

## Tribute to R. G. Boutilier: The effect of size on the physiological and behavioural responses of oscar, *Astronotus ocellatus*, to hypoxia

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### Summary

The physiological and behavioural responses of two size groups of oscar (*Astronotus ocellatus*) to hypoxia were studied. The physiological responses were tested by measuring  $\dot{M}_{O_2}$  during decreasing environmental oxygen tensions. Larger oscars were better able to maintain oxygen consumption during a decrease in  $P_{O_2}$ , regulating routine  $\dot{M}_{O_2}$  to a significantly lower  $P_{O_2}$  threshold (50 mmHg) than smaller oscars (70 mmHg). Previous studies have also demonstrated a longer survival time of large oscars exposed to extreme hypoxia, coupled with a greater anaerobic enzymatic capability. Large oscars began aquatic surface respiration (ASR) at the oxygen tension at which the first significant decrease in  $\dot{M}_{O_2}$  was seen (50 mmHg). Interestingly, smaller oscars postponed ASR to around 22 mmHg, well beyond the  $P_{O_2}$  at which they switched from oxyregulation to oxyconformation.

Additionally, when given the choice between an hypoxic environment containing aquatic macrophyte shelter and an open normoxic environment, small fish showed a greater preference for the hypoxic environment. Thus shelter from predators appears particularly important for juveniles, who may accept a greater physiological compromise in exchange for safety. In response to hypoxia without available shelter, larger fish reduced their level of activity (with the exception of aggressive encounters) to aid metabolic suppression whereas smaller oscars increased their activity, with the potential benefit of finding oxygen-rich areas.

Key words: oxygen, Amazon, predation, social, aquatic surface respiration (ASR), oscar, *Astronotus ocellatus*.

### Introduction

The aquatic environment is subject to many environmental variations and one of the most important parameters affecting non-air breathing vertebrates is dissolved oxygen. Many fish species encounter hypoxic conditions and in consequence a plethora of adaptations to environmental hypoxia have evolved. Extreme examples of these adaptations include the derivation of air-breathing organs from intestine, swim bladder, stomach, opercular cavity or skin (Graham, 1997), allowing the use of air as a respiratory medium. Breathing air can be advantageous as it contains a higher percentage of oxygen relative to volume than water (Dejours, 1994), but there are also many problems associated with air-breathing in aquatic organisms as their gills need to remain functional for gas exchange, nitrogen excretion and ionoregulation (Gilmour, 1998).

Thus many fish respond differently to falling oxygen tensions and non air-breathing fish demonstrate a suite of

physiological, biochemical and behavioural strategies. For example, changes in gill morphology (Laurent and Perry, 1991) allow more efficient extraction of oxygen from the water that can be coupled with increased oxygen carrying capacity (Powers, 1980) to combat hypoxia. Many vertebrates and invertebrates have an inherent ability to downregulate their metabolic rate to balance ATP demand and ATP supply pathways, a subject that was explored in great depth by Bob Boutilier and his colleagues (Boutilier and St-Pierre, 2000; Boutilier, 2001a; Boutilier, 2001b; Staples et al., 2003). This downregulation can be brought about at the cellular level by decreasing energy-consuming processes and/or by increasing the efficiency of energy-producing pathways (Boutilier, 2001b). Depression of metabolic rate can significantly increase survival time of fish exposed to hypoxia (Almeida-Val et al., 2000). Additionally, changes in fish behaviour can complement metabolic depression by minimising non-essential activities (Israeli and Kimmel, 1996; Crocker and Cech, 1997;

Dalla Via et al., 1998) or by exploiting spatial heterogeneity of dissolved oxygen through increased activity (Dizon, 1977; Domenici et al., 2000).

Another behaviour performed in response to hypoxia is aquatic surface respiration (ASR), where fish choose to move to surface waters where oxygen diffusion from the air results in a thin layer of well-oxygenated water (Kramer and Mehegan, 1981; Kramer and McClure, 1982; Verheyen et al., 1994; Shingles et al., 2005). Rather than a 'last gasp' attempt to escape hypoxia, ASR is a clear behavioural adaptation displayed by many fish species (Kramer and Mehegan, 1981; Kramer, 1987). Although an efficient method of maintaining oxygen consumption (Val, 1995), ASR necessitates excursions to the water surface to ventilate in the oxygen-rich surface layer, which can dramatically increase susceptibility to aerial predators (Kramer et al., 1983; Randle and Chapman, 2005) and air-breathing predatory fish (Wolf, 1985).

So, on the one hand ASR can decrease the physiological demands imposed by hypoxia but may increase susceptibility to predation. On the other hand, remaining in hypoxic waters necessitates either physiological or biochemical responses but can decrease the risk of both aerial predation and predation by aquatic predators less tolerant of hypoxia (McIntyre and McCollum, 2000; Robb and Abrahams, 2003). The idea of hypoxia as an ecological refuge in which animals can avoid predators less tolerant of low oxygen levels is based on the premise that many larger predatory fish are physiologically excluded from hypoxic conditions available to the smaller fish that they eat (Kolar and Rahel, 1993; Chapman et al., 1996; Robb and Abrahams, 2003). Indeed, it has been documented within several fish species that smaller individuals are more tolerant of hypoxia than larger individuals (Smale and Rabeni, 1995; Bursleson et al., 2001; Robb and Abrahams, 2003).

