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Adaptive Responses of Branchial Morphology to Hypoxia in the Neotropical Electric Fish Genus Brachyhypopomus

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ADAPTIVE RESPONSES OF BRANCHIAL MORPHOLOGY TO HYPOXIA IN THE NEOTROPICAL ELECTRIC FISH GENUS *BRACHYHYPOPOMUS*

by

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ABSTRACT

Many tropical aquatic environments worldwide are characterized by intermittent or prolonged hypoxia (low dissolved oxygen). Nevertheless, many tropical freshwater fishes are able to inhabit these challenging environments via a range of morphological, physiological and behavioral adaptations. *Brachyhypopomus* is a diverse genus of weakly electric fishes represented by 28 known species distributed from Panama to Argentina. 17 species are restricted to permanently normoxic habitats (blackwater rivers and terra firme streams), eight species are restricted to seasonally or perennially hypoxic habitats (whitewater floodplains of large tropical rivers or permanent swampy habitats), and three species are eurytopic (occur in both seasonally hypoxic and normoxic habitats). These habitat distributions offer the opportunity to explore both species- and population-level variation in adaptive responses to hypoxia. Across 25 of the 28 known species in the genus (for which specimens were available), one- and two-way ANOVA was used to correlate total gill filament length (a metric of gill surface area) with lifestyle – divided into four categories: 1) stenotopic species (i.e. species occurring in a narrow range of habitats) restricted to hypoxic habitats; 2) stenotopic species restricted to normoxic habitats; 3) populations of eurytopic species from hypoxic habitats, and; 4) populations of eurytopic species from normoxic habitats. One-way ANOVA revealed that populations of eurytopic species from hypoxic habitats had significantly larger total gill filament lengths than stenotopic species from the same habitat ($P = 0.0169$). Likewise, populations of eurytopic from normoxic habitats had significantly larger total gill filament lengths than stenotopic species from normoxic habitats ($P < 0.005$). Two-way ANOVA showed that eurytopic species had significantly larger total gill filament lengths than stenotopic species, independent of the disparity in total gill filament length

associated with either hypoxic or normoxic habitats. Results indicate a strong correlation between gill surface area and oxygen-habitat among species and populations, which supports the hypothesis that an enlarged gill surface area increases oxygen uptake and serves as an adaptive response to seasonal hypoxia.

For my parents and husband

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

AIC	Akaike's Information Criterion
AGL	Average Gill hemibranch Length
ASR	Aquatic Surface Respiration
ANCOVA	Analysis of Covariance
ANOVA	Analysis of variance
DO	Dissolved Oxygen
GFL	Grand average Filament Length
PC	Principal Component
PCA	Principal Component Analysis
TGFL	Total Gill Filament Length
TGSA	Total Gill Surface Area
THA	Total Hemibranch Area
TNF	Total Number of Gill Filaments
TP	Total perimeter of hemibranchs

CHAPTER 1: INTRODUCTION

Most animals are highly dependent upon the availability of oxygen in their environment. In atmospheric air, oxygen is freely and constantly available – declining to the point that it poses physiological challenges only at high altitudes (Dejours 1981). In contrast, the concentration of oxygen dissolved in water (dissolved oxygen, DO) can fluctuate greatly – even over short periods of time. DO concentration is determined by multiple processes, including levels of diffusion from the atmosphere, oxygenesis by photosynthesis, and consumption by plant, animal, and microbial respiration (Dejours 1981; Graham 1990). Further, with increasing temperature, water becomes saturated at lower concentrations of oxygen (Graham 1990). In some aquatic habitats, DO concentrations are highly variable, and may decline to a point at which they pose physiological challenges (hypoxia), or become depleted entirely (anoxia). Hypoxia poses extreme selective pressures that have resulted in a variety of adaptations in aquatic animals (Val 2000; Bickler and Buck 2007). Because of the variability of oxygen concentrations in water, and because oxygen is a critically limiting resource, adaptations to hypoxia in aquatic animals have been used as model systems for understanding natural selection and adaptive evolution – both at the level of species, and at the level of populations (Kramer and McClure 1982; Almeida-Val et al. 1993; Almeida-Val and Farias 1996; Val et al. 1998; Chapman et al. 1999; Chapman and Hulen 2001; Diaz and Breitburg 2009).

The freshwater habitats of the tropics are generally characterized by much lower DO concentrations than cooler temperate systems. This is not only because water is saturated at lower DO concentrations in warmer water, but also because tropical waters typically receive large inputs

of organic material (especially in forest systems), which further deplete oxygen concentrations when subjected to microbial decomposition (Carter and Beadle 1930; Lowe-McConnell 1975; Junk and Furch 1985; Hamilton et al. 1995, 1997, 2004). However, in many tropical rainforest systems, such as the Amazon, oxygen regimes can vary dramatically among habitats, even over the geographical scale of a few kilometers (Junk 1983; Crampton 1996; Val 1996). Some habitats, such as large rivers or small rainforest streams, are typically permanently close to saturation (usually 3-7 mg l^{-1}) or normoxic; while other habitats, such as floodplain water bodies or swamps, exhibit intermittent or prolonged seasonal anoxia (unmeasurable DO concentrations), or hypoxia (My working definition of hypoxia in this thesis is DO concentrations below 0.5 mg l^{-1}) (Val and Almeida-Val 1995; Crampton 1996; 1998a). Nonetheless, despite the heterogeneity of DO in tropical river basins, fish diversity is exceptionally high. For instance, the Amazon basin may host as many as 4,000 species (Vari and Malabara 1998; Lundberg et al. 2000; Reis et al. 2003) and the Congo at least 650 (Lowe-McConnell 1993).

The distributions and the diversity of many tropical fish lineages are strongly affected by dissolved oxygen (Saint-Paul and Soares 1987; Chapman and Liem 1995; Crampton 1998a, b; McKinsey and Chapman 1998; Chapman et al. 2002; Kobza et al. 2004). Several studies have also documented the influence of DO on the structuring of local or regional tropical fish assemblages (Carter and Beadle 1930; Carter 1955; Junk 1983; Crampton 1996; Crampton 1998a; Petry et al. 2003). For instance, many fishes avoid hypoxic floodplain environments by undergoing temporary migrations into neighboring normoxic rivers (Val and Almeida-Val 1995; Fernandes 1997), although when oxygen levels decline rapidly, these migratory fishes may not have time to escape to neighboring systems – leading to mass fish kills (Henderson and Robertson 1999; Townsend and

Edwards 2003). However, there are tremendous advantages for fishes that are able to persist in these challenging environments – which are typically rich in food resources and relatively free of large piscivorous fishes (many of which are themselves excluded by an inability to tolerate hypoxia).

Tropical floodplain fishes exhibit a wide variety of physiological, behavioral, and morphological adaptations in response to hypoxia (Almeida-Val et al. 1993; Val and Almeida-Val 1995; Val 2000; Bickler and Buck 2007; Diaz and Breitburg 2009). Physiological adaptations include a reduction in metabolic rate (Guppy and Withers 1999; Bickler and Buck 2007), elevation of blood hemoglobin and hermatocrit concentrations (Martinez et al. 2004), and transitions to anaerobic cellular respiration (Wasserma et al. 1973). Behavioral adaptations include movements away from localized pockets of hypoxia, reductions in activity levels, and "aquatic surface respiration" (ASR), in which fishes move to the surface and channel the superficial layer of the water column across their gills; this surface skin is usually rich in oxygen, even in otherwise anoxic waters (Kramer and Mehegan 1981; Kramer and McClure 1982; Chapman et al. 1995; Chapman et al. 2002). ASR has evolved independently in many Neotropical fish taxa, including osteoglossiforms, characiforms, and cichlids (Val 1996; Chippari-Gomes et al. 2003). Many fishes also possess suites of morphological adaptations to hypoxia, foremost among these being accessory air breathing structures, which include modified swimbladders, stomachs, pharynxes, buccal cavities, and skin. These accessory structures are sometimes used in conjunction with conventional gill breathing (facultative air-breathing), but represent the dominant gas-exchange surface in obligate air breathers, which die if they cannot reach the surface to breathe air (reviews in Graham 1997; Chapman and McKenzie 2009). An expansion of the respiratory surface area of the gills is

another common morphological adaptation to hypoxia (see 1.1 Gill morphology and dissolved oxygen availability, below) (Hughes and Morgan 1973). Although gills are usually used to extract oxygen from water, some fishes use their gills to breathe atmospheric oxygen (Carter and Beadle 1931). These include *Symbranchus marmoratus* (Johansen 1966), the common eel (*Anguilla anguilla*) (Berg and Steen 1965), and also some species of Neotropical electric knifefishes (Johansen 1970; Crampton 1998a).

