Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region ☆

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Abstract

In Lake Nabugabo, Uganda, a satellite of Lake Victoria, $\approx 50\%$ of the indigenous fishes disappeared from the open waters subsequent to the establishment of the introduced predatory Nile perch, *Lates niloticus*. This pattern is similar to the faunal loss experienced in the much larger Lake Victoria. Several of these species persisted in wetland refugia (e.g. ecotonal wetlands, swamp lagoons); however, deep swamp refugia (habitats lying well within the dense interior of fringing wetlands), are available only to a subset of the basin fauna with extreme tolerance to hypoxia. Although air-breathers are common in deep swamp refugia; we also documented a surprisingly high richness and abundance of non-air-breathing fishes. We describe several mechanisms that may facilitate survival in deep swamp refugia including high hemoglobin concentration, high hematocrit, large gill surface area and a low critical oxygen tension (P_c). In addition, swamp-dwelling fishes showed lower P_{O_2} thresholds for onset of aquatic surface respiration than the lake-dwelling fishes. This suggests higher tolerance to hypoxia in the swamp fishes because they are able to withstand a lower oxygen tension before approaching the surface. We suggest that physiological refugia may be important in modulating the impact of Nile perch and indigenous fishes in the Lake Nabugabo region; this highlights the need to evaluate relative tolerance of introduced predators and indigenous prey to environmental stressors.
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1. Introduction

The introduction of the predatory Nile perch (*Lates niloticus*) into the Lake Victoria basin of East Africa has been followed by dramatic changes in fish faunal structure and diversity. In the late 1950s and early 1960s, the Nile perch was introduced into the Lake Victoria basin in an attempt to increase depleted commercial fisheries (Fryer, 1960; Ogutu-Ohwayo, 1990a,b, 1993, 1994). Although many fish stocks declined prior to expansion of the introduced Nile perch population, the dramatic increase in Nile perch in the 1980s in Lake Victoria roughly coincided with a dramatic decline in populations of many species (Ogutu-Ohwayo, 1990a; Witte et al., 1992a,b; Kaufman, 1992; Kaufman et al., 1997). Most notable was the disappearance of over 50% of the non-littoral haplochromine cichlids or approximately 40% of

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the endemic haplochromine cichlid assemblage in Lake Victoria (Kaufman, 1992; Witte et al., 1992a; Seehausen et al., 1997a,b). Many non-cichlid fishes (e.g. some catfishes, Goudswaard and Witte, 1997) also declined or disappeared. Other changes in the Lake Victoria system, including increasing eutrophication and deoxygenation of deeper waters (Hecky, 1993; Kaufman and Ochumba, 1993; Hecky et al., 1994; Seehausen et al., 1997a), may also have contributed to the faunal collapse. Similar changes occurred with the introduction of Nile perch into Lake Kyoga (Ogutu-Ohwayo, 1994), central Ugandan satellite lakes (Kaufman et al., 1997) and Lake Nabugabo (Ogutu-Ohwayo, 1993; Chapman et al., 1996a,b).

This tremendous loss of biodiversity led to studies directed at identification of faunal refugia. Within Lake Victoria, rocky shores and offshore rocky islands serve as important refugia because of their high structural complexity and relatively clear waters that facilitate coexistence of many fish species (Seehausen, 1996, 1999; Seehausen et al., 1997b). Wetland areas also protect prey species by offering both low-oxygen and structural refugia (Chapman et al., 1996a,b; Schofield and Chapman, 1999; Rosenberger and Chapman, 1999). Schofield and Chapman (2000) found relatively low tolerance to hypoxia in Nile perch, which may explain why Nile perch are unable to exploit these habitats.

The community composition of wetland habitats has been well studied in Lake Nabugabo and demonstrates the significance of these habitats in minimizing or eliminating interaction with Nile perch. Lake Nabugabo (31°50′E and 31°56′30″E to 0°20′S and 0°25′5″S) is a small satellite lake (24 km^2) of Lake Victoria, lying within a former bay of Lake Victoria that was isolated from the main lake approximately 4000 years ago (Greenwood, 1965). The lake is surrounded by an extensive wetland, the Lwamunda Swamp. This is a predominantly marsh system with the grass *Miscanthidium violaceum* as the primarily emergent plant. Nile perch were introduced into Lake Nabugabo in the early 1960s. Thirty species of fishes (including five endemic haplochromines) were recorded by a Cambridge Expedition (Cambridge Nabugabo Biological Survey, 1962) that sampled the fish community prior to demographic expansion of the Nile perch population. A survey conducted in 1991–1992 by the Fisheries Resources Research Institute of Uganda, showed that 16 of these species had disappeared or declined in the

main lake waters since the Cambridge Expedition (Ogutu-Ohwayo, 1993).

Despite faunal loss in the open waters, surveys of wetland areas surrounding Lake Nabugabo (1994–1999) showed that over 50% of the species reported rare or missing from the main lake waters could be found in wetland refugia (Chapman et al., 1996a,b). However, there were strong differences in community composition and physico-chemical conditions between wetland ecotones and the dense swamp interior. We refer to habitats in the interior swamp where there is little interaction with the main lake as deep swamp refugia. In ecotonal wetlands, interaction with main lake waters increases dissolved oxygen, allowing relatively high faunal abundance and diversity (Chapman et al., 1996a; Rosenberger and Chapman, 1999; Schofield and Chapman, 1999). Nile perch occur in low densities in or near wetland ecotones, but clearly these habitats minimize interaction between Nile perch and prey, probably due both to moderate hypoxia and high structural complexity. Deep swamp refugia in the Lake Nabugabo region exhibit much more extreme conditions that wetland ecotones. In a survey of small lagoons within the Lwamunda Swamp in the early dry season (May 1999), dissolved oxygen in the upper 20 cm averaged only 0.6 mg l^{-1} ($\approx$11 mmHg) in the early morning and 1.3 mg l^{-1} ($\approx$24 mmHg) in the mid-day period (Fig. 1). In samples taken just above the sediments, dissolved oxygen averaged 0.3 mg l^{-1} ($\approx$5 mmHg) in the early morning and 0.4 mg l^{-1} ($\approx$7 mmHg) in the mid-day (Fig. 1). The lagoons in the Lwamunda Swamp are separated from the main lake by approximately 100–3000 m of dense fringing wetland, and we have never captured Nile perch in any lagoons. Thus, the deep swamp refugia seem to effectively eliminate interaction with Nile perch.

Deep (interior) swamps are clearly important refugia, but are available only to a subset of the basin fauna with tolerance to extreme hypoxia. One would predict a high percentage of air-breathing fishes in the dense swamp interior. However, in quantitative sampling of 12 lagoons in the Lwamunda Swamp over a 3-year period (1997–1999), we found that the majority of fishes (with respect to numerical abundance and species richness) in these extremely hypoxic waters were non-air-breathing fishes (Fig. 2). Our objective in this study was to quantify several indicators of hypoxia tolerance that may be useful in predicting persist-
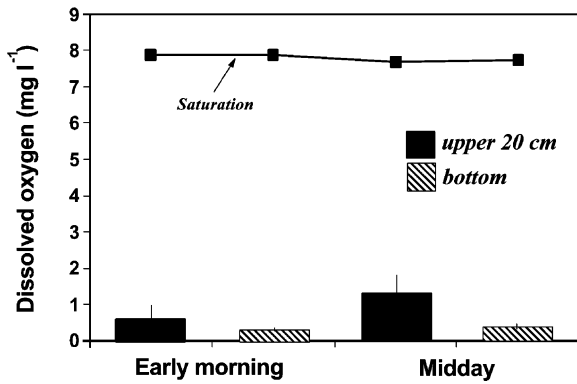
Fig. 1. The mean dissolved oxygen level (mg l^{-1}, ±S.E.) in a series of 12 lagoons in the Lwamunda Swamp that surrounds Lake Nabugabo, Uganda. In each lagoon, dissolved oxygen was measured at a series of microsites in the upper 20 cm of the water column and just above the bottom substrate (for sites greater than 50 cm). Data were collected in May 1999 (early dry season) and averaged for each lagoon. The dissolved oxygen saturation values for each mean were estimated based on the average water temperature of the lagoons in the early and mid-day periods.

ence of non-air-breathing fishes in deep swamp refugia. To achieve this objective, we compared a series of respiratory traits between fishes collected from the dense interior of wetlands in the Lake Nabugabo region to open-water species. We selected a suite of characters that represent the cascade of oxygen from the environment to the respiratory pigments. These included: respiratory behavior; respiratory organ morphology; hemoglobin concentration; hematocrit; metabolic rate; and critical oxygen tension.

