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Implications of hypoxia tolerance for wetland refugia use in Lake Nabugabo, Uganda

Andrea J. Reid[1], Maxwell J. Farrell[1], Max N. Luke[1], Lauren J. Chapman[1,2]
[1]Department of Biology, McGill University, 1205 Docteur Penfield, Montreal, Quebec H3A 1B1, Canada
[2]Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, New York 10460, USA

Abstract – Aquatic hypoxia can affect predator-prey interactions by altering the success rate of the predator and/or the vulnerability of prey. For example, in the Lake Victoria basin of East Africa, native prey exploit hypoxic wetlands as refugia from predation by introduced Nile perch (*Lates niloticus*). Here, it is predicted that species exploitation of wetlands depends on their hypoxia tolerance relative to the heterogeneity of wetland hypoxia. In this study, we compared the hypoxia tolerance of four fish taxa that differ in their use of hypoxic wetlands in Lake Nabugabo, Uganda: the cichlid *Pseudocrenilabrus multicolor victoriae* that inhabits the dense swamp interior; and three taxa that inhabit wetland ecotones including Nile tilapia (*Oreochromis niloticus*), *L. niloticus* and juvenile endemic haplochromine cichlids. We characterised hypoxia tolerance by exposing fish to progressive hypoxia and quantifying variation in aquatic surface respiration (ASR). The effect of body size on tolerance was explored in *L. niloticus* by quantifying ASR behaviour across a range of size classes. ASR behaviour was also compared between *O. niloticus* groups from wetland versus open-water habitats to detect habitat-associated intraspecific variation. The most tolerant taxon was the swamp specialist *P. multicolor*, indicated by its low ASR thresholds and small percentage of fish using ASR during the final sample interval. The other three taxa did not differ in ASR behaviour, and no differences were detected between *O. niloticus* groups. Body size effects were present for *L. niloticus* suggesting a lower tolerance to hypoxia in larger-bodied individuals, thus limiting their ability to penetrate wetlands.

Key words: aquatic surface respiration; habitat use; introduced species; native fish; Nile perch

Introduction

Given the current and unprecedented rate at which species are being introduced into ecosystems (Ricciardi 2007), it is critical that ecologists are able to manage and predict their impacts on recipient communities. In the context of introduced predators, this requires a thorough understanding of characteristics of refugia that protect native prey and/or hinder predator dispersal, and of the traits of prey that enable their use of refugia (Rosenberger & Chapman 1999). This knowledge can mitigate biodiversity loss through effective management of refugia, and improves our ability to predict future ecological interactions. These are two issues of particular importance in the Lake Victoria basin of East Africa, where the introduction of an aquatic predator coincided with the extinction of hundreds of native fish species.

Lake Victoria's endemic flock of haplochromine cichlids (>500 species) represents one of the most recent, rapid and extensive vertebrate radiations (Greenwood 1980; Kaufman et al. 1997). The lake also contains a great diversity of native noncichlids (Greenwood 1966), and a similar faunal assemblage, although less speciose, is shared by neighbouring lakes in the region (e.g., lakes Kyoga and Nabugabo; Ogutu-Ohwayo 1993, 1994). Since the introduction of the large, piscivorous Nile perch (*Lates niloticus*) in the late 1950s and early 1960s, a staggering amount of this diversity has been lost (Kaufman 1992). In Lake Victoria, an estimated 50% of the native fish species (99% haplochromine cichlids) declined or disappeared coincident with a substantial increase in *L. niloticus* numbers in the early 1980s (Ogutu-Ohwayo 1990; Kaufman 1992; Witte et al. 1992; Kaufman et al. 1997). Although intense fishing

pressure and other anthropogenic stressors (e.g., eutrophication) have contributed to changes in the faunal assemblage, predation by *L. niloticus* seems to have played a key role in the faunal collapse. Similar loss of fish diversity has been observed following *L. niloticus* introductions into lakes Kyoga (Ogutu-Ohwayo 1994) and Nabugabo (Ogutu-Ohwayo 1993). Interestingly, Lake Nabugabo has not undergone the dramatic eutrophication characteristic of Lake Victoria, highlighting the significance of the *L. niloticus* introduction as a driver of change in this system (Paterson & Chapman 2009).

Remarkably, some native fish species in lakes with invasive *L. niloticus* have persisted in small numbers in faunal refugia, which may represent the basis of future biodiversity recovery (Chapman et al. 2003, 2008). Refugia have been identified in rocky habitats (Witte et al. 1992; Seehausen 1996; Seehausen et al. 1997; Witte et al. 2007) and satellite lakes (e.g., lakes Nwampassa, Manywa and Kayugi; Ogutu-Ohwayo 1993; Kaufman et al. 1997). In addition, wetlands have been shown to serve as structural and low-oxygen (hypoxic) refugia for native fish; the dense macrophytic growth may inhibit the hunting efficiency and dispersal of this large predator, and the low levels of dissolved oxygen (DO) limit its exploitation since this species has a low tolerance to hypoxia (Schofield & Chapman 2000; Chapman et al. 2002).

