yellow perch *Perca flavescens* responded to three different dissolved oxygen concentrations. All fish were monitored continuously for any indications of stress in response to these manipulations. Within and between species, smaller individuals were the most tolerant of hypoxic environments. A species effect, however, did contribute to this observation, with fathead minnows being more tolerant of hypoxic environments than similar-sized yellow perch. In aquatic ecosystems where smaller fishes are more tolerant to hypoxia than their larger predators, hypoxic environments may have the potential to act as a refuge from predators.

Key words: body size; dissolved oxygen; *Perca flavescens*; physiological exclusion; *Pimephales promelas*.

INTRODUCTION

A literature survey of tolerance values to limited dissolved oxygen (hypoxia) for teleosts depicts a large degree of variation among and within some species (Doudoroff & Shumway, 1970; Gee *et al.*, 1978; Smale & Rabeni, 1995). This variation traditionally has been explained by environmental variables, metabolic requirements, activity levels of individuals and physiological and biochemical strategies (Holeton, 1980; Powers, 1980; Johansen, 1982; Palzenberger & Pohla, 1992). In non air-breathing fishes one or a combination of physiological mechanisms can alter tolerance to hypoxia. These include increased minute ventilation (amount of oxygen flowing over the gills per unit time) (Johansen, 1982; Randall, 1982; Smith & Jones, 1982), increased gill perfusion and subsequent increase in functional gill-surface area (Booth, 1979; Johansen, 1982) and increased blood oxygen carrying capacity and affinity (Powers, 1980). In addition to these responses, many teleosts are able to withstand hypoxia as a result of enlarged gill-surface area (Hughes & Morgan, 1973; Palzenberger &

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Pohla, 1992) and use of anaerobic metabolism (Blažka, 1958; Holeton, 1980; Hochachka, 1986). Thus, in chronically hypoxic environments, individuals often exhibit enlarged gill-surface area (Palzenberger & Pohla, 1992) or higher blood oxygen affinity (Larsson *et al.*, 1976; Powers, 1980). Fast swimming teleosts may also have greater oxygen extracting capacities than less active fishes (Holeton, 1980). Alternatively, body size has also been found to limit physiological response of an individual in oxygen-poor environments (Doudoroff & Shumway, 1970; Zanuy & Carrillo, 1985; Tonn & Paszkowski, 1986; Fox & Keast, 1990).

Indeed, several studies have found a size-sensitive tolerance to hypoxia with smaller fishes being more tolerant of low oxygen environments. Most comparisons were within a single species and among fish of various ages (Doudoroff & Shumway, 1970; Lowe-Jinde & Niimi, 1983; Zanuy & Carrillo, 1985; Smale & Rabeni, 1995). Of the physiological mechanisms known to increase oxygen extracting efficiency, both ventilation frequency and oxygen carrying capacity have been found to be influenced by body size (Jones, 1971; Lowe-Jinde & Niimi, 1983; Zanuy & Carrillo, 1985). Increasing minute ventilation is energetically expensive for fishes and this expense increases with an increase in body mass (Jones, 1971; Boutilier *et al.*, 1988; Yamamoto, 1991, 1992). Blood oxygen carrying capacity as determined by the number of red blood cells and haemoglobin concentration may also affect the ability of a teleost to tolerate hypoxic conditions (Powers, 1980; Gallaugher & Farrell, 1998). Although Schmidt-Nielsen (1984) states that blood parameters are not scaleable, several studies have found some effect of body size (Doudoroff & Shumway, 1970) including two that demonstrated an inverse relationship between body size and haematocrit within a species (Lowe-Jinde & Niimi, 1983; Zanuy & Carrillo, 1985). Winter mortality provides additional evidence of a scaling effect on tolerance. The reduced dissolved oxygen (DO) in benthic regions of ice-covered lakes often results in a differential mortality of larger individuals within a species (Klinger *et al.*, 1982; Fox & Keast, 1990). The death of predominantly larger individuals subjected to low temperatures and reduced oxygen levels also occurs in lakes with communities such as the *Umbra-Perca* assemblages of Wisconsin (Tonn & Paszkowski, 1986, 1987). Not all findings, however, suggest this advantage for smaller individuals (Doudoroff & Shumway, 1970; Stanley, 1973; Holeton, 1980; Brown & Maurer, 1986; Almeida-Val *et al.*, 2000).

Two possible mechanisms that might explain the limitations of body size on respiratory function include an allometric and fractal scaling relationship with body size. Both of these relationships predict that smaller individuals will have a greater tolerance of hypoxic conditions, although for different reasons. A negative allometric relationship exists for mass-specific gill-surface area, suggesting that smaller individuals may have more efficient gas exchange with their environment (Muir, 1969; Hughes, 1984). The fractal scaling model argues that, independent of body size, fishes are limited by the fixed size of red blood cells to exchange gasses with their environment (West *et al.*, 1997). Therefore, larger fishes require greater branching of blood vessels and subsequently greater time and effort for red blood cells to reach tissues.