For non air-breathing species that perform ASR, it therefore seems possible that smaller individuals, which by virtue of their size are more vulnerable to predation (Abrahams, 2005), should have evolved a greater physiological capacity for using hypoxia as an escape from predation. In contrast, larger individuals, less susceptible to predation, could supplement their oxygen uptake by performing ASR. It is then perhaps surprising to find examples of fish species known to perform ASR, where hypoxia tolerance is greater in larger individuals. Interestingly, in the oscar *Astronotus ocellatus*, characterised as an extremely hypoxia-tolerant species (Almeida-Val and Hochachka, 1995), measurements of two key glycolytic and oxidative flux enzymes suggest that anaerobic potential actually increases during growth (Almeida-Val et al., 2000). Thus a scaling effect on hypoxia tolerance has been proposed where larger oscars are better physiologically equipped for coping with hypoxic conditions. By reducing standard metabolic rates and postponing anaerobic glycolysis, adult oscars are able to tolerate complete anoxia for up to 4 h (Muusze et al., 1998), unlike smaller juvenile oscars.

The present study therefore aimed to investigate the interplay between size and the physiological and behavioural responses of oscars to hypoxia. The physiological responses of

oscars to hypoxia were assessed by measuring the rate of oxygen consumption of fish in relation to decreasing environmental oxygen and the behavioural responses by quantifying movements with regard to ASR, hypoxia avoidance, activity levels and social interaction.

### Materials and methods

Oscars (*Acará-açu*; *Astronotus ocellatus*; Cichlidae; Cuvier 1829), ranging in mass from 5 to 310 g, were obtained from Sítio dos Rodrigues (Km 35, Rod. AM-010, Brazil). The fish were transferred to the Ecophysiology and Molecular Evolution laboratory at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil and held in 500 l tanks at  $28 \pm 3^\circ\text{C}$ . The tanks were continually aerated and 50% of the water replaced every 2 days. In each experiment described below comparisons were made between the physiology and behaviour of the two different sized groups of fish, referred to within the text as 'large' and 'small'. The fish in these groups were selected for size (Experiment 1: large,  $230 \pm 11$  g; small,  $16.2 \pm 1.9$  g; Experiments 2–4: large,  $147.6 \pm 6.4$  g; small,  $16.3 \pm 1.0$  g). Experimental temperature was  $28 \pm 1.5^\circ\text{C}$ . Oxygen tensions were measured throughout all the experiments by injecting water samples into a thermostatted cell containing a Cameron OM-200 oxygen electrode (Port Aransas, TX, USA) connected to a Cameron OM-200 oxygen meter. The oxygen electrode was calibrated using air-saturated water at the appropriate temperature and pressure, and a solution of 5% sodium metabisulphite (0 mmHg; 1 mmHg=133.3 Pa).

#### *Experiment 1: The effect of declining $P_{\text{O}_2}$ on $\dot{M}_{\text{O}_2}$*

Experiments were performed on 35 oscars covering the mass range 8.8–308 g. Approximately 12 h before an experiment, the fish were placed in sealable Nalgene® containers, and allowed to settle overnight. The containers were placed in a water-bath to control temperature and were covered with black plastic to minimize visual disturbance. The containers were chosen so as to have a volume equivalent to 50–80 times the mass of the fish, and were vigorously aerated to maintain air-saturated conditions during the settling period. The following morning, the water was renewed with minimal disturbance, and the experiment started 2 h later by ceasing aeration and sealing the containers. Water samples (0.5 ml) were drawn by syringe at 15 min intervals until the  $P_{\text{O}_2}$  had fallen below 10 mmHg, a process that took 4–8 h. The small volume of water that was removed at each sample time was replaced. Plots of  $P_{\text{O}_2}$  versus time were constructed, and  $\dot{M}_{\text{O}_2}$  values calculated from the slopes over 20 mmHg intervals interpolated to  $P_{\text{O}_2}$ =150, 130, 110, 90, 70, 50, 30 and 10 mmHg, factored by fish mass, water volume and  $\text{O}_2$  solubility coefficient at the exact temperature, the latter taken from Boutilier et al. (Boutilier et al., 1984).

#### *Experiment 2: Relationship between oxygen tension and ASR*

Fish were placed individually into a glass tank (34 l) containing water that was continuously aerated through an air

stone situated at the bottom of the tank. The fish were allowed 1 h to acclimate to the tank prior to the start of the experiment. A piece of opaque plastic floating on the water surface largely eliminated any contact of the water with the air. The tank was divided into vertical sections marked on the outside of the tank. The first section was from the bottom of the tank (0%) to a quarter of the way to the surface (25%), the second from 25–50%, the third from 50–75%, the fourth from 75% to 90% and the final section consisted of the water within 10% of the surface (90–100%). Following acclimation, the air supply to the tank was switched to nitrogen and the oxygen tension gradually reduced. Oxygen tensions were measured as above every 5 min in each vertical section (see Fig. 3A in the Results section). No significant vertical zonation of oxygen occurred ( $P=0.771$ ). Throughout the experiment the vertical movements of the fish were recorded and every 5 min the breathing frequency of the fish was recorded as beats  $\text{min}^{-1}$ . When the snout of the fish touched the water surface this was considered the end of the experiment, the gas supply was switched back to air and the fish allowed to recover.