1.1 Gill morphology and dissolved oxygen availability

The larger the surface area of the gills relative to body size, the more oxygen can be extracted from the environment per unit time, all else being equal (Palzenberger and Pohla 1992; Nilsson and Sundin 1998; Fernandes and Mazon 2003). Larger gill surface areas are therefore expected in fishes that frequent hypoxic environments (Chapman et al. 2000, 2002,2007, 2008). Studies of adaptations of gill surface area to oxygen availability in fishes have focused on three broad questions. First, is gill surface area correlated to DO regime (generally divided for simplicity into hypoxic versus normoxic) among different species (Galis and Barel 1980; Chapman and Hulen 2001; Chapman et al. 2002; Crampton et al. 2008)? – i.e. do species from hypoxic environments have larger gills than those in normoxic environments, when corrected for body size? Second, is there intraspecific variation in gill surface area among populations that occur in habitats characterized by different DO regimes (Chapman and Liem 1995; Chapman et al. 1999, 2007; Timmerman and Chapman 2004: Paterson et al. 2010)? Third, is there evidence for developmental plasticity of gill morphology - i.e. do individuals that are raised in hypoxic conditions develop larger gills than those in normoxic

conditions (e.g. Chapman et al. 2000, 2007, 2008; Saroglia et al. 2002)?

Although many studies have explored the first of the questions above (do species that occur in hypoxic habitats have larger gill surface areas than those in normoxic habitats?), no study has compared gill surface area between closely related species that are eurytopic (i.e. occur in both hypoxic and normoxic habitats) and those that are stenotopic, i.e. occur exclusively in either hypoxic or normoxic habitats. For instance, do populations of eurytopic species that occur in hypoxic habitats exhibit gill surface areas similar to stenotopic species that are restricted to hypoxic habitats? And, likewise, do populations of eurytopic species that occur in normoxic habitats exhibit gill surface areas similar to stenotopic species in normoxic habitats? Alternatively, do populations of eurytopic species always exhibit large gill surface areas regardless of oxygen regime? Or do populations of eurytopic species exhibit intermediate gill surface areas between those of stenotopic species in hypoxic habitats and stenotopic species in normoxic habitats? These questions have so far gone unanswered, and yet exploring how trait conditions in eurytopic species compare to species that are limited to hypoxic or normoxic environments is a valuable exercise because it may shed light on the evolutionary lability of traits at both the intraspecific and the interspecific level.

Studies of adaptations of gill morphology to hypoxia in tropical fishes have also focused almost entirely on African fishes – both in interspecific comparisons (Galis and Barel 1980; Chapman and Hulen 2001; Chapman et al. 2002) and in population-level studies (Chapman and Liem 1995; Chapman et al. 1999; Chapman et al. 2000; Schaack and Chapman 2003; Paterson et al. 2010). In contrast, relatively few studies have utilized Neotropical freshwater fishes, despite their considerably higher species diversity, the abundance of hypoxic and normoxic ecosystems in Neotropical waters, and the increasing availability of species-level taxonomic revisions (formerly a

major constraint on comparative studies of morphology) and ecological studies. Exceptions include Mazon et al. (1998), which considered two riverine Neotropical species (a species of Sciaenidae and a species of Prochilodontidae), and Crampton et al. (2008), which compared four species of the electric knifefish *Brachyhypopomus* (two from seasonally hypoxic habitats and two from permanently normoxic habitats in the Central Amazon of Brazil).

1.2 Model taxon *Brachyhypopomus*

This thesis seeks to explore correlations between DO regime and gill morphology among members of the Neotropical freshwater electric knifefish genus *Brachyhypopomus* (Teleostei, Ostariophysi, Gymnotiformes) (See Figure 1 for a photograph of a representative of the genus). Here I go beyond the simple four-species study of Crampton (2008) to embrace 25 of the 28 known species of *Brachyhypopomus,* including representatives distributed across the entire range of the genus, from Panama to Uruguay. *Brachyhypopomus* are small (mostly < 30g), nocturnally active, cryptically pigmented predators of aquatic invertebrates in lowland lakes, streams and river margins, and often form species-rich (10+ species) local communities. Unlike other Neotropical fishes, gymnotiforms possess a combined electrogenic and electrosensory system, which permits both the location of objects in the dark, and also communication (Bullock et al. 2005). Of the 28 species that are currently known (Table 1), the majority occur exclusively in the tropics. Only three species (*B. bombilla*, *B. draco,* and *B. gauderio*) occur partially or exclusively in sub-tropical habitats, to as far south as the Rio de La Plata, Uruguay (Crampton 2011).