In Lake Nabugabo, a satellite of the larger Lake Victoria, a small subset of fish species thrive in wetland refugia, many of which largely disappeared from the open waters following the *L. niloticus* introduction (Chapman et al. 1996a,b; Rosenberger & Chapman 1999). A recent study (Reid et al. In Press) quantified the fish assemblage structure across the lake-wetland edge in Lake Nabugabo to identify peaks in native fish abundance and richness, termed peak refugia. This study found strong clines in water temperature, pH, dissolved oxygen (DO), depth and vegetation density along transects extending from the wetland edge to the swamp interior, and found peak refugia in a narrow band surrounding the lake-wetland edge. This peak refuge lies very close to waters where *L. niloticus* are both abundant and feeding primarily on fish, in particular endemic haplochromine cichlids (Paterson & Chapman 2009), suggesting high pressure on native fish to penetrate wetland refugia. We predict that one factor limiting the width of the peak refugium in Lake Nabugabo is the ability of native species to withstand the extreme hypoxia that characterises the swamp interior (mean DO = 14.0 PO_2; Reid et al. In Press).

This study builds on previous research in the Lake Victoria basin that explores the role of hypoxia tolerance on variation in wetland habitat use. Here, we focus on the hypoxia tolerance of two of the most abundant non-air-breathing wetland refuge inhabitants in Lake Nabugabo, Uganda (juvenile endemic haplochromine cichlids and the widespread haplochromine cichlid *Pseudocrenilabrus multicolor*), and the two predominant invaders in the lake (*Oreochromis niloticus* and *L. niloticus*; Reid et al. In Press). We quantify the distribution of these four taxa across wetland gradients in Lake Nabugabo, and characterise their hypoxia tolerance by exposing fish to progressive hypoxia and quantifying variation in aquatic surface respiration (ASR). ASR is a behaviour that enables fish to ventilate their gills with water from the air-water interface where diffusion produces a thin layer of well-oxygenated water (Kramer & McClure 1982), however, this surfacing behaviour may also heighten the risk of avian predation (Kramer et al. 1983). We make intra-specific comparisons to explore the effects of body size on hypoxia tolerance, and to examine variation in hypoxia tolerance between *O. niloticus* from wetland versus open-water habitats. Although previous studies have characterised the ASR behaviour of *L. niloticus* (Schofield & Chapman 2000) and haplochromine cichlids in Lake Nabugabo (Rosenberger & Chapman 2000), this study is the first to directly link ASR behaviour to edge-related gradients, and the first to quantify effects of body size on the hypoxia tolerance of *L. niloticus*.

Methods

Site and study species

Lake Nabugabo is a small satellite lake (area = 33 km^2; mean depth = 4.5 m) of Lake Victoria located in southern Uganda. Formerly a bay on Lake Victoria's western shore, the extensive Lwamunda Swamp (*c.* 4 km wide) that surrounds Lake Nabugabo was created during water-level fluctuations approximately 5000 years ago (Greenwood 1965; Stager et al. 2005). The Juma River is Lake Nabugabo's primary tributary, and outflow seeps eastward into Lake Victoria *via* the Lwamunda Swamp (Greenwood 1965). Its periphery is primarily wetland habitat dominated by emergent macrophytes; they include hippo grass (*Vossia cuspidata*), *Miscanthidium violaceum*, water lilies (*Nymphaea caerulea* and *N. Lotus*) and small stands of papyrus (*Cyperus papyrus*).

Fish species were selected for ASR studies to shed light on the relationship between hypoxia tolerance and distribution across the wetland refugium. Although one species of endemic cichlid (*Prognathochromis venator*) was extirpated from Lake Nabugabo coincident with the introduction of *L. niloticus*, other populations of haplochromines declined, but persisted, with juveniles primarily captured in wetland ecotonal refugia (Chapman et al. 1996a,b; Schofield & Chapman 1999;

Chapman et al. 2003). In this study, juvenile endemic haplochromines could not be reliably identified to species level in the field, and were thus grouped *sensu lato* as 'endemic haplochromines', but were most likely dominated by *Astatotilapia velifer*. The widespread cichlid *P. multicolor* naturally occurs across a broad range of habitats in the Nile basin from hypoxic swamps to well-oxygenated rivers, and is found throughout the dense swamp interior in the Lake Nabugabo system (Reid et al. In Press). Standard minnow traps (0.6 cm mesh) were set overnight in the interior of the Lwamunda Swamp to target *P. multicolor* and at the ecotone to target endemic haplochromines. Because *O. niloticus* occupy both wetland and open-water habitats in Lake Nabugabo (Bwanika et al. 2006), minnow traps were set overnight at the ecotone to target wetland *O. niloticus*, while lake-dwelling *O. niloticus* were collected concurrently with *L. niloticus* using a small mesh seine (30 m) in a near shore, open-water habitat. Although juvenile *L. niloticus* do occur occasionally in minnow traps set near the wetland edge (Paterson & Chapman 2009), we were not able to capture wetland-associated *L. niloticus* in sufficient numbers during our survey to perform ASR trials; and therefore, we focused on *L. niloticus* from exposed (no wetland) inshore areas.