For teleosts, the ecological implications of a size effect on hypoxia tolerance would be most obvious between a predator and its prey. The relative size of a

predator to its prey has already been established as an important variable in predator–prey relationships by affecting probability of capture (Mittelbach, 1981; Werner & Gilliam, 1984). In an environment with variable DO, refuges can be created by eliminating larger piscine predators that are unable to tolerate conditions available to their smaller piscine prey (Wright & Shapiro, 1990; Kolar & Rahel, 1993; Chapman *et al*., 1996*a*, *b*). This can be termed 'physiological exclusion'. To compare physiological tolerance between a teleost predator and prey variables were measured which were expected to increase in response to hypoxia, including gill ventilation rate and two blood parameters (haematocrit and haemoglobin concentration). Continuous monitoring was also undertaken for any obvious signs of stress. It is predicted that smaller prey would have a greater ability to compensate for reduced DO.

MATERIALS AND METHODS

STUDY ANIMALS

The fathead minnow *Pimephales promelas* Rafinesque and its predator the yellow perch *Perca flavescens* (Mitchill) were used in the experiments. The fathead minnow, with a standard length (L_S) of 51 mm, is commonly found in many ponds, streams and freshwater lakes of western and central North America (Scott & Crossman, 1973). Yellow perch, 102–254 mm L_S, are also common in many freshwater lakes across western Canada (Scott & Crossman, 1973). Both species are likely to encounter abrupt diurnal changes in DO; however, they are unlikely to encounter chronic hypoxia during summer months.

Fathead minnows (mean ± S.E. wet mass, 2·73 ± 0·07 g) were collected using minnow traps at the University of Manitoba Field Station, Delta Marsh, at the southern tip of Lake Manitoba (50°11′ N; 98°23′ W) in early and late September 1998. They were held in 200 l tanks at room temperature (*c*. 22° C) in normoxic water, fed Nutrafin flakes and kept at a constant photoperiod of 12L : 12D. Yellow perch (mean ± S.E. wet mass, 34·07 ± 1·28 g) were angled using barbless hooks from Stephenfield Lake in Stephenfield Provincial Recreation Park, Manitoba (50°23′ N; 98°10′ W) in April 1999. The yellow perch were held in groups of 10 in 200 l tanks, fed artificial pellets and worms and held at the same photoperiod, water temperature and DO concentration as the fathead minnows. In September 2000, smaller yellow perch (2·29 ± 0·25 g) were collected using a beach seine from the southern tip of Lake Manitoba. They were held in 200 l tanks, fed pellets, brine shrimp (*Artemia* spp.) and Nutrafin flakes and maintained in the same environmental conditions as the fathead minnows. Approximately 24 h prior to a trial, a group of fathead minnows, a group of small yellow perch or a single large yellow perch was placed in an isolated tank without food.

ENVIRONMENTAL CONDITIONS

The apparatus consisted of a 50 l aquarium (80 × 12 × 14 cm), but fishes were restricted to an experimental area (9 × 12 × 8 cm) by 0·5 cm netting, allowing an even DO concentration in the tank and the fishes to be continuously monitored with a video camera. The tank provided a large enough volume of water to maintain a consistent DO concentration for the duration of the experiment as determined by pre-experiment trials. The video camera was used to monitor fishes for signs of stress (loss of equilibrium) and to measure their ventilation frequency. To lower the DO concentration, nitrogen gas was bubbled through five air stones evenly spaced throughout the tank. For control trials, air rather than nitrogen was bubbled at a similar rate. The DO was measured using a YSI model 33 DO meter (Yellow Springs Instruments, Yellow Springs, Ohio, U.S.A.) calibrated daily with air-saturated tank water. The DO probe was placed in the tank within a perforated 10 cm long tube over a magnetic stirrer to facilitate water movement over the probe.

After the pre-specified DO level was reached the air stones were removed and plastic was placed over the water to prevent diffusion of atmospheric oxygen into the water. The plastic was fitted as closely as possible to the surface of the water, providing no opportunity for aquatic surface respiration. Beyond preventing early attempts at aquatic surface respiration, the presence of this plastic had no obvious effect on the behaviour of these fishes for the duration of these trials. The DO was recorded for the duration of the trial with a Linear model 2030 chart recorder (Linear, Reno, Nevada, U.S.A.).

Each fathead minnow and small yellow perch was individually identified by an acrylic tag for measurement of ventilation frequency over the trial duration (Abrahams & Sutterlin, 1999). All fishes were allowed at least 2 days for recovery from the tagging procedure and any individual showing signs of lethargy or haematoma was excluded from the experiment.