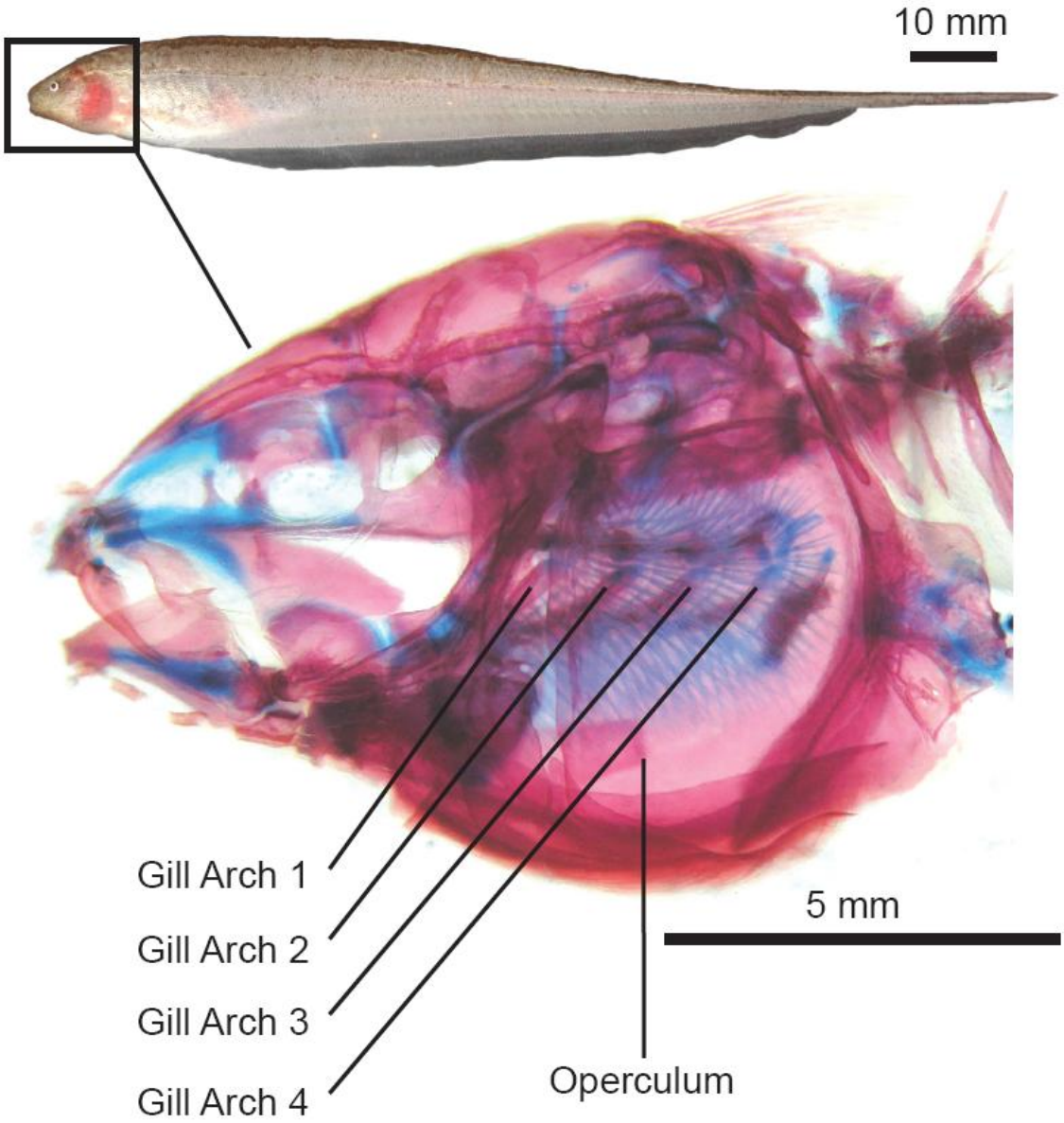

Figure 1. Location of the four gill arches in a specimen of the gymnotiform electric fish *Brachyhypopomus pinnicaudatus* from the Central Amazon of Brazil. Upper photograph shows a live specimen: note that the gills are visible as a red patch through the thin operculum. Lower photograph shows a cleared and stained preparation of the cranium, with bone stained red and cartilage stained blue. The cartilaginous gill filaments attached to the bony gill arches are visible through the opercular bone.

Brachyhypopomus is ideally suited for comparative studies of respiratory morphology for a number of reasons. First, the genus is likely monophyletic (pending the outcome of an ongoing systematic revision, de Santana, Crampton, and Lovejoy in prep.). Second, it exhibits relatively high species diversity (11 species described and 17 more in preparation for description; Crampton and de Santana in prep.). Third, the genus is morphologically exceptionally conservative – exhibiting no obvious morphological variation in jaw structure, or the surrounding elements, associated with a diversity of feeding habits. Therefore, variation in gill morphology is unlikely to be a consequence of incidental correlations with ecological selection on jaw morphology (or other cranial morphological suites). Fourth, some members of the genus are known to occur exclusively in hypoxic habitats, and others exclusively in normoxic habitats, while some are known to be eurytopic (Crampton 2011). Fifth, *Brachyhypopomus* species are known to spend their entire life-cycle near their natal sites, without undertaking lateral or up-river migrations through multiple habitats (Crampton 2011); this simplifies an understanding of correlations between habitat and gill morphology. Finally, due to the extremely wide geographical range of the genus, complex geomorphological history of aquatic systems in the Neotropics, and the polyphyletic structure of local assemblages in all lowland Neotropical fish assemblages (Albert and Reis 2011), there have likely been opportunities for multiple independent evolutionary transitions between hypoxic and normoxic habitats – which will ultimately permit phylogenetically independent comparative tests of correlations between character state and habitat. Moreover, like many Neotropical taxa, gymnotiforms are a relatively ancient group. They diverged from their sister taxon, the Siluriformes (catfishes), in the Jurassic, and molecular clocks and a (scant) fossil record (Gayet et al. 2001) indicate that they probably attained phenotypic modernity by at least the Miocene (Lundberg 1998;

Reis 1998; Crampton 2006). Paleoecological studies have also demonstrated that the division of the lowland Amazon basin into permanently normoxic and seasonally hypoxic environments has persisted at least since the Miocene, when formations ascribable to modern whitewater floodplains were clearly present, as were formations resembling contemporary terra firme stream systems (Nuttall 1990; Hoorn 2006; Wesselingh 2006; Crampton 2011). Hence, there has been ample evolutionary time for oxygen to act as a selective pressure on species of *Brachyhypopomus* – and ample time for these pressures to shape community composition, ecological distributions, and morphological adaptive responses.

There is an additional, final, reason to explore correlations between gill morphology and dissolved oxygen in electric fishes, and specifically *Brachyhypopomus*. All gymnotiforms are nocturnal (Steinbach 1970) – which means that their period of peak activity coincides to a period of rapidly declining oxygen levels, when aquatic vegetation switches from photosynthesis to respiration. Hence, unlike diurnally active fishes, gymnotiform fishes are unable to escape the persistently anoxic conditions in floodplains by simply seeking local pockets of normoxia during their periods of activity. Adaptations to tolerate hypoxia are therefore expected to be especially critical to the ecological success of electric fishes in these environments. This is especially the case in the highly oxygen-depleted floodplains of major whitewater rivers where *Brachyhypopomus* is common in "floating meadows" of macrophytes. Dissolved oxygen concentrations in floating meadows during the high water season decline immediately after sunset to unmeasurable levels and remain so until the next day (Crampton 1998, 2008, 2011).

Some aspects of the respiratory biology of *Brachyhypopomus* have already been studied. Based on field observations, Crampton (1998a) noted that several *Brachyhypopomus* species that

inhabit whitewater floodplains employ their gills not only for extracting oxygen from water, but also as an air breathing structure. Crampton (1998a) noted two forms of aerial respiration: "air-gulping" and "skulking", both of which are employed as oxygen levels approach depletion, and also during anoxic conditions. In air-gulping, bubbles of atmospheric air are forced into the gill chamber. The fish then retreats from the surface and oxygen is absorbed through the gills over the course of a few minutes while the fish continues with its normal activity. In air-skulking, the individuals wedge themselves into vegetation near the surface and/or inflate their opercular chambers with air and hang from the surface of the water with their mouths open. The gills are constantly irrigated with air during this behavior, and are kept moist by periodic submersion. In both forms of aerial respiration, the important point is that gills serve as the air-breathing organ, and this adaptation supplements oxygen intake. In species that undertake this form of air-breathing, a large gill surface not only should permit increased oxygen uptake during normal underwater gill respiration, but should also enhance air breathing performance during air-gulping and skulking. Crampton (1998a) also conducted an experiment in which DO was depleted from an aquarium by controlled addition of sodium sulfite. Seven of eight species from whitewater floodplains of the Tefé region were able to survive a decline in DO from hypoxia (defined in this paper as 0.5 mg l^{-1}) to anoxia ($\approx$ 0 mg l^{-1}) followed by several hours of uninterrupted anoxia. Of these seven species only one, *B. brevirostris*, was unable to survive these conditions by air breathing. This species instead switched off its electric signal, reduced the rate and depth of opercular ventilation to almost imperceptible levels, and went into a state of apparent metabolic quiescence.

The one species (*B.* n. sp. REGA) that was unable to survive gradual hypoxia exposure in addition to several hours of anoxia, appeared to exhibit a form of aquatic surface respiration

(described earlier in the chapter), but not air breathing. Crampton (1998a) also noted that the most abundant species in hypoxic environments exhibited the least amount of reduction in their electrical and motor activity during hypoxic conditions. Nonetheless, during complete anoxia, all species exhibited a severe reduction of their activity and electric organ discharge (EOD) pulse rate (sometimes extending to a complete cessation of the EOD).

In a second study, Crampton et al. (2008) compared gill surface area from two species that occur only in seasonally hypoxic whitewater floodplain systems (*B.* n. sp. BENN and *B.* n. sp. FLAV), with two species that occur only in normoxic blackwater systems (*B.* n. sp. HEND from blackwater lakes, and *B.* n. sp. ROYE from blackwater streams). These authors noted that gill surface areas in the species restricted to hypoxic habitats are substantially larger than those from normoxic habitats.