Fish distribution

To characterise the distribution and average DO levels of the habitats occupied by wetland taxa, we sampled eighteen 110 m long wetland transects in Lake Nabugabo between June and August of 2011. Transects extended 10 m into the open water from lake-wetland interface, and 100 m into the wetland habitat. Minnow traps were set at 10-m intervals with additional traps placed at 2 and 5 m on either side of the lake-wetland edge (n = 16 traps). At each trap sampling site, we measured DO and recorded data on the species captured in each trap. From this we were able to derive mean DO levels and standardised catch data for distance into the wetland from the edge. Diel variation in DO is generally low in the dense interior of the Lwamunda Swamp. For swamp pools at the landward side of the Lwamunda swamp, Reardon & Chapman (2008) reported DO (1 year of monthly samples) that averaged 19.3 PO_2 in the morning and 47.9 PO_2 in the afternoon. Because of the low diel variation in DO and the logistics of traversing through the swamp interior, we sampled only in the morning, which should reflect the lower range of the daily variation.

ASR behaviour

Captured fish were placed in well-oxygenated holding tanks (mean DO = 130.5 ± 15.9 PO_2, SD) for a minimum of 12 hours in a small structure on the shores of Lake Nabugabo that provided shade and shelter from wind and rain. Lake water was collected daily (0600–0900 hours) and sponge-filtered before use to remove some of the suspended particulate matter. Behavioural trials were performed on small groups (for *P. multicolor* and endemic haplochromines) and individuals (for wetland *O. niloticus*, and lake-dwelling *O. niloticus* and *L. niloticus*), because the latter two taxa were larger in size, and wetland *O. niloticus* were rare in our transects. No individuals were subject to more than a single trial. Prior to behavioural observations, experimental fish were transferred to a Plexiglas aquarium (depth = 20 cm; volume = 11,300 cm^3) and acclimated for 4 to 8 h. Two LED lamps were placed on either side of the aquarium, and a blind with a small viewing port was placed in front of the aquarium to minimise observer disturbance. After the acclimation period, oxygen was lowered slowly through the addition of sodium sulphite (Na_2SO_3; following Chapman & Liem 1995; Olowo & Chapman 1996) for an average 2.8-h (±30 min, SD) period, and then held at <5.3 PO_2 for 30 min (refer to Table 1 for trial details). Due to the remoteness of Lake Nabugabo, nitrogen gas could not be used to lower DO levels, however, Lewis (1970) found negligible differences in the behavioural responses of fish exposed to water deoxygenated using sodium sulphite versus nitrogen gas.

Behavioural responses to progressive hypoxia were recorded at 15-min intervals. For group trials, the number of fish performing ASR was assessed by scan counting at 10-s intervals for 100 s. For trials on individuals, the presence or absence of ASR behaviour was recorded every 10 s for 100 s. If any individual lost equilibrium, it was immediately removed from the experimental aquarium and placed in well-oxygenated water to recover. Temperature and DO were measured before, after and during the trial at 3-min intervals using a YSI ProDO optical probe.

The level of oxygen at which 10% (ASR_{10}) and 50% (ASR_{50}) of fish performed ASR was calculated by fitting cubic curves to plots of PO_2 (mmHg) and per cent ASR for each trial. For group trials, per cent ASR was calculated as the number of fish using ASR divided by the total number of individuals, averaged over the 10 observations in a given interval. For trials on individuals, this was calculated as the number of observations where ASR was present over the 10 observations in a given interval. A threshold of 0 PO_2 was assigned when there was no point at which 50% of fish used ASR. For all trials, the per cent ASR at the end of each trial was derived as the percentage of fish using ASR in the final sample interval (always less than 5.3 PO_2).

Table 1. A description of hypoxia tolerance trials for five species groups from Lake Nabugabo, Uganda [*Ln* – *Lates niloticus*, *On*$_L$ – lake *Oreochromis niloticus*, *On*$_W$ – wetland *Oreochromis niloticus*, Haps – endemic haplochromines, *Pm* – *Pseudocrenilabrus multicolor*].