2. Materials and methods

This paper synthesizes our previously published data on fishes from the Lake Nabugabo region with our more recent studies. We therefore present general methods and refer to specific publications for details. We consider two major habitat types within the Lake Nabugabo region: lake waters; and deep (interior) swamp refugia. For our study of fishes from the lake waters, we include the open waters of Lake Nabugabo and the nearby Lake Kayanja. The latter system is smaller than Lake Nabugabo, but has a similar morphometry, no Nile perch and a fish faunal assemblage similar to the pre-Nile perch community in Lake Nabugabo. We also include in our open-water (lake) habitat category fishes found in the ecotonal regions because these habitats interact closely with adjacent main lake waters and thus do not exhibit
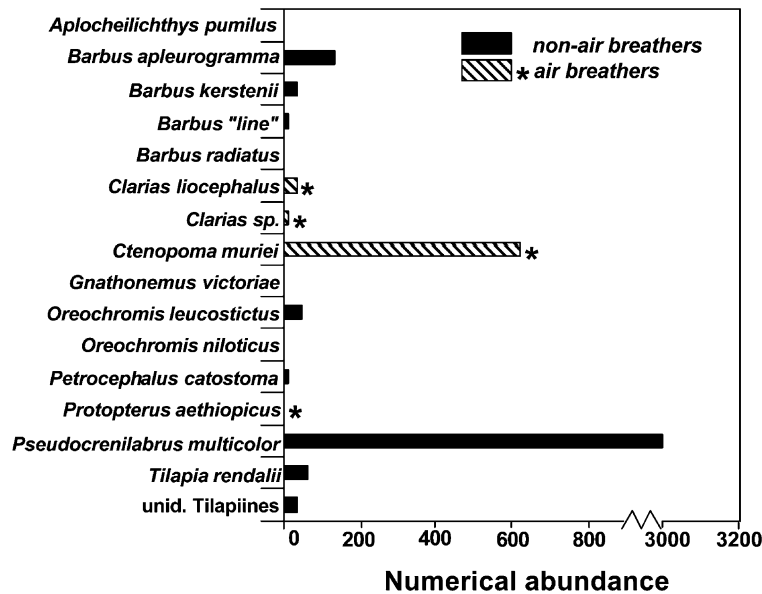

Fig. 2. The numerical abundance of fishes captured in minnow traps in a series of 12 lagoons in the Lwamunda Swamp that surrounds Lake Nabugabo, Uganda. The lagoons were sampled once each summer dry season between 1997 and 1999 using a series of approximately 10 minnow traps set in representative habitats within each lagoon. The numerical abundance of each species represents the total number captured over the 3-year period. All species listed in the figure were captured in the lagoons during the study. Traps captured fishes greater than ≈2.2 cm in total length.

the extreme physico-chemical conditions that characterize the dense interior of surrounding wetlands. For our examination of fishes from deep swamp refugia, we include fishes collected from the lagoons of the Lwamunda Swamp, the papyrus-choked Juma River and the dense papyrus/*Miscanthidium* swamp that surrounds Lake Manywa. The latter system is a small satellite of the larger Lake Nabugabo that has no introduced Nile perch and is surrounded by a very extensive wetland. Although we do not control for phylogenetic constraints in our statistical analyses, we do include fish from more than one lineage in our treatment groups (swamp vs. open water) for most characters considered.

2.1. Respiratory behavior

Many non-air-breathing fishes are known to use aquatic surface respiration (ASR, Kramer and Mehegan, 1981) in response to hypoxia, ventilating their gills with water from the air–water interface where diffusion produces a thin layer of well-oxygenated water (Kramer and McClure, 1982; Chapman et al., 1995). We collated data on the response of six fishes to progressive hypoxia. These included three species from deep swamp refugia (the cichlid *Pseudocrenilabrus multicolor victoriae* and two mormyrids, *Gnathonemus victoriae* and *Petrocephalus catostoma*) and three species from the main lake waters of either Lake Nabugabo (*Astatotilapia velifer*,[1] *Lates niloticus*) or the open waters of nearby Lake Kayanja (*Prognathochromis venator*). *Pseudocrenilabrus multicolor* is a widespread fish in the dense interior of swamps in the Lake Victoria basin, but also occurs in rivers and the open waters of lakes. The two mormyrid species disappeared from the open waters of Lake Nabugabo coincident with the increase in Nile perch, but have persisted as swamp refugees in very localized populations within the Lwamunda Swamp (Chapman et al., 1996b; Chapman and Chapman, 1998). *Astatotilapia velifer* declined in the open waters of Lake Nabugabo

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[1] The genus *Astatotilapia* includes a fluviatile group that forms a monophyletic lineage (Lippitsch, 1993). The lacustrine species previously assigned to *Astatotilapia* are unrelated to the riverine group as has been shown in a study of scale and squamation characters (Lippitsch, 1993). Lippitsch states that it seems probable that the lacustrine species are closely related to other endemic species of the Lake Victoria system rather than to riverine *Astatotilapia* and suggests that new genera be established for these.

following the establishment of Nile perch, but persisted in ecotonal wetlands and other nearshore habitats during the peak of Nile perch abundance in the lake (Chapman et al., 1996a,b; Rosenberger and Chapman, 1999). It is not an inhabitant of deep swamp refugia. *Prognathochromis venator* has been extirpated from Lake Nabugabo but can be found in the open waters of the nearby Lake Kayanja. Nile perch were collected by Schofield and Chapman (2000) from the open waters of Lake Nabugabo. To quantify behavioral response of fishes to progressive hypoxia, fish were held in aerated, large plastic containers. Groups of fish (10 *P. catostoma*; eight *G. victoriae*, *A. velifer*, *P. multicolor* and *P. venator*; and four *L. niloticus*) were transferred to an aquarium ($50 \times 22 \times 30$ cm) for behavioral observations. Oxygen was slowly lowered with the addition of small amounts of sodium sulfite (following Chapman and Liem, 1995) over approximately 3.5 h, and then held at levels averaging < 0.02 mg l^{-1} for 30 min. Lewis (1970) found no observable differences in the behavioral responses of fishes to water freed of oxygen with sulfite and water freed of oxygen by bubbling with nitrogen. A blind positioned in front of the aquarium permitted observations through a small viewing port. At 15-min intervals we recorded the number of fish using aquatic surface respiration (recorded every 10 s for 100 s). Dissolved oxygen concentration and water temperature were also recorded every 15 min. Percent ASR was calculated as the number of fish in a group using ASR divided by the total number of fish, averaged over the 10 observations in a given sample. The level of oxygen at which 10% (ASR_{10}), 50% (ASR_{50}) and 90% (ASR_{90}) of the fish performed ASR was estimated by fitting curves to plots of P_{O_2} and percent ASR. This produced a value for each threshold for each trial, and the number of trials per species ranged between two and eight. Further details are provided in Chapman and Chapman (1998), Rosenberger and Chapman (2000) and Schofield and Chapman (2000). A *t*-test was used to detect a difference in ASR thresholds between lake-dwelling fishes and species from deep swamp refugia, with the mean for each species considered as one independent observation.