1.3 Objectives

Here I present the first comprehensive study of species-level variation in gill morphology involving more than just four congeners – embracing instead a much broader diversity of species from a species-rich genus: 25 of the 28 known species in the Neotropical electric fish genus *Brachyhypopomus*. This study is also one of the first to explore gill morphological adaptations in a Neotropical taxon, and the first to encompass a variety of ecosystems and geographical distributions from the Neotropics. This thesis is organized around three key questions:

1. Do species of *Brachyhypopomus* that are restricted to hypoxic habitats, when corrected for body

size, exhibit larger gill surface areas than species restricted to normoxic habitats? Such a disparity would be consistent with the hypothesis that an enlarged gill surface area facilitates enhanced oxygen uptake. I will address this question in Chapter 2.

2. For eurytopic species of *Brachyhypopomus* (i.e. those that occur in both hypoxic and normoxic habitats), do *populations* from hypoxic habitats exhibit larger gill surface areas than *populations* from normoxic habitats? Again, such a disparity would be consistent with the hypothesis that an enlarged gill surface area permits increased oxygen uptake and arose as an adaptive response to hypoxia – either by local adaptation, or by phenotypic plasticity during ontogeny at the population-level. I will address this question in Chapter 3.

3. Do populations of eurytopic species of *Brachyhypopomus* that occur in hypoxic habitats exhibit gill surface areas that approximately match those of *stenotopic species* (that is, those present in only one oxygen habitat) that are restricted to hypoxic habitats, or do they have larger (or smaller) gill surface areas? Likewise, how do the gill surface areas of populations of eurytopic species that occur in normoxic habitats compare to the gill surface areas of stenotopic species that are restricted to normoxic habitats? I will address these question and the implications of the outcomes in Chapter 3.

CHAPTER 2: INTERSPECIFIC VARIATION IN GILL SURFACE AREA IS CORRELATED TO DISSOLVED OXYGEN IN THE NEOTROPICAL ELECTRIC FISH GENUS *BRACHYHYPOPOMUS* (GYMNOTIFORMES: HYPOPOMIDAE)

2.1 Introduction

Several authors have noted that hypoxia can act as a divergent selective pressure, generating interspecific diversity of gill surface areas in fishes (see Gibbs and Hurwitz 1967, for stomiatoids; see Mazon et al. 1998, for a species of Sciaenidae and a species of Prochilodontidae; see Chapman and Hulen 2001, for Mormyridae). Generally, species or populations that occur in poorly oxygenated environments exhibit larger gill surface areas, and these are hypothesized to have evolved as an adaptation to improve the efficiency of oxygen uptake (Palzenberger and Pohla 1992; Nilsson and Sundin 1998; Fernandes and Mazon 2003). Larger gill surface areas are therefore expected in fishes that frequent hypoxic environments, and indeed numerous studies have observed such patterns (Gibbs and Hurwitz 1967; Galis and Barel 1980; Chapman and Liem 1995; Chapman et al. 1999, 2000, 2007, 2008; Chapman and Hulen 2001; Timmerman and Chapman 2004; Crampton et al. 2008; Paterson et al. 2010).

Nonetheless, gill surface area may also be under selection from other environmental factors, notably temperature (Hughes 1966; Mangum et al. 1978; Potts 1984; Palzenberger and Pohla 1992; McDonald et al 1991; Sollid et al. 2003). Like most ectotherms, teleost fishes increase their metabolic rate at higher water temperatures (Crawshaw 1984; Lemons and Crawshaw 1985; Johnston and Dunn 1987; Claireaux and Lagardère 1999). The greater demand for oxygen can be met by increasing gill surface area (as shown in crucian carp and

gold fish, Sollid et al. 2005; Sollid and Nilsson 2006). At lower temperatures, a large gill surface area is less critical for gas exchange because: first, oxygen dissolves into water at much higher concentrations (Dejours 1981; Graham 1990), and second the demand for oxygen is reduced commensurate with the decline in metabolic rate (Crawshaw 1984; Lemons and Crawshaw 1985). An additional influence on gill surface area is activity level (Gray 1954; Hughes 1966; Palzenberger and Pohla 1992) – active moving species typically exhibit larger gill surface areas than more sedentary species (Gray 1954; Hughes 1966). Nonetheless, despite the importance of temperature, Chapman (2007) concluded that among oxygen, temperature, water flow, and depth, oxygen is usually by far the most significant predictor of gill surface area.

Other aquatic parameters that may co-vary strongly with dissolved oxygen in Neotropical waters and yet have independent influences on gill surface area include pH and conductivity (Crampton 2011). Low pH is predicted to favor small gill-size in fishes, because ion channels involved in the osmoregulatory passage of sodium and chloride ions are impeded under conditions of extremely low pH (Potts 1984; Booth et al. 1988; Freda and McDonald 1988; McDonald 1983). Nonetheless, empirical studies have yet to demonstrate that low pH favors small gill surface areas. For instance, McDonald et al. (1991) found no correlation between gill lamellar surface area and environmental pH in natural populations of five temperate freshwater fish species. Nonetheless, none of these environments involved the exceptionally low pH conditions (< pH 4) found in Amazonian blackwater habitats. Low conductivity is also predicted to influence gill size because low conductivity (i.e. low dissolved salt content), in theory, introduces a greater osmotic differential between the external and internal environment of a fish, such that smaller gills in principle should be

favored to limit excessive water-gain by osmosis (Mangum et al. 1978; Potts 1984). Nonetheless, while low conductivity conditions have been shown to affect gill morphology by increasing chloride cells in rainbow trout (Perry 1997, 1998), this change occurs at the cellular level and has, as yet, been shown to affect gill surface area independently of other environmental variables.

In this chapter I explore interspecific variation in gill surface area in relation to dissolved oxygen in the Neotropical electric fish *Brachyhypopomus*. All studies involving interspecific comparisons of gill surface area in relation to DO to date (e.g. Gibbs and Hurwitz 1967; Fernandes et al. 1994; Chapman and Hulen 2001; Crampton et. al 2008), have only used a small number of species from the same taxonomic group (< 5). The objective of this study is to conduct a more exhaustive comparison, involving 25 out of 28 known species, all from the same genus. I specifically test the first of my three questions: In *Brachyhypopomus,* do stenotopic species restricted to hypoxic habitats, when corrected for body size, exhibit larger gill surface areas than stenotopic species from normoxic habitats? This disparity is expected to arise as an adaptive response, permitting increased oxygen uptake in conditions of hypoxia and anoxia.

2.2 Methods

i. Collections

Representatives of 25 of the 28 currently known species of *Brachyhypopomus* (see list in Table 1) were collected from South America by William G.R. Crampton and colleagues, or

received as loans from museums. All specimens had been fixed in 10% formalin and preserved in 70% ethanol.

ii. Classification of habitats

For the purpose of correlating gill metrics to oxygen regime, the extreme variability of oxygen levels in Neotropical aquatic systems precludes using "one-off" dissolved oxygen measurements taken at the time each specimen was captured (also museum specimens are very rarely accompanied by such information). Instead, habitats need to be categorized by long-term trends in oxygen concentrations, which encompass seasonal changes. In this thesis, aquatic habitats are divided into two categories, which I refer to as "*normoxic*" and "*hypoxic*". **Normoxic habitats** are defined as those that are typically close to saturation (usually 3-7 mg l^{-1}), which decline to less than 0.5 mg l^{-1} and only rarely, and which never become anoxic (defined here as unmeasurably low). **Hypoxic habitats** are defined as those subject to intermittent or protracted periods during which DO declines well below 0.5 mg l^{-1} (often reaching complete anoxia) either on a year-round basis (e.g. in permanent swamps), or for prolonged periods - typically weeks or months - on a seasonal basis (e.g. in seasonally inundated floodplains of major rivers). The categorization of all *Brachyhypopomus* species included in this thesis is detailed in Table 1.