#### Experiment 3: Position choice in an oxygen gradient

An oxygen gradient was generated in a long shallow tank (length: 2 m 16 cm; width: 44 cm; depth: 17 cm) by bubbling nitrogen into one end and air into the other. The gradient was measured throughout the experiment (Fig. 4A). At the most hypoxic end of the tank, shelter was created by the addition of some floating plants, *Pistia stratiotes* (shaded area; see Fig. 4A in the Results section). Once the gradient was established, a fish was placed into the middle of the gradient (at  $\sim 65$  mmHg). The fish was then observed from a distance and the time spent under the shelter (i.e. in the lowest oxygen tension) was noted. Each trial lasted for 10 min. After this time the fish was removed, the gradient allowed to re-establish and the next fish tested. Four different experimental treatments of fish ( $N=7$  for each treatment) were tested. The first two treatments were either large or small oscars taken directly from their respective stock tanks. For the third and fourth treatments, large or small oscars were held in hypoxic water (30 mmHg) for 1 h prior to testing in the experimental set-up. Within each size group, different individuals were used to test for the prior effects of holding in normoxia or hypoxia, so that each individual was naïve to the system. However, there were no significant intra-group differences in size between these treatments ( $P=0.9$ ).

#### Experiment 4: Group activity during hypoxia

Groups of four oscars (all large or all small) were transferred to glass tanks (77 l). Three sides of the tank were covered with black plastic to minimise disturbance to the fish. Water within the tanks was continually aerated and a piece of opaque plastic floating on the water surface reduced the contact of water with the air. Each glass tank was divided into four sections with opaque partitions so that the four fish were initially separated. Fish were allowed to acclimate to the tanks for 24 h before the partitions were removed to allow group interaction. Following 1 h of interaction observations were made on group behaviour.

Prior to each observation the observer sat for 10 min in front of the tank to allow the fish to acclimate to the observer's presence. Fish were then watched for 10 min. For the recording of activity, the tank was divided horizontally into quarters (drawn on the outside of the tank). Each time the snout of a fish crossed into another section of the tank this was counted as one horizontal movement. The tank was also divided into three vertical sections (marked on the outside). The first section was from the bottom of the tank (0%) to half way to the surface (50%), the second from 50–90% and the final section consisting of the water within 10% of the surface (90–100%). Each time the snout of a fish crossed into another vertical zone this was counted as one vertical movement. The number of horizontal and vertical movements of each fish was therefore calculated throughout the observation periods. Aggression was also calculated, with an attempted or actual bite of one fish against another counting as one aggressive action.

Following the initial 10 min observation period, tanks were allocated to either control or experimental treatments. Control tanks were left for 1 h, and in the experimental tanks oxygen tensions were reduced from normoxia ( $136.4 \pm 1.3$  mmHg) to a nominal concentration of 80 mmHg ( $79.8 \pm 0.8$  mmHg) by bubbling nitrogen into the tank. The required  $P_{\text{O}_2}$  in experimental tanks was achieved by a steady reduction over the 1 h period. Behavioural observations were then repeated in both treatments. Oxygen levels were measured during the observation time with water samples being taken from all three vertical zones. No significant vertical zonation of  $P_{\text{O}_2}$  was noted within the tanks ( $P>0.1$ ).

Following the second behavioural observation the control tanks were left for a further 1 h while the oxygen level in the experimental tanks was gradually reduced as before to a nominal  $P_{\text{O}_2}$  of 40 mmHg ( $39.7 \pm 0.6$  mmHg). A final set of behavioural observations was then made on both the control and experimental tanks.

To investigate whether the socially mediated differences in plasma cortisol concentrations documented in other fish species (Sloman and Armstrong, 2002; Gilmour et al., 2005) occurred among social groups of oscars, six control groups of large oscars were sampled for cortisol at the end of the experiment. Fish were killed by a lethal dose of anaesthetic ( $0.5$  mg  $\text{ml}^{-1}$  benzocaine) and a blood sample withdrawn by caudal venipuncture. Blood samples were centrifuged, the plasma removed and stored at  $-80^\circ\text{C}$  for later analysis of cortisol by radioimmunoassay (ICN Pharmaceuticals, Costa mesa, CA, USA).

#### Statistical analyses

Data were checked for normality using the Kolmogorov–Smirnov test. Where data were normally distributed, parametric analyses of variance (ANOVA: *post hoc* Tukey and Student's *t*-test) were used to test for statistical differences among treatments in physiological and behavioural parameters. In some cases significant interaction effects in two-way ANOVA analyses masked differences between size groups so the data were subsequently analysed separately for

*post hoc* effects. Where data were not normally distributed the non-parametric Kruskal–Wallis test was used to look for statistical differences among treatments.

In experiment 1, the relationships describing  $\dot{M}_{O_2}$  as a function of body mass and water  $P_{O_2}$  were analysed by multiple regression, analysis of covariance (ANCOVA; logarithmic and linear models) and also by a two step-procedure in which  $\log \dot{M}_{O_2}$  was first regressed against  $\log$  mass [ $\log \dot{M}_{O_2} = \log(a) + b(\log \text{mass})$ ], and an asymptotic equation was fitted to the residuals ( $m$ ) from the first equation which described them as a function of  $P_{O_2}$  [ $m = x - y \cdot z^{P_{O_2}}$ ]. The two equations were then combined [ $\log \dot{M}_{O_2} = [\log(a) + x] + b[\log(\text{mass})] - y \cdot z^{P_{O_2}}$ ]. This latter approach yielded the highest  $r^2$  and was the procedure chosen to derive the reported model.