2.2. Gill morphometrics

Gill surface area data were derived from the raw data synthesized in Chapman and Chapman

Table 1
Adjusted mean total gill surface area (cm^2, adjusted to a common body mass of 3.4 g and a common regression line using ANCOVA) and linear regression analyses for the relationship between body mass and total gill surface area for five species of fishes from the Lake Nabugabo region of Uganda

Species (family)	Habitat	r^2	P	Adjusted mean TGSA$\pm$S.E. (antilog adj. mean)	Not different from @ $P < 0.05$
Gnathonemus victoriae (Mormyridae) [1]	Lwamunda Swamp	0.96	<0.001	1.53$\pm$0.02 [33.9]	5
Petrocephalus catostoma (Mormyridae) [2]	Lwamunda Swamp	0.95	<0.001	1.40$\pm$0.02 [25.1]	3
Pseudocrenilabrus multicolor (Cichlidae) [3]	Manywa Swamp and Juma River	0.89	<0.001	1.35$\pm$0.01 [22.4]	2
Astatotilapia velifer (Cichlidae) [4]	Lake Nabugabo (ecotone)	0.96	0.003	1.64$\pm$0.03 [43.7]	5
Prognathochromis venator (Cichlidae) [5]	Lake Kayanja	0.99	<0.001	1.56$\pm$0.03 [36.3]	1,4

Both gill surface area and body mass (g) were log transformed, and log values are presented with their standard errors as well as antilogged means. Sidak a posteriori comparisons were run to detect differences among species at $P < 0.05$. The last column indicates species that did not differ from the species identified in the row. Data for *P. multicolor* (Juma River), *Astatotilapia velifer* and *Prognathochromis venator* have been previously synthesized in Rosenberger and Chapman (2000); *P. multicolor* (Manywa Swamp) in Chapman et al. (2000); and *Gnathonemus victoriae* and *Petrocephalus catostoma* in Chapman and Hulen (2001).

(1998), Rosenberger and Chapman (2000), Chapman et al. (2000) and Chapman and Hulen (2001). In this paper, we use two analyses. First, we compare gill surface area between swamp-dwelling populations of three species to two lake-dwelling species. Our swamp-dwelling species included: two populations of the swamp-dwelling cichlid *P. multicolor* (one from the papyrus swamp in the Juma River and other from the dense interior of the Lake Manywa wetland); and two mormyrid species (*P. catostoma* and *G. victoriae*) from the hypoxic lagoons of the Lwamunda Swamp. Our lake-dwelling species included two cichlids: *A. velifer* (Lake Nabugabo); and *P. venator* (Lake Kayanja, Table 1). Second, we compared gill size between swamp and open-water populations for three species to evaluate interdemic variation in gill size. These comparisons included: *P. multicolor* (Manywa swamp vs. Lake Kayanja); *G. victoriae* (Lwamunda Swamp vs. Lake Kayanja); and *P. catostoma* (Lwamunda Swamp vs. Lake Kayanja). All studies used comparable methods. Total gill filament length was measured using standard methods modified after Muir and Hughes (1969) and Hughes (1984). For each fish, the branchial basket was removed, and the four gill arches from the left side of the basket were separated. For each

hemibranch of the gill arches, the length of every 5th gill filament was measured. Two successive measurements along a hemibranch were averaged and multiplied by the number of filaments in the section between the two filaments. Filament lengths were summed for the four hemibranches and multiplied by 2 to produce an estimate of total gill filament length (TFL). Lamellar density (ALD) was measured in the dorsal, middle and ventral parts of every 10th filament of the second gill arch on the left side. For every 10th or 20th filament (depending on the number of filaments), the length of the secondary lamellae were measured a number of times over the filament (Galis and Barel, 1980). Lamellar height was also measured on specimens where it could be accurately determined. Average values of these characters for each filament were converted to estimates of lamellar area using a regression determined through the dissection of 30–60 lamellae from various sections of the second gill arch from two or more specimens for each species. The sum of the total lamellar area for all sections of the second arch was divided by the total number of lamellae and multiplied by 2 to produce a weighted average bilateral surface area on one side of the filament (ALA). Our estimate of total gill surface area

Table 2
The hemoglobin concentration ([Hb], g dl^{-1}) and hematocrit (Hct%) for several species of lake- and swamp-dwelling fishes from Lake Nabugabo and the hypoxic waters of the surrounding Lwamunda Swamp

Species	Air breather	Habitat	[Hb] (g dl^{-1})	N	Mass (g)	Hct (%)	n	Mass (g)
Synodontis afrofischeri (Synodontidae)	No	Lake	7.43 ± 0.05	24	34.0	42.1 ± 0.8	25	34.4
Paralabidochromis beadlei (Cichlidae)	No	Lake	6.35 ± 0.25	15	52.5	38.0 ± 1.6	10	54.1
Oreochromis niloticus (Cichlidae)	No	Lake	7.15 ± 0.14	13	89.5	37.5 ± 1.6	13	71.5
Brycinus sadleri (Characidae)	No	Lake	6.96 ± 0.27	17	12.3	41.8 ± 1.1	14	12.6
Lates niloticus (Centropomidae)	No	Lake	5.93 ± 0.27	7	89.5	36.5 ± 3.6	6	90.1
Clarias werneri (Clariidae)	Yes	Swamp	7.14 ± 0.38	14	21.5	40.2 ± 1.1	17	21.5
Clarias liocephalus (Clariidae)	Yes	Swamp	8.36 ± 0.79	5	31.6	32.9 ± 2.2	6	18.3
Ctenopoma muriei (Anabantidae)	Yes	Swamp	11.66 ± 0.44	3	8.5	38.3 ± 2.2	21	5.6
Protopterus aethiopicus (Protopteridae)	Yes	Swamp	6.86 ± 0.38	8	91.0	27.8 ± 1.6	9	91.0
Petrocephalus catostoma (Mormyridae)	No	Swamp	9.52 ± 0.27	2	2.8	–	–	3.1
Pseudocrenilabrus multicolor (Cichlidae)	No	Swamp	10.94 ± 0.46	7	5.9	45.7 ± 1.5	22	4.7
Oreochromis leucostictus (Cichlidae)	No	Swamp	10.45 ± 0.23	3	9.6	46.7 ± 1.6	16	6.5

(TGA) was calculated using the formula: TGA = TFL×2×ALD×ALA.

We used analysis of covariance (ANCOVA) to compare total gill surface area among species and populations with body mass (total mass of preserved specimens) as the covariate. Variables were log$_{10}$ transformed. Adjusted means and their standard errors were calculated from the ANCOVA analysis, and the a posteriori Sidak test was used to test for significant differences between pairs of species. Palzenberger and Pohla (1992) reviewed literature data available on the gill morphology of fishes. From their data set for 28 non-air-breathing freshwater species, they extracted the mean slope of significant regressions for total gill filament length, lamellar density and gill surface area vs. body mass. They set the lowest and highest mean values within each parameter range to 0 and 100%, respectively, to create a range of values for each gill parameter. This permitted us to express the gill surface area of a species as a percentile within the range of freshwater fishes.

2.3. Hemoglobin concentration and hematocrit

To consider the role of hematological characters in facilitating persistence in deep swamp refugia, we compared blood hemoglobin concentration [Hb] and hematocrit (Hct) among three groups of fishes: three species of non-air breathers from deep swamp refugia; four species of air-breathing fishes from swamp habitats; and five species of fishes from the open waters or ecotonal waters of Lake Nabugabo (Table 2). Sample size averaged 9.8

(range=2–24) for [Hb], and 14.5 (range=6–24) for Hct (Table 2). Fish were live captured in June 2001 and held in coolers of water from the habitat of origin. All hematological samples were collected within 3 days of capture on post-absorptive fish. After being carefully netted from experimental aquaria, the fish was anesthetized with buffered MS 222 (Smit et al., 1979). Blood was immediately taken from the dorsal aorta in the region of the caudal peduncle using two heparinized microhematocrit tubes. For hemoglobin analyses we used either 44.7 or 22.4 μl of blood depending on the size of the fish. Hematocrit analyses were carried out on blood collected in 20-μl tubes. After blood samples were taken, fish were measured (mass and total length).