Seasonal changes in oxygen concentrations are best known from the Tefé region of the Upper Amazon, where 12 of the 25 species analyzed here were collected (marked with an asterisk in Table 1). Here, habitats were defined as hypoxic or normoxic based on direct multi-season field samples and water-quality measurement collections (reported in Crampton

1996; 1998a,b, 1999, 2001, 2006, 2007, 2008, 2011; Crampton & Albert 2006; Crampton et al. 2008). Broadly speaking, the seasonally inundated floodplain systems of large, nutrient-rich whitewater rivers exhibit protracted anoxia or severe hypoxia (lasting 2-5 months) during the flood season due to large-scale decomposition of leaf litter and other organic debris on the flooded forest floor. At this time of the year *Brachyhypopomus* are found in floating rafts of macrophytes, or in leaf litter of newly flooded forest. During the low-water season whitewater floodplain fishes are confined to lakes or channels, and *Brachyhypopomus* are at this time found in floating or marginal macrophytes. Surface dissolved oxygen levels fluctuate at low water – ranging from fully saturated to anoxic, although usually normoxic. In summary, whitewater floodplain systems are completely or almost completely deoxygenated at high water and usually normoxic (but with transient hypoxia) at low water.

In contrast, small terra firme forest creeks and the marginal floodplains of blackwater rivers are permanently normoxic. The fact that oxygen levels remain high, despite the large volumes of organic detritus and debris in streams and blackwater floodplains, is largely a consequence of the low pH and low nutrient content of blackwaters, which serves to limit bacterial decomposition of organic matter. In addition, the floodplains of most blackwater rivers are relatively small in expanse when compared to those of whitewater rivers and so receive a proportionally greater input of well-oxygenated river water (Goulding et al. 2003).

Crampton (2011) provided a broad classification of Neotropical aquatic habitats but commented that a more detailed approach is limited by the paucity of water quality data in the ecological and taxonomic literature, and because water quality data are typically not appended to museum vouchers. Therefore, assigning the 13 species of *Brachyhypopomus* from outside the Tefé region to hypoxic or normoxic habitats is less straightforward. The

oxygen regimes of whitewater and blackwater systems in the forested lowland Amazon and Orinoco systems can are similar to those of the Tefé region (review in Crampton 2011). Less well known in the Amazon basin are the oxygen regimes of large clearwater rivers derived from Shield formations – although due to their low acidity, low-nutrient loads, and small floodplains, these are likely permanently normoxic. Outside the forested regions of the Amazon and Orinoco, several major savanna and semi-forested floodplain systems of the Neotropics are known to exhibit prolonged seasonal hypoxia during the flood season. These include the Orinoco Llanos (Lasso et al. 1997, Machado-Allison 1990; Hamilton et al. 2004), the Rupununi savanna of Guyana (Lowe-McConnell 1964), the Brazilian Pantanal (Hamilton et al. 1995, 1997), the Bolivian Llanos de Mojos (Hamilton et al. 2004), and the Paraguayan Chaco (Carter and Beadle 1931). Permanent swampland, with documented perennial hypoxia, is also found in some parts of the Neotropics, including coastal swamps of the Guianas (Hopkins 1991). Moreover, recent satellite studies have indicated that some 1 million km^2 of terra firme lowlands of the Amazon basin (ca. 15% of the total area of the basin) lying above the maximum extent of seasonal flooding are instead inundated intermittently by local rainstorms. These swamps form in low-lying depressions near streams and are typically hypoxic due to leaf litter decomposition (Crampton pers obs). These swamps contain a low-diversity of fishes (Saul 1975, Pazin et al. 2006). Crampton has observed the presence of *Brachyhypopomus beebei* and other electric fishes in these systems in the Tefé region (Crampton pers. com.).

Oxygen regimes are therefore well circumscribed for the 12 species in the Tefé region, but only approximately so for the 13 species outside the Tefé region – either by extrapolation from similar systems in the Tefé region based on habitat structure,

geomorphology, and flood dynamics, or from reports of water chemistry in the literature, or from direct measurements of DO in the field (usually taken over a relatively short time span with incomplete seasonal coverage). Despite these limitations, the categorizations used here are likely to prove robust to future improvements in our knowledge of the water chemistry of these systems.

Based on this division of Neotropical habitats into hypoxic and normoxic systems, all 25 species of *Brachyhypopomus* (for which sufficient numbers of specimens were available for dissection) were categorized as occurring in either of these two systems (stenotopic species) or both (eurytopic species).

iii. Dissection and measurements

Teleost fishes possess four gill-arches on each side of the branchial basket. Anchored to a gill arch are two hemibranchs, each of which supports multiple cartilaginous gill filaments. The gill filaments are divided into numerous, thin, blade-like lamellae, which form the surface area for gas exchange. All four-gill arches from the left side of a specimen were ablated and separated. Both hemibranchs from each of these four gill-arches were then laid flat and photographed under a Meiji Techno RZ stereo-microscope with a SDCM-3 3.1 Megapixel digital camera. Measurements were made from these digital photographs as point-to-point distances using Motic 2.0 software (Motic Images 2000).

The primary gill metric used in this thesis is total gill filament length (TGFL), which is the estimated summed length of all filaments in a fish. TGFL is a common measure of gill size and it has been shown to be positively correlated to total gill surface area (TGSA) in a

wide range of freshwater fishes (Palzeberger and Pohla 1992; Chapman et al. 2000). Although it would be ideal to base measurements solely on TGSA and then to further correlate TGFL to TGSA, I was unable to adequately measure TGSA, and thus TGFL was used in lieu of TGSA. Numerous attempts were made to dissect lamellae from *Brachyhypopomus* with the view of recording TGSA. However, in the majority of specimens, the lamellae were fused together and tended to disintegrate when attempts were made to tease them apart (this is a common problem with small fish that cannot be remedied, Chapman, pers. com.). It was therefore impossible to discern where one lamellae started and another ended, and likewise impossible to measure individual lamellae. Nonetheless, the filament rods used to obtain TGFL were readily discernable, even among sub-optimally preserved specimens.

The technique described by Muir and Hughes (1969) was used to estimate TGFL. This technique utilizes both hemibranchs of each of the four gills on the left side of an individual. In brief, for each hemibranch, the length of the 1st, 3rd, every 5th, and the last filament was measured. Filament lengths between these landmarks were interpolated as the average of the two adjacent measured filaments. The TGFL of the entire fish is the added filament lengths across all eight hemibranchs from the left side, multiplied by two to represent both the left and right side of the fish (see Figure 2 for gill filament length example).

Five additional gill metrics were used for multivariate analysis (see Principal Component Analysis, below): 1) Total hemibranch area (THA), the area of all gill filaments, was obtained by outlining all filaments with image digitizing software (not including the bony arch), and then calculating the area within each outline (see Figure 2 for an illustrated

outline of the hemibranch area). The areas for all eight hemibranchs on the left side of the fish were then added and multiplied by two to estimate THA for the entire fish. 2) Total number of gill filaments (TNF), the total number of filaments counted on the left side of the fish (for all eight hemibranchs), multiplied by two. 3) Grand average filament length (GFL) the mean of all filament lengths across the eight hemibranches of the left side. 4) Total perimeter of the hemibranchs (TP), the sum of the perimeters of all eight hemibranchs from the left side multiplied by two. 5) Average gill hemibranch length (AGL) the mean of all hemibranch lengths on the left side, where hemibranch length is the distance from the base of the first filament to the base of the last filament (see Figure 2 for an illustrated example of hemibranch length).