EXPERIMENTAL PROTOCOL

A group of five to six fathead minnows and six individually identified small yellow perch or a single large yellow perch was placed into the apparatus at *c*. 1700 hours the day before the trial. Groups of smaller fishes were used to approximate the total oxygen consumption of a single large yellow perch. Thirty minutes prior to the trial, the DO concentration was lowered. Once the desired level was reached the trial started and ventilation rates were measured. Individuals exhibiting any obvious signs of stress (disequilibrium) were immediately removed from the apparatus and placed in well-oxygenated water until they fully recovered. The time they spent in the test chamber was recorded. The apparatus was drained and cleaned following each trial. Fish were subjected to one of a total of four DO ranges, including three hypoxia levels [extreme (1·57–1·96 mg l^{-1}), moderate (2·35–2·74 mg l^{-1}) and mild (3·13–3·92 mg l^{-1})] and one normoxia (control) level (6·27–8·22 mg l^{-1}). These levels were chosen to reflect the fluctuating levels found under natural conditions where both the fathead and yellow perch are found together (Fig. 1). Six replicates of each of the experimental DO ranges were conducted on a total of 24 groups of fathead minnows, and on 24 large and small yellow perch. Fishes were used only once in these experiments. All experiments were performed between 1000 and 1600 hours with the water temperature maintained at 22° C.

MEASUREMENTS

Over the course of each trial, opercular movements were recorded for 28 s at 30 min intervals. The time intervals were reduced to 5 min for the large yellow perch at extreme hypoxia levels due to the short length of these trials. Mean ventilation frequency (opercular beats s^{-1}) was calculated for each time period for each large yellow perch and three randomly selected fathead minnows of each trial. At the end of each trial, fishes were anaesthetized with a 0·125-ml l^{-1} dose of 2-phenoxyethanol. The masses of all fishes were measured (Table I) and blood samples obtained. Due to size limitations, blood sampling required sacrificing all of the fathead minnows with an overdose of 2-phenoxyethanol and combining blood samples of individuals within each group for analysis. For both species, blood was taken from a puncture of a caudal vessel using a heparinized 27 gauge 1·3 cm hypodermic needle. At least 10 µl was used to determine the haemoglobin concentration using Sigma Kit 525, and the remaining blood volume was drawn into a heparinized microcapillary tube for haematocrit determination. Microcapillary tubes were centrifuged for *c*. 5 min and the percentage of red blood cells was calculated (haematocrit). From these variables the mean cellular haemoglobin content (MCHC) was determined, as the concentration of haemoglobin divided by the haematocrit times 100. In a small portion of trials (no more than one for each DO range) blood haemolysis was apparent in blood samples and for these individuals blood haematological variables were not used in the analysis. Fathead minnows removed from the apparatus prior to the end of the trial were not included in blood analyses since individuals provided insufficient blood volume for these measures. They were also not included for ventilation analysis since the random sampling procedure meant that by chance there were no data for these

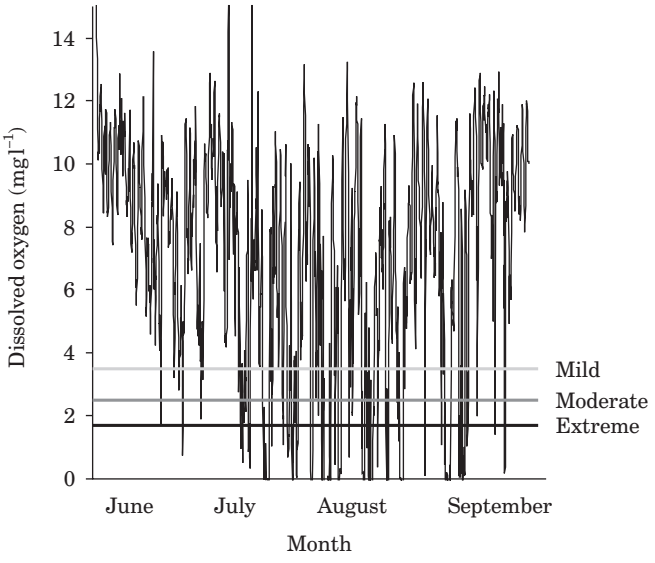

FIG. 1. Dissolved oxygen concentrations within Delta Marsh, Manitoba during the summer of 2000. Data were acquired using a YSI 6920 sonde equipped with a dissolved oxygen probe (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Readings were taken every 10 min and averaged to provide a 1 h value. The horizontal lines represent the middle dissolved oxygen levels used for experiments.

individuals. Based on the results of earlier experiments, neither ventilation frequency nor blood parameters were measured on the small yellow perch.

Means ± S.E. are given in the results. For statistical analyses the GLM procedure of SPSS was used unless otherwise noted. All comparison of means were conducted using least significant difference (LSD). Individuals represented statistically independent observations except for some measures of the fathead minnows, where pooled blood samples and randomly selected individuals for ventilation frequency meant that groups were the level of independent observation.