Hemoglobin concentration [Hb] was measured spectrophotometrically by conversion to the cyanomethemoglobin derivative (Blaxhall and Daisley, 1973; Innes and Wells, 1985; Murad et al., 1990; Brown, 1993). This method has been used reliably by several researchers (Blaxhall and Daisley, 1973; Innes and Wells, 1985) and is well suited to small fish because it requires only very small quantities of blood. To determine the packed cell volume of erythrocytes, within 15 min of sampling, microcapillary tubes of blood were sealed and centrifuged (5000×g) for 10 min to separate plasma from formed elements (Wells et al., 1980; Brown, 1993). A microcapillary reader was used to determine the hematocrit (% erythrocytes).

We used the mean values for each species in our analyses. A linear regression analysis was used to test for a significant relationship between blood

hemoglobin concentration and hematocrit across all 12 species. ANOVA was used to test for a difference in [Hb] and Hct among air breathers, non-air-breathing lake fishes and non-air-breathing fishes from deep swamp refugia. The Scheffe test was used to detect a posteriori differences in [Hb] among the three groups, and the Tamhane's test, which is appropriate when variances are unequal, was used to detect differences in Hct among groups.

2.4. Metabolic rates and critical oxygen tensions

Tolerance to low oxygen may relate in part to the metabolic rate of a fish (Verheyen et al., 1994). Fishes will regulate their metabolic rate over a range of dissolved oxygen concentrations, however, at some point, a further reduction in oxygen tension will produce a shift from a metabolic rate that is independent of oxygen concentration to one that is dependent on oxygen level. This point is referred to as the critical oxygen tension (P_c, Ultsch et al., 1978, 1999). To consider the role of energetics in facilitating persistence in deep swamp refugia, we compared both metabolic rate and P_c between two groups of fishes: six species of non-air breathers from deep swamp refugia (including two separate populations of *P. multicolor*); and seven species of fishes from the open waters or ecotonal waters of Lake Nabugabo (including a lake-dwelling population of *P. multicolor*, Table 3). Each group includes species from more than one phylogenetically distinct fish lineage (Table 3). Sample size averaged 12.1 (range = 5–34) for metabolic rate and 9.7 (range = 3–27) for P_c (Table 3). Metabolic rate and critical oxygen tension estimates for several species in the Nabugabo region were derived from our original unpublished data and from raw data that were previously synthesized in Chapman and Chapman (1998), Rosenberger and Chapman (2000) and Schofield and Chapman (2000). All data were collected under field conditions using comparable methods. Metabolic rate was determined as routine oxygen consumption (rates during random movement under experimental conditions, Saint-Paul 1984) for a range of body sizes for each species. We considered the mean of the decrease in oxygen partial pressure during an interval to approximate the P_{O_2} at which the metabolic rate was measured (Crowder et al., 1998). All calculations of routine metabolic rate were of mean values over a period

of time within which the final oxygen measurement showed a P_{O_2} value that remained equal to or higher than the P_c value. We were also careful to exclude any initial values in a run where there was evidence of an elevated metabolic rate that may reflect disturbance to the probe sealing procedure. Winberg (1956, 1961) derived a standard curve for the relationship between total metabolic rate (at 20 °C) and body size for freshwater fishes and Q_{10} values based on literature data. We adjusted the observed metabolic rates to 20 °C using Q_{10} values in Winberg (1956) to permit comparison among species. The critical oxygen tension (or critical partial oxygen pressure, P_c) was estimated by visual interpretation of the relationship between metabolic rate and P_{O_2} (mmHg) and quantitatively using the SEGFIT program, a BASIC program described by Yeager and Ultsch (1989) designed to fit two regression lines to the data set by least squares method.

The closed respirometer setup was designed for use at remote sites with no electricity. An individual fish was placed in a dark chamber with a battery-operated bubbler at least 1 h before an experimental trial. The chamber was held in a larger water-filled cooler in a shaded facility to minimize variation in water temperature. Water temperature among all of the runs reported in this paper averaged 19.5 °C and ranged among species between 18.1 and 20.4 °C. At the start of each experiment, the chamber was sealed with an oxygen probe (YSI Model 600) connected to a data collection system run on solar power. The meter was programmed to take measurements of water temperature and dissolved oxygen at either 2- or 10-min intervals and to display plotted values throughout the trial. Once the P_c was detected on the computer-generated plots, the experiment was terminated, and the water in the chamber was quickly returned to normoxia. Following each trial, the total length and body mass of the fish were recorded. Metabolic rates were measured on post-absorptive fish within 3 weeks of capture. During this time the fish were held in large plastic containers or small outdoor ponds under normoxia (4–6 mg l^{-1}) and at temperatures that varied between approximately 18 and 21 °C.

We used linear regression to examine relationships between metabolic rate and body size within species. Both metabolic rate and body size were log$_{10}$ transformed. ANCOVA was used to test for differences in bilogarithmic relationship of meta-

Table 3

Species (Family)	Habitat	Log mean MO$_2$ ±S.E. (mg O$_2$ h^{-1}) [antilog mean]	Mean P_c ±S.E. (mmHg)	n	Mean mass (g) (range)	r	P
Synodontis afrofischeri (Mochokidae)	Lake	−0.188±0.045 [0.649]	19.87±2.49	10/10	25.3 (1.53–57.3)	0.79	0.006
Aethiomastacembelus frenatus (Mastacembelidae)	Lake	−0.393±0.048 [0.405]	20.64±2.80	9/9	22.1 (13.0–40.5)	0.96	<0.001
Lates niloticus (Centropomidae)	Lake	−0.050±0.039 [0.893]	26.72±1.72	11/9	8.9 (3.9–28.0)	0.95	<0.001
Astatotilapia velifer (Cichlidae)	Lake	−0.083±0.040 [0.826]	15.98±1.04	10/8	5.4 (0.8–12.9)	0.81	0.005
Pseudocrenilabrus multicolor (Cichlidae)	Lake	−0.426±0.057 [0.375]	13.58±1.56	5/4	4.3 (2.2–8.9)	0.93	0.024
Prognathochromis venator (Cichlidae)	Lake	−0.358±0.042 [0.439]	14.94±1.08	9/9	4.3 (0.7–8.5)	0.82	0.007
Oreochromis niloticus (Cichlidae)	Lake	−0.214±0.040 [0.611]	24.13±3.11	10/8	9.3 (1.4–65.6)	0.96	<0.001
Petrocephalus catostoma (Mormyridae)	Swamp	−0.323±0.032 [0.475]	11.50±0.56	16/15	2.4 (1.4–3.7)	0.70	0.003
Gnathonemus victoriae (Mormyridae)	Swamp	−0.174±0.030 [0.670]	12.46±1.22	18/14	5.8 (1.0–11.9)	0.91	<0.001
Pseudocrenilabrus multicolor (Cichlidae)	Swamp (Juma River and Manywa Swamp)	−0.451±0.028 [0.353]	8.51±0.67	25/17	1.9 (0.6–4.4)	0.65	<0.001
Astatoreochromis alluaudi (Cichlidae)	Swamp	−0.428±0.058 [0.373]	9.50±1.76	5/3	1.9 (0.8–3.7)	0.61	0.279
Oreochromis leucostictus (Cichlidae, juveniles)	Swamp	−0.183±0.022 [0.656]	13.93±0.93	34/27	5.4 (0.9–49.8)	0.86	<0.001
Tilapia rendalli (Cichlidae, juveniles)	Swamp	−0.247±0.052 [0.566]	15.53±1.51	6/4	2.8 (0.8–6.3)	0.99	<0.001