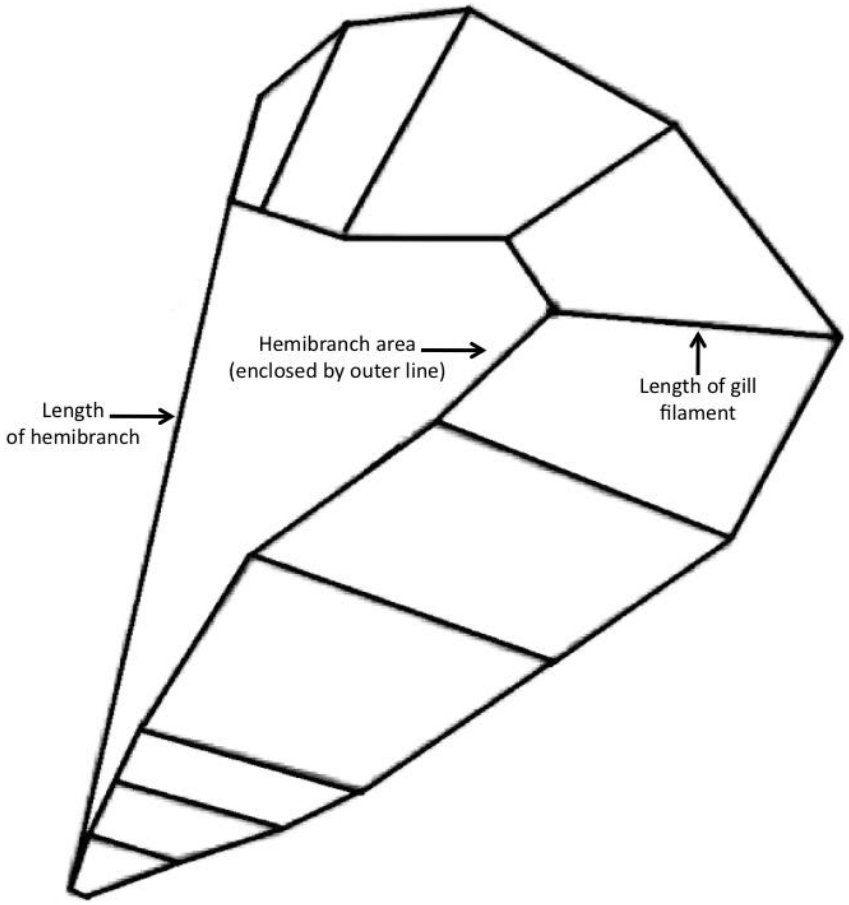

Figure 2. Illustration of one side of a gill hemibranch with primary measurements used to calculate the gill metrics utilized in this study.

iv. Statistical analyses

One-tailed t-test

Analysis of covariance (ANCOVA) has been used in the literature (Chapman and Hulen 2001; Chapman et al. 2002; Crampton et al. 2008) on gill morphology, because it allows body size to be incorporated as a covariate in statistical comparisons of gill metrics among species. However, ANCOVA is a tool that works only for comparisons of small numbers of groups. For instance, Crampton et al. (2008) compared four species of *Brachyhypopomus* from the Tefé region of the Brazilian Amazon: two from hypoxic habitats (*B.* n. sp. FLAV and *B.* n. sp. BENN), and two from normoxic habitats (*B.* n. sp HEND, and *B.* n. sp ROYE). Here, ANCOVA was used to demonstrate that the two species in hypoxic habitats did not exhibit significantly different intercepts or slopes to each other, but did exhibit significantly different intercepts and slopes to the two species from normoxic habitats. One of the strengths of ANCOVA is its ability to break down relationships among groups into direct comparisons, and in this example, because there were only six pair-wise combinations of four species, ANCOVA could generate meaningful results, that is, in this case, results that are indicative of significant differences in the intercept and or slope of species from hypoxic vs. normoxic environments. However, when multiple comparisons are undertaken, ANCOVA is expected to lose much of its utility due to the large number of available pair-wise combinations of species (for example, for a comparison of 25 species there are 300 combinations). Faced with multiple comparisons, not only is the number of comparisons overwhelming from the perspective of presenting the results of the statistical analysis, but

also the Bonferroni corrections required to correct for multiplicity would undoubtedly render many of the interspecific comparisons of gill surface area insignificant - despite an important biological difference (i.e. cause type-II error).

Due to these limitations of ANCOVA, I adopted an alternative approach to explore disparities in gill surface areas between stenotopic species restricted to normoxic and hypoxic habitats. Instead I derived a procedure to characterize each species with a single value of TGFL – i.e. a single value representing gill surface area in each of the 22 stenotopic species restricted to either hypoxic or normoxic habitats. These values were then compared between stenotopic species in hypoxic versus normoxic systems using a one-tailed t-test, with the prediction that gill size in species restricted to hypoxic habitats should be larger than in species restricted to normoxic habitats. This single value was derived using the following three steps (see Figure 3), using a custom-script written in R (R Development Core Team 2008):

1. Specimens were selected from each species belonging only to a body mass range within which most species overlap (0.5 - 3.5 g). This avoided the necessity to extrapolate beyond empirical data sets outside the range of some groups of individuals (e.g. in adults of species that mature at large size versus adults of small sizes). Some species only grow to approximately 3 g (e.g. *B.* n. sp. ROYE), and thus representatives in this size range are mostly adults. In contrast, some species grow to much larger sizes (e.g. up to 20 g in *B. brevirostris*), and thus representatives in the 0.5-3.5 g range are all juveniles. This would be problematic if there were substantial ontogenetic changes in the relationship between log size and log TGFL. This was not the case, for example in *B. beebei* and *B. brevirostris*, which

attain among the largest body sizes for the genus, with adults typically exceeding 3.5 g. In both these species the relationship between log size and log TGFL through all development stages remained a close linear fit (*B. beebei* r^2 = 0.97 and *B. brevirostris* r^2 = 0.93). Moreover, gill surface area has previously been repeatedly demonstrated in numerous fish species to increase with body mass through all development stages in a linear manner (i.e. linear allometry) (Price 1931; see for review Palzenberger and Pohla 1992).

2. All 22 stenotopic species (the three eurytopic species were excluded from this analysis but are later revisited in Chapter 3) were entered into an ANCOVA model with TGFL as the response variable and body mass as the predictor variable. Both the response and predictor variables were log transformed because a bilogarithmic relationship represents the best fit, for each species, based on Akaike's Information Criterion (AIC) (Akaike 1992; Burnham and Anderson 2002). Finally, for the ANCOVA model I demonstrated homogeneity of slopes – evidenced through non-significance of the interaction term between slopes. This yielded a common slope for all of the 22 species.

3. The size adjusted TGFL is the TGFL calculated from the common slope when the logged body mass is equivalent to the common mean logged body mass of all individuals in the comparison (see Figure 3 for schematic explanation). This was equivalent to a log body mass of to 0.118239 g (untransformed body mass of 1.56 g)

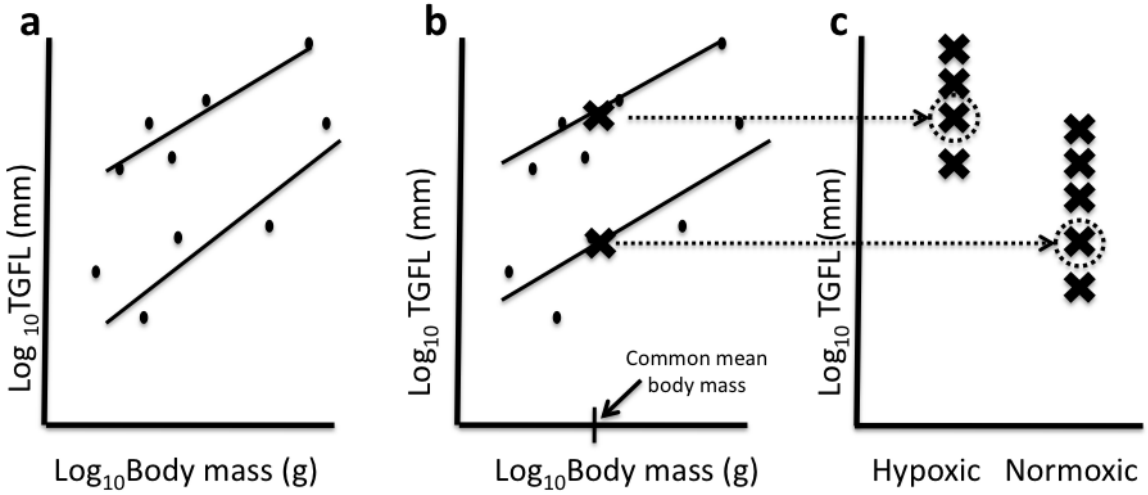

Figure 3. Illustrated method for obtaining the size adjusted total gill filament length (TGFL) for each species. a) ANCOVA is performed for all species (two are shown here for simplicity) and the interaction term is tested for significance; b) The non-significant interaction term is removed, a common slope is obtained, and adjusted means (symbolized by X) are calculated for each species by measuring the response variable (TGFL) when the body mass equals the common mean body mass (among all individuals in the comparison); c) adjusted means from (b) are then used to compare species restricted to hypoxic habitats to species restricted to normoxic habitats via a one-tailed t-test.