TABLE I. Mean ± S.E. wet masses of the fathead minnows and yellow perch used for analysis at each of the dissolved oxygen ranges (see text for values) used in the study. The number of fathead minnows used in each treatment is indicated in parentheses; for all perch, six different individuals were used for each treatment

	Dissolved oxygen range			
	Normoxia	Mild hypoxia	Moderate hypoxia	Extreme hypoxia
Species	Mean ± S.E. mass (g)			
Fathead minnow	2·70 ± 0·16 (30)	2·73 ± 0·13 (32)	2·71 ± 0·12 (30)	2·82 ± 0·14 (32)
Yellow perch – small	2·46 ± 0·41	2·25 ± 0·67	2·21 ± 0·53	2·33 ± 0·69
Yellow perch – large	34·11 ± 3·55	31·52 ± 1·96	37·81 ± 2·54	32·83 ± 1·65

RESULTS

Of the fathead minnows, <5% (five of 129) showed signs of lethargy or haematoma after tagging and therefore were not used in the experiment. The two main treatments in this experiment, type of fish (fathead minnow, small yellow perch or large yellow perch) and DO range significantly affected the total time spent in the chamber or the time to loss of equilibrium (Fig. 2 and Table II). As predicted, there was a significant interaction between these factors, whereby both the small yellow perch and fathead minnows were able to spend a significantly greater amount of time in the chamber at the extreme hypoxia DO range than the large yellow perch (Table II and Fig. 2; LSD, $P < 0{\cdot}001$). A size and species effect was also evident under moderate hypoxia as the proportion of time spent in the chamber was the same for fathead minnows and small yellow perch (Fig. 2; LSD, $P = 0{\cdot}918$), both groups of which differed from the large yellow perch (LSD, $P < 0{\cdot}001$). No large yellow perch lost equilibrium during mild hypoxia and normoxia trials and a single small yellow perch was removed prior to the end of the mild hypoxia trial. A single fathead minnow at each of the moderate and mild hypoxia levels was removed before the end of the trial. During the extreme hypoxia trial, four ($3{\cdot}83 \pm 0{\cdot}44$ g) of the 32 fathead minnows lost equilibrium. The fathead minnows were larger than those that survived ($2{\cdot}68 \pm 0{\cdot}13$ g) the full duration of the trial (*t*-test, $t = 2{\cdot}41$, d.f. $= 4$, $P = 0{\cdot}037$).

To determine the degree of physiological response of fathead minnows and large yellow perch to hypoxic conditions, blood parameters were compared to the mean values observed at normoxia. After the normoxia treatment the mean haematocrit and haemoglobin values for the fathead minnows were $26{\cdot}32 \pm 1{\cdot}12\%$ ($n = 5$) and $5{\cdot}79 \pm 1{\cdot}29\%$ g dl^{-1} ($n = 6$) respectively. In response

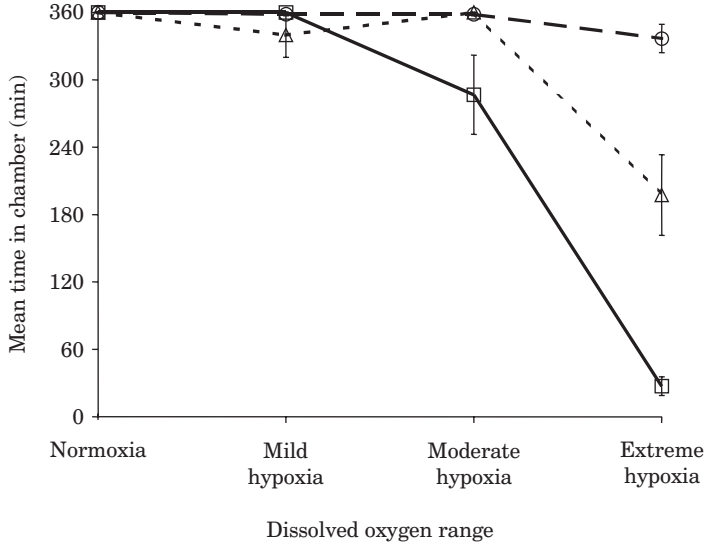

FIG. 2. Mean ± S.E. time spent in the chamber for fathead minnow (○, $2{\cdot}73 \pm 0{\cdot}07$ g), small yellow perch (Δ, $2{\cdot}29 \pm 0{\cdot}25$ g) or large yellow perch (□, $34{\cdot}07 \pm 1{\cdot}28$ g) at each of the dissolved oxygen ranges tested (see text for values). Times <360 min indicate individuals were removed when they lost equilibrium.