In experiment 4, an overall behaviour score for individual fish was calculated by combining each behavioural parameter measured (i.e. vertical activity, horizontal activity, aggression) in a Principal Components Analysis and a correlation was used to analyse the relationship among behaviour scores of fish at varying levels of  $P_{O_2}$ . All data are presented as means  $\pm$  s.e.m.

## Results

### Experiment 1: The effect of declining $P_{O_2}$ on $\dot{M}_{O_2}$

Both fish size ( $P < 0.001$ ) and environmental  $P_{O_2}$  ( $P < 0.001$ ) significantly influenced  $\dot{M}_{O_2}$  and the response of  $\dot{M}_{O_2}$  to declining  $P_{O_2}$  varied with fish size ( $P < 0.001$ ). In large oscars, mass-specific  $\dot{M}_{O_2}$  remained unchanged until a  $P_{O_2}$  of 50 mmHg, where it fell significantly to 74% of the value in air-saturated water (Fig. 1). By 10 mmHg,  $\dot{M}_{O_2}$  had declined to about 30%. In small oscars, mass-specific  $\dot{M}_{O_2}$  was much greater, but fell gradually with  $P_{O_2}$ , the first significant decline occurring at a higher threshold, 70 mmHg, where it was 73%

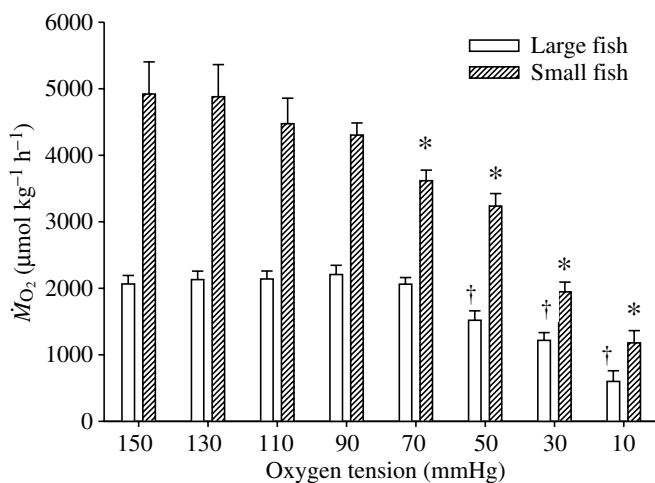


Fig. 1. The influence of oxygen tension (mmHg) on mass-specific  $O_2$  consumption rate ( $\dot{M}_{O_2}$ ) in large ( $N=13$ ; open bars) and small ( $N=15$ ; hatched bars) oscars. Values are means  $\pm$  1 s.e.m. Measurements were made over 20 mmHg intervals. Asterisks and daggers indicate significant differences ( $P < 0.05$ ) from the respective rates at the highest  $P_{O_2}$ .

of the value in air-saturated water (Fig. 1). By 10 mmHg,  $\dot{M}_{O_2}$  had fallen to about 20%. The overall range in body mass was sufficient to construct a model (Fig. 2) describing the three-dimensional response surface relating mass-specific  $\dot{M}_{O_2}$  to body mass and water  $P_{O_2}$ , which explained 94.8% and 92.8% of the variance, respectively, for fish of mass 10–50 g and 50–300 g. In both small and large fish, mass-specific  $\dot{M}_{O_2}$  explained 81.6% of the variability in the data, while water  $P_{O_2}$  explained the additional 13.2% and 11.2%, respectively.

### Experiment 2: Relationship between oxygen tension and ASR

Size significantly affected the  $P_{O_2}$  at which oscars made an attempt to break the water surface with their snouts ( $P=0.02$ ; Fig. 3B). Large fish surfaced at a higher  $P_{O_2}$  ( $49.6 \pm 9.8$  mmHg) than small fish ( $22.3 \pm 3.7$  mmHg). Fish mass was positively correlated with the  $P_{O_2}$  at which the fish broke the water surface with its snout ( $P=0.04$ ,  $r^2=0.207$ ). In general, small oscars showed a greater ( $48.1 \pm 5.3\%$ ) decrease in breathing frequency than large oscars ( $22.3 \pm 4.3\%$ ;  $P=0.002$ ) during hypoxia, probably due to the earlier surfacing of larger fish.

### Experiment 3: Position choice in an oxygen gradient

For fish previously held in normoxia ( $\sim 130$  mmHg), size significantly affected time spent under the plant shelter, with smaller individuals spending significantly more time (essentially the entire 10 min test period) in the hypoxic, sheltered water (Fig. 4B;  $P < 0.01$ ). However, small oscars reduced the amount of time under the shelter when held in hypoxic water (30 mmHg) for 1 h prior to testing in the experimental set-up (Fig. 4B;  $P < 0.01$ ). Large oscars did not.