	Ln	*On*$_L$	*On*$_W$	Haps	*Pm*
No. of trials	9	8	5	6	5
Fish per trial	1	1	1	5[†]	5
Total no. of fish	9	8	5	29	25
Individual mass (g)	11.7 ± 10.2	6.5 ± 2.1	4.5 ± 1.6	4.3 ± 2.7	4.3 ± 1.1
Individual TL (cm)	9.34 ± 3.1	7.2 ± 0.6	6.1 ± 0.6	6.4 ± 1.2	6.2 ± 0.6
Trial time (min)[‡]	181 ± 35	151 ± 29	170 ± 30	169 ± 20	179 ± 39
DO drop (mg l^{-1})[‡]	7.4 ± 0.7	7.6 ± 0.4	7.7 ± 0.3	7.1 ± 0.9	7.2 ± 0.6
Temperature (°C)[‡]	20.8 ± 1.4	21.9 ± 0.7	21.4 ± 1.4	21.9 ± 0.9	21.0 ± 0.9
Na$_2$SO$_3$ added (g)[‡]	3.1 ± 0.9	3.2 ± 0.7	3.9 ± 1.9	2.7 ± 0.6	2.8 ± 0.7
Equilibrium loss	Y	N	N	N	N

[†]One group of four individuals was used when one individual died during the acclimation period.
[‡]Mean ± SD. No significant difference between species groups ($P > 0.05$).

Statistical analysis

Prior to testing for differences in hypoxia tolerance among species groups, Pearson correlation analysis was used to detect relationships between body mass (log-transformed) and three ASR traits for each species group. Traits included: ASR$_{10}$ and ASR$_{50}$ thresholds and per cent ASR at the final sample interval. For *P. multicolor* and endemic haplochromines, we used the mean body mass of fish within each trial because trials were run on groups of similarly sized individuals; for tilapias and *L. niloticus*, individual body mass was used because trials were run on individual fish.

Analysis of variance (ANOVA) was used to compare trial conditions (e.g., water temperature, trial length) among species groups to detect any strong deviations from the average experimental conditions. Because of the body-size variation in *L. niloticus* and effects of body mass on some ASR traits (see Results), we used analysis of covariance (ANCOVA) to detect interspecific differences in the ASR$_{10}$ and ASR$_{50}$ thresholds and per cent ASR at the end of each trial, using log-transformed body mass as a covariate. For all three traits, we found no evidence for slope heterogeneity (body mass × species interaction term, $P > 0.05$), therefore the interaction term was removed from the model. To compare the same three respiratory traits between lake and wetland *O. niloticus* groups, we used ANOVA, since body-size variation was small and effects on ASR were not detected (see Results).

Results

Fish distribution

Transect data revealed very distinct species distributions and a strong DO gradient across the wetland habitat (Fig. 1). We found 134 juvenile endemic haplochromines in the ecotone (extending 5 m on either side of the lake-wetland edge), and did not recover any from the swamp interior (from 10 to 100 m inshore). In contrast, *P. multicolor* was found in comparatively low abundance in the ecotone ($n = 31$) and high abundance in the swamp interior ($n = 157$). Extremely few juvenile *L. niloticus* and *O. niloticus* were found in the ecotone, and neither invader was captured inside the swamp. Their absence from minnow traps in offshore sampling sites may be explained by their low density in open-water environments, or perhaps their body size in these habitats simply exceeds that which can be captured by standard minnow traps (minnow traps set nearshore in Lake Nabugabo capture *L. niloticus* between 3 and 13.5 cm TL; L. Chapman, unpublished data).
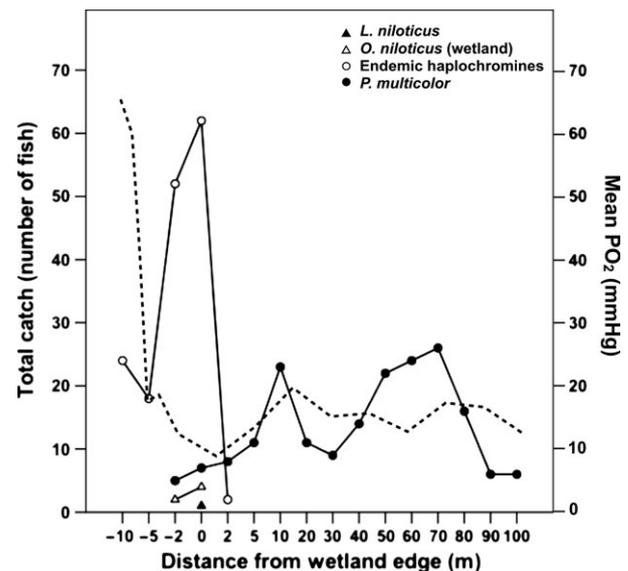

Fig. 1. The distribution and abundance of four fish taxa along a negative dissolved oxygen gradient (dashed line) in the Lwamunda Swamp of Lake Nabugabo, Uganda. Positive distances are indicated from the wetland-lake interface into the wetland, negative distances are from the interface towards the open water of the lake.