TABLE II. Two-way ANOVA results with proportion of time spent until loss of equilibrium for all fish types used (fathead minnow, small yellow perch and large yellow perch) and at all dissolved oxygen levels (normoxia, mild hypoxia, moderate hypoxia and extreme hypoxia)

Source	d.f.	F	P
Group	2	39·98	<0·001
Dissolved oxygen range	3	76·08	<0·001
Group × dissolved oxygen range	6	23·46	<0·001
Error	156		

to reduced DO levels, fathead minnows increased the number of red blood cells from the mean normoxia values at both the extreme and moderate hypoxia ranges (Table III; LSD $P < 0{\cdot}01$). At the extreme hypoxia range fathead minnows increased haemoglobin concentrations to nearly 1·5 times that of the mean normoxic value [extreme hypoxia $7{\cdot}76 \pm 1{\cdot}00\,\mathrm{g\,dl^{-1}}$ ($n = 5$)]. This, however, was a marginal influence ($P = 0{\cdot}068$). In addition there was no significant change in the MCHC values of the fathead minnows in response to hypoxic conditions (Table III). Mean haematocrit and haemoglobin values for yellow perch at normoxia were $28{\cdot}67 \pm 1{\cdot}81\%$ ($n = 6$) and $6{\cdot}00 \pm 0{\cdot}54\,\mathrm{g\,dl^{-1}}$ ($n = 6$) respectively. None of the haematological variables measured in yellow perch were affected by change in DO level (Table III). For fathead minnows, haematocrit and haemoglobin concentration across all DO ranges were positively correlated (Pearson's product correlation: $r = 0{\cdot}497$, $n = 20$, $P = 0{\cdot}004$). No such correlation was found for the yellow perch ($P = 0{\cdot}061$).

The mean difference from the mean normoxic value at each of the three hypoxia ranges was used to compare species response [(Fig. 3(a), (b)]. Although

TABLE III. ANOVA results for all haematological variables and ventilation frequency

Species	Variable	Source	d.f.	F	P
Fathead minnow	Haematocrit	DO	3	6·65	0·004
		Error	17		
	Haemoglobin concentration	DO	3	2·80	0·068
		Error	19		
	MCHC	DO	3	2·25	0·121
		Error	16		
	Ventilation frequency	DO	3	25·33	<0·001
		Error	68		
Yellow perch	Haematocrit	DO	3	0·09	0·963
		Error	18		
	Haemoglobin concentration	DO	3	0·48	0·698
		Error	19		
	MCHC	DO	3	0·46	0·715
		Error	18		
	Ventilation frequency	DO	3	7·95	0·001
		Error	23		

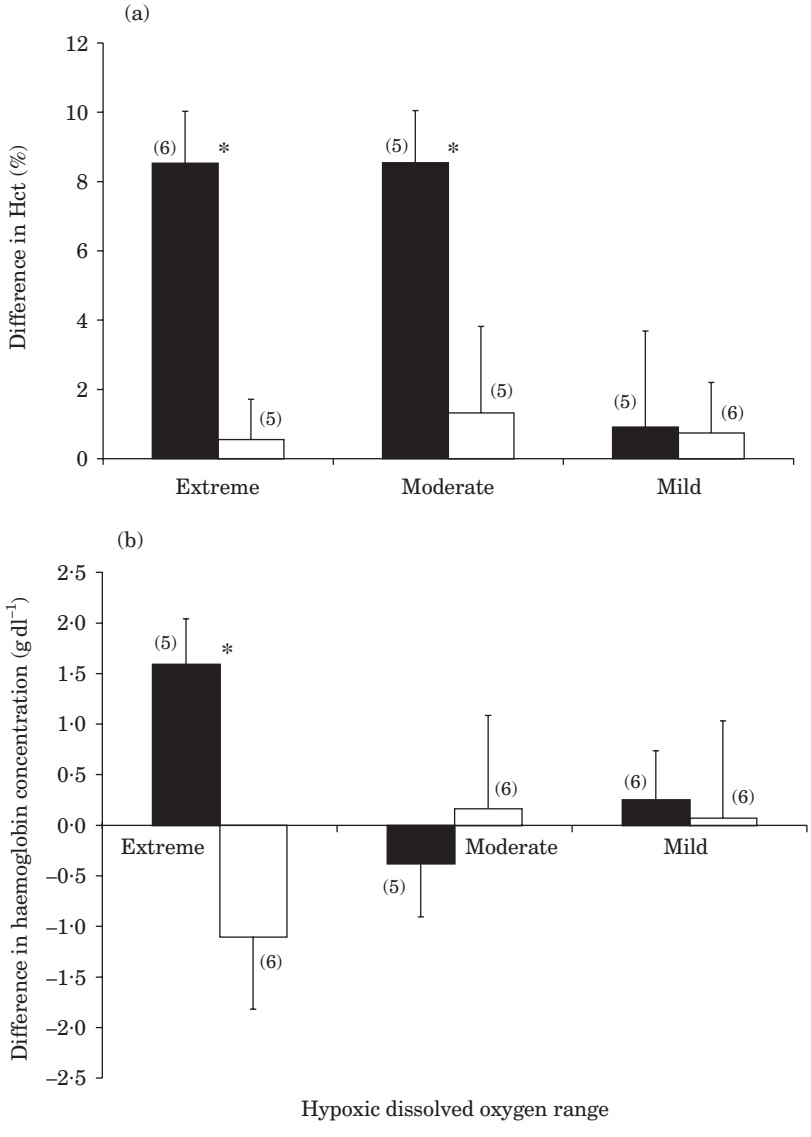

FIG. 3. Mean ± S.E. difference from normoxic values of (a) haematocrit and (b) haemoglobin concentration for fathead minnow (■) and yellow perch (□) at hypoxic dissolved oxygen ranges tested (see text for values). Sample sizes used for comparison are indicated in parentheses. *, Significantly different between species at $P < 0{\cdot}05$.