2.3 Results

i. Distributions

The distribution of *Brachyhypopomus* species among hypoxic and normoxic habitats is summarized in Figure 4. Eurytopic species (which I will include in the analyses reported in Chapter 3) are those that occur in the area of overlap of the Venn circles.

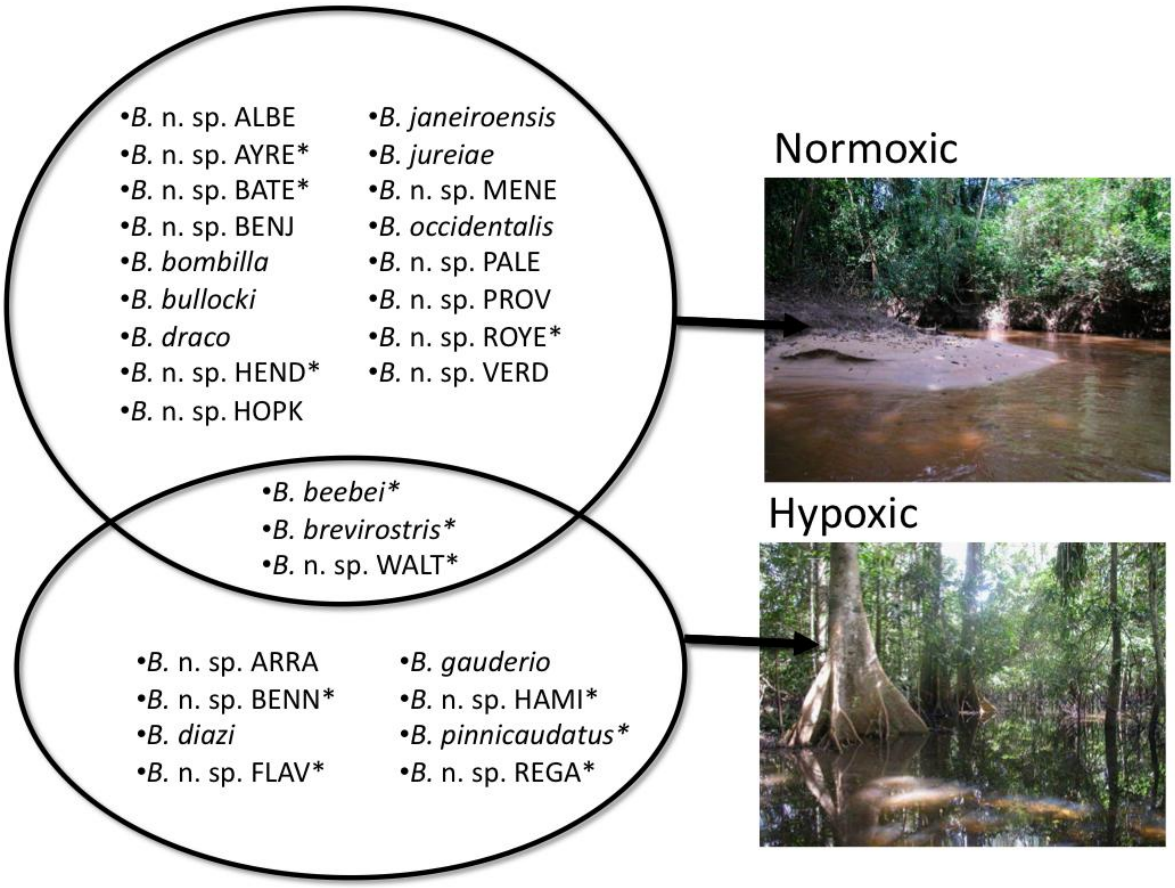

Figure 4. Venn diagram of the distribution of 28 known species of *Brachyhypopomus* found among hypoxic and normoxic habitats of the lowland Neotropics. Areas of overlap indicate species that are eurytopic (occur in both hypoxic and normoxic habitats). Four-letter codes are cheironyms for undescribed species pending descriptions (de Santana and Crampton in prep.). Asterisks denote 12 species collected from Tefé region of the Upper Amazon basin. Photographs (courtesy W. Crampton) show examples of a normoxic habitat (terra firme rainforest stream) and a hypoxic habitat (flooded forest habitat), both from the Central Amazon.

ii. Gill metrics

Summary statistics for the gill metrics TGFL, THA, TNF, GFL, TP, and AGL, are presented in Table 1.

Table 1. Summary of six gill metrics [total gill filament length (TGFL), total hemibranch area (THA), total gill filament number (TNF), grand average filament length (GFL), total hemibranch perimeter (TP), and average gill hemibranch length (AGL)] measured from 25 species of *Brachyhypopomus.* Taxa are categorized into four dissolved oxygen categories: 1) stenotopic species restricted to hypoxic habitats (Stenotopic-Hypoxic); 2) stenotopic species restricted to normoxic environments (Stenotopic-Normoxic); 3) populations of eurytopic species from hypoxic habitats (Eurytopic-Hypoxic); and 4) populations of eurytopic species from normoxic environments (Eurytopic-Normoxic). Asterisks denote 12 species collected from Tefé region of the Upper Amazon basin.