ASR behaviour

No differences were detected in trial conditions among species groups, and *L. niloticus* was the only taxon that lost equilibrium during trials (Table 1). For lake-dwelling and wetland *O. niloticus*, endemic haplochromines and *P. multicolor*, no significant relationships were detected between ASR traits (ASR_{10}, ASR_{50}, Percent ASR at final interval) and log-transformed body mass (ASR_{10}: $0.13 < P < 0.89$; ASR_{50}: $0.13 < P < 0.63$; %ASR at final interval: $0.12 < P < 0.85$).

Body-size effects on ASR behaviour were detected for *L. niloticus,* most probably because the size range of individuals was larger than for all other taxa (Fig. 2). We found similar, positive correlations between log-transformed body mass of *L. niloticus* and their ASR_{50} thresholds ($r = 0.81$; $P = 0.008$) and per cent ASR at end of trial ($r = 0.71$; $P = 0.03$); the relationship between body mass and ASR_{10}, however, showed the same trend but was not significant ($r = 0.53$; $P = 0.141$; Fig. 2).

The ASR behaviour of the four taxa differed during their exposure to progressive hypoxia (Fig. 3). *Lates niloticus*, *O. niloticus* and endemic haplochromines all differed from *P. multicolor* in their ASR behaviour, but did not differ from one another (Fig. 4). *Pseudocrenilabrus multicolor* was the most tolerant of hypoxia, exhibiting the lowest ASR_{10} (12.7 PO_2) and ASR_{50} (1.8 PO_2) thresholds, and the lowest percentage (39%) of fish performing ASR in the final sample interval (Fig. 4). The similarly high ASR thresholds and per cent ASR at end of trial for the other three taxa suggest a shared, low tolerance to low-oxygen conditions. Interestingly, we detected no
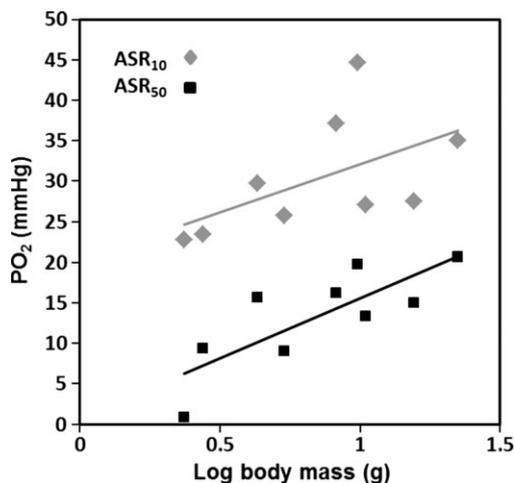

Fig. 2. Relationship between the log-transformed body mass of *L. niloticus* individuals ($n = 9$) and the oxygen levels at which 10% and 50% of fish used aquatic surface respiration (ASR) during hypoxic exposure.

differences in the ASR behaviour of lake and wetland *O. niloticus* groups (ASR_{10}: $F_{df} = 4.48_{2,11}$, $P = 0.058$; ASR_{50}: $F_{df} = 2.62_{2,11}$, $P = 0.134$;%ASR at final interval: $F_{df} = 1.49_{2,11}$, $P = 0.25$).

Discussion

We evaluated relative hypoxia tolerance among taxa using two indices: ASR thresholds and per cent ASR at the final sample interval. Hypoxia tolerance was greatest in *P. multicolor*, a species that persists in high abundance in the deep interior of wetland refugia (Chapman et al. 1996b; Rosenberger & Chapman 1999) and that was numerous across the interior of our transects where recorded levels of DO were extremely low. The low ASR thresholds for *P. multicolor* and the small percentage of fish that used ASR during the final sample interval indicate that this species can withstand hypoxia without continuous use of ASR. In an earlier study of *P. multicolor* from Lake Nabugabo, Rosenberger & Chapman (2000) reported an average threshold of 14.7 PO_2 for ASR_{10} and 8.3 PO_2 for ASR_{50} for fish of a similar size range (0.63–9.68 vs. 2.73–6.46 g in this study). The lower thresholds found in this study, especially at the ASR_{50} level, may be explained by the point of origin of trial specimens. We collected individuals from severely hypoxic waters beyond the marginal swamps, whereas Rosenberger & Chapman (2000) obtained specimens from the upstream regions of the Juma River where oxygen conditions are more moderate (mean DO = 26.5 PO_2; Rosenberger & Chapman 1999, 2000).