there was a time difference spent in the experimental chamber for yellow perch and fathead minnow, blood variables at the extreme and moderate hypoxia ranges were still compared. This was because it was assumed that loss of equilibrium was due to the inability of individuals to increase physiological mechanisms to obtain sufficient oxygen. The response by fathead minnows was greater than yellow perch at the extreme hypoxia range for haematocrit [Fig. 3(a); ANOVA, $F_{1,9} = 16{\cdot}501$, $P = 0{\cdot}003$] and haemoglobin concentration

[Fig. 3(b); ANOVA, $F_{1,9} = 10{\cdot}073$, $P = 0{\cdot}013$] but not for MCHC ($P = 0{\cdot}286$). The differences from normoxic mean haematocrit values were significantly greater for fathead minnows than yellow perch at moderate hypoxia levels (ANOVA, $F_{1,9} = 6{\cdot}106$ $P = 0{\cdot}039$). The fathead minnow response to moderate hypoxia levels, however, was no different from yellow perch when comparing haemoglobin concentration and MCHC [Fig. 3(b); haemoglobin, $P = 0{\cdot}620$; MCHC, $P = 0{\cdot}368$]. At mild hypoxia ranges there was no species difference in change of haematological variables from the mean normoxic value (ANOVA, haematocrit, $P = 0{\cdot}955$; haemoglobin, $P = 0{\cdot}869$; MCHC, $P = 0{\cdot}731$).

At all DO ranges measured, no significant change in ventilation frequency (number of opercular beats s^{-1}) was observed over the course of a single trial for either large yellow perch or fathead minnows (regression analyses revealed no slopes different from zero). Therefore the mean ventilation frequency for each yellow perch and three fathead minnows was used for all statistical analyses. The normoxic trial mean ventilation frequencies for the fathead minnow and yellow perch were $3{\cdot}02 \pm 0{\cdot}05$ beats s^{-1} ($n = 18$) and $0{\cdot}79 \pm 0{\cdot}04$ beats s^{-1} ($n = 6$) respectively. A negative linear relationship described the fathead minnow ventilation rate and experimental DO concentration (Fig. 4). The yellow perch also significantly increased ventilation frequency in response to hypoxia when compared to the ventilation response at normoxia (Table III). Mean ventilation frequency values at the extreme, moderate and mild hypoxia ranges, however, were not significantly different from each other (LSD, $P > 0{\cdot}05$). Within each species, body mass did not influence ventilation frequency (Table III).

In comparing ventilation responses between species, only at mild and moderate hypoxia ranges were yellow perch able to maintain a ventilation frequency increase similar to the fathead minnow response (Fig. 5; ANOVA, mild, $P = 0{\cdot}84$; moderate, $P = 0{\cdot}109$). At the extreme hypoxia range the difference

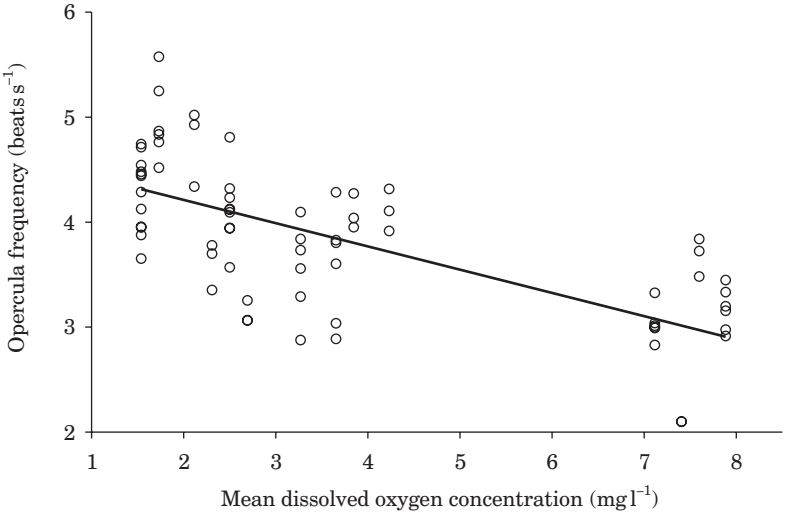

FIG. 4. Influence of mean dissolved oxygen on the mean ventilation frequency for each fathead minnow observed ($n = 72$). The line was fitted by $y = -0{\cdot}2215x + 4{\cdot}6546$ ($r^2 = 0{\cdot}4734$, $P < 0{\cdot}05$).

