Biol. Rev. (2008), **83**, pp. 173–189.
doi:10.1111/j.1469-185X.2008.00038.x

Does size matter for hypoxia tolerance in fish?

Göran E. Nilsson[1]* and Sara Östlund-Nilsson[2]

[1] *Physiology Programme, Department of Molecular Biosciences, University of Oslo, P.O. Box* 1041, *N*-0316 *Oslo, Norway*
[2] *Department of Biology, University of Oslo, P.O. Box* 1066*; N*-0316 *Oslo, Norway*

(*Received* 13 *August* 2007; *revised* 18 *February* 2008; *accepted* 20 *February* 2008)

ABSTRACT

Fish cover a large size range, from milligrams to tonnes, and many of them are regularly exposed to large variations in ambient oxygen levels. For more than half a century, there have been various, often divergent, claims regarding the effect of body size on hypoxia tolerance in fish. Here, we attempt to link old and new empirical data with the current understanding of the physiological mechanisms behind hypoxia tolerance. Three main conclusions are drawn: (1) body size *per se* has little or no impact on the ability to take up oxygen during hypoxic conditions, primarily because the respiratory surface area matches metabolic rate over a wide size range. If size-related differences are seen in the ability for oxygen uptake in a species, these are likely to reflect adaptation to different life-styles or habitat choice. (2) During severe hypoxia and anoxia, where fish have to rely on anaerobic ATP production (glycolysis) for survival, large individuals have a clear advantage over smaller ones, because small fish will run out of glycogen or reach lethal levels of anaerobic end-products (lactate and H^+) much faster due to their higher mass-specific metabolic rate. (3) Those fish species that have evolved extreme adaptations to hypoxia, including haemoglobins with exceptionally high oxygen affinities and an alternative anaerobic end-product (ethanol), reveal that natural selection can be a much more powerful determinant of hypoxia tolerance than scaling of physiological functions.

Key words: Anoxia, hypoxia, scaling, aerobic metabolism, anaerobic metabolism, lactate

CONTENTS

* Address for correspondence (Tel: +47-22855807; Fax: +47-22856041; E-mail: g.e.nilsson@imbv.uio.no)

I. INTRODUCTION

There are at least two obvious reasons why scaling of hypoxia tolerance in fish is of interest. Firstly, the range of body masses of fish covers at least nine orders of magnitude (eight orders of magnitude for teleost fishes alone), which is larger than in any other vertebrate group. Teleost fishes include the smallest of all vertebrates: the recently described *Paedocypris progenetica* from swamps in Sumatra, and *Schindleria brevipinguis*, from the Great Barrier Reef. These minute fish mature at a body length of around 8 mm (Watson & Walker, 2004; Kottelat *et al.*, 2006) and a body mass of 10–20 mg. The largest teleosts include the sunfish (*Mola mola*) and the beluga sturgeon (*Huso huso*), which reach 2000 kg (Novák, 1982; Frimodt, 1995), while the whale shark (*Rhincodon typus*) can weigh 34 000 kg (Chen, Liu & Young, 1999). Also during their life span, fish often cover very large ranges in body mass. Numerous species that start off life weighing one or a few milligrams can reach body masses of 10 kg or more.

Secondly, fish live in an environment that often shows great variation in oxygen levels. The major reasons for this are that the oxygen concentration of air is some 30 times higher than that of air-saturated water, and that oxygen diffuses nearly 10 000 times faster in air than in water (Schmidt-Nielsen, 1997). Thus, the small amount of molecular oxygen present in water can be rapidly used up by aquatic organisms, and it is then only slowly replenished by diffusion. This means that hypoxia is a common phenomenon in stagnant water, where currents and convection do not introduce oxygenated water, and especially at night, when plants do not photosynthesize. Consequently, hypoxia is regularly encountered by fish living in tropical freshwater habitats, in tide pools, and even on coral reefs (Val, Almeida-Val and Randall, 2006). However, the longest and most severe hypoxia is encountered by fish in ponds and small lakes in the northern hemisphere, where the short day length in combination with thick ice cover completely stops photosynthesis and oxygen diffusion from the atmosphere. In Northern Europe, such small bodies of water often become completely devoid of oxygen (anoxic) for several months during the winter (Blazka, 1958; Nilsson & Renshaw, 2004; Vornanen & Paajanen, 2006).

Although numerous studies for more than half a century have examined the effect of body size on various indices of hypoxia tolerance in a wide range of fish species, there has been no comprehensive review on the subject. One reason for this may be the great divergence in published results, which, at least partly, can be attributed to an equally great divergence in methods and species. The primary aim of this review is to clarify the basic principles and mechanisms that are likely to determine the scaling of hypoxia tolerance in fish. In addition, we examine a recent data set on hypoxia tolerance that covers a particularly large range of body sizes of fish from one teleost family in one habitat, which allow us to test some of our predictions and reach basic conclusions.

II. MECHANISMS OF LIFE AND DEATH IN HYPOXIA

In order to understand how body size influences survival in hypoxia, we first need to consider the reasons why fish die in hypoxic conditions. Animals need oxygen to generate ATP through oxidative phosphorylation. A reduction in ATP levels is immediately life-threatening because ion pumping cannot be maintained, leading to depolarization of cells, which initiates both necrotic and apoptotic mechanisms (Lipton, 1999). Neural tissue has the highest obligatory rate of ATP use, and will therefore be the first to experience a decrease in ATP levels when oxygen supply is reduced (Lutz, Nilsson & Prentice, 2003, for review). Thus, the high ATP turnover rate in the brain, about 10 times faster than that of the average body tissue of vertebrates (Mink, Blumenschine & Adams, 1981), including fish (Nilsson, 1996), means that the brain is particularly sensitive to hypoxia. Key detrimental events in the oxygen-deficient brain, such as depolarization and neurotransmitter release appear to be very similar in fish and mammals, and take place within a similar time frame if temperature differences are taken into account (Nilsson *et al.*, 1993; Hylland, Nilsson & Johansson, 1995).

However, even before the brain has become irreversibly damaged by hypoxia, the lack of oxygen will make it electrically silent (Hansen, 1985). A cessation of brain electrical activity will normally set the time limit for hypoxic survival, because electrical signals arising in the brain are responsible for breathing movements. Thus, an electrically silenced brain will be a point of no return, even if water oxygen levels are restored, as blood re-oxygenation will be prevented by the cessation in breathing (an exception may be fish with a particularly large capacity for cutaneous oxygen uptake independent of gill ventilation).

ATP levels in fish tissues vary between 0.5 and 5 mmol kg^{-1}, with levels in brain tissue generally being below 2 mmol kg^{-1} (DiAngelo & Heath, 1987; Van Raaij *et al.*, 1994; DeBoeck *et al.*, 1995; Van Ginneken *et al.*, 1996; Ishibashi *et al.*, 2002). Estimated rates of ATP synthesis in fish brain vary between 1.3 and 5 mmol kg^{-1} min^{-1} at 12 to 26 °C (Johansson, Nilsson & Törnblom, 1995; Nilsson, 1996), which means that the ATP pool is turned over about once every minute, and that ATP levels will decrease immediately if ATP synthesis is slowed down or stopped.

The first option for fish to accommodate a decrease in ambient oxygen levels is to increase the ventilation of the gills and the blood perfusion through the gills (Nilsson, 2007, for review). Thereby, fish become "oxygen regulators" (Ultsch, Jackson & Moalli, 1981), because they regulate their oxygen uptake so that oxygen consumption ($\dot{V}O_2$) is maintained at a steady level over a more or less wide range of ambient oxygen concentrations. Typically, fish adapted to hypoxic habitats can maintain $\dot{V}O_2$ down to much lower water oxygen levels than species that do not normally encounter severe hypoxia. The lowest level at which a fish can maintain its $\dot{V}O_2$ is termed the critical

oxygen concentration ($[O_2]$crit), or critical oxygen tension ($P O_2$crit) when the oxygen level is expressed as partial pressure (Prosser & Brown, 1961; Ultsch *et al.*, 1981). (When oxygen concentration is given as % of air saturation, this can be translated to $P O_2$ in mmHg or kPa by multiplying the concentration with 1.5 or 0.2, respectively, since 100% air saturation normally refers to $[O_2]$ in water that has been equilibrated with humid air near sea level, where $P O_2$ is about 150 mmHg or 20 kPa).

If water oxygen levels fall below $[O_2]$crit, the fish will have to start making ATP anaerobically. Phosphocreatine (PCr) can rapidly regenerate ATP from ADP, but since fish brain PCr levels range from about 0.5 to 5 mmol kg^{-1} (e.g. DiAngelo & Heath, 1987; Van Raaij *et al.*, 1994; Van Ginneken *et al.*, 1996), this pathway cannot maintain ATP levels for more than a few minutes. Therefore, anaerobic glycolysis is the only main ATP-generating pathway that can function during longer periods of severe hypoxia (Hochachka & Somero, 2002).

The most viable strategy for long-term hypoxic survival is to have a $[O_2]$crit low enough to avoid using on anaerobic metabolism. This is because anaerobic glycolysis leads to a build-up of anaerobic end-products and rapid depletion of glucose stored as glycogen. The ATP yield of anaerobic glycolysis is approximately 10% of that of aerobic metabolism, since most of the chemical energy remains in the anaerobic end-product (Hochachka & Somero, 2002; Brand, 2003).

Results suggest that acidosis caused by anaerobic metabolism, rather than an inability to produce enough ATP, is the cause of anoxic death in some fish. ATP levels are maintained or show only a modest decrease in dying rainbow trout (*Oncorhynchus mykiss*) and bullhead catfish (*Ictalurus nebulosus*) during anoxia, while lactate levels reach 12 – 20 mmol kg^{-1}, which may be intolerable for the brain (DiAngelo & Heath, 1987; Van Raaij *et al.*, 1994). On the other hand, in severely hypoxic common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*), considerable reductions in brain ATP levels have been detected (Van Raaij *et al.*, 1994; Ishibashi *et al.*, 2002). It is not clear if the reduction in ATP levels seen in some fish brains is caused by a metabolic inability to produce enough ATP, a run down of glycogen stores, or metabolic dysfunction caused by lactic acidosis.

A fish therefore has two main options for maintaining ATP synthesis during hypoxia: to take up as much O_2 as possible from the water, thereby allowing oxidative phosphorylation to continue; or to utilize anaerobic glycolysis to compensate for reduced oxidative phosphorylation. As we shall see, it is likely that the ability to extract oxygen from the water is relatively independent of body mass, while the scaling of metabolic rate puts significant size-related constraints on the ability of fish to use anaerobic glycolysis for hypoxic survival.

In addition to boosting oxygen uptake and producing ATP anaerobically, some fish may also increase their hypoxia survival time by depressing ATP demand (see section VI), thereby matching ATP supply and demand for a longer period or during more severe hypoxia.

III. DIVERGENT RESULTS

There have been various suggestions regarding the influence of body size on the ability of fish to endure low oxygen levels. Doudoroff & Shumway (1970) summarized early results, the first studies being from the 1940s. These early studies often gave conflicting results that are difficult to interpret due to poor experimental design, including a lack of temperature control. For example, in contrast to a previous study by Privolnev (1947), Bishai (1960) claimed that hypoxia tolerance in Atlantic salmon (*Salmo salar*) decreases with age. He followed a batch of newly hatched salmon from hatching to 132 days post-hatching and found that the lowest oxygen level that they could survive increased from 2.8% to 27% of air saturation. On the other hand, during the 132 day period, the water temperature rose from 5 to 16 °C, which would have resulted in a several-fold increase in metabolic rate that could explain the increased demand for oxygen. A better-controlled study by Rombough (1988) on steelhead trout (*Oncorhynchus mykiss*) found a decrease in $[O_2]$crit during the first 20 days after hatching, after which it stabilizes (Table 1).