### Experiment 4: Group activity during hypoxia

The behaviour of control groups of fish was observed over time in the absence of hypoxia. For control groups of both small and large oscars, there was no observed change in behaviour among observation periods ( $P > 0.5$ ). However, there were differences in control group behaviours according to size. Groups of large fish displayed more horizontal activity than groups of smaller fish and were more aggressive ( $P < 0.001$ ). Indeed no aggressive acts were observed among control groups of small oscars in comparison with a total of 147 acts in control groups of large oscars. Among groups of large oscars, clear social hierarchies were observed, with high levels of aggression generally displayed by one fish within the group. However, no socially mediated differences in cortisol were observed [ $P=0.8$ ; mean cortisol value ( $\pm$  s.e.m.) =  $6.35 \pm 0.85$  ng ml<sup>-1</sup>].

Hypoxia significantly affected the group behaviour of both large and small oscars, but with large and small oscars responding in different ways ( $P < 0.05$ ). Groups of large oscars showed a decrease in horizontal activity with decreasing  $P_{O_2}$  (Fig. 5A;  $P < 0.01$ ) while small oscar did not ( $P > 0.1$ ). In contrast groups of small oscars showed an increase in vertical activity (up to the 50% line) with decreasing  $P_{O_2}$  (Fig. 5B;  $P < 0.05$ ) unlike larger oscar ( $P > 0.1$ ). Aggression was not significantly affected by hypoxia in either size group ( $P > 0.1$ )

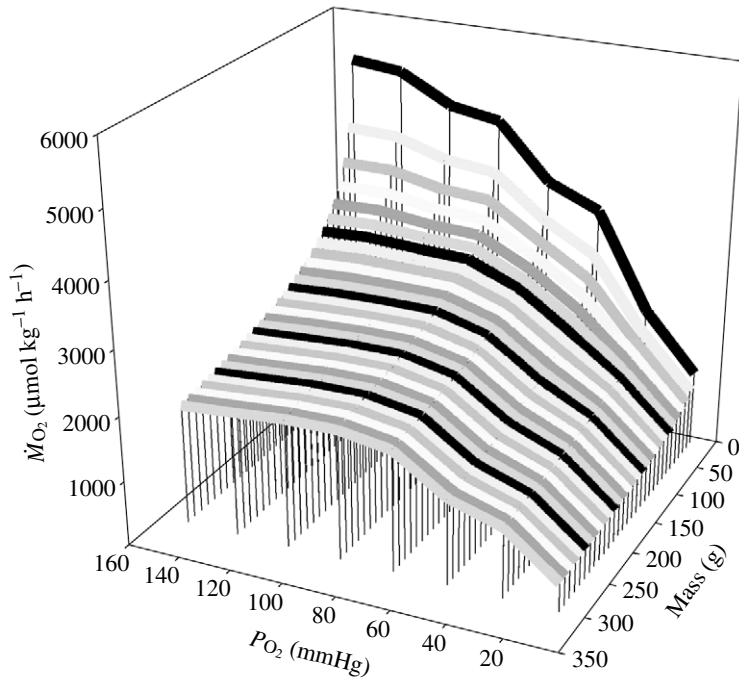


Fig. 2. A response surface describing mass-specific  $\dot{M}_{O_2}$  as a function of body mass and  $P_{O_2}$  based on data from 35 oscars ranging in mass from 8.8 to 308 g. The model is:  $\log \dot{M}_{O_2} = 3.20686 + 0.786 \times \log(\text{mass}) - 0.88913 \times 0.96371^{P_{O_2}}$  (0.05–0.3 kg fish) ( $r^2 = 0.948$ );  $\log \dot{M}_{O_2} = 3.28076 + 0.786 \times \log(\text{mass}) - 0.92331 \times 0.97024^{P_{O_2}}$  (0.01–0.05 kg fish) ( $r^2 = 0.928$ ), where  $\dot{M}_{O_2}$  is in  $\mu\text{mol O}_2 \text{ h}^{-1}$ , mass is in kg, and  $P_{O_2}$  is in mmHg.

## Discussion

Small oscars had a significantly higher  $\dot{M}_{O_2}$  than large oscars in experiment 1 and initially both small and large oscars displayed independent respiration (Hughes, 1981), with rate of oxygen consumption ( $\dot{M}_{O_2}$ ) remaining constant despite a fall in the oxygen tension of the water ( $P_{O_2}$ ). However, at 70 mmHg, the  $\dot{M}_{O_2}$  of small oscars ceased to be independent of environmental oxygen tension and a significant decrease in  $\dot{M}_{O_2}$  was noted. At 50 mmHg a similar effect was seen in large oscars, suggesting that large oscars are able to regulate their oxygen consumption by adjustments in respiration and circulation to a lower oxygen threshold than small oscars. Large oscars also have a much greater ability to survive exposure to extreme hypoxia, as previously demonstrated (Almeida-Val et al., 2000). Fish around 16 g in mass (equivalent to 'small' in our study) survived extreme hypoxia for about 9 h (Almeida-Val et al., 2000) and the respective larger individuals of 230 g survived for approximately 35 h. Thus while larger oscars appear to tolerate falling  $P_{O_2}$  levels slightly better than their smaller counterparts, this difference is magnified considerably once

although, as for the control groups, aggression was significantly higher among groups of larger fish ( $P < 0.001$ ; Fig. 5C).

Interestingly, a positive correlation existed among the individual activity scores for each fish at varying levels of  $P_{O_2}$  ( $P < 0.001$ ; Fig. 5D). Therefore the activity of each individual fish in relation to its group members did not vary with changing oxygen tensions (i.e. the most active member of each group remained the most active at each oxygen level).