Endemic haplochromines, wetland *O. niloticus* and *L. niloticus* all inhabit wetland ecotones, but vary in their abundances and are extremely rare within the swamp interior (Chapman et al. 1996a,b; Rosenberger & Chapman 1999; Schofield & Chapman 1999). Indeed, across our wetland transects none of these taxa were recovered beyond the wetland edge. They shared similarly high ASR thresholds and per cent ASR during the final sample interval, suggesting a common low tolerance to hypoxia. Earlier studies have yielded similar thresholds for the haplochromine cichlid *A. velifer* (ASR_{10}: 27.2 PO_2; ASR_{50}: 19.1 PO_2; Rosenberger & Chapman 2000), captive-bred *O. niloticus* (ASR_{10}: 34.8 PO_2; ASR_{50}: 6.8 PO_2; Chapman & Liem 1995) and lake-dwelling *L. niloticus* from Lake Nabugabo (ASR_{10}: 25.3 PO_2; ASR_{50}: 17.1 PO_2; Schofield & Chapman 2000), over equivalent size ranges for *A. velifer* (0.78–12.87 vs. 0.77–12.46 g in this study) and *L. niloticus* (3.91–27.99 vs. 2.35–22.34 g), however, *O. niloticus* were markedly larger than in the present study (40.07 ± 12.87 vs. 4.5 ± 1.6 g). In their review of the respiratory traits of lake and swamp-dwelling fish in the Nabugabo region, Chapman et al. (2002) demonstrated that
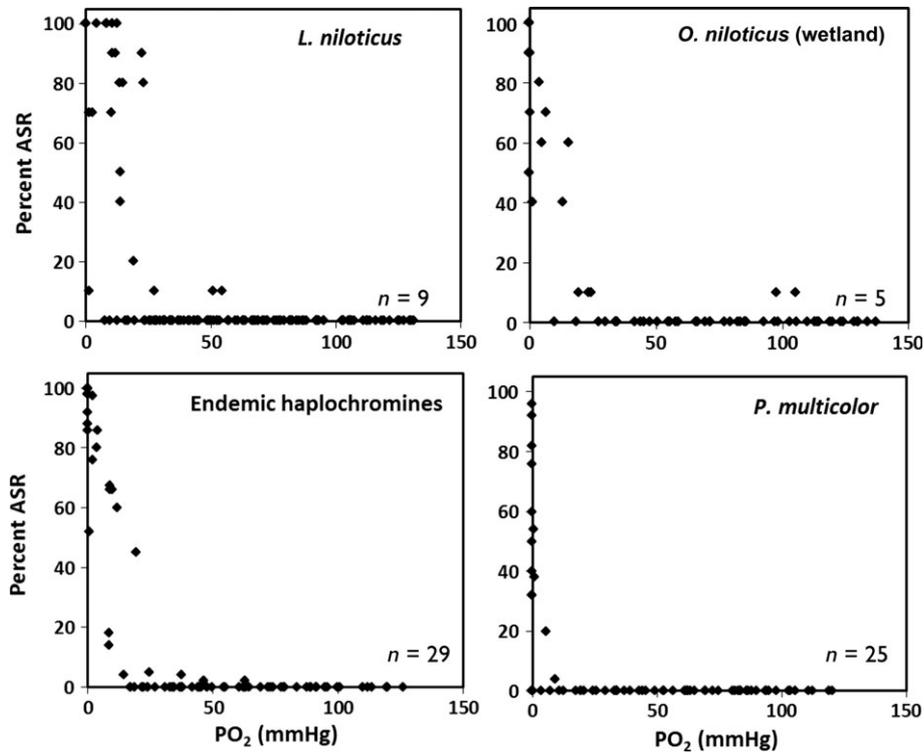
Fig. 3. The aquatic surface respiration (ASR) response expressed as the per cent time using ASR during exposure to progressive hypoxia for four fish taxa from Lake Nabugabo, Uganda.
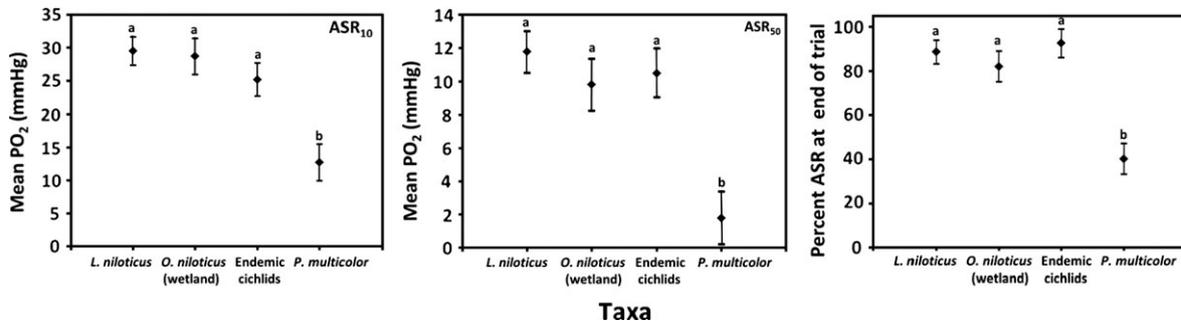

Fig. 4. The aquatic surface respiration (ASR) thresholds of four hypoxia-exposed fish taxa from Lake Nabugabo, Uganda. ASR_{10} and ASR_{50} indicate the oxygen thresholds at which 10% and 50% of fish performed ASR. Values are adjusted means with their standard errors calculated from the ANCOVA analyses (sample means adjusted for a common mean body mass and a common regression line).

the ASR thresholds of endemic haplochromines (*A. velifer* and *P. venator*) were matched by *L. niloticus*, and both were significantly higher than those of *P. multicolor*.