Rombough (1988) also found that $[O_2]$crit increases with age during the embryonic stage in steelhead trout. This has also been observed in other fish (see Kamler, 1992, for review) and most likely reflects the inability of the embryo to regulate its O_2 uptake, which largely depends upon O_2 diffusion over the surface of the egg. As the embryo grows its O_2 requirements will increase while the respiratory surface area remains constant. The result is often a steady decrease in the ability to tolerate hypoxia until hatching, although embryos of some species appear to survive hypoxia by slowing down development, or almost totally shutting down metabolism (Johansen & Krogh, 1914; Podrabsky *et al.*, 2007). Here, we do not consider further the embryonic stage, but focus on how size influences hypoxia tolerance of free-swimming fish.

More recent studies indicate that in some species, the young individuals are the least hypoxia tolerant, while in others the opposite situation occurs (Table 1). For example, in the oscar cichlid (*Astronotus ocellatus*) of the Amazon river, small individuals are significantly less hypoxia tolerant than larger ones, when measured both as survival time (Almeida-Val *et al.*, 2000) and $[O_2]$crit (Sloman *et al.*, 2006). Interestingly, small oscar cichlids seem to occupy different, possibly better oxygenated, habitats than larger individuals (Junk, Soares & Carvalho, 1983; Botero, 2000). By contrast, in a study comparing behavioural hypoxia avoidance in yellow perch (*Perca flavescens*) with that of its prey, fathead minnow (*Pimephales promelas*, approximate mass 3 g), Robb & Abrahams (2003) concluded that larger perch (30 g) were significantly less likely to remain in hypoxic water than smaller perch (3 g) and minnows, a behaviour that was suggested to allow the smaller fish to use hypoxic areas as a refuge from the larger predatory perch. Similarly, in largemouth bass (*Micropterus salmoides*), small fish selected lower oxygen levels than large fish (Burleson, Wilhelm & Smastresk, 2001). However, preference or avoidance studies do not necessarily reveal the physiological hypoxia tolerance of the fish. For example, although large oscar cichlids can

Table 1. Overview of published data on the effect of body size on hypoxia tolerance in teleost fishes

Family/Species	Method	Size	Size effect detected	Reference
Cichlidae				
Oscar cichlid	Lethal time	3–350g	Large is better	Almeida-Val *et al.* (2000)
(*Astronotus ocellatus*)	$[O_2]$crit	16–230g	Large is better	Sloman *et al.* (2006)
Nile tilapia (*Oreochromis niloticus*)	$[O_2]$crit	2.1–250.3g	None	Verheyen. Blust & Decleir (1994)
Cyprinidae				
Striped shiner (*Luxilus chrysocephalus*)	Lethal $[O_2]$	1.1–10.5g	None	Smale & Rabeni (1995)
Bigmouth shiner (*Notropis dorsalis*)	Lethal $[O_2]$	0.2–2.9g	None	Smale & Rabeni (1995)
Red shiner (*Cyprinella lutrensis*)	Lethal $[O_2]$	0.2–3.1g	None	Smale & Rabeni (1995)
Central stoneroller (*Campostoma anomalum*)	Lethal $[O_2]$	0.3–5.8g	None	Smale & Rabeni (1995)
Creek chub (*Semotilus atromaculatus*)	Lethal $[O_2]$	1.0–10.1g	None	Smale & Rabeni (1995)
Bluntnose minnow (*Pimephales notatus*)	Lethal $[O_2]$	0.4–4.5g	None	Smale & Rabeni (1995)
Centrarchidae				
Longear sunfish (*Lepomis megalotis*)	Lethal $[O_2]$	0.7–22.4g	None	Smale & Rabeni (1995)
Green sunfish (*Lepomis cyanellus*)	Lethal $[O_2]$	0.6–29.8g	None	Smale & Rabeni (1995)
Bluegill sunfish (*Lepomis macrochirus*)	Lethal $[O_2]$	1.2–23.6g	None	Smale & Rabeni (1995)
Largemouth bass (Micropterus salmoides)	Avoidance	23–3000g	Large avoid hypoxia	Burleson *et al.* (2001)
Percidae				
Yellow perch (*Perca flavescens*)	Avoidance	2.3–34g	Large avoid hypoxia	Robb & Abrahams (2003)
Sparidae				
Red seabream (*Pagrus major*)	Lethal $[O_2]$	9.5–35 mm total length	Large is better	Ishibashi *et al.* (2005)
Salmonidae				
Rainbow trout (*Oncorhynchus mykiss*)	$[O_2]$crit	1–40 day embryos	Small is better	Rombough (1988)
	$[O_2]$crit	1–40 days after hatching	Large is better	Rombough (1988)

Better means that they have a lower critical oxygen concentration ($[O_2]$crit), survive a lower $[O_2]$, or tolerate a longer hypoxia exposure. Of the species studied by Smale & Rabeni (1995) we have only included data covering at least a 10-fold mass range, which is still probably too narrow to reveal scaling effects. Earlier studies, reviewed by Doudoroff & Shumway (1970), gave an equally variable picture of the scaling of hypoxia tolerance in fishes.

survive more severe hypoxia than small ones, they are still more inclined to resort to aquatic surface breathing to escape hypoxia (Sloman *et al.*, 2006). Moreover, as we shall see, physiological measures of hypoxia tolerance, such as $[O_2]$crit (related to oxygen uptake capacity) and lethal hypoxia levels (involving the capacity for anaerobic metabolism), are likely to differ considerably in their dependence on body mass.

IV. SCALING OF OXYGEN UPTAKE IN HYPOXIA

(1) Metabolic rate and respiratory surface area

Scaling relationships (i.e. the change in a physiological variable in relation to body mass) have been used as arguments for why both small and why large fish should be the most hypoxia tolerant.

Like all organisms, fish show a reduction in mass-specific metabolic rate (i.e. oxygen uptake per unit of body mass) with increasing size. For more than a century, various theories have been presented to explain this relationship, including a relative decrease in body surface area with body mass (Rubner, 1883), the balance between energy metabolism and heat loss (Kooijman, 1993), constraints associated with branched distribution networks like blood vessels (West, Brown & Enquist, 1997), the sum of all processes making up energy metabolism (Darveau *et al.*, 2002), and the suggestion that no universal model can explain it (White, Phillips & Seymour, 2006). Although there is no consensus regarding the mechanisms behind scaling of metabolic rate, it is clear that $\dot{V}O_2$ does not rise linearly with body mass (M), but follows an exponential equation:

$$\dot{V}O_2 = a \cdot M^b, \quad (1)$$

(Fig. 1A) where the factor a is often different for different animal groups or species, while the scaling exponent b shows a striking consistency among animals, although phylogenetic differences occur (White *et al.*, 2006). The corresponding equation for mass-specific metabolic rate is:

$$\dot{V}O_2 \cdot M^{-1} = k \cdot M^{b-1}, \quad (2)$$

where k is the mass-specific factor derived from a ($k = a \cdot M^{-1}$) and $b-1$ is the mass specific scaling exponent.

From large data sets of teleost fishes, the scaling exponent (b) for $\dot{V}O_2$ has been estimated to be between 0.79 and 0.88

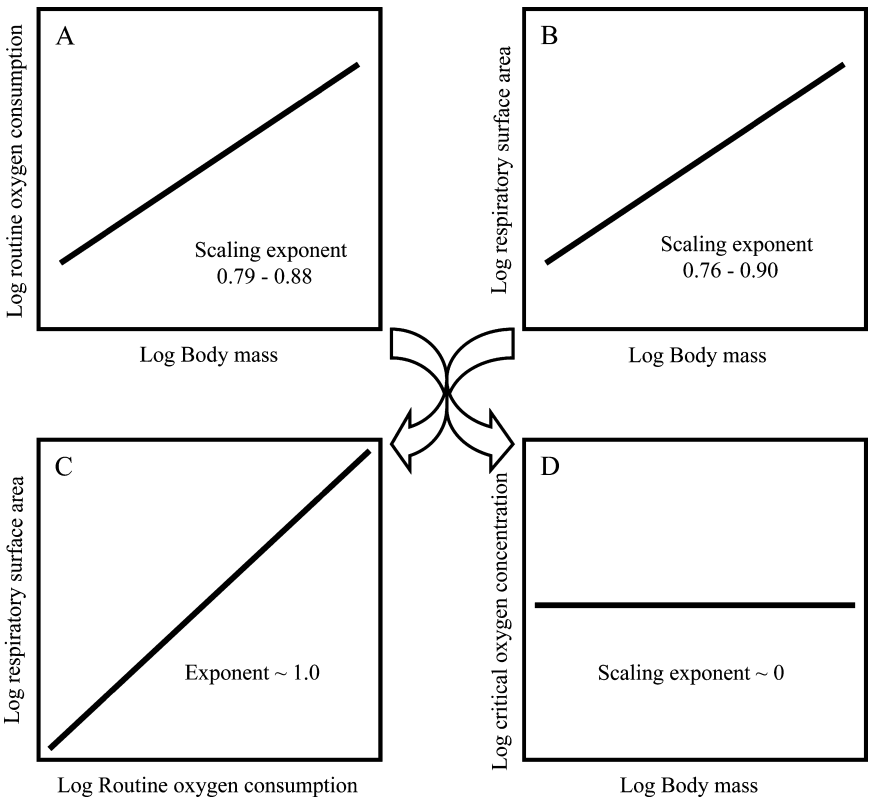

Fig. 1. A series of logaritmic plots showing that oxygen consumption (A) and respiratory surface area (B) scales similarly with mass. Consequently, respiratory surface area and oxygen consumption are tightly matched (C), and as a result, oxygen uptake ability in hypoxia (measured as critical oxygen concentration, $[O_2]$crit) becomes independent of size (D). $[O_2]$crit is the lowest water $[O_2]$ where the rate of oxygen consumption can be maintained. Note that in a log-log plot, the slope of the line is the scaling exponent because a scaling equation ($y = a \cdot x^b$) is $\log y = \log a + b \log x$ in its logarithmic form.

(Winberg, 1960; Goolish, 1995; Clarke & Johnston, 1999; White *et al.*, 2006), which corresponds to a mass-specific scaling exponent ($b-1$) of -0.12 to -0.21. Thus, a 100-fold increase in body mass only leads to a 40- to 60-fold increase in oxygen uptake. Consequently, Almeida-Val *et al.* (2000) and Sloman *et al.* (2006) suggested that the relatively low metabolic rate of large oscar cichlids could in part explain their higher degree of hypoxia tolerance.

Conversely, it has been argued that the allometric relationship between respiratory surface area and body mass means that hypoxia tolerance will decrease with size (Robb & Abrahams, 2003). Thus, small fish have a larger gill area in relation to body size than large ones: authors that have measured the total gill area (A_{gill}) for a large number of species (Muir, 1969; De Jager & Dekkers, 1975; Palzenberger & Pohla, 1992) have found that it follows the equation:

$$A_{gill} = a \cdot M^b, \quad (3)$$

(Fig. 1B), with a scaling exponent (b) between 0.76 and 0.90. It is striking how similar this is to the scaling exponent for $\dot{V}O_2$ (0.79 – 0.88, see above), implying that gill area has a linear relationship with metabolic rate (Fig. 1C). In other words, gill area appears to be closely matched to the requirement for oxygen.

Another variable linking gill morphology to the capacity for oxygen uptake is the diffusion distance between water and blood, i.e. the thickness of the cell layers separating water from blood in the gill lamellae. However, this seems to be relatively unaffected by body size: a study on tilapia (*Oreochromis niloticus*) indicated a scaling exponent of 0.077 over a size range of 0.1 to 1000 g, suggesting that the diffusion distance may be only slightly greater in large individuals (doubling over a 10 000-fold size range) (Kisia & Hughes, 1992). This is a minor difference compared to the 50-fold difference in diffusion distance found among species, and which can be attributed to phylogeny and life-style (Kisia & Hughes, 1992).

It is therefore hard to see how scaling arguments could be used to link the respiratory surface area to any size dependence of hypoxia tolerance. In fact, the similar scaling of oxygen consumption and gill area indicates that the capacity for oxygen uptake measured as $[O_2]$crit should be independent of size (Fig. 1D). [An exception may be the larval stage of some fish, where cutaneous respiration occurs in addition to gill respiration, resulting in increased gas exchange capacity (Rombough & Moroz, 1997), although this effect may be abolished by underdeveloped gills at these early life stages (Oikawa & Itazawa, 1985)].