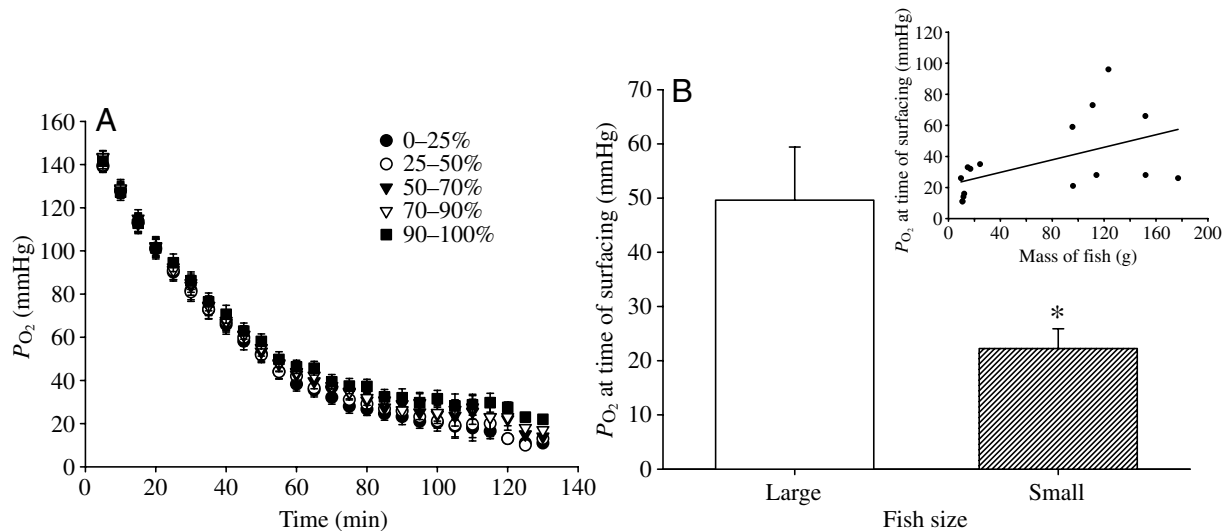


Fig. 3. (A) Decrease in oxygen tensions (mmHg) over time as nitrogen is bubbled into a glass tank. Oxygen tensions were measured every 5 min in each vertical zone (see key) within the tank and there was no significant zonation of oxygen tension within the tank ( $P = 0.771$ ). (B) The oxygen tension at the time of surfacing of large (open bar) and small oscar (hatched bar). The asterisk indicates a significant difference ( $t = 2.14$ ,  $P = 0.02$ ,  $N = 8$ ); values are means  $\pm$  s.e.m. The insert shows the correlation between mass of individual fish (g) and the oxygen tension at time of surfacing ( $P = 0.04$ ;  $r^2 = 0.207$ ).

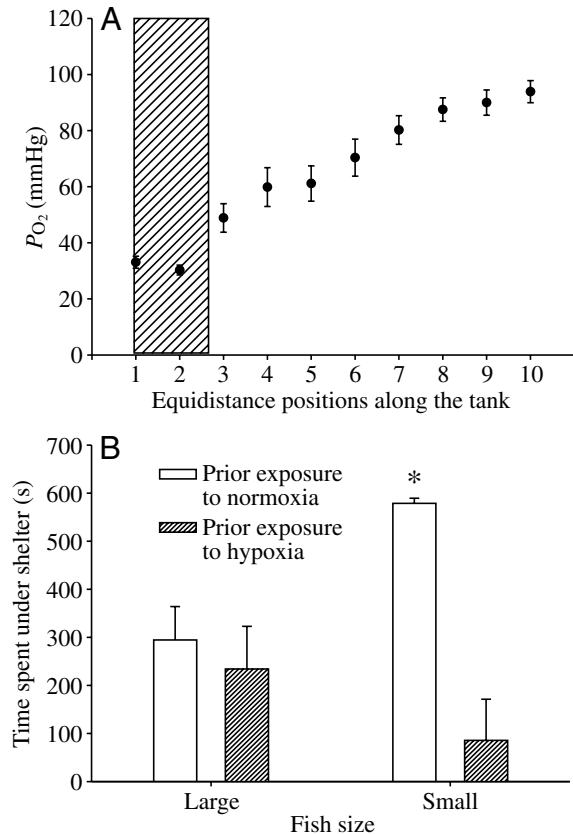


Fig. 4. (A) Gradient of oxygen tension (mmHg) in a long (2.16 m) shallow tank. Each data point is equidistant along the tank. The shaded area represents the area covered by the floating plant, *Pistia stratiotes*. (B) Time spent under the shelter of the floating plant, *Pistia stratiotes*, at the most hypoxic end of the gradient by fish from the large and small groups of oscars, held in either normoxic (~130 mmHg; open bars) or hypoxic (~40 mmHg; hatched bars) water for 1 h prior to being placed into the experimental set-up. There was a significant interaction between hypoxia and size (two-way ANOVA;  $P < 0.01$ ), with the asterisk indicating a significant effect of both size and hypoxia treatment ( $t$ -test:  $P < 0.01$ ,  $N = 7$  fish per group). All values are means  $\pm$  s.e.m.

extreme hypoxia is reached and anaerobic metabolism becomes necessary. The greater anaerobic potential of large oscar, as indicated by higher concentrations of lactate dehydrogenase and malate dehydrogenase (Almeida-Val et al., 2000), also fits with this scenario. In summary, unlike many other species, e.g. yellow perch *Perca flavescens* (Robb and Abrahams, 2003) and largemouth bass *Micropterus salmoides* (Burlerson et al., 2001), the oscar shows a positive relationship between physiological tolerance of hypoxia and size.