Species	TGFL				THA				TNF				GFL				TP				AGL			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Stenotopic-Hypoxic																								
Brachyhypopomus n. sp. ARRA	581.2	1236.2	844.7	241.3	78.2	228.1	132.0	55.7	556.0	652.0	600.7	36.0	1.0	1.9	1.4	0.3	168.4	277.5	212.6	41.4	2.1	4.2	3.1	0.8
Brachyhypopomus n. sp. BENN*	662.79	2093.3	1298.4	557	104.82	491.48	264.18	152	332	832	614.56	204	1.39	4.24	2.7	1	138.51	436.66	288.93	119	1	6.35	3.7	2.14
Brachyhypopomus diazi	502.5	2044.3	1127.7	535.8	63.3	378.8	173.4	111.1	614.0	968.0	773.3	119.4	0.8	2.1	1.4	0.4	162.2	398.2	251.6	83.9	2.3	6.0	3.9	1.3
Brachyhypopomus n. sp. FLAV*	513.9	674.9	588.1	57.8	65.6	137.1	86.8	29.0	528.0	568.0	549.2	15.5	1.0	1.2	1.1	0.1	157.5	223.0	178.8	26.8	2.3	3.4	2.6	0.5
Brachyhypopomus gauderio	552.2	1099.7	919.1	218.9	73.1	189.5	145.0	46.6	586.0	686.0	658.0	45.7	0.9	1.6	1.4	0.3	170.8	264.1	232.5	39.4	2.6	4.3	3.6	0.7
Brachyhypopomus n. sp. HAMI*	470.7	739.1	609.4	109.7	52.8	96.0	73.8	17.8	524.0	606.0	569.0	34.6	0.9	1.2	1.1	0.1	140.7	190.4	164.2	20.3	2.1	2.7	2.4	0.2
*Brachyhypopomus pinnicaudatus**	537.1	1292.9	975.2	293.5	75.5	237.5	160.9	60.6	560.0	700.0	648.3	52.1	0.9	1.8	1.5	0.4	175.5	294.3	240.1	44.6	2.4	4.1	3.4	0.6
Brachyhypopomus n. sp. REGA*	555.9	1391.1	1026.7	284.7	64.0	257.0	173.5	65.9	592.0	738.0	669.6	52.3	0.9	1.9	1.5	0.3	172.6	328.1	259.6	53.4	2.3	4.4	3.5	0.7
Stenotopic-Normoxic																								
Brachyhypopomus n. sp. ALBE	337.3	651.3	546.1	141.8	29.2	77.6	62.3	22.8	502.0	612.0	560.5	46.1	0.7	1.1	1.0	0.2	117.0	180.3	158.7	28.4	1.7	3.0	2.4	0.5
Brachyhypopomus n. sp. AYRE*	345.1	587.9	439.9	94.5	38.4	77.9	51.7	13.9	476.0	562.0	504.7	30.1	0.7	1.0	0.9	0.1	123.3	173.5	141.8	17.1	2.0	2.4	2.1	0.2
Brachyhypopomus n. sp. BATE*	325.8	338.0	331.4	6.2	33.6	38.9	36.4	2.7	402.0	498.0	436.0	53.8	0.7	0.8	0.8	0.1	120.8	132.3	128.0	6.3	1.8	2.1	1.9	0.1
Brachyhypopomus n. sp. BENJ	496.4	798.6	651.3	120.7	55.9	117.2	82.5	23.9	526.0	588.0	552.4	27.0	1.0	1.4	1.2	0.2	151.7	218.5	179.2	27.6	2.1	2.8	2.5	0.3
Brachyhypopomus bombilla	314.4	508.9	400.5	70.7	32.0	74.3	50.0	14.1	450.0	556.0	502.7	37.7	0.6	0.9	0.8	0.1	121.6	186.2	150.7	21.5	1.9	2.7	2.2	0.3
Brachyhypopomus bullocki	485.3	643.2	550.4	59.8	61.8	103.5	82.7	15.7	532.0	580.0	561.3	20.1	0.9	1.1	1.0	0.1	164.8	207.5	188.6	15.3	2.5	2.9	2.7	0.1
Brachyhypopomus n. sp. HEND*	481.2	658.4	571.2	82.3	71.5	104.1	85.6	16.4	520.0	574.0	548.4	22.6	0.9	1.2	1.0	0.1	175.2	208.5	189.9	15.4	2.3	2.7	2.5	0.2
Brachyhypopomus n. sp. HOPK	346.7	452.2	405.3	39.3	39.1	54.8	47.5	5.2	466.0	542.0	516.3	29.7	0.7	0.8	0.8	0.1	124.9	143.5	135.0	6.6	2.0	2.5	2.3	0.2
Brachyhypopomus janeiroensis	559.0	1138.8	840.1	237.6	64.4	163.6	113.4	41.1	648.0	804.0	731.0	64.0	0.9	1.4	1.1	0.2	164.1	255.6	213.2	38.1	2.2	4.1	3.1	0.8
Brachyhypopomus occidentalis	347.8	1641.5	991.5	432.2	39.1	318.3	152.0	91.4	570.0	906.0	753.6	107.3	0.6	1.9	1.3	0.4	139.0	375.2	249.9	73.6	2.3	5.6	3.7	1.0
Brachyhypopomus n. sp. PALE	223.0	1175.4	567.7	303.6	22.1	200.7	76.6	57.0	464.0	780.0	614.8	114.1	0.5	1.5	0.9	0.3	104.5	308.4	177.9	64.9	1.6	4.1	2.7	0.8
Brachyhypopomus n. sp. PROV	274.6	426.3	356.5	60.0	30.7	63.5	43.2	10.4	402.0	506.0	457.6	33.1	0.7	0.9	0.8	0.1	111.0	160.3	133.6	15.2	1.7	2.8	2.0	0.3
Brachyhypopomus n. sp. ROYE*	208.9	395.1	335.8	66.7	16.4	52.4	39.2	12.3	460.0	542.0	501.3	37.3	0.5	0.8	0.7	0.1	93.5	161.3	138.3	23.4	1.5	2.7	2.2	0.4
Brachyhypopomus n. sp. VERD	440.0	593.2	516.3	62.3	49.7	73.5	59.1	9.6	546.0	634.0	581.2	32.9	0.8	1.0	0.9	0.1	140.9	166.0	151.3	10.5	2.1	2.8	2.4	0.3
Eurytopic-Hypoxic																								
*Brachyhypopomus beebei**	536.2	2961.7	1479.3	785.6	68.7	692.9	279.0	201.3	564.0	900.0	751.5	114.6	0.9	3.3	1.9	0.8	165.3	507.3	308.2	112.0	2.0	9.6	4.4	2.2
*Brachyhypopomus brevirostris**	1015.9	3435.1	2056.0	626.2	133.2	896.3	407.6	182.7	768.0	1048.0	912.9	84.6	1.3	3.3	2.2	0.5	247.8	600.7	408.8	89.2	2.9	8.3	5.2	1.3
Brachyhypopomus n. sp. WALT*	494.7	1595.3	1028.8	407.6	70.7	305.1	174.7	86.4	538.0	786.0	658.2	79.5	0.9	2.0	1.5	0.5	167.9	3387.4	640.1	977.5	2.5	5.0	3.7	0.9
Eurytopic-Normoxic																								
*Brachyhypopomus beebei**	381.7	2031.4	1233.7	548.6	40.7	447.6	220.3	142.0	556.0	902.0	772.0	115.4	0.7	2.3	1.5	0.5	133.1	425.1	281.8	99.2	1.9	7.0	4.3	1.7
*Brachyhypopomus brevirostris**	401.4	2519.4	1618.7	692.5	32.7	500.9	278.3	160.6	606.0	1016.0	876.5	125.6	0.7	2.6	1.8	0.6	137.1	459.3	333.7	108.3	1.9	6.3	4.4	1.5
Brachyhypopomus n. sp. WALT*	449.5	1622.6	823.2	302.8	57.3	333.1	127.6	76.3	530.0	744.0	606.9	55.5	0.8	2.2	1.3	0.3	155.1	350.2	217.8	56.5	2.2	5.0	3.1	0.8

One-tailed t-test

1. Generating the adjusted means: For all 22 stenotopic species, AIC selected the bilogarthmic model (AIC = -669.59) as the best model that represents the relationship between total gill filament length (TGFL) and body mass. Alternative models considered were: untransformed response and covariate (AIC = 3100.63), only covariate log transformed (AIC = 3133.34), and only response log transformed (AIC = -487.02).

The results of the ANCOVA on total gill filament lengths with body mass as the covariate, and 22 stenotopic species restricted to either hypoxic or normoxic habitats as individual treatments, showed a strong covariate effect, i.e. there was a strong relationship between body mass and TGFL (ANCOVA; DF = 1; F-value = 1363.162; $P < 0.0001$). There was a strong treatment effect ($P < 0.05$). However, the interaction term between slopes of each treatment was insignificant ($P = 0.853$). This allowed for the slopes of each treatment to be homogenized and an adjusted mean value for TGFL to be calculated for each species.

2. The one-tailed t-test: A comparison of the size adjusted TGFL of stenotopic species from hypoxic versus normoxic habitats confirmed the expectation that species from hypoxic habitats exhibited significantly higher TGFL (Mean log transformed TGFL = 2.88 mm, SD = 0.03, N = 8) than species from normoxic habitats (Mean log transformed TGFL = 2.73 mm, SD = 0.06, N = 17) (df = 19.45, t = -7.52, $P < 0.0001$) (see Figure 5).