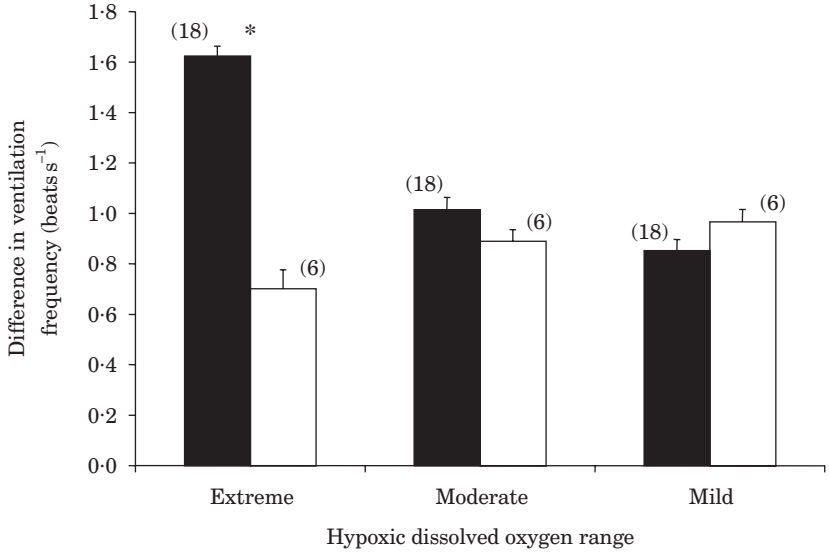

FIG. 5. Mean ± S.E. difference from normoxic values of ventilation frequency for fathead minnow (■) and yellow perch (□) at hypoxic dissolved oxygen ranges tested (see text for values). Sample sizes used for comparison are given in parentheses. *, Significantly different between species at $P < 0{\cdot}05$.

from normoxic value was significantly greater for the fathead minnow than yellow perch (Fig. 5; ANOVA, $F_{1,22} = 21{\cdot}704$, $P < 0{\cdot}001$).

DISCUSSION

Reduction in environmental DO influenced both the haematological variables and the ventilation responses of fathead minnow and yellow perch in this study. At extreme and moderate hypoxia ranges the physiological response varied between species in that only the large yellow perch exhibited signs of respiratory distress. Fathead minnows were able to survive more extreme hypoxic conditions and had a greater oxygen carrying capacity than yellow perch. At extreme hypoxia they were able to increase their ventilation frequency at a greater rate than yellow perch. The few fathead minnows that failed to endure extreme hypoxic conditions for the duration of the trial tended to be larger individuals. Despite the lower mass-specific metabolic rate for larger yellow perch, the increase in ventilation frequency was not sufficient to compensate for the environmental change in DO at the extreme (and in some cases the moderate) hypoxia levels. Smaller yellow perch, however, were able to withstand extreme hypoxic conditions longer than larger yellow perch.

As with many teleosts subjected to hypoxic conditions, fathead minnows responded with an increase in haematocrit and haemoglobin concentration (Larsson *et al.*, 1976; Lowe-Jinde & Niimi, 1983; Nikinmaa *et al.*, 1984; Peterson, 1990; García *et al.*, 1992). In a previous study, fathead minnows subjected to various levels of pulp fibres (and a subsequent drop in DO levels) increased haematocrit by *c.* 1·4 fold for each 1 mg l^{-1} drop in DO (MacLeod & Smith,

1966). Despite the increase in both haematocrit and haemoglobin concentration in this study, there was no change in the calculated MCHC when fathead minnows were subjected to hypoxia. The stable MCHC indicates that the increase in haematocrit was not entirely due to red blood cell swelling and therefore represented an increase in the number of red blood cells (Gallaugher & Farrell, 1998). This was further supported by a positive correlation of haematocrit and haemoglobin concentration among all DO levels. Yellow perch response to lower DO levels was limited, and there was no detectable increase in haematological variables measured in response to hypoxia in this study. Several yellow perch lost equilibrium prior to the end of the extreme and moderate hypoxia trials, probably as a result of the limited haematological response. The inability of yellow perch to increase haematocrit and haemoglobin concentration in response to hypoxia may be attributed to the limitations of one or a combination of physiological mechanisms including splenic red blood cell production, cardiac work or ventilation abilities (Wells & Weber, 1991). It may simply have been due to their limited time in these environmental conditions to change their blood parameters. Previous studies have found yellow perch tolerance of limited DO to be much greater than observed in this study (Petit, 1973; Petrosky & Magnuson, 1973; Suthers & Gee, 1986). The yellow perch in these previous studies, however, were juveniles (Suthers & Gee, 1986) acclimated to lower DO levels prior to being used in experiments (Petit, 1973) or subjected to a concurrent decrease in temperature with reduced DO levels (Petrosky & Magnuson, 1973). It is important to note that fishes used in the present experiments were held under, and therefore acclimated to, normoxic conditions for different lengths of time. The most hypoxia tolerant individuals were the fathead minnows that had the longest exposure to chronic normoxia. The influence of temperature and maturity along with season, nutrition, handling time and sampling methods have also been found to alter haematological values (Larsson *et al.*, 1976; Lowe-Jinde & Niimi, 1983; Saint-Paul, 1984; Zanuy & Carrillo, 1985; Gallaugher & Farrell, 1998). One or more of these factors may explain the variation of intraspecific tolerance values between the present study and others.