(2) Circulatory and haematological parameters

The blood flow generated by the heart (cardiac output) is an important determinant of oxygen delivery to tissues and may also influence oxygen uptake through the gills. Cardiac output correlates with heart mass (Franklin & Davie, 1992), and heart mass increases linearly with body mass in fish, as in other vertebrates (the scaling exponent is about 1.0) (Farrell *et al.*, 1988; Cerra *et al.*, 2004, for review). However, during their life time, fish often show changes in the muscular composition of the heart that could influence cardiac performance, and a study on eel (*Anguilla anguilla*) revealed improved cardiac performance during growth from 100 g to 650 g (Cerra *et al.*, 2004). Still, it is not clear whether high cardiac output would allow greater hypoxia tolerance, particularly since cardiac output is either unchanged or reduced in teleost fishes exposed to hypoxia (Wood & Shelton, 1980; Bushnell & Brill, 1992; Gamperl, Pinder & Grant, 1994; Axelsson, Altimiras & Claireaux, 2002; Stecyk *et al.*, 2007). Thus, the current state of knowledge does not indicate any major role of cardiac output in the scaling of hypoxia tolerance in fish.

The ability to take up oxygen in hypoxia will also be influenced by the oxygen affinity of haemoglobin in blood. There are results suggesting that the half-saturation pressure for oxygen binding to haemoglobin (the P_{50} value) increases with body mass in mammals (Schmidt-Nielsen & Larimer, 1958), birds (Lutz, Longmuir & Schmidt-Nielsen, 1974) and lizards (Pough, 1977*b*), while it decreases with body mass in snakes (Pough, 1977*a*). However, it has been suggested that the apparent scaling of P_{50} in mammals is an experimental artefact caused by inappropriate PCO_2 and pH values during measurements and that the oxygen affinity of blood *in vivo* is independent of body size (Steen, 1971; Lahiri, 1975). Unfortunately, no multi-species study on the scaling of blood oxygen affinity in fish appears to exist, but a single study on piranha (*Serrasalmus rhombius*) suggests that P_{50} decreases from approximately 15 to 10 mmHg when fish mass increases from 250 to 600 g (Wood, Weber & Powers, 1979). Comparing published records of P_{50} from various species of different sizes is unlikely to be very informative since P_{50} is greatly influenced by life-style (degree of hypoxia tolerance and activity level), as well as sample treatment and analytical procedure which often differ between studies. Moreover, the P_{50} of fish haemoglobin is also influenced by factors that can change during hypoxia, such as blood pH, blood PCO_2, and erythrocyte ATP content (Jensen, Fago & Weber, 1998, for review). Until further studies are available, we will assume that this factor does not have a major influence on how body size affects hypoxia tolerance in fish.

Another haematological variable that could be considered relevant is the amount of haemoglobin in blood (i.e. the oxygen carrying capacity of blood). This correlates to the haematocrit of the blood and there are data suggesting that haematocrit increases with body size in fish (Goolish, 1995). However, an initial assessment of the large number of studies that include measurements of haematocrit and haemoglobin levels in fish suggests that these haematological parameters do not correlate with hypoxia tolerance. While hypoxia-tolerant species like goldfish (*Carassius auratus*), common carp, tench (*Tinca tinca*), Nile tilapia, tambaqui (*Colossoma macropomum*), and black scorpionfish (*Scorpaena porcus*) have haematocrit and haemoglobin levels of 19–34% and 5–10 g dl^{-1}, respectively (Murad, Houston & Samson, 1990; Ishibashi *et al.*, 2002; Harikrishnan, Rani & Balasundaram, 2003; da Costa *et al.*, 2004; Silkin & Silkina, 2005; Shah, 2006), the same values for hypoxia-intolerant species like rainbow trout, salmon (*Salmo salar*), whitefish (*Coreogonus lavaretus*) largemouth bass, cobia (*Rachycentron canadum*), and annular seabream (*Diplodus annularis*) are 32–47% and 3–9 g dl^{-1}, respectively (Rehulka, Minarik & Rehulkova, 2004; Lappivaara & Marttinen, 2005; Linton, Scuton & McKinley, 2005; Silkin & Silkina, 2005; Subhadra *et al.*, 2006; Zhoua *et al.*, 2006).

A final haematological variable that may correlate with hypoxia tolerance is the ability to increase the blood haemoglobin content by spleen contraction in response to hypoxia. Data on spleen size in relation to body size indicate a scaling exponent close to 1 [0.95 in a multi-species survey (Crile & Quiring, 1943), and 1.2 in a study on channel catfish (*Ictalurus punctatus*) (Schultz et al., 1999)], suggesting that the ability to increase blood haemoglobin content during hypoxia is relatively independent on body size.

(3) Scaling of oxygen uptake capacity in damselfishes

In recent years, we have investigated hypoxia tolerance of coral reef fishes at Lizard Island, Great Barrier Reef, Australia (Nilsson & Östlund-Nilsson, 2004; Östlund-Nilsson & Nilsson, 2004; Nilsson *et al.*, 2004, 2007*b,c*), collecting data on over 240 individuals representing 50 species from 10 families over a 4000-fold size range (Nilsson, Hobbs & Östlund-Nilsson, 2007*a*). While most families are only represented by a few individuals and species, or cover a relatively small size range (< 100 fold), the damselfish (Pomacentridae) data include over 100 juvenile and adult individuals from 15 species covering a size range of approximately 10 mg to 40 g. These represent measures of hypoxia tolerance from the widest range of fish sizes on one fish family in one geographic location available to date. Moreover, habitat preferences for these damselfishes appear to be relatively similar; they use spaces between branches in coral colonies as nocturnal shelters. At night, this microhabitat can become severely hypoxic: in coral colonies kept in outdoor tanks, $[O_2]$ between the branches decreases to about 20% of air saturation (Nilsson *et al.*, 2004), and field measurements on calm nights confirm that such low oxygen levels occur *in situ* (Nilsson *et al.*, 2007*a*).

Using closed respirometry, where the fish is placed in a sealed chamber and exposed to a continuous decrease in $[O_2]$ due to its own oxygen consumption, we recorded two indices of hypoxia tolerance in damselfishes from the Lizard Island reef: $[O_2]$crit and the oxygen level where the fish showed the first signs of distress or problems with maintaining equilibrium, denoted $[O_2]$out (Nilsson & Östlund-Nilsson, 2004). $[O_2]$out was always lower than $[O_2]$crit, and was usually reached an hour or more after

$[O_2]$crit. $[O_2]$out should be influenced by the anaerobic (glycolytic) capacity of the fish; further decreases in $[O_2]$ will result in death. Although the damselfish data-set reveals a size dependence of anaerobic survival (see section V), it was not originally collected with the present questions in mind and it is not ideal for clarifying the underlying mechanisms. This is because the end point ($[O_2]$out) is likely to be influenced by both time spent in hypoxia (below $[O_2]$crit) and the tolerable level of hypoxia.

In closed respirometry, PCO_2 will increase in the chamber as $[O_2]$ decreases, similar to many natural situations. However, in the case of coral reef fishes, the rise in PCO_2 will be small, because warm seawater is well buffered and has a pH well above 8 (see Kalle, 1972). Even in marine tide pools that are supersaturated with O_2 (200–300% of air saturation) during the day, PCO_2 does exceed about 2 mmHg, and pH remains above 7.5, at night when organisms have used up all the O_2 (Truchot & Duhamel-Jouve, 1980). In closed respirometry with warm seawater, PCO_2 values should not exceed approximately 1 mmHg, which is unlikely to significantly affect normal CO_2 excretion of fish, which normally have a blood PCO_2 near 4 mmHg (Ishimatsu *et al.*, 2005).

For the damselfishes from the Lizard Island reef, hypoxia tolerance measured as $[O_2]$crit was strikingly constant over a 4000-fold range in mass (Fig. 2A). Although a small but

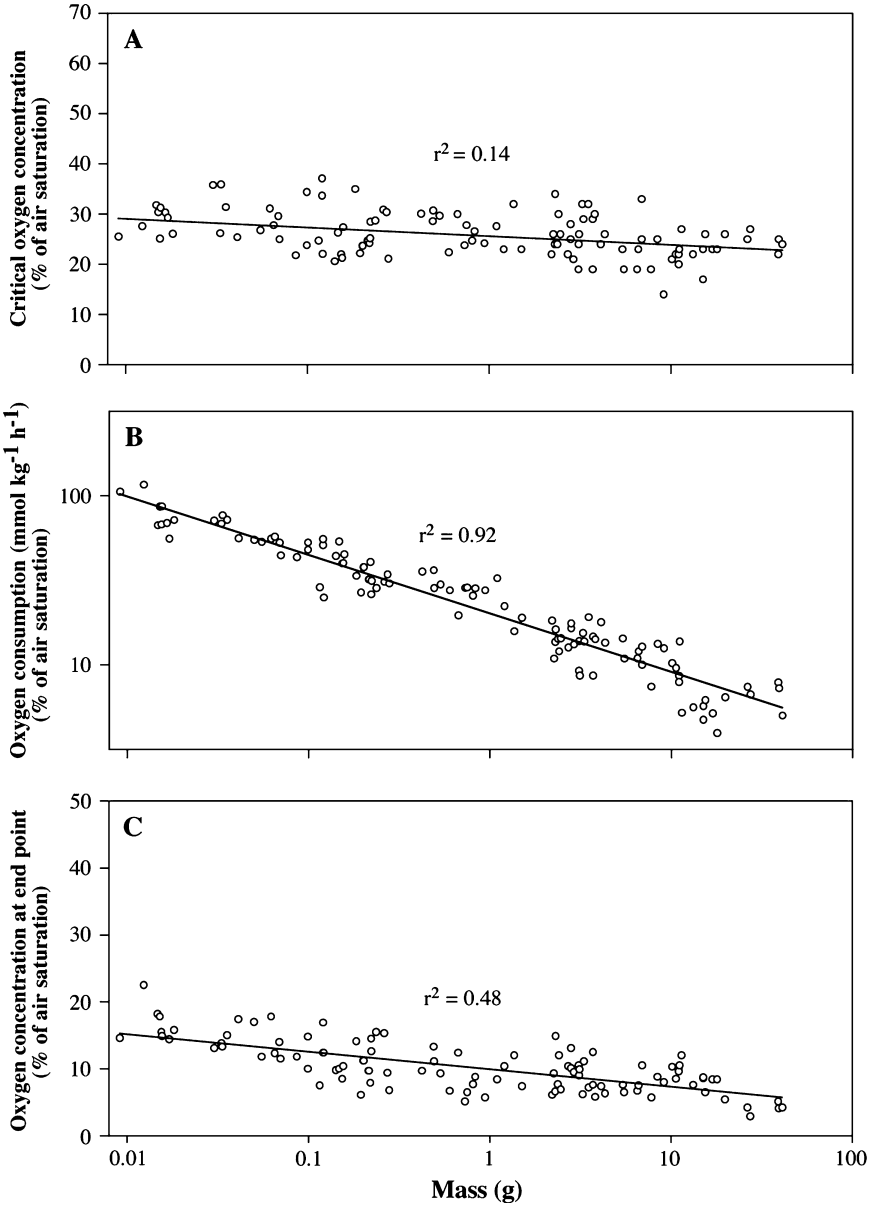

Fig. 2. The scaling of (A) critical oxygen concentration, (B) oxygen consumption, and (C) near lethal oxygen concentration ($[O_2]$out) with mass 117 Great Barrier Reef damselfishes (Pomacentridae) belonging to 15 species. See Table 2 for details and references. The regression lines (all $P < 0.001$) are described by the following equations: (A) $y = 25.6 - 1.73x$; (B) $y = 1.30 - 0.35x$; (C) $y = 9.97 - 2.61x$.