Mean critical oxygen tension (mmHg, ±S.E.), and adjusted mean total metabolic rate (MO$_2$, mg O$_2$ h^{-1}, adjusted to a common body mass (3.9 g) and common regression line using ANCOVA). Both body mass and total metabolic rate were log transformed, and log mean values are presented with their standard errors as well as antilogged means. For each species, the sample size (metabolic rate/P_c), mean body mass and mass range are presented for the total sample, as well as the correlation coefficient and P value for the bilogarithmic relationship between metabolic rate and body mass.
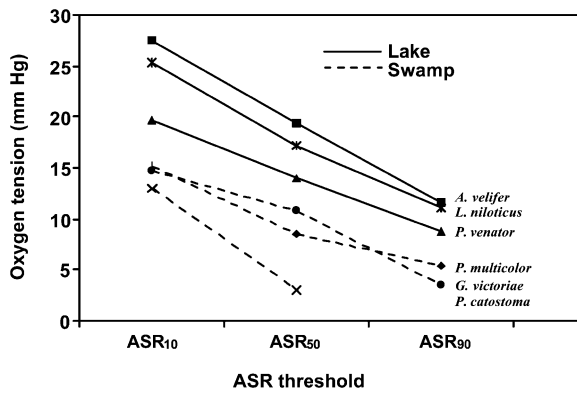
Fig. 3. Aquatic surface respiration (ASR) thresholds (10, 50, 90%) for six species of fishes from the Lake Nabugabo region of Uganda. Three species were captured in the open or ecotonal waters of the main lake and three species were collected in deep swamp refugia. ASR_{90} thresholds could not be estimated for *Petrocephalus catostoma*, because this species always spent less than 90% of its time at the surface. ASR thresholds were derived from Chapman and Chapman (1998) for *Petrocephalus catostoma* and Schofield and Chapman (2000) for *Lates niloticus*. Thresholds for *Astatotilapia velifer, Pseudocrenilabrus multicolor* and *Prognathochromis venator* are abridged from Rosenberger and Chapman (2000).

bolic rate and body mass among the three populations of *P. multicolor*, and ANOVA was used to detect differences in critical oxygen tension among the same three populations. ANCOVA was used to produce adjusted mean metabolic rates for each species. We then used a *t*-test to detect differences in mass-adjusted mean metabolic rate between swamp and lake-dwelling species. Linear regression was used to test for a relationship between critical tension and metabolic rate (bilogarithmic data) across species; and ANCOVA was used to detect differences in P_c between lake- and swamp-dwelling fishes with metabolic rate as the covariate.

3. Results

3.1. Respiratory behavior

All six species (three lake species and three swamp species) used aquatic surface respiration (ASR) in response to progressive hypoxia, however, they differed in the oxygen partial pressure at which they spent 10, 50 and 90% of their time at the surface. The lake-dwelling group of fish species showed higher ASR_{10}, ASR_{50} and ASR_{90} thresholds than the swamp-dwelling group (*t*-tests,

ASR_{10}: $t = 4.08$, $P = 0.015$; ASR_{50}: $t = 3.38$, $P = 0.028$; ASR_{90}: $t = 4.58$, $P = 0.020$, Fig. 3).

3.2. Gill morphometrics

In our interspecific comparison of swamp-dwelling and open-water fishes, all five species (including two populations of *P. multicolor*) showed a positive bilogarithmic relationship between gill surface area and body mass (Fig. 4, Table 2), and all species fell in the upper range for freshwater fishes (Fig. 4). Gill surface area expressed as a range for freshwater fishes varied from the 66 percentile in *P. multicolor* to 98 percentile in *A. velifer* (Fig. 4). There was no difference in either the slopes ($F = 1.65$, $P = 0.217$) or the intercepts ($F = 0.05$, $P = 0.824$) between the two swamp-dwelling populations of *P. multicolor*, so we combined these two populations to test for differences among the five species. The slopes did not differ significantly among the five species (ANCOVA, $F = 1.01$, $P = 0.412$); however, the intercepts did differ ($F = 30.66$, $P < 0.001$), indicating significant variation in adjusted mean gill surface area among species. Interestingly, *P. multicolor*, a widespread swamp-dwelling fish and the swamp-dwelling refugee, *P. catostoma* exhibited the smallest gill
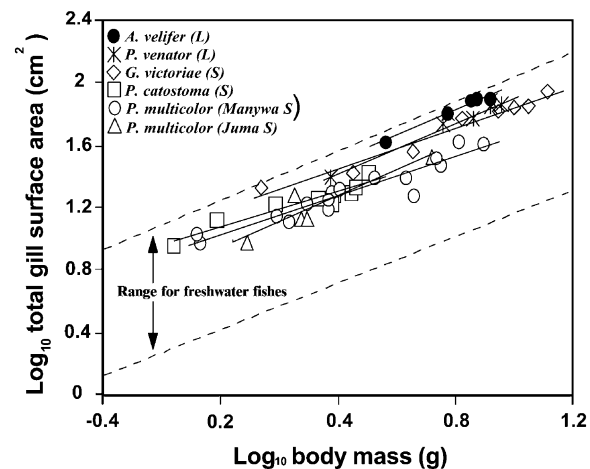

Fig. 4. The bilogarithmic relationship between gill surface area (cm²) and body mass (g) for five species of fishes from the Lake Nabugabo region of Uganda. For *Pseudocrenilabrus multicolor*, we present data for two populations (the papyrus-choked Juma River and the wetland surrounding Lake Manywa). Gill surface area data were derived from the raw data synthesized in Chapman and Chapman (1998), Rosenberger and Chapman (2000), Chapman et al. (2000) and Chapman and Hulen (2001).

surface area of the species considered. The open-water cichlids, *A. velifer* and *P. venator*, and the swamp-dwelling mormyrid *G. victoriae* had the largest gill surface areas (Fig. 4, Table 1).

Although, there was no overall trend for larger gill surface area in swamp-dwelling species, we did find evidence for larger gill size in swamp-dwelling populations than in open-water populations of the same species. We compared the gill morphology of *Pseudocrenilabrus multicolor*, *Gnathonemus victoriae* and *Petrocephalus catastoma* from dense swamps near Lake Nabugabo to populations of the same species from the well-oxygenated open waters of lakes nearby that do not harbor Nile perch. In both *G. victoriae* and *P. multicolor*, gill surface area was significantly larger in the swamp-dwelling populations (Fig. 5). For *P. catostoma* from Lake Kayanja, the gills were contaminated with a high silt load or were cloudy, and we were only able to consider total gill filament length. However, we found gill filament length to be significantly higher in the swamp population than in the open-water population (Fig. 5).

3.3. Hemoglobin concentration and hematocrit

We compared hematological characters among three groups of fishes: (1) three species of non-air breathers from deep swamp refugia (only two species for Hct); (2) four species of air-breathing fishes from swamp habitat; and (3) five species of lake-dwelling fishes. The three groups differed in both hemoglobin concentration (ANOVA, $F = 7.98$, $P = 0.011$) and hematocrit (ANOVA, $F = 5.74$, $P = 0.028$, Table 2). The non-air breathing species from deep swamp refugia, as a group, showed higher levels of [Hb] (Scheffe, $P = 0.013$) and Hct (Tamhane, $P = 0.008$) than the non-air-breathing lake fishes. The air-breathing species, most of which are swamp specialists did not differ significantly with respect to either [Hb] (Scheffe, $P > 0.05$) or Hct (Tamhane, $P > 0.05$) from the lake-dwelling fishes or the swamp-dwelling fishes.