Both fathead minnows and yellow perch increased ventilation frequency above normoxic values in response to lowered DO. This mechanism was similar to previous studies where both the fathead minnow and yellow perch increased gill ventilation rates in response to depleted oxygen levels at low temperatures (Petrosky & Magnuson, 1973; Klinger *et al.*, 1982). Claireaux & Dutil (1992) also found an initial increase in number of opercular beats in Atlantic cod *Gadus morhua* L. in response to both severe and mild hypoxia. Increases in ventilation frequency maintain a steep oxygen partial pressure (P_{O_2}) gradient between blood and water at the gill to promote gas diffusion in addition to the maintenance of oxygen at the tissue (Johansen, 1982; Smith & Jones, 1982; Nikinmaa & Salama, 1998). There are costs associated with the increase in frequency, however, as an individual must provide enough oxygen for normal life processes in addition to the muscular activity required to move the opercula. The limited ability of yellow perch to increase ventilation frequency in response to extreme and moderate hypoxia levels in this study suggests that these costs were greater than the benefits. Other studies have found non-linear rates of

ventilation increase as breathing slows near critical oxygen tensions (Larsson *et al.*, 1976; Saint-Paul, 1984; Rantin *et al.*, 1992). This increased ventilation frequency near critical oxygen tensions may not be feasible in the long-term as it reduces the net amount of oxygen extracted (Jones & Randall, 1978).

A negative relationship of body size and hypoxia tolerance has been suggested as the key mechanism in the variation of response to hypoxic conditions by the fathead minnow and yellow perch. One basis for this explanation is the negative allometric relationship of gill-surface area and body size. This relationship applies to many freshwater fishes (Palzenberger & Pohla, 1992). Additional evidence of intraspecific size-sensitive variation in tolerance to hypoxic conditions include ventilation frequencies (Jones, 1971), blood oxygen carrying capacity (Lowe-Jinde & Niimi, 1983; Zanuy & Carrillo, 1985), gill uptake rates (Sijm *et al.*, 1995) and winterkill (Tonn & Paszkowski, 1986). Significant variation in tolerance between small and large yellow perch was found, corroborating other studies that suggest that body size plays a role in this relationship. In addition, a size effect in fathead minnow tolerance to extreme hypoxia was observed, where larger individuals had to be removed early due to loss of equilibrium. Variation in performance between similar-sized fathead minnows and yellow perch, however, also indicated a strong species effect. Indeed, species differences are reported in the literature with a diversity of morphological and physiological adaptations to the habitats occupied (Doudoroff & Shumway, 1970; Gee *et al.*, 1978; Kramer, 1987; Chapman *et al.*, 1999). Interestingly, where species differences exist, prey species are often more tolerant to hypoxia than their predators (Gee *et al.*, 1978; Smale & Rabeni, 1995)

The present findings and other studies provide ecological implications for variation in hypoxia tolerance among predator and prey species. Despite different mechanisms responsible for interspecific differences in hypoxia tolerance, the consequences of prey species being more tolerant than their predators is the same. From the study it is concluded that fathead minnows would have an advantage in habitats in which DO fluctuate from normoxia to hypoxia in short time periods. Formation of temporary hypoxic habitats spatially and temporally may create refuges for smaller fathead minnows to temporarily evade predators. Indeed, temporary hypoxic habitats were found to occur under natural conditions (Fig. 1) and some evidence to suggest that the fathead minnows are using these areas exclusive of their larger predators (unpubl. data). In addition there is evidence that at critical DO levels the predator (yellow perch) may exhibit behaviours that are not interpreted as being risky to the prey (fathead minnows), thus allowing prey to use even moderate hypoxic habitats (Robb & Abrahams, 2002). Physiological exclusion of predators may also have some limitations. For example, Rahel & Nutzman (1994) found the central mudminnow *Umbra limi* (Kirtland) made 'dives' into lethal hypoxic environments when capturing prey.

Small individuals are often out-competed through domination of resource use by larger individuals (Brown & Maurer, 1986). Being small also implies greater costs related to feeding energetics, survival and reproduction (Peters, 1983; Brown & Maurer, 1986). In addition, smaller individuals are often subject to a high risk of predation (Werner & Gilliam, 1984). The use of hypoxic (piscine predator-free) refuges, however, can provide prey with an advantage in these

short-term and possibly long-term situations. The findings of Chapman *et al.* (1996*a*, *b*) suggested that hypoxic refuges exist. The present study further supports their conclusions by suggesting that body size may be a factor that makes use of hypoxic habitats possible. In addition, McIntyre & McCollum (2000) found a similar relationship with larval bullfrogs *Rana catesbeiana* and predatory larval salamanders *Ambystoma tigrinum*, suggesting this relationship may exist in other aquatic predator–prey systems.

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