Table 2. Indices for hypoxia tolerance in damselfishes from the lagoon at Lizard Island Research Station, Great Barrier Reef

Species	N	Body mass (g)	Normoxic $\dot{V}O_2$ (mmol kg^{-1} h^{-1})	$[O_2]$crit (% of air saturation)	$[O_2]$out
Acanthochromis polyacanthus	23	0.010–15.4	6.1–116.0	21–37	7–23
Chromis atripectoralis	35	0.033–11.0	7.9–47.8	19–30	7–14
Chromis viridis	6	1.2–4.1	13.2–22.2	21–24	6–10
Chrysiptera flavipinnis	1	2.4	12.0	30	12
Dascyllus aruanus	3	3.1–5.5	8.6–10.9	19	6–10
Dascyllus reticulatus	1	1.4	15.8	32	12
Neoglyphidodon melas	10	2.3–41.0	5.0–7.8	22–27	3–8
Neoglyphidodon nigroris	6	11.4–17.9	3.9–5.7	17–27	8–12
Neopomacentrus azysron	1	3.2	15.4	32	6
Pomacentrus amboinensis	13	0.18–15.0	5.9–67.3	17–35	5–16
Pomacentrus bankanensis	1	7.8	7.4	19	6
Pomacentrus coelestis	6	2.8–11.0	8.6–16.4	14–25	8–11
Pomacentrus lepidogenys	5	2.8–3.8	13.7–19.1	28–34	6–15
Pomacentrus moluccensis	4	2.7–11.1	8.6–14.7	22–29	9–10
Pomacentrus philippinus	2	2.2–6.9	10.0–10.9	26–33	9–10

Normoxic $\dot{V}O_2$ = routine rate of O_2 consumption at a water $[O_2]$ >70% air saturation; $[O_2]$crit = critical $[O_2]$, below this level $\dot{V}O_2$ starts falling and is no longer independent of ambient $[O_2]$; $[O_2]$out = $[O_2]$ at which the fish showed signs of distress or problems with maintaining equilibrium. Taxonomic nomenclature follows Randall *et al.* (1997). Values are from Nilsson & Östlund-Nilsson (2004), Nilsson *et al.* (2007c), and from unpublished measurements made under identical conditions by G. E. Nilsson and S. Östlund-Nilsson. Measurements were made at 28–30 °C.

significant ($P < 0.001$) decrease in $[O_2]$crit with size was indicated for the damselfishes, the shallow slope of the regression line gives predicted mean $[O_2]$crit values below 29% of air saturation over the whole size range (23% in the largest and 29% in the smallest fish). The r^2 value of 0.14 for the regression line indicates that only 14% of the variability in hypoxia tolerance can be ascribed to an effect of body mass. For the damselfishes, the scaling of mass-specific routine rate of oxygen consumption against mass is described by the equation:

$$\dot{V}O_2 \cdot M^{-1} = 20 \cdot M^{-0.35}, \quad (4)$$

i.e., the mass-specific scaling exponent ($b-1$) is -0.35 (the slope of the log-log plot in Fig. 2B), and the scaling exponent (b) is 0.65. Note that this is lower than the mean scaling exponent found in larger data sets of fish species from a wide range of environments (0.79–0.88; see Section IV.1). However, it falls within the range of values (0.59–0.73) that have been determined previously for tropical fish, and have been argued to characterize fish in warm climates (Almeida-Val, Gomes & Lopes, 2006).

We believe that the available data show that size *per se* has a very little effect on the ability for oxygen uptake during hypoxia. The relatively constant $[O_2]$crit values for damselfishes indicate that natural selection equips fish with the oxygen uptake capacities needed to survive in their habitat, in this case a coral reef, virtually irrespective of their body mass.

V. SCALING OF ANAEROBIC SURVIVAL

When water $[O_2]$ continues to fall below $[O_2]$crit, the fish must start to produce ATP through anaerobic glycolysis. The ability to survive on anaerobic ATP production will depend on processes that differ from those determining aerobic capacity. Stores of anaerobic fuel, anaerobic enzyme activities, tolerance to anaerobic end products, and rate of anaerobic ATP use can be expected to determine the capacity for anaerobic survival.

There are few data available on the effect of size on anaerobic survival in fish. A study on the ontogeny of hypoxia tolerance in juvenile red sea bream (*Pagrus major*) (Table 1) showed that the oxygen level causing 50% mortality decreased from approximately 2.3 mg l^{-1} at the flexion stage (9.5 mm body length) to 1.2 mg l^{-1} at a length of 35 mm (Ishibashi *et al.*, 2005). There was a particularly high ability to survive hypoxia at the earliest larval stages (< 5 mm body length), which may be related to an ability of embryonic and larval fish to deeply depress metabolism in response to hypoxia (see Section VI). In a survey of 35 species of headwater stream fishes in Missouri (Smale & Rabeni, 1995), which determined the oxygen level that resulted in cessation of breathing movements (i.e. death), an end-point that should be influenced by anaerobic capacity, no influence of size was seen. However, the 10-fold maximum ranges in body mass examined (Table 1) were probably too small to detect scaling effects against considerable individual variation.

By contrast, our damselfishes, covering a size range of 0.01–40 g, exhibited $[O_2]$out values (likely to be close to the lethal $[O_2]$) that decreased significantly with body mass (Fig. 2C): from approximately 15% of air saturation in the smallest juvenile damselfishes to about 5% in the largest adults. The r^2 of 0.48 for this regression line suggests that nearly 50% of the variation in $[O_2]$out was related to body mass, indicating that size places a significant constraint on the anaerobic abilities of damselfishes.

We start this section by letting the damselfish data illustrate the problem of being small in anoxia, and subsequently arrive at some conclusions that should apply to fish in general.

(1) Lactate load as a limiting factor

Glycolysis is the only major ATP producing pathway used under anaerobic conditions, and yields three ATP molecules per molecule of glucose (including one ATP from glycogen breakdown), much less than the approximately 29 ATP molecules produced from each glucose molecule during aerobic metabolism (Brand, 2003). With few exceptions, fish, like other vertebrates, produce lactate as the main end-product of anaerobic glycolysis. This process leads to the production of equimolar amounts of lactate and H^+ (Hochachka & Somero, 2002). Lactate and H^+ accumulate in the fish (Milligan & Wood, 1986) and total exhaustion occurs at body lactate levels around 12–40 mmol kg^{-1} (Turner, Wood & Höbe, 1983; Milligan & Wood, 1986; DiAngelo & Heath, 1987), when intracellular pH has decreased to around pH 6.8 (Van Waarde *et al.*, 1990), although one species of Amazonian armoured catfish (*Glyptoperichthyes gibbceps*) has been documented to survive 66 mmol lactate kg^{-1} (MacCormack *et al.*, 2003). For simplicity, and because lactate levels are easily measured, we will mainly discuss lethal lactate levels below, although the accompanying equimolar production of H^+ may be more detrimental than lactate *per se*.

Simple calculations reveal that hypoxic survival through lactate producing glycolysis is a much less viable option for small fish with relatively high metabolic rates. The oxidation of 1 mol glucose by 6 mol O_2 results in the production of approximately 29 mol ATP, i.e. 4.8 mol ATP/mol O_2 (Brand, 2003), while the anaerobic production of 1 mol lactate only yields 1.5 ATP (2 mol of lactate produced for every mol of glucose consumed). Consequently, if aerobic ATP production is fully replaced by anaerobic glycolysis (i.e. ATP turnover is maintained), $4.8/1.5 = 3.2$ mol lactate will be produced to equal the consumption of 1 mol of O_2. We can use equation 4 to predict the scaling of mass-specific lactate production ($V_{\mathrm{lactate}} \cdot M^{-1}$) in damselfishes during anaerobic conditions:

$$\begin{aligned} V_{\mathrm{lactate}} \cdot M^{-1} &= 3.2 \cdot VO_2 \cdot M^{-1} \\ &= 3.2 \cdot 20 \cdot M^{-0.35} = 64 \cdot M^{-0.35}. \end{aligned} \quad (5)$$

Thus a large (40 g) damselfish with an oxygen consumption of 6 mmol O_2 kg^{-1} h^{-1}, will have to produce 19 mmol lactate kg^{-1} h^{-1} to compensate fully for aerobic ATP production with anaerobic glycolysis. Consequently, it could maintain ATP production for 1–2 h before reaching a lethal lactate level of 12–40 mmol kg^{-1}. The same calculation for a small damselfish (40 mg) with its 10-fold higher rate of oxygen consumption (60 mmol O_2 kg^{-1} h^{-1}; Fig 2B) shows that it would have to produce lactate at 10 times the rate (190 mmol lactate kg^{-1} h^{-1}) and would therefore reach a lethal lactate level within approximately 5–10 min.

(2) Glycogen depletion as a limiting factor

Whole-body glycogen levels in fish generally vary between 5 and 20 mmol glycosyl units kg^{-1} (wet mass) (Scarabello, Heigenhauser & Wood, 1992; Van den Thillart & Van Raaij, 1995; Wilkie *et al.*, 2001; Begg & Pankhurst, 2004). A body glycogen store that can yield 5–20 mmol glucose kg^{-1} means that the fish can maximally produce 10–40 mmol lactate kg^{-1} since each mol of glucose yields 2 mol lactate. Consequently, a 40 mg damselfish producing 190 mmol lactate kg^{-1} h^{-1} to maintain its normoxic ATP production rate, would run out of glycogen after approximately 3–13 minutes, a similar period to that calculated for lactate poisoning above. A 40 g damselfish producing 19 mmol lactate kg^{-1} h^{-1} would take approximately 30–130 min to run out of glycogen. In fact, a glycogen store of 10 mmol glycosyl units kg^{-1} would theoretically put the same time limit on anaerobic survival as a maximum tolerable lactate level of 20 mmol kg^{-1}, due to the 1:2 relationship between glucose consumed and lactate produced. Like lactate poisoning, glycogen depletion will rapidly terminate anaerobic ATP generation in a small fish, while glycogen stores would last much longer in larger fish.

It makes sence that glycogen stores and maximum tolerable lactate levels are well matched: there is no reason to have glycogen stores that would allow a lactate production that exceeds the tolerable level. This view is supported by some exceptional fish that produce end-products other than lactate, and thus do not experience high lactate levels: these fish have glycogen stores that are several times larger than in other fish (see Section VII).

(3) Scaling equations for anaerobic survival

An equation for anaerobic survival time (T_a, in hours) can be derived as follows:

$$T_a = L_{\mathrm{lactate}} / \left(V_{\mathrm{lactate}} \cdot M^{-1}\right), \quad (6)$$

where L_{lactate} is the lethal lactate level (in mmol kg^{-1}) and $V_{\mathrm{lactate}} \cdot M^{-1}$ is the mass-specific rate of lactate production in anoxia (in mmol kg^{-1} h^{-1}). The latter was given for damselfishes by equation 5, but in its general form it will be:

$$V_{\mathrm{lactate}} \cdot M^{-1} = 3.2 \cdot k \cdot M^{b-1}, \quad (7)$$

where k is the same as in equation 2. Substituting equation 7 into equation 6 gives:

$$\begin{aligned} T_a &= L_{\mathrm{lactate}} / (3.2 \cdot k \cdot M^{b-1}) \\ &= (L_{\mathrm{lactate}} / 3.2 \cdot k) \cdot M^{-(b-1)}. \end{aligned} \quad (8)$$

For damselfishes in the size range 10 mg–100 g we estimate the following values: $k = 20$, $b - 1 = -0.35$, and $L_{\mathrm{lactate}} = 20$ mmol kg^{-1}, giving the relationship:

$$T_a = 0.31 \cdot M^{0.35} \quad (9)$$

shown in Fig. 3. Anaerobic survival times would be different if ATP turnover was reduced by metabolic depression, or if the glycogen store or if lethal lactate levels differ, although the overall shape of the relationship would remain the same.