A behavioural ecology approach might predict that when faced with decreasing oxygen, fish should choose the response that minimises the costs of obtaining the required amount of oxygen (Kramer, 1987). So, one might expect that if ASR, like the air-breathing response in other species, is evoked by the environmental oxygen tension at which respiratory mechanisms fail to compensate for environmental hypoxia

(Takasusuki et al., 1998), then smaller oscars would perform ASR at a higher oxygen tension than adults. In experiment 2, larger oscars did perform ASR at approximately 50 mmHg, the tension at which the first significant drop in their  $\dot{M}_{O_2}$  was seen. Surprisingly, smaller oscars postponed ASR to around 22 mmHg, well beyond the  $P_{O_2}$  at which they switched from oxyregulation to oxyconformation (70 mmHg).

Claireaux et al. (Claireaux et al., 1995) stated that in responding to environmental factors fish may simply be constrained into choosing the lesser of two evils, and for smaller oscars it appears that, at least at oxygen tensions above 22 mmHg, ASR has greater negative consequences for fitness than remaining in a hypoxic environment. Susceptibility to aerial predators (Kramer et al., 1983; Randle and Chapman, 2004) and other predatory air-breathing fish (Wolf, 1985) is a likely explanation, as supported by the results of experiment 3. In an oxygen gradient where shelter from floating plants was only available in hypoxic (30 mmHg) water, small oscars chose to move under the shelter for virtually the entire experimental period and accept the associated physiological cost of exposure to hypoxia. In contrast, large oscars spent approximately 50% of their time in normoxia and 50% under the shelter. However, if small oscars were held for 1 h at 30 mmHg prior to placing in the gradient, then they were forced to choose more oxygenated waters that did not contain shelter. As oscar are known to accumulate significant amounts of lactate during hypoxia (Muusze et al., 1998) it is likely that these small oscar were no longer able to physiologically tolerate hypoxia. Predators of oscar include examples of air-breathing fish (e.g. pirarucu; *Arapaima gigas*) and other vertebrates such as alligators (V.M.F.A.-V., personal observations). It is not yet known whether there is a difference in predator species that prey on large and small oscar.

Oxygen chemoreceptors on the gill epithelia mediate physiological changes in response to hypoxia (Reid and Perry, 2003). Similar receptors are believed to stimulate air-breathing fish to surface in hypoxia (McKenzie et al., 1991; Taylor et al., 1996) and it has been suggested that they also play a role in eliciting ASR (Shingles et al., 2005). In the flathead grey mullet *Mugil cephalus*, Shingles and colleagues (Shingles et al., 2005) demonstrated that ASR is a behavioural  $O_2$ -chemoreflex that can be modified by the risk of predation. The choice of juvenile oscars to remain in hypoxic waters appears to be another example of behavioural modulation of hypoxic chemoreflexes affording them a reduction in susceptibility to potential predators.

ASR is but one behavioural adaptation in a suite of many and a complex combination of behavioural responses to hypoxia should be expected, designed to match oxygen supply and demand by the least costly means (Kramer, 1987). In experiment 4, changes in activity were considered as another behavioural response to hypoxia, both as the simple amount of horizontal and vertical movement and also as more complex social interactions. The effects of hypoxia on spontaneous locomotor activity documented within the literature appear to be species- and situation-specific (Kramer, 1987). Here a clear