For wetland inhabitants, the chronic hypoxia that characterises the swamp interior of Lake Nabugabo may necessitate prolonged ASR to maintain routine metabolic activity. We found an average DO level of 14.0 PO_2 (±12.5, SD) across the wetland interior, which approaches the level at which juvenile endemic haplochromines, *O. niloticus* and *L. niloticus* spend 50% of their time performing ASR at the water surface. Extended time spent at the air-water interface could increase the risk of aerial predation (Kramer et al. 1983), especially in Lake Nabugabo, a

known hub for hundreds of migratory and resident bird species, including the Pied kingfisher (*Ceryle rudis*) that has been observed feeding intensively in pools within the Lwamunda Swamp (Randle & Chapman 2004; Bikangaga et al. 2007). In this study, juvenile endemic haplochromines, *O. niloticus* and *L. niloticus* were shown capable of handling the marginally hypoxic conditions characteristic of wetland ecotones. It is possible that juveniles avoid the dense interior of the swamp to minimise exposure to the surface. At times when DO levels drop below their ASR thresholds, access to the better-oxygenated main lake may minimise time at the surface.

Intraspecific variation in ASR thresholds has been observed in some fish species that cross strong DO

gradients in the field, including the African cyprinid *Barbus neumayeri* (Olowo & Chapman 1996) and the New World poeciliid *Poecilia latipinna* (Timmerman & Chapman 2004). We therefore predicted that juvenile *O. niloticus* captured from wetland ecotones would exhibit higher tolerance to hypoxia (lower ASR thresholds) than *O. niloticus* captured from open-water sites. However, we found no difference in ASR thresholds or per cent ASR during the final sample interval between the two groups. Both lake-dwelling and wetland specimens, however, exhibited a unique behaviour during trials that may point to a heightened anaerobic capacity in this species. Individuals were observed refraining from ASR as oxygen levels approached extreme hypoxia (<5.3 PO_2) to settle at the base of the experimental aquarium in an apparent inactive state. We propose that *O. niloticus* relies on some other compensatory mechanism to reduce time spent at the surface. Several recent physiological studies have demonstrated an exceptional tolerance to hypoxia in the *Oreochromis* heart (e.g., Farrell & Stecyk 2007; Speers-Roesch et al. 2010; Lague et al. 2012), which may provide *O. niloticus* from both habitats with an alternative to ASR, thus minimising the costs associated with surfacing behaviour (e.g., travel, aerial predation) and providing the ability to exploit both normoxic and hypoxic habitats.

For *L. niloticus*, the strong positive correlations between body mass and both the ASR_{50} threshold and per cent ASR in the final sample interval indicate that larger individuals initiate ASR at higher DO levels than smaller conspecifics, which suggests a lower tolerance to hypoxia. Smaller-bodied individuals may therefore be better equipped to exploit hypoxic environments, and are thus more wetland-resistant. Several studies have found a similar size-dependent tolerance to hypoxia, with smaller fish being more tolerant of low-oxygen environments (e.g., Smale & Rabeni 1995; Robb & Abrahams 2003; Sloman et al. 2006). It has been argued that this size sensitivity may be due to a fractal scaling relationship whereby larger fish are limited by the fixed size of red blood cells for gas exchange and/or a negative allometric relationship for mass-specific gill-surface area (reviewed by Robb & Abrahams 2003). However, the latter argument has been questioned in a recent study by Nilsson & Östlund-Nilsson (2008) that presents a similar scaling relationship between metabolic rate and gill respiratory surface. Although the mechanism remains unclear, evidence for higher tolerance to hypoxia in smaller *L. niloticus* is also supported by Nyboer & Chapman (2012) who explored the movement of individual *L. niloticus* in Lake Nabugabo using radio telemetry. They observed a positive relationship between the total length of their tagged

specimens and concentration of surface DO used by these individuals.