3.4. Metabolic rate and critical oxygen tension (P_c)

There was no difference in the adjusted mean metabolic rate among the three populations (two swamp-dwelling and one lake-dwelling) of *P. multicolor* (slopes: $F = 0.23$, $P = 0.797$; intercepts: $F =$
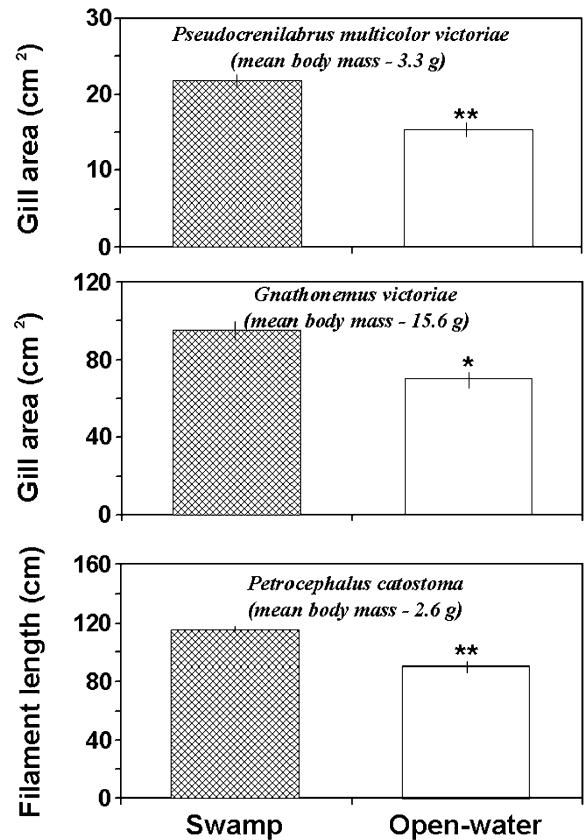

Fig. 5. Adjusted mean gill size ($\pm$S.E.) for open-water and swamp-dwelling populations of three species of fishes from the Lake Nabugabo region. Gill characters were adjusted to the mean size of the two populations for each species using ANCOVA. Data were abridged from Chapman et al. (2000) for the two populations of *Pseudocrenilabrus multicolor* and Chapman and Hulen (2001) for both open-water and swamp populations of the two mormryids (*Petrocephalus catostoma* and *Gnathonemus victoriae*).

0.16, $P = 0.849$); however, critical oxygen tension was higher in the lake-dwelling population (mean $P_c = 13.6$ mmHg) than in the two swamp-dwelling populations (Juma River mean $P_c = 8.1$ mmHg; Manywa Swamp mean $P_c = 9.0$ mmHg, $F = 9.23$, $P = 0.002$, Scheffe, $P < 0.05$). P_c did not differ between the two swamp populations (Scheffe, $P = 0.671$). We therefore combined the two swamp populations for further analyses.

We compared both metabolic rate and P_c between two groups of fishes: six species of non-air breathers from deep swamp refugia; and seven species of fishes from the open waters or ecotonal waters of Lake Nabugabo. All species showed a positive bilogarithmic relationship between meta-
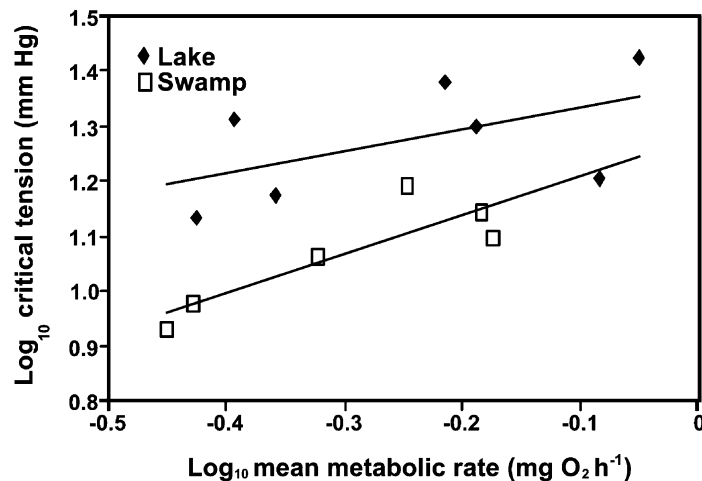
Fig. 6. The bilogarithmic relationship between P_c (mmHg) and metabolic rate (mg O_2 h^{-1}) for seven species of lake-dwelling fishes, and six species of swamp-dwelling fishes (each group contains one population of *Pseudocrenilabrus multicolor*). Metabolic rate and critical oxygen tension estimates for several species in the Nabugabo region were derived from our original unpublished data and from raw data that was previously synthesized in Chapman and Chapman (1998), Rosenberger and Chapman (2000) and Schofield and Chapman (2000). All data were collected under field conditions using comparable methods.

bolic rate and body mass, and this relationship was significant for all but one species (Table 3). ANCOVA indicated no difference in the slopes of the relationships between metabolic rate and body size among the groups ($F = 0.69$, $P = 0.761$). We therefore used ANCOVA to calculate the mean metabolic rates for all groups adjusted to a mean body size of 3.9 g. Adjusted mean metabolic rate ranged from 0.35 mg O_2 h^{-1} in swamp-dwelling *P. multicolor* to 0.89 mg O_2 h^{-1} in Nile perch from the open waters of Lake Nabugabo (Table 3). Overall, there was no difference in adjusted mean metabolic rate between lake- (mean metabolic rate = 0.60 mg O_2 h^{-1}) and swamp-dwelling fishes (mean metabolic rate = 0.52 mg O_2 h^{-1}, *t*-test, $n = 13$, $t = 0.85$, $P = 0.416$). However, it is notable that the two species with the lowest metabolic rates were swamp-dwelling fishes, and the two species with the highest metabolic rates were lake-dwelling species (Table 3), even though the mean metabolic rate of the two groups did not differ.

Critical oxygen tension showed no relationship with body size in 11 of the 12 species considered and no relationship with metabolic rate in 10 of the species considered; mean values ranged from 8.5 mmHg in *P. multicolor* from the Juma River swamp to 26.7 in Nile perch (*L. niloticus*, Table 3). However, across the range of species considered, P_c was positively related to metabolic rate

(bilogarithmic plot, $r = 0.60$, $P = 0.032$). We therefore used ANCOVA to test for a difference in P_c's among lake- and swamp-dwelling fishes with metabolic rate as a covariate. There was no difference between lake- and swamp-dwelling fishes in the slope of the relationship between P_c and metabolic rate ($F = 0.48$, $P = 0.505$), however, the intercepts differed ($F = 14.96$, $P = 0.003$). The adjusted mean P_c was significantly higher in the lake-dwelling fishes (antilog adjusted mean = 18.3 mmHg), than in the swamp-dwelling fishes (adjusted mean = 12.2 mmHg, Fig. 6).

4. Discussion

4.1. Mechanisms compensating for hypoxia in deep swamp refugia

Dense swamps in the Lake Victoria region have been critical refugia for fishes since the expansion of introduced Nile perch. Although they harbor only a small subset of the basin fauna, the extreme conditions that characterize the dense swamp interior seem to eliminate interaction between indigenous fishes and Nile perch. Our results support the idea that low tolerance to hypoxia in Nile perch may be a major factor in explaining this pattern, though clearly the high structural complexity of dense wetlands may also affect the hunting efficiency of the visual predator. Overall, swamp-

dwelling fishes were characterized by higher hematocrit and hemoglobin concentration than lake-dwelling fishes, and both a lower critical oxygen tension and a lower threshold for aquatic surface respiration. Although, there were no overall differences in gill size between swamp and open-water species, we did detect strong differences between swamp-dwelling and open-water populations of the same species.