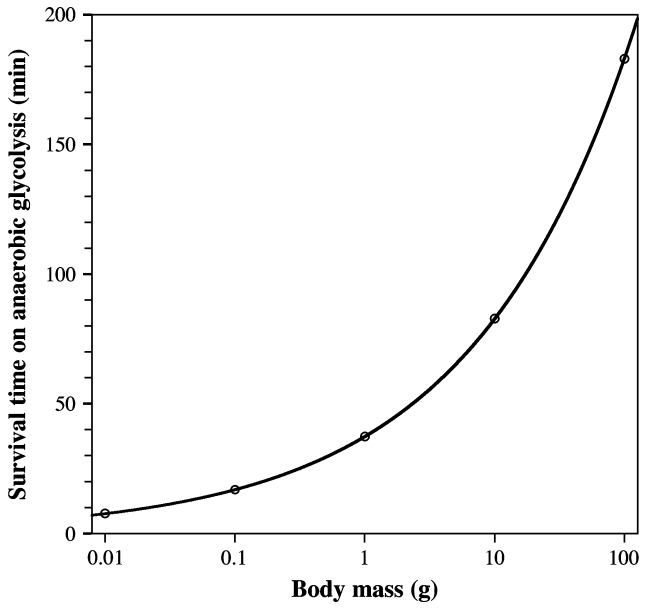

Fig. 3. A semi-log plot illustrating the expected relationship between body mass and survival time on anaerobic glycolysis for damselfishes in the size range 10 mg–100 g. The anoxic survival time (T_a, in h) is described by the equation $T_a = 0.31 \cdot M^{0.35}$. The equation is based on the measured metabolic rate of damselfishes, and on the assumption that this metabolic rate is maintained in anoxia and that either a lactate level of 20 mmol kg^{-1} is lethal or that the glycogen store contains 10 mmol glycosyl units kg^{-1}.

Because mass-specific metabolic rate decreases exponentially with body mass, survival time on anaerobic glycolysis will increase exponentially with body mass. The exponent will have the same value as for the equation describing metabolic rate but due to the inverse relationship of these processes, the exponent will be positive for survival time and negative for mass-specific metabolic rate.

We can expand equation 8 to account for metabolic depression:

$$T_a = m^{-1} \cdot (L_{lactate}/3.2 \cdot k) \cdot M^{-(b-1)}, \qquad (10)$$

where m is the relative metabolic depression (ATP turnover in anoxia / ATP turnover in normoxia). If glycogen depletion rather than lactate poisoning limits anoxic survival, the corresponding equation would be:

$$T_a = m^{-1} \cdot (S_{glycogen}/1.6 \cdot k) \cdot M^{-(b-1)}, \qquad (11)$$

where $S_{glycogen}$ is the glycogen store (in mmol glucose kg^{-1}) and 1.6 is derived from the assumption that one mole glucose from glycogen yields 3 ATP anaerobically while the consumption of one mole O_2 yields 4.8 ATP (i.e. $4.8/3 = 1.6$).

These anaerobic survival time equations will obviously not apply to fish that cannot sustain ATP levels through anaerobic metabolism, and die due to ATP depletion, which will probably first occur in brain. For those species anoxic survival time will be considerably shorter and depend on the metabolic rate of the brain, which is relatively independent of body mass (Mink *et al.*, 1981; Nilsson, 1996), brain PCr stores, rate of glycolytic ATP production, and factors such as neuronal ion permeability and regional differences in brain ATP use. It is likely that many fish experiencing decreasing oxygen levels may initially be able to maintain energy balance through increased glycolytic ATP production, but eventually ATP levels fall when the sum of aerobic and anaerobic ATP production no longer meets ATP demands. For these fish, body size should affect survival time because of the limitations it puts on anaerobic capacity.

In the above analysis we did not consider the heterogeneous distribution of glycogen within the body: the liver glycogen being responsible for glucose supply to many tissues, while that of striated muscle is probably exclusively used for anaerobic muscle activity (Hochachka & Somero, 2002). Similarly, lactate levels are likely to increase faster in organs with the highest ATP turnover, such as the brain. Moreover, one may expect the brain to be more sensitive to lactate and H^+ loads than for example white muscle, which presumably is adapted to the presence of lactate and H^+ produced during burst swimming. Indeed, while salmonids can tolerate muscle lactate levels of 25–40 mmol kg^{-1} during exhaustive exercise (Kieffer, 2000), a brain lactate level of about 12 mmol kg^{-1} is fatal to rainbow trout in anoxic conditions (DiAngelo & Heath, 1987). However, these factors are unlikely to vary with body mass and should therefore not affect our general conclusions.

(4) Scaling of glycolytic enzyme activity

The maximal rate at which an animal can produce ATP anaerobically will depend on the catalytic rates of the enzymes involved. If anaerobic enzyme activities scale with body mass, this could be an additional mechanism by which body mass affects survival in hypoxia and anoxia.

Results show that the activity of glycolytic enzymes such as lactate dehydrogenase (LDH) and pyruvate kinase (PK) are either relatively independent of body mass or, in most cases, increase in specific activity with body mass in fish (Somero & Childress, 1980; Childress & Somero, 1990; Davies & Moyes, 2007) as well as in other vertebrates (Moyes & LeMoine, 2005), whereas both mass-specific rate of aerobic metabolism and the activity of aerobic enzymes decrease with body mass. In muscle and heart, the increase in anaerobic enzyme activity with body mass is suggested to compensate for the increased drag of large fish during anaerobic burst swimming (Somero & Childress, 1980; Childress & Somero, 1990).

The increase in LDH activity with body mass seen in various organs of oscar cichlids has been suggested to contribute to the greater hypoxia tolerance of larger individuals (Almeida-Val *et al.*, 2000), although LDH activities measured in even the smallest oscar cichlids appear to far exceed the requirements of anaerobic ATP production. LDH activity in small (15 g) oscar cichlids is 2600 mmol kg^{-1} h^{-1} in brain and 200 mmol kg^{-1} h^{-1} in liver at 25 °C (Almeida-Val *et al.*, 2000). In vertebrates, including fish, the mass-specific metabolic rate of the brain

is about 4–10 times higher than that of the whole body, while metabolic rate of the fish liver is about twice that of the body (Itazawa & Oikawa, 1986; Johansson *et al.*, 1995; Nilsson, 1996; Gallagher *et al.*, 1998). The normoxic $\dot{V}O_2$ of a 15 g oscar cichlid is 4.5 mmol O_2 kg^{-1} h^{-1} (Sloman *et al.*, 2006), which in terms of ATP synthesis corresponds to an anaerobic production of 14 mmol lactate kg^{-1} h^{-1} (see Section V.1). Thus the anoxic brain and liver would maximally need to produce 140 and 28 mmol lactate kg^{-1} h^{-1}, respectively, to maintain ATP supply at aerobic levels. Clearly, LDH activities in these tissues of small oscar cichlids are at least some 7–20 times higher than needed for maintaining the ATP production in anoxia. Similarly high activities of LDH and PK have been measured in brain tissue of Californian anchovy (*Engraulis mordax*), kelp bass (*Paralabrax clathratus*), and barred sand bass (*P. nebulifer*) (Somero & Childress, 1980), none of which are known to be particularly hypoxia tolerant, as well as in the brain of more hypoxia-tolerant species like short-spine thornyhead (*Sebastolobus alascanus*) and spotted scorpionfish (*Scorpaena guttata*) (Yang & Somero, 1993). Recently, Martínez *et al.* (2006) measured the activity of all glycolytic enzymes, including regulatory or potentially rate-limiting enzymes such as hexokinase and phosphofructokinase, in the brain and liver of the hypoxia-tolerant Gulf killifish (*Fundulus grandis*). Their data revealed high enzyme activities that easily meet requirements for ATP production during anoxia. The same study showed that a four-week hypoxia exposure caused minor and inconsistent changes in glycolytic enzyme activities, indicating that upregulation of activity was not needed. Another study showed that hypoxia only causes minor changes in the transcription of glycolytic enzymes in the hypoxia-tolerant goby (*Gillichthys mirabilis*) (Gracey, Troll & Somero, 2001). Although these fishes appear to have sufficient anaerobic enzyme activities to sustain ATP production under anaerobic conditions, processes like particle binding, phosphorylation and allosteric regulation of glycolytic enzymes probably play a role in stimulating their activity during hypoxia (Storey, 1987; Duncan & Storey, 1991).

To conclude, anoxia sensitive tissues in fish appear to have glycolytic enzyme activities that are more than sufficient for anaerobic needs, even in small fish, and the levels of glycolytic enzymes does not seem to correlate with hypoxia tolerance or to be significantly up-regulated by hypoxia.

VI. METABOLIC DEPRESSION

Survival time at oxygen levels below $[O_2]$crit can be significantly increased if the fish can reduce its rate of ATP use (metabolic depression). Some hypoxia- and anoxia-tolerant fish, including goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), tilapia (*Oreochromis mossambicus*) and the oscar cichlid depress their rate of ATP use in anoxia by 40–70% (Van Waversveld, Addink & Van den Thillart, 1989; Johansson *et al.*, 1995; Van Ginneken *et al.*, 1996; Muusze *et al.*, 1998), while other relatively hypoxia-tolerant species, like the common carp, do not undergo metabolic depression in hypoxia (Van Ginneken *et al.*, 1998). Metabolic depression in fish appears to be induced by increased levels of the inhibitory neurotransmitter gamma-amino butyric acid (GABA) functioning as an endogenous tranquillizer (Nilsson, Lutz & Jackson, 1991; Nilsson, 1992; Van Ginneken *et al.*, 1996; Hylland & Nilsson, 1999), and by production of the inhibitory neuromodulator adenosine (derived from a net break-down of ATP) (Nilsson, 1991; Bernier *et al.*, 1996; Renshaw, Kerrisk & Nilsson, 2002).

We found no evidence for metabolic depression in damselfishes, the largest species in our survey (*Neoglyphidodon melas*) could not cope with oxygen levels lower than $[O_2]$crit for more than about an hour or tolerate an ambient oxygen level lower than about 5% of air saturation, in line with predictions from their aerobic metabolic rates (see Section V).

There are no published data showing that metabolic depression is size dependent at a cellular or physiological level, and it is difficult to see any reason for why it should be directly dependent upon size. However, in light of the scaling of anaerobic survival, one may speculate that small fish species or juveniles may need to resort to metabolic depression to survive hypoxia. For embryonic fish, anoxia may slow down or stop development (Johansen & Krogh, 1914; Rombough, 1988; Kamler, 1992), which could be regarded as deep metabolic depression; embryos of the annual killifish (*Austrofundulus limnaeus*), can enter a state of metabolic arrest that enables them to survive two months of anoxia at 25°C (Podrabsky *et al.*, 2007).

On the other hand, the low capacity for anaerobic metabolism in small individuals could force them to increase their physical activity to escape hypoxia by seeking oxygen-rich areas, a strategy that could be successful if hypoxic habitats are heterogeneously distributed. Indeed, while large oscar cichlids lower their level of activity in hypoxia, thereby reducing their ATP requirements, smaller conspecifics actually increase their activity (Sloman *et al.*, 2006), presumably causing exaggerated size-related differences in hypoxic metabolic rate in this species. This behaviour would lead to a more pronounced effect of size on anoxic survival time. Indeed, Almeida-Val *et al.* (2000) found that survival time in severe hypoxia (20% of air saturation, $[O_2]$crit = 30 – 50%) increased with size in this species, from 5 h in 5 g fish to 50 h in 350 g individuals, a scaling exponent of 0.59. This exponent is higher than the 0.41 predicted if survival time is inversely related to mass-specific metabolic rate in oscar cichlids (Almeida-Val *et al.*, 2006). However, the difference can be explained by an increase in metabolic depression with body mass in this species.

VII. ADAPTATION TO SPECIFIC NICHES

Self-poisoning from build up of lactate and H^+ during severe hypoxia and anoxia is avoided in some fish by the production of alternative anaerobic end-products. The only vertebrates known to be able to do this are three cyprinid fish: the goldfish, the crucian carp and the bitterling (*Rhodeus amarus*) (Shoubridge & Hochachka, 1980; Johnston & Bernard, 1983; Nilsson, 1988; Wissing & Zebe, 1988),

which produce ethanol, which readily passes biological membranes and leaves the fish *via* the gills. The evolution of an alternative metabolic end product has lead to an extraordinary capacity for long-term anoxic survival (Nilsson, 2001, for review): the crucian carp can survive days to months of anoxia, depending on temperature (Blazka, 1958; Piironen & Holopainen, 1986; Nilsson, 1990). To survive wintertime anoxia, it stores enormous amounts of glycogen, approximately 350 mmol glycosyl units kg^{-1} of body. Of these, nearly 2000 mmol kg^{-1} are found in the liver, which can constitute 15% of the body mass (Hyvärinen, Holopainen & Piironen, 1985). During the summer, when anoxia is not encountered, crucian carp liver size decreases to approximately 2% of body mass with a glycogen content of approximately 100 mmol glycosyl units kg^{-1} (Hyvärinen *et al.*, 1985), a value comparable to that of other fish (Moon & Foster, 1995).