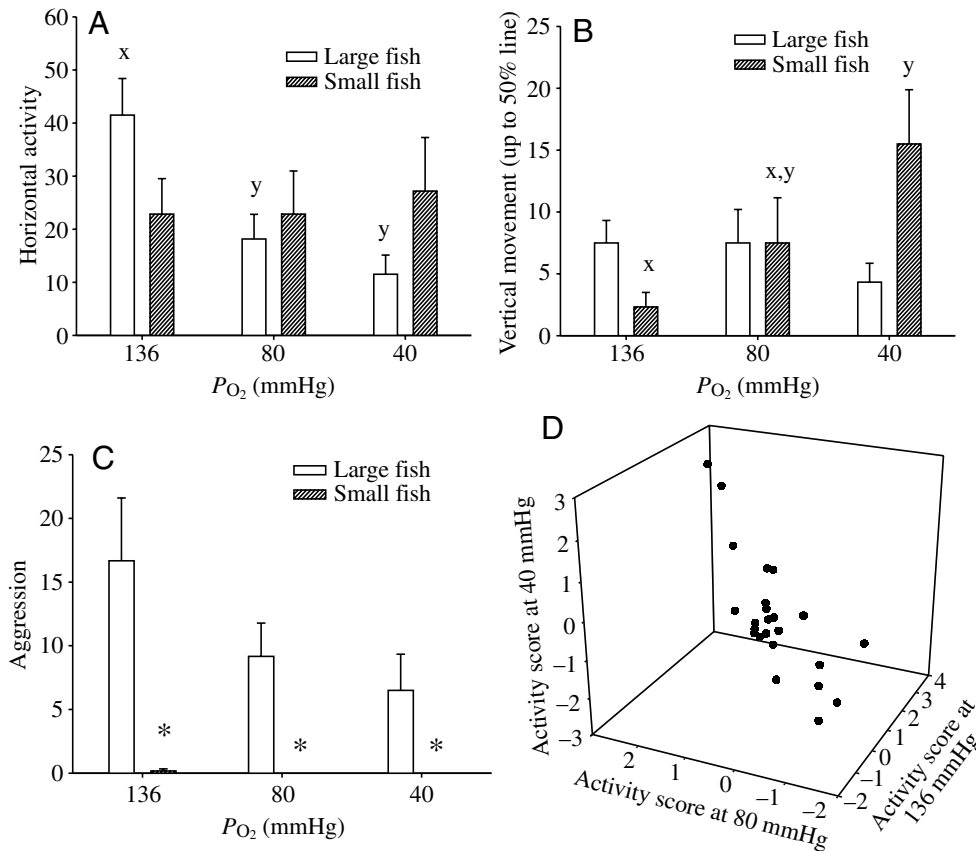


Fig. 5. Changes in behaviour in large and small oscar during progressive hypoxia. Where significant interactions ( $P < 0.05$ ) between hypoxia and size masked changes in behaviour within size classes, one-way ANOVA analyses were performed *post hoc* to test for statistical differences. (A) Horizontal activity, (B) vertical activity and (C) aggression. Values are means  $\pm$  s.e.m. Letters denote significant differences within size groups where bars sharing the same letter are not significantly different (one-way ANOVA:  $P < 0.05$ ) and asterisks denote a significant difference between size groups (two-way ANOVA:  $P < 0.001$ ). (D) Relationship between the activity scores of individual fish at different oxygen tensions (Spearman's Rank Correlation:  $r_s = 0.667, 0.698, 0.718$  for  $P_{O_2} = 40, 80$  and  $136$  mmHg, respectively;  $P < 0.001$ ).

difference between sizes was seen in the effect of hypoxia on activity level. Large oscars were more active than small oscars under control conditions and showed a decrease in movement in the horizontal plane with decreasing oxygen tensions, whereas small fish showed an increase in activity during hypoxia. The increase in activity in smaller fish was recorded in the vertical plane although it should be noted that the fish only increased their number of excursions around the 50% line, not up to the water surface (as might be expected at tensions less than 22 mmHg). Thus it seems likely that larger fish reduce their level of activity to aid metabolic suppression (Boutillier and St-Pierre, 2000) whereas smaller fish increase their activity, potentially in the hope of finding areas less devoid of oxygen (Domenici et al., 2000).

Oscars live in small schools forming monogamous pairs for reproduction, where both sexes will establish and defend breeding sites (Santos et al., 1984; Beeching, 1995). Aggressive interactions between mature adults have been documented (Beeching, 1997), with combat defeat eliciting a colour pattern change from the normal olive-green-brown body colouration to a near black colour interrupted with irregular white barring (Beeching, 1995). Aggressive interactions were noted among the groups of large fish whereas no instances of aggression were noted among the groups of small fish. The lack of aggression among smaller oscar is supportive of their known schooling behaviour prior to reproductive maturity (Santos et al., 1984). Among large fish, although activity was seen to

decrease with falling oxygen tensions the level of aggression remained constant. Additionally, the activity of each individual fish in relation to its group members was not affected by hypoxia, suggesting that group social structure remained intact. Hypoxia is known to decrease stability of dominance hierarchies in stickleback *Gasterosteus aculeatus* (Sneddon and Yerbury, 2004) and decrease the duration of aggressive encounters in the shore crab *Carcinus maenas* (Sneddon et al., 1999). However, in large oscars, although a general decline in activity would have allowed a reduction in metabolic activity, aggression and defence seemed to be behaviours worthy of persistence, at least down to 40 mmHg.

In conclusion, unlike many other species, the oscar shows a positive relationship between physiological tolerance to hypoxia and size, with large oscars withstanding the effects of falling  $P_{O_2}$  better than smaller oscar, supported by a greater anaerobic potential to allow prolonged survival in extreme hypoxia. Contrary to physiological predictions, small oscars chose to remain in hypoxic waters to lower oxygen tensions than large oscars affording the former a reduction in susceptibility to potential predators. We have clearly demonstrated in the present study the need to consider both the behavioural and physiological response of these fish to hypoxia to fully understand their mechanisms of adaptation. Little is known about the distribution of these fish within their Amazonian habitat, although some preliminary data suggests that large and small oscar may occupy different areas of the

water column (Junk et al., 1983) with juveniles tending to associate with floating plants (Botero, 2000). Oscars live mostly in Amazon lakes and at the margins of rivers, with a strong preference for lentic environments (Santos et al., 1984). Oxygen gradients are known to develop, particularly at night, between hypoxic flooded areas and the normoxic main river, which are likely to influence distribution of oscars. Kramer et al. (Kramer et al., 1978) highlight the amazing diversity of aquatic habitats within the Amazonian rainforest, from 'strongly flowing rivers' to 'stagnant puddles', and future work should now strive to take our knowledge of the behavioural and physiological responses of oscars into the field to understand how they are executed within an ecological context.

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