In our comparison of aquatic surface respiration in six species of fishes from the Lake Nabugabo region, we found that the lake-dwelling species showed higher ASR_{10}, ASR_{50} and ASR_{90} thresholds than the swamp-dwelling fishes. This suggests higher tolerance to hypoxia in the latter group because they are able to withstand a lower oxygen concentration before approaching the surface. ASR is a widespread behavioral response to extreme aquatic hypoxia in fishes (Gee et al., 1978; Kramer and McClure, 1982; Chapman et al., 1994, 1995; Val and de Almeida-Val, 1995; Olowo and Chapman, 1996; Rosenberger and Chapman, 2000), however, the thresholds for aquatic surface respiration for the three swamp-dwelling species (14.3 and 7.4 mmHg for ASR_{10} and ASR_{50}, respectively) were very low relative to other tropical freshwater fishes. Kramer and McClure (1982) found an average ASR_{10} of 21 mmHg and an ASR_{50} of 16 mmHg in their study of 26 species of non-air-breathing tropical fishes. The ASR thresholds for our lake-dwelling fishes (24.2 and 16.9 mmHg for ASR_{10} and ASR_{50}, respectively) fall much closer to the average values in the study by Kramer and McClure (1982).

The averaged dissolved oxygen level in the lagoons of the Lwamunda Swamp during dry season conditions was above the average ASR_{50} thresholds for the swamp dwellers during the day, but not above the average ASR_{10} threshold during the early morning, suggesting that ASR may be required, but only for short periods. ASR may occur more frequently associated with nocturnal oxygen reduction. Increased time at the surface may be energetically expensive, particularly for a species like *P. catostoma* that swims continuously during ASR (Chapman and Chapman, 1998) and may increase exposure to predators (Kramer et al., 1983). Aerial predation on fishes by pied kingfishers (*Ceryle rudis*) is very common in the lagoons of the Lwamunda Swamp (Randle, 2001). Therefore, a low dependence on ASR to cope with extreme chronic hypoxia, particularly during the

day, should not be surprising. In these fishes, a low metabolic rate, a low P_c, high [Hb] and Hct, and relatively large gills may permit a low threshold for ASR minimizing both risk of aerial predation and energetic costs associated with surface swimming.

Gill surface area was high in all species that we considered, and fishes from deep swamp refugia did not show consistently higher values than lake-dwelling species. This pattern may reflect the interaction of environment factors (e.g. hypoxia) and metabolic requirements. For example, the mormyrid *P. catostoma* exhibited a gill surface area that was 26% lower than the second swamp-dwelling mormyrid, *G. victoriae*. Mormyrids are well known for their exceptionally large brains and have a higher brain mass to body mass ratio than do other fishes (Nieuwenhuys and Nicholson, 1967, 1969; Chapman and Hulen, 2001). The large brain size reflects the extraordinary hypertrophy of their cerebellum (Nilsson, 1996), a character that is probably related to their remarkable electrogenic and electroreceptive abilities (Hopkins, 1974; Moller, 1995). These large brains may incur a relatively high oxygen demand (Nilsson, 1996). The metabolic rate of *P. catostoma* was 29% lower than that of *G. victoriae*, and in an earlier study, Chapman and Hulen (2001) found that the brain size of *P. catostoma* was also smaller than *G. victoriae*. It is possible that the much larger gill surface area in *G. victoriae* than in *P. catostoma* may relate to both its higher metabolic rate and larger brain size. Among the cichlids, gill surface area was highest in the lake-dwelling *A. velifer* and lowest in the two swamp populations of *P. multicolor*. Again, this may relate to the metabolic requirements of the two species. The routine metabolic rate in *P. multicolor* was 58% lower than the metabolic rate observed in *A. velifer*. Nonetheless, it is notable that, even in *P. multicolor* and *P. catostoma*, gill surface area falls within the upper range for freshwater fishes. In these two swamp-dwelling fishes, increased oxygen uptake efficiency afforded by large gills may reflect a requirement of the environment, rather than sustaining high activity levels. However, data on the active metabolic rate of these species will be necessary to fully understand the interaction of gill size, metabolic rate and oxygen availability among lake- and swamp-dwelling fishes.

Although there was no overall trend towards larger gill surface area in swamp-dwelling fishes,

our interdemic comparisons of *P. multicolor*, *G. victoriae* and *P. catostoma* from dense swamps near Lake Nabugabo to populations from well-oxygenated waters of nearby perch-less lakes showed gill size (either gill surface area or total gill filament length) to be larger in the swamp-dwelling populations. This suggests that increase in gill surface area may be an adaptive response to hypoxic stress. Interdemic variation in gill size has been reported in other comparisons of populations from hypoxic vs. normoxic habitats. Chapman et al. (1999) found that total gill filament length varied among populations of the African cyprinid *Barbus neumayeri* and was negatively related to the dissolved oxygen availability of the environment. Similarly, Timmerman (2001) reported significantly larger gill surface area in sailfin mollies (*Poecilia latipinna*) from the periodically hypoxic waters of a Florida salt marsh than in a population from the well-oxygenated waters of a nearby river. It is unknown whether this variation in gill size is due to heritable response to selection and/or due to environmentally-induced phenotypic plasticity. However, Chapman et al. (2000) raised the African cichlid *P. multicolor* from a single brood under normoxic and hypoxic conditions to detect plasticity in gill size. Total gill surface area was 18% greater in the hypoxia group demonstrating plasticity in gill size, but the difference between field populations (swamp vs. open water) was greater (29%) suggesting both genotypic and phenotypic effects. Phenotypic plasticity in gill size has also been documented in response to hypoxia in the internal gills (number of gill filaments and filament size) of larval bullfrogs (*Rana catesbeiana*, Burggren and Mwalukoma, 1983) and in the size of gills and shape and structure of gill filaments in two larval salamanders (*Salamandra maculosa* and *Ambystoma jeffersonianum*, Bond, 1960).

Swamp-dwelling species were characterized by a lower average P_c (12.2 mmHg) than lake-dwelling fishes (18.3 mmHg). This reflects high oxygen uptake efficiency even at very low levels of dissolved oxygen for fishes persisting in deep swamp refugia. Based on field data of oxygen availability in the Lwamunda lagoons, these species may only experience levels below their P_c during the night and early morning in some lagoons. Mid-day values fell above the mean P_c. There was no overall difference in the routine metabolic rate between swamp- and lake-dwelling fishes. However, it was notable that the two species with the lowest metabolic rates were from deep swamp refugia, and the two species with the highest metabolic rates were lake-dwelling fishes. In addition, we observed a positive relationship between metabolic rate and P_c among the fishes in Lake Nabugabo. The swamp-dwelling fishes with the lowest P_c's were also characterized by very low metabolic rates. This seems consistent with the idea that fishes of oxygen-poor areas have evolved reduced oxygen demands and improved means of extracting what oxygen is available.

Critical oxygen tension is an integrative character representing the manifestation of many potential physiological and morphological mechanisms. In the swamp-dwelling fishes, a large gill surface area, high hemoglobin and high hematocrit may facilitate the low P_c's observed. The P_c's that we measured in the swamp-dwelling fishes fall below the range of other tropical fishes that inhabit well-oxygenated tropical habitats (Timmerman, 2001). Our values for the two of the open-water fishes (*L. niloticus* and *O. niloticus*) fall within the range of this group, but the P_c's of the other lake-dwelling fishes are lower (Timmerman, 2001). The very low P_c's that we report for our deep swamp refugia fishes may relate, in part, to the use of a closed respirometry system, but may also reflect a strategy for living under chronically low hypoxia. A reduction in P_c in response to hypoxia also occurs as a short-term response to chronic hypoxia acclimation (e.g. *Carassius auratus*, Lomholt and Johansen, 1979; *Poecilia latipinna*, Timmerman, 2001), supporting the role of oxygen availability as one factor that may influence metabolic response to hypoxia.