Apparently, if an alternative metabolic end product like ethanol is produced, and lactate/H^+ loads no longer set a limit to how much ATP can be produced anaerobically, then it is well worth while to store vast amounts of glycogen. It has been shown that in crucian carp, it is the total depletion of the glycogen store that finally limits its anoxic survival (Nilsson, 1990).

Crucian carp and goldfish also display other features that illustrate that adaptation to a particular niche can be a more powerful determinant of hypoxia tolerance than scaling of respiratory and metabolic variables. They have haemoglobins with the highest known oxygen affinities in fish (P_{50} for O_2 = 0.8 mmHg in crucian carp at 10°C, Sollid, Weber & Nilsson, 2005), and a capacity to remodel the gills to increase the respiratory surface area during hypoxia, resulting in a very low [O_2]crit (5% of air saturation; Sollid *et al.*, 2003; Sollid & Nilsson, 2006).

The relatively constant [O_2]crit values we measured in damselfishes in a coral reef environment again indicates that natural selection result in appropriate oxygen uptake capacities for the habitat, virtually irrespective of body mass. This conclusion is further supported by the recent finding that late-stage pelagic damselfish larvae have much higher [O_2]crit values (40–60% of air saturation) than after settlement (Fig. 4) (Nilsson *et al.*, 2007*c*). Late in the pelagic stage, damselfish larvae probably have no need for hypoxia tolerance, but instead require a respiratory system that is optimized for rapid oxygen delivery to aerobic muscles used for fast sustained swimming. However, within a few days after they settle on the reef, the young damselfishes become hypoxia tolerant, showing a clear decrease in [O_2]crit (Nilsson *et al.*, 2007*c*) and swimming capacity (Bellwood & Fisher, 2001). Their ability to make such a rapid respiratory transition suggests there is strong selection pressure for hypoxia tolerance on coral reefs, illustrating that natural selection rather than size is the primary determinant of the respiratory properties of fish.

VIII. CONCLUSIONS

(1) The scaling of oxygen consumption with body mass and that of gill surface area with body mass both have been suggested to constrain hypoxia tolerance in fish. However, oxygen consumption and gill surface area show virtually identical scaling exponents, suggesting that gill surface area

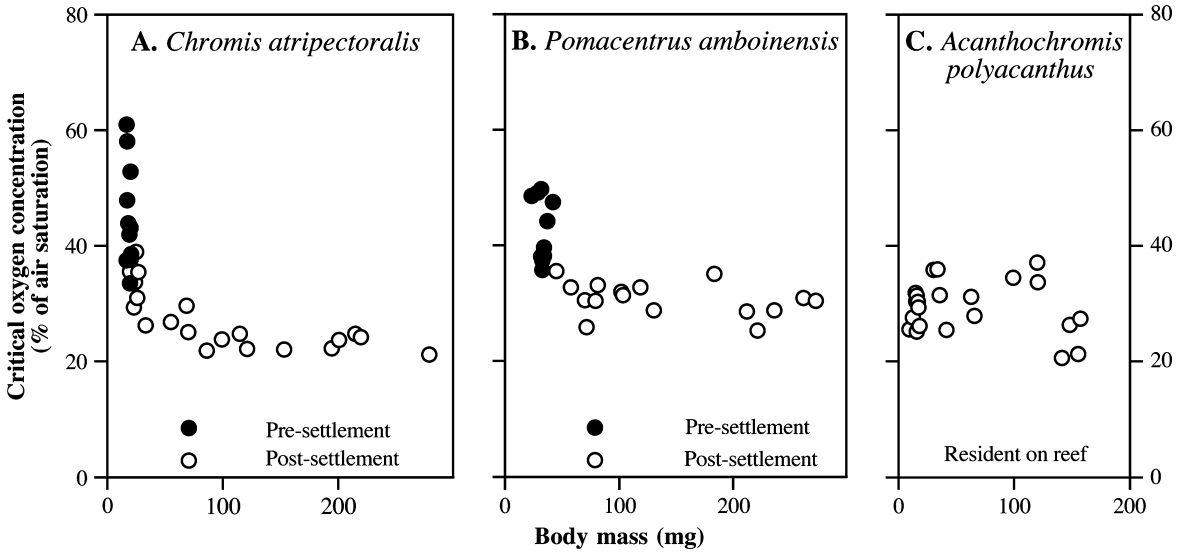

Fig. 4. Relationship between body mass and the critical oxygen concentration ([O_2]crit) of pre-settlement larvae and post-settlement juveniles of three damselfish species: (A) *Chromis atripectoralis* (mass 17 – 280 mg), (B) *Pomacentrus amboinensis* (23 – 280 mg) and (C) *Acanthochromis polyacanthus* (9 – 157 mg). The latter species lacks a planktonic larval stage, living its whole life on the reef. Note that at the pre-settlement stage, the larvae of *C. atripectoralis* and *P. amboinensis* have a higher [O_2]crit that decreases rapidly when they settle on the reef, where they need to be hypoxia tolerant to survive. Since they were caught near the reef, the transition to a life on the reef may already have started in some individuals in the pre-settlement group (explaining the large variability in [O_2]crit in this group). Data from Nilsson *et al.* (2007*c*).

is matched with the requirement for oxygen uptake over a large body size range, and that the ability to take up oxygen during hypoxia is independent of size. This conclusion is supported by data on $[O_2]$crit values from juvenile and adult damselfishes, ranging in size from 10 mg to 40 g, from the coral reef at Lizard Island: for these damselfishes, size *per se* has a negligible influence on oxygen uptake capacity in hypoxia. If $[O_2]$crit is seen to change with size of a species, this is likely to reflect adaptation to changes in life-style or habitat.

(2) The ability to tolerate oxygen levels below $[O_2]$crit, where fish have to rely on anaerobic glycolysis, shows a strong positive correlation with body mass in damselfishes, indicating that their capacity for to meet ATP demands through anaerobic ATP production increases with body size. The relatively high metabolic rate of a small fish cannot be sustained through anaerobic metabolism for more than a few minutes due to the build up of lactate and H^+ and/or the depletion of glycogen. Anaerobic metabolism is therefore of limited value for hypoxic survival in small fish. Exceptions are provided by some embryonic fish that can reduce ATP consumption during anoxia, and some cyprinids that produce ethanol as a non-accumulating anaerobic end-product.

(3) Natural selection has resulted in haemoglobins with high oxygen affinities, increased respiratory surface areas, and alternative anaerobic end products in some hypoxia-tolerant species. These adaptations can be more powerful determinants of hypoxia tolerance than scaling of various physiological variables.

IX. ACKNOWLEDGEMENTS

We thank the Research Council of Norway for financial support.

X. REFERENCES

Almeida-Val, V. M. F., Gomes, A. R. C. & Lopes, N. P. (2006). Metabolic and physiological adjustments to low oxygen and high temperature in fishes of the Amazon. In *Fish Physiology Vol. 21. The Physiology of Tropical Fishes* (eds. A. L. Val, V. M. F. Almeida-Val and D. J. Randall), pp. 443–500. Elsevier/ Academic Press, London.

Almeida-Val, V. M. F., Val, A. L., Duncan, W. P., Souza, F. C. A., Paula-Silva, M. N. & Land, S. (2000). Scaling effects of hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes: Cichlidae): Contribution of tissue enzyme levels. *Comparative Biochemistry and Physiology* **125B**, 219–226.

Axelsson, M., Altimiras, J. & Claireaux, G. (2002). Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. *Journal of Experimental Biology* **205**, 2891–2896.

Begg, K. & Pankhurst, N. W. (2004). Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus*. *Journal of Fish Biology* **64**, 133–145.

Bellwood, D. R. & Fisher, R. (2001). Relative swimming speeds in reef fish larvae. *Marine Ecology Progress Series* **211**, 299–303.

Bernier, N. J., Harris, J., Lessard, J. & Randall, D. J. (1996). Adenosine receptor blockade and hypoxia-tolerance in rainbow trout and Pacific hagfish. 1. Effects on anaerobic metabolism. *Journal of Experimental Biology* **199**, 485–495.

Bishai, H. M. (1960). The effect of gas content of water on larval and young fish. *Zeitschrift für Wissenschaftliche Zoologie* **163**, 37–64.

Blazka P. (1958). The anaerobic metabolism of fish. *Physiological Zoology* **31**, 117–128.

Botero, J. I. S. (2000). Distribuição espatial da lctiofauna associada às raizes de macrófitas em relação ao oxigênio dissolvido, temperatura e tipo de planta na Amazônia Central, pp. 47. MSc thesis, Instituto Nacional de Pesquisas da Amazonia/Universidade Federal do Amazonas.

Brand, M. (2003). Approximate yield of ATP from glucose, designed by Donald Nicholson. *Biochemistry and Molecular Biology Education* **31**, 2–4.

Burleson, M. L., Wilhelm, D. R. & Smatresk, N. J. (2001). The influence of fish size on the avoidance of hypoxia and oxygen selection by largemouth bass. *Journal of Fish Biology* **59**, 1336–1349.

Bushnell, P. G. & Brill, R. W. (1992). Oxygen-transport and cardiovascular-responses in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*). *Journal of Comparative Physiology B* **162**, 131–143.

Cerra, M. C., Imbrogno, S., Amelio, D., Garofalo, F., Colvee, E., Tota, B. & Icardo, J. M. (2004). Cardiac morphodynamic remodelling in the growing eel (*Anguilla anguilla* L.). *Journal of Experimental Biology* **207**, 2867–2875.

Chen, C. T., Liu, K. W. & Young, S. J. (1999). Preliminary report on Taiwan's whale shark fishery. In *Elasmobranch biodiversity, conservation and management* (eds. S. L. Fowler, T. Reid, and F. A. Dipper). Proc. Int. Seminar and Workshop in Sabah, Malaysia, pp. 162–167. IUCN, Gland, Switzerland.

Childress, J. J. & Somero, G. N. (1990). Metabolic scaling: a new perspective based on scaling of glycolytic enzyme activities. *American Zoologist* **30**, 161–173.

Clarke, A. & Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**, 893–905.

Crile, G. & Quiring, D. P. (1940). A record of the body weight and certain organ and gland weights of 3690 animals. *Ohio Journla of Science* **40**, 219–259.

Da Costa, O. T. F., Ferreira, D. J. D., Mendonca, F. L. & Fernandes, M. N. (2004). Susceptibility of the Amazonian fish, *Colossoma macropomum* (Serrasalminae), to short-term exposure to nitrite. *Aquaculture* **232**, 627–636.

Davies, R. & Moyes, C. D. (2007). Allometric scaling in centrarchid fish: origins of intra- and inter-specific variation in oxidative and glycolytic enzyme levels in muscle. *Journal of Experimental Biology* **210**, 3798–3804.

Darveau, C. A., Suarez, R. K., Andrews, R. D. & Hochachka, P. W. (2002). Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166–170.

DeBoeck, G., Nilsson, G. E., Elofsson, U., Vlaeminck, A. & Blust, R. (1995). Brain monoamine levels and energy status in common carp after exposure to sublethal levels of copper. *Aquatic Toxicology* **33**, 265–277.

DeJager, S. & Dekkers, W. J. (1975). Relation between gill structure and activity in fish. *Netherlands Journal of Zoology* **25**, 276–308.

DiAngelo, C. R. & Heath, A. G. (1987). Comparison of in vivo energy metabolism in the brain of rainbow trout, *Salmo gairdneri*,

and bullhead catfish, *Ictalurus nebulosus*, during anoxia. *Comparative Physiology and Biochemistry* **88B**, 297–303.