Similar to Schofield & Chapman (2000), we focused our respiratory behaviour study of *L. niloticus* on juveniles captured in open-water inshore zones where we could reliably capture specimens. There is recent evidence to suggest, however, that *L. niloticus* may show strong patterns of habitat-associated ecological divergence in Lake Nabugabo that we did not capture in this study. Paterson et al. (2010) compared the gill size of juvenile *L. niloticus* captured in open-water areas with a small number of juveniles captured from wetland ecotones. They reported larger gills in wetland *L. niloticus* than conspecifics from exposed habitats and suggested that larger gills may facilitate use of wetland ecotones both as refugia from larger *L. niloticus* and as a foraging habitat, since small *L. niloticus* captured near wetland ecotones constitute a high proportion of fish in their diet (Paterson & Chapman 2009; Paterson et al. 2010). In their study of fish distribution in ecotonal wetlands, Paterson & Chapman (2009) captured 16 juvenile *L. niloticus* in 100 minnow traps in the edge habitat (equivalent to our '0' site), a catch per unit effort (CPUE) of one fish per 6.25 traps. We live-captured only one juvenile *L. niloticus* in our transect survey (a CPUE of 1/18 fish per trap set at '0'), precluding a comparison of ASR behaviour between wetland and open-water juveniles, but also suggesting a decline in juvenile *L. niloticus* penetrating the ecotonal wetlands between 2007 and 2011. We speculate that the development of a 'light-capture' fishing technique for *Rastrineobola argentea* in 2011, a method whereby fish are lured into fine-meshed nets using a powerful light source that attracts prey items (e.g., insects), may have driven a decline in *L. niloticus* recruitment in Lake Nabugabo during that period. Qualitative survey of *R. argentea* bycatch at the time of our transect survey revealed a large number of juvenile *L. niloticus*. In any case, *L. niloticus* use of wetland ecotones beyond the edge continues to be extremely rare, but could be facilitated by habitat-specific divergence in hypoxia tolerance, a trend that needs to be at the forefront of studies of habitat refugia in this region.

Aquatic surface respiration is a widespread behavioural response to extreme aquatic hypoxia in fish throughout the globe (e.g., Kramer & McClure 1982; McNeil & Closs 2007). Chapman & McKenzie (2009) provide a global review of species that have been observed using ASR and for which thresholds have been determined ($n = 81$). Comparing the ASR behaviour of the four taxa examined in this study with the thresholds collated by Chapman & McKenzie (2009), it is apparent that these fish from Lake Nabugabo fall within a comparable range as fish from

around the world (Fig. 5). *P. multicolor* exhibits high hypoxia tolerance at a global scale, having an average ASR_{10} threshold in the lower 25th percentile of the distribution, and exhibiting the lowest ASR_{50} threshold of all fish examined. Endemic haplochromines, wetland *O. niloticus* and *L. niloticus*, in contrast, lie in the mid-range of ASR_{10} thresholds, between the 50th and 75th percentile, and cluster closely together towards the lower end of the ASR_{50} spectrum, between the 25th and 50th percentile.

Conclusions

Aquatic surface respiration is a common response in fish exposed to extreme aquatic hypoxia. The four fish taxa examined in this study differed in their use of ASR, but shared relatively high tolerances when compared on a global scale. ASR served as a useful index of hypoxia tolerance as it corresponded strongly to the wetland distribution of taxa in Lake Nabugabo. The finding of higher tolerance in smaller *L. niloticus* specimens than in larger individuals is especially interesting in light of increasing fishing pressure in open-water habitats and the concentration of preferred prey in wetland ecotones (*viz.* haplochromines), which may be exerting a selective pressure for more wetland-resistant *L. niloticus*.
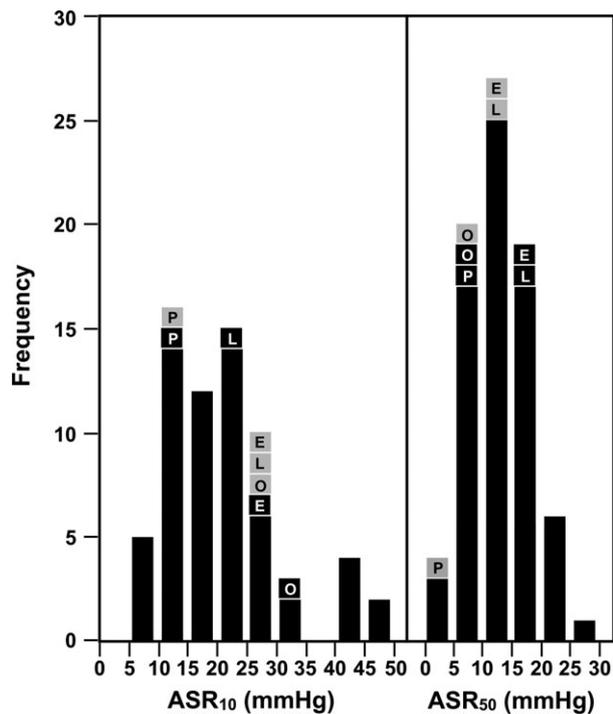

Fig. 5. The aquatic surface respiration thresholds (ASR_{10} and ASR_{50}) for four fish taxa from Lake Nabugabo, Uganda (this study; grey bars) and 81 fish species from around the world (data derived from Table 2.1 in Chapman & McKenzie 2009; black bars). P: *Pseudocrenilabrus multicolor*; E: Endemic haplochromines; O: *Oreochromis niloticus*; L: *Lates niloticus*.

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