P_c also differed between populations of *P. multicolor*. The population from the well-oxygenated waters of Lake Kayanja showed a higher P_c than the two swamp-dwelling populations that were examined. This interdemic variation in P_c may reflect differences among the populations in other respiratory traits. For example, the larger gill surface area that we observed in swamp-dwelling *P. multicolor* than in fishes from the ecotonal areas of Lake Kayanja may facilitate a lower tension in the swamp-dwelling fish. Interdemic variation in P_c was also observed by Timmerman (2001) in her study of sailfin mollies (*P. latipinna*). She found a lower P_c in mollies collected from the periodically hypoxic waters of a tidal marsh than

in mollies from well-oxygenated waters of a nearby river.

Blood hemoglobin concentration and hematocrit (%) were higher in swamp-dwelling fishes than lake-dwelling fishes from the Lake Nabugabo region, and this may contribute to the low critical tensions and ASR thresholds that characterized the swamp-dwelling species. Gallaugher and Farrell (1998) derived a standard curve for the relationship between hemoglobin concentration and Hct for fish species that included a phylogenetically diverse suite of species and covered a range of ecological groupings. The derived relationship accounted for 83% of the variation in [Hb] among fishes. The swamp-dwelling species that we considered in our study have high [Hb] and Hct relative to the range for fishes reviewed by Gallaugher and Farrell. However, for the range of Hct values we report, our [Hb] values generally fall below the curve. Gallaugher and Farrell (1998) point out that Hct measured with acute techniques may overestimate Hct values; and this may have contributed to the high Hct/[Hb] ratio that we see in this study. However, many of the species incorporated into Gallaugher and Farrell's line were also measured using acute techniques.

It is noteworthy that two of the swamp-dwelling species that we examined (*P. multicolor* and *P. catostoma*) have relatively low routine metabolic rates that may reflect low activity levels, suggesting that the high [Hb] in both species and the Hct in *P. multicolor* reflects, at least in part, respiratory constraints of their hypoxic environment. Relatively high [Hb] and Hct values in fishes inhabiting hypoxic habitats have been reported in other tropical fishes. For example, Val et al. (1992) reported Hct ranging from 37 to 40% and [Hb] ranging from 10.3 to 11.0 g dl^{-1} for the Amazonian characid *Prochilodus* cf. *nigricans*. This species experiences moderate hypoxia (3 mg l^{-1}) at certain times of the year (Val et al., 1992). In their study of the endemic cichlid fauna of the crater lake Barombi Mbo in Cameroon, Green et al. (1973) found much higher blood hemoglobin concentration (16.55 g dl^{-1}) in a species that periodically fed in the deoxygenated hypolimnion than in 10 other endemic cichlids ([Hb] range=5.55–8.70 g dl^{-1}), that were restricted to the upper lake stratum. Graham (1985) reported a negative relationship between [Hb] and the dissolved oxygen content of the habitat among three populations of the catfish *Hypostomus plecostomus*.

Our hematological data were collected during the early dry season when hypoxia was very severe in the Lwamunda Swamp. It is certainly possible that lower levels of both [Hb] and Hct would be observed in the swamp-dwelling fishes during the rainy season when oxygen levels in the Lwamunda system can be higher (Chapman, Nordlie and Seifert, unpublished data). Short-term increases in both Hct and [Hb] in response to seasonal or acclimation-induced hypoxia have been reported in several non-air-breathing and air-breathing fishes representing a phylogenetically diverse suite of taxa and ecological groupings (Timmerman, 2001). Examples include: Hct in the characids *Prochilodus* cf. *nigricans* (Hct only, Val et al., 1992); [Hb] in the characids *Piabucina festae* (Graham, 1985) and the loricariid catfishes *Hypostomus plecostomus* and *Ancistrus chagresi* (Graham, 1985); Hct and [Hb] in the nototheniid *Pagothenia borchgrevinki* (Wells et al., 1989); [Hb] in the sand goby *Pomatoschistus minutus* (Petersen and Petersen, 1990); and Hct and [Hb] in the poeciliid *Poecilia latipinna* (Timmerman, 2001).

Johansen et al. (1978) noted that many air-breathing fishes from the Amazon have high hemoglobin concentrations and hematocrit relative to non-air-breathing species. Some of the air-breathing species that we examined had high [Hb] relative to some of the lake-dwelling non-air breathers, although the overall differences between the two groups was not significant. Johansen et al. (1978) suggested that the high blood oxygen capacities of many derived air breathers might relate to the need to overcome circulatory inefficiencies; loss of oxygen transport efficiency is associated with shunting of blood draining the air-breathing organs into systematic venous blood before it enters systemic arteries. It is interesting to note that three of the four fishes that we examined were facultative air breathers. The fourth species, the African lungfish, *Protopterus aethiopicus* is a primitive air breather with a partitioning of the circulatory system that results in separation of pulmonary and systemic venous returns (Farmer, 1997). This produces an efficient circulation with less shunting (Johansen et al., 1968, 1978; Graham, 1997). This species showed the lowest [Hb] and Hct of all of the air breathers that we considered, perhaps due in part to its efficient circulatory anatomy.

The suite of respiratory strategies that permit exploitation of deep swamp refugia are complex and interactive, but clearly facilitate persistence under chronic, extreme hypoxia. In light of the devastating loss of species coincident with the introduction of Nile perch into waters of the Lake Victoria basin, the importance of wetlands cannot be overestimated. Deep swamp refugia eliminate interaction between indigenous fishes and piscivorous Nile perch and serve as barriers to the dispersal of the introduced predator.

4.2. Swamp refugia and the seeds of resurgence

The remnant populations of species that have persisted with Nile perch are extremely important because they are the seeds for resurgence should predator pressure be reduced. In some lakes of the Victoria basin and some sections of Lake Victoria, overfishing has reduced the numbers of large Nile perch; this seems to have coincided with a resurgence of indigenous species. In Lake Nabugabo, there has been a dramatic recovery of a subset of the pre-Nile perch fauna that had largely disappeared subsequent to the establishment of introduced Nile perch (Chapman and Chapman, unpublished data). Recovery has been most pronounced in the haplochromine cichlids that have both increased in abundance and moved offshore over the past few years. The resurgence of indigenous fishes that we have seen in Lake Nabugabo represents a picture of the faunal recovery that is evident in other areas of the Lake Victoria basin (Seehausen et al., 1997b; Witte et al., 2000). In areas of the basin where faunal resurgence is evident, it represents a suite of species that have both persisted with Nile perch in habitat refugia and have the reproductive capacity to respond quickly to a change in environmental pressures. Although the habitat derivation of the recovering populations is unclear, key refugia in Lake Nabugabo are likely wetland areas, as rocky boulder refugia are absent in the system. The endemic haplochromine cichlids were largely restricted to inshore areas, primarily wetland ecotones during the pre-resurgence period. Other species that have reappeared in the open waters of the main lake were primarily recovered in deep swamp refugia in the early 1990s (e.g. *P. catostoma*, *G. victoriae*, *Barbus radiatus*, *Barbus kerstenii*, Chapman et al., 1996b). In Lake Victoria, both wetland refugia and rocky refugia are likely to have been key source areas for the resurging faunas.

In summary, for non-air-breathing fishes that inhabit deep swamp refugia in the Lake Victoria region there seems to be a suite of adaptations to the extreme environmental conditions including: high hemoglobin concentration and hematocrit; a large gill surface area; a low P_c; and a low threshold for aquatic surface respiration. The ability of these species to persist under the extreme conditions of the dense swamp interior eliminates interaction with Nile perch. Physiological refugia have clearly been important in modulating the impact of Nile perch on indigenous fish communities, and this highlights the need to evaluate relative tolerance of introduced predators and indigenous prey to environmental stressors.

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