Doudoroff, P. & Shumway, D. L. (1970). Dissolved oxygen requirements of freshwater fishes. *FAO Technical Paper* 86.

Duncan, J. A. & Storey, K. B. (1991). Role of enzyme binding in muscle metabolism of the goldfish. *Canadian Journal of Zoology* **69**, 1571–1576.

Farrell, A. P., Hammons, A. M., Graham, M. S. & Tibbits, G. (1988). Cardiac growth in rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* **66**, 2368–2373.

Franklin, C. E. & Davie, P. S. (1992). Sexual maturity can double heart mass and cardiac power output in male rainbow trout. *Journal of Experimental Biology* **171**, 139–148.

Frimodt, C. (1995). *Multilingual illustrated guide to the world's commercial coldwater fish*. Fishing News Books, Osney Mead, Oxford.

Gallagher, D., Belmonte, D., Deurenberg, P., Wang, Z., Krasnow, N., Pi-Sunyer, F. X. & Heymsfield, S. B. (1998). Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. *American Journal of Physiology – Endocrinology and Metabolism* **275**, E249–E258.

Gamperl, A. K., Pinder, A. W. & Grant, R. R. (1994). Influence of hypoxia and adrenaline administration on coronary blood-flow and cardiac performance in seawater rainbow-trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **193**, 209–232.

Goolish, E. M. (1995). The metabolic consequences of body size. In *Biochemistry and Molecular Biology of Fishes, vol. 4, Metabolic Biochemistry* (eds. P. W. Hochachka and T. P. Mommsen), pp. 335–366. Elsevier, Amsterdam.

Gracey, A. Y., Troll, J. V. & Somero, G. N. (2001). Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis*. *Proceedings of the National Academy of Scienes USA* **98**, 1993–1998.

Hansen, A. J. (1985). Effect of anoxia on ion distribution in the brain. *Physiological Reviews* **65**, 101–148.

Harikrishnan, R., Rani, M. N. & Balasundaram, C. (2003). Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture* **221**, 41–50.

Hochachka, P. W. & Somero, G. N. (2002). *Biochemical Adaptation*. Oxford University Press, New York.

Hylland, P. & Nilsson, G. E. (1999). Extracellular levels of amino acid neurotransmitters during anoxia and forced energy deficiency in crucian carp brain. *Brain Research* **823**, 49–58.

Hylland, P., Nilsson, G. E. & Johansson, D. (1995). Anoxic brain failure in an ectothermic vertebrate: release of amino acids and K+ in rainbow trout thalamus. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **269**, R1077–R1084.

Hyvärinen H., Holopainen I. J. & Piironen, J. (1985). Anaerobic wintering of crucian carp (*Carassius carassius* L.) – I. Annual dynamics of glycogen reserves in nature. *Comparative Biochemistry and Physiology* **82A**, 797–803.

Ishibashi, Y., Ekawa, H., Hirata, H. & Kumai, H. (2002). Stress response and energy metabolism in various tissues of Nile tilapia *Oreochromis niloticus* exposed to hypoxic conditions. *Fisheries Science* **68**, 1374–1383.

Ishibashi, Y., Inoue, K., Nakatsukasa, H., Ishitani, Y., Miyashita, S. & Murata, O. (2005). Ontogeny of tolerance to hypoxia and oxygen consumption of larval and juvenile red sea bream, *Pagrus major*. *Aquaculture* **244**, 331–340.

Ishimatsu, A., Hayashi, M., Lee, K. S., Kikkawa, T. & Kita, J. (2005). Physiological effects on fishes in a high-CO_2 world. Journal of Geophysical Research Oceans, **110**, C09S09

Itazawa, Y. & Oikawa, S. (1986). A quantitative interpretation of the metabolism-size relationship in animals. *Experientia* **42**, 152–153.

Jensen, F. B., Fago, A. & Weber, R. E. (1998). Hemoglobin structure and functions. In *Fish Physiology Vol. 17, Fish Respiration*. (eds. S. F. Perry and B. L. Tufts), pp. 1–40. Academic Press, San Diego.

Johansen, A. C. & Krogh, A. (1914). The influence of temperature and certain other factors upon the rate of development of the eggs of fishes. *Publication Circonstantial du Conseil Permenent International pour L'Exploration du Mer* **68**, 1–44.

Johansson, D., Nilsson, G. E. & Törnblom, E. (1995). Effects of anoxia on energy metabolism in crucian carp brain slices studied with microcalorimetry. *Journal of Experimental Biology* **198**, 853–859.

Johnston, I. A. & Bernard, L. M. (1983). Utilization of the ethanol pathway in carp following exposure to anoxia. *Journal of Experimental Biology* **104**, 73–78.

Junk, W. J., Soares, G. M. & Carvalho, F. M. (1983). Distribution of fish species in a lake of the Amazon river floodplain near Manaus (Lago Camaleão), with special reference to extreme oxygen conditions. *Amazoniana* **7**, 397–431.

Kalle, K. (1972). Dissolved gasses. In *Marine Ecology*, Vol. 1, part 3, *Environmental Factors* (ed. O. Kinne), pp. 1452–1457. Wiley-Interscience, London.

Kamler, E. (1992). *Early Life History of Fishes, An Energetics Approach*. Chapman & Hall, London.

Kieffer, J. D. (2000). Limits to exhaustive exercise in fish. *Comparative Biochemistry and Physiology* **126A**, 161–179.

Kisia, S. M. & Hughes, G. M. (1992). Estimation of oxygen-diffusing capacity in the gills of different sizes of a tilapia, *Oreochromis niloticus*. *Journal of Zoology (London)* **227**, 405–415.

Kooijman, S. A. L. M. (1993). *Dynamic Energy Budgets in Biological Systems*. Cambridge University Press, Cambridge.

Kottelat, M., Britz, R., Hui, T. H. & Witte, K.-E. (2006). *Paedocypris*, a new genus of Southeast Asian cyprinid fish with a remarkable sexual dimorphism, comprises the world's smallest vertebrate. *Proceedings of the Royal Society Series B* **273**, 895–899.

Lahiri, S. (1975). Blood oxygen affinity and alveolar ventilation in relation in body weight in mammals. *American Journal of Physiology* **229**, 529–536.

Lappivaara, J. & Marttinen, S. (2005). Effects of waterborne iron overload and simulated winter conditions on acute physiological stress response of whitefish, *Coregonus lavaretus*. *Ecotoxicology and Environmental Safety* **60**, 157–168.

Linton, E. D., Scuton, D. A. & McKinley, R. S. (2005). Physiological effects of thermomechanical newsprint mill effluent on Atlantic salmon (*Salmo salar* L.). *Ecotoxicology and Environmental Safety* **62**, 317–330.

Lipton, P. (1999). Ischemic cell death in brain neurons. *Physiological Reviews* **79**, 1431–1568.

Lutz, P. L., Longmuir, I. S. & Schmidt-Nielsen, K. (1974). Oxygen affinity of bird blood. *Respiration Physiology* **20**, 325–330.

Lutz, P. L., Nilsson, G. E. & Prentice, H. (2003). *The Brain Without Oxygen*. 3rd Ed. Kluwer Academic Publishers/Springer, Dordrecht.

MacCormack, T. J., McKinley, R. S., Roubach, R., Almeida-Val, V. M. F., Val, A. L. & Driedzic, W. R. (2003). Changes in ventilation, metabolism, and behaviour, but not bradycardia, contribute to hypoxia survival in two species of Amazonian armoured catfish. *Canadian Journal of Zoology* **81**, 272–280.

Martínez, M. L., Landry, C., Boehm, R., Manning, S., Cheek, A. O. & Rees, B. B. (2006). Effects of long-term hypoxia on

enzymes of carbohydrate metabolism in the Gulf killifish, *Fundulus grandis*. *Journal of Experimental Biology* **209**, 3851–3861.

Milligan, C. L. & Wood, C. M. (1986). Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* **123**, 93–121.

Mink, J. W., Blumenschine, R. J. & Adams, D. B. (1981). Ratio of central nervous system activity to body metabolism in vertebrates: its constancy and functional basis. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **241**, R203–R212.

Moon, T. W. & Foster, G. D. (1995). Tissue carbohydrate metabolism, gluconeogenesis and hormonal environmental influences. In *Biochemistry and Molecular Biology of Fishes, vol. 4, Metabolic Biochemistry* (eds. P. W. Hochachka and T. P. Mommsen), pp. 65–100. Elsevier, Amsterdam.

Moyes, C. D. & LeMoine, C. M. R. (2005). Control of muscle bioenergetic gene expression: implications for allometric scaling relationships of glycolytic and oxidative enzymes. *Journal of Experimental Biology* **208**, 1601–1610.

Muir, B. S. (1969). Gill dimensions as a function of fish size. *Journal of the Fisheries Research Board of Canada* **26**, 165–170.

Murad, A., Houston, A. H. & Samson, L. (1990). Haematological response to reduced oxygen-carrying capacity, increased temperature and hypoxia in goldfish. Journal of Fish Biology **36**, 289–305.

Muusze, B., Marcon, J., Van den Thillart, G. & Almeida-Val, V. (1998). Hypoxia tolerance of Amazon fish, respirometry and energy metabolism of the cichlid *Astronotus ocellatus*. *Comparative Biochemistry and Physiology* **120A**, 151–156.

Nilsson, G. E. (1988). A comparative study of aldehyde dehydrogenase and alcohol dehydrogenase activity in crucian carp and three other vertebrates: apparent adaptations to ethanol production. *Journal of Comparative Physiology* **158B**, 479–485.

Nilsson, G. E. (1990). Long term anoxia in crucian carp: changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. *Journal of Experimental Biology* **150**, 295–320.

Nilsson, G. E. (1991). The adenosine receptor blocker aminophylline increases anoxic ethanol production in crucian carp. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiolog* **261**, R1057–R1060.

Nilsson, G. E. (1992). Evidence for a role of GABA in metabolic depression during anoxia in crucian carp (*Carassius carassius* L.). *Journal of Experimental Biology* **164**, 243–259.

Nilsson, G. E. (1996). Brain and body oxygen requirements of *Gnathonemus petersii*, a fish with an exceptionally large brain. *Journal of Experimental Biology* **199**, 603–607.

Nilsson, G. E. (2001). Surviving anoxia with the brain turned on. *News in Physiological Sciences* **16**, 218–221.

Nilsson, G. E. (2007). Gill remodeling in fish – a new fashion or an ancient secret? *Journal of Experimental Biology* **210**, 2403–2409.

Nilsson, G. E., Hobbs, J.-P., Munday, P. L. & Östlund-Nilsson, S. (2004). Coward or braveheart: extreme habitat fidelity through hypoxia tolerance in a coral-dwelling goby. *Journal of Experimental Biology* **207**, 33–39.

Nilsson, G. E, Hobbs, J.-P. & Östlund-Nilsson, S. (2007*a*). Tribute to P.L. Lutz: Respiratory ecophysiology of coral-reef teleosts. *Journal of Experimental Biology* **210**, 1673–1686.

Nilsson, G. E, Hobbs, J.-P., Östlund-Nilsson, S., & Munday, P. L. (2007*b*). Hypoxia tolerance and air breathing ability correlate with habitat preference in coral-dwelling fishes. *Coral Reefs* **26**, 241–248.

Nilsson, G. E., Lutz, P. L., & Jackson, T. L. (1991). Neurotransmitters and anoxic survival of the brain: a comparison between anoxia-tolerant and anoxia-intolerant vertebrates. *Physiological Zoology* **64**, 638–652.

Nilsson, G. E. and Östlund-Nilsson, S. (2004). Hypoxia in paradise: widespread hypoxia tolerance in coral reef fishes. *Proceedings of the Royal Society Series B (Biology Letters Supplement)* **271**, S30–S33.

Nilsson, G. E., Östlund-Nilsson, S., Penfold, R. & Grutter, A. S. (2007*c*). From record performance to hypoxia tolerance – respiratory transition in damselfish larvae settling on a coral reef. *Proceedings of the Royal Society Series B* **274**, 79–85.

Nilsson, G. E., Pérez-Pinzón, M., Dimberg, K. & Winberg, S. (1993). Brain sensitivity to anoxia in fish as reflected by changes in extracellular potassium-ion activity. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **264**, R250–R253.

Nilsson, G. E. & Renshaw, G. M. C. (2004). Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. *Journal of Experimental Biology* **207**, 3131–3139.

Novák, J. (1982). Comparison of two different methods of growth values computation illustrated on rudd scales (Pisces: Cyprinidae). *Vestník Ceskoslovenské Spolecnosti Zoologické* **46**, 25–32.

Oikawa, S. & Itazawa, Y. (1985). Gill and body surface areas of the carp in relation to body mass, with special reference to the metabolism-size relationship. *Journal of Experimental Biology* **117**, 1–14.

Östlund-Nilsson, S. & Nilsson, G. E. (2004). Breathing with a mouth full of eggs: respiratory consequences of mouthbrooding in cardinalfishes. *Proceedings of the Royal Society Series B* **271**, 1015–1022.

Palzenberger, M. & Pohla, H. (1992). Gill surface area of water-breathing freshwater fish. *Reviews in Fish Biology and Fisheries* **2**, 187–216.

Piironen, J. & Holopainen, J. (1986). A note on seasonality in anoxia tolerance of crucian carp (*Carassius carassius* (L.)) in the laboratory. *Annales Zoologici Fennici* **23**, 335–338.

Podrabsky, J. E., Lopez, J. P., Fan, T. W. M., Higashi, R. & Somero, G. N. (2007). Extreme anoxia tolerance in embryos of the annual killifish *Austrofundulus limnaeus*: insights from a metabolomics analysis. *Journal of Experimental Biology* **210**, 2253–2266.

Pough, F. H. (1977*a*). The relationship between body size and blood oxygen affinity in snakes. *Physiological Zoology* **50**, 77–87.

Pough, F. H. (1977*b*). The relationship of blood oxygen affinity to body size in lizards. *Comparative Biochemistry and Physiology* **57A**, 435–441.

Privolnev, T. I. (1947). Critical oxygen tension in water for young salmon (Salmo salar) of different ages (in Russian). *Doklady Akademii nauk SSSR* **58**, 1179–1181.

Prosser, C. L. & Brown, F. A. (1961). *Comparative Animal Physiology*. W. B. Saunders Co., Philadelphia.

Randall, J. E., Allen, G. R. & Steene, R. C. (1997). *Fishes of the Great Barrier Reef and Coral Sea*. 2nd ed. Crawford House Press, Bathurst.

Rehulka, J., Minarik, B. & Rehulkova, E. (2004). Red blood cell indices of rainbow trout *Oncorhynchus mykiss* (Walbaum) in aquaculture. *Aquaculture Research* **35**, 529–546.

Renshaw, G. M. C., Kerrisk, C. B. & Nilsson, G. E. (2002). The role of adenosine in the anoxic survival of the epaulette shark, *Hemiscyllium ocellatum*. *Comparative Biochemistry and Physiology B – Biochemistry and Molecular Biology* **131**, 133–141.

Robb, T. & Abrahams, M. V. (2003). Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? *Journal of Fish Biology* **62**, 1067–1081.

Rombough, P. J. (1988). Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead trout (*Salmo gairdneri*). *Canadian Journal of Zoology* **66**, 651–660.

Rombough, P. J. & Moroz, B. M. (1997). The scaling and potential importance of cutaneous and branchial surfaces in respiratory gas exchange in larval and juvenile walleye *Stizostedion vitreum*. *Journal of Experimental Biology* **200**, 2459–2468.

Rubner, M. (1883). Über den Einfluss der Körpergrösse auf Stoff und Kraftwechsel. *Zeitschrift für Biologie* **19**, 535–562.

Scarabello, M., Heigenhauser, G. J. & Wood, C. M. (1992). Gas exchange, metabolite status and excess post-exercise oxygen consumption after repetitive bouts of exhaustive exercise in juvenile rainbow trout. *Journal of Experimental Biology* **167**, 155–169.

Schmidt-Nielsen, K. (1997). *Animal Physiology*. 5th ed. Cambridge University Press, Cambridge.

Schmidt-Nielsen, K. & Larimer, J. L. (1958). Oxygen dissociation curves of mammalian blood in relation to body size. *American Journal of Physiology* **195**, 424–428.

Schultz, I. R., Barron, M. G., Newman, M. C. & Vick, A. M. (1999). Blood flow distribution and tissue allometry in channel catfish. *Journal of Fish Biology* **54**, 1275–1286.

Shah, S. L. (2006). Hematological parameters in tench *Tinca tinca* after short term exposure to lead. *Journal of Applied Toxicology* **26**, 223–228.

Shoubridge, E. A. & Hochachka, P. W. (1980). Ethanol: novel endproduct in vertebrate anaerobic metabolism. *Science* **209**, 308–309.

Silkin, Y. A. & Silkina, E. N. (2005). Effect of hypoxia on physiological-biochemical blood parameters in some marine fish. *Journal of Evolutionary Biochemistry and Physiology* **41**, 527–532.

Sloman, K. A., Wood, C. M., Scott, G. R., Wood, S., Kajimura, M., Johannsson, O. E., Almeida-Val, V. M. F. & Val, A. L. (2006). Tribute to R. G. Boutilier: The effect of size on the physiological and behavioural responses of oscar, *Astronotus ocellatus*, to hypoxia. *Journal of Experimental Biology* **209**, 1197–1205.

Smale, M. A. & Rabeni, C. F. (1995). Hypoxia and hyperthermia tolerances of headwater stream fishes. *Transactions of the American Fisheries Society* **124**, 698–710.

Sollid, J., De Angelis, P., Gundersen, K. & Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross-morphological changes in crucian carp gills. *Journal of Experimental Biology* **206**, 3667–3673.

Sollid, J. & Nilsson, G. E. (2006). Plasticity of respiratory structures – adaptive remodeling of fish gills induced by ambient oxygen and temperature. *Respiratory Physiology and Neurobiology* **154**, 241–251.

Sollid, J., Weber, R. E. & Nilsson, G. E. (2005). Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *Journal of Experimental Biology* **208**, 1109–1116.

Somero, G. N. & Childress, J. J. (1980). A violation of the metabolism-size scaling paradigm: Activities of glycolytic enzymes in muscle increase in larger size fishes. *Physiological Zoology* **53**, 322–337.

Stecyk, J. A. W., Stensløkken, K.-O., Nilsson, G. E. & Farrell, A. P. (2007). Adenosine does not save the hearts of anoxia-tolerant vertebrates during prolonged oxygen deprivation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **147**, 961–973.

Steen, J. B. (1971). *Comparative Physiology of Respiratory Mechanisms*. Academic Press, London.

Storey, K. B. (1987). Tissue-specific controls on carbohydrate catabolism during anoxia in goldfish, *Physiological Zoology* **60**, 601–607.

Subhadra, B., Lochmann, R., Rawles, S. & Chen, R. (2006). Effect of fish-meal replacement with poultry by-product meal on the growth, tissue composition and hematological parameters of largemouth bass (*Micropterus salmoides*) fed diets containing different lipids. *Aquaculture* **260**, 221–231.

Truchot, J.-P. & Duhamel-Jouve, A. (1980). Oxygen and carbon dioxide in the marine environment: Diurnal and tidal changes in rockpools. *Respiration Physiology* **39**, 241–254.

Turner, J. D., Wood, C. M. & Höbe, H. (1983). Physiological consequences of severe exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*): a comparison with the active pelagic rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **104**, 269–288.

Ultsch, G. R., Jackson, D. C. & Moalli, R. (1981). Metabolic oxygen conformity among lower vertebrates: The toadfish revisited. *Journal of Comparative Physiology B* **142**, 439–443.

Val, A. L., Almeida-Val, V. M. F. & Randall, D. J. (2006). *Fish Physiology Vol. 21. The Physiology of Tropical Fishes*. Elsevier/Academic Press, London.

Van den Thillart, G. & Van Raaij, M. (1995). Endogenous fuels; non-invasive versus invasive approaches. In *Biochemistry and Molecular Biology of Fishes, vol. 4, Metabolic Biochemistry* (eds. P. W. Hochachka and T. P. Mommsen), pp. 33–63. Elsevier, Amsterdam.

Van Ginneken, V., Nieveen, M., VanEersel, R., Van den Thillart, G. & Addink, A. (1996). Neurotransmitter levels and energy status in brain of fish species with and without the survival strategy of metabolic depression. *Comparative Biochemistry and Physiology* **114A**, 189–196.

Van Ginneken, V. J. T., Van Caubergh, P., Nieveen, M., Balm, P., Van den Thillart, G. & Addink, A. (1998). Influence of hypoxia exposure on the energy metabolism of common carp (*Cyprinus carpio* L.). *Netherland Journal of Zoology* **48**, 65–82.

Van Raaij, M. T. M., Bakker, E., Nieveen, M. C., Zirkzee, H. & Van den Thillart, G. E. E. J. M. (1994). Energy status and free fatty acid patterns in tissues of common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* L.) during severe oxygen restriction. *Comparative Physiology and Biochemistry* **109A**, 755–767.

Van Waarde, A., Van den Thillart, G., Erkelens, C., Addink, A. & Lugtenburg, J. (1990). Functional coupling of glycolysis and phosphocreatine utilization in anoxic fish muscle. *Journal of Biological Chemistry* **265**, 914–923.

Van Waversveld, J., Addink, A. D. F. & Van den Thillart, G. (1989). Simultaneous direct and indirect calorimetry on normoxic and anoxic goldfish. *Journal of Experimental Biology* **142**, 325–335.

Verheyen, R., Blust, R. & Decleir, W. (1994). Metabolic rate, hypoxia tolerance and aquatic surface respiration of some lacustrine and riverine African cichlid fishes. *Comparative Biochemistry and Physiology* **107A**, 403–411.

Vornanen, M. & Paajanen, V. (2006). Seasonal changes in glycogen content and Na+-K+-ATPase activity in the brain of crucian carp. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **291**, R1482–R1489.

Watson, W. & Walker Jr, J. J. (2004). The world's smallest vertebrate, *Schindleria brevipinguis*, a new paedomorphic species in

the family Schindleriidae (Perciformes: Gobioidei). *Records of the Australian Museum* **56**, 139–142.

West, G. B., Brown, J. H. & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126.

White, C. R., Phillips, N. F. & Seymour, R. S. (2006). The scaling and temperature dependence of vertebrate metabolism. *Biology Letters* **2**, 125–127.

Wilkie, M. P., Bradshaw, P. G., Joanis, V., Claude, J. F. & Swindell, S. L. (2001). Rapid metabolic recovery following vigorous exercise in burrow-dwelling sea lampreys (*Petromyzon marinus*). *Physiological and Biochemical Zoology* **74**, 261–272.

Winberg, G. G. (1960). Rate of metabolism and food requirements of fishes. *Fisheries Research Board of Canada Translation Series* **194**, 1–202.

Wissing, J. & Zebe, E. (1988). The anaerobic metabolism of the bitterling Rhodeus amarus (Cyprinidae, Teleostei). *Comparative Biochemistry and Physiology* **89B**, 299–303.

Wood, C. M. & Shelton, G. (1980). The reflex control of heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage, and systemic vasomotor tone. *Journal of Experimental Biology* **87**, 271–284.

Wood, S. C., Weber, R. E. & Powers, D. A. (1979). Respiratory properties of blood and hemoglobin solutions from the piranha. *Comparative Biochemistry and Physiology* **62A**, 163–167.

Yang, T. H. & Somero, G. N. (1993). Effects of feeding and food deprivation on oxygen consumption, muscle protein concentration and activities of energy metabolism enzymes in muscle and brain of shallow-living (*Scorpaena guttata*) and deep-living (*Sebastolobus alascanus*) scorpaenid fishes. *Journal of Experimental Biology* **181**, 213–232.

Zhoua, Q.-C., Wua, Z.-H., Tanb, B.-P., Chia, S.-Y. & Yanga, Q.-H. (2006). Optimal dietary methionine requirement for Juvenile Cobia (*Rachycentron canadum*). *Aquaculture* **258**, 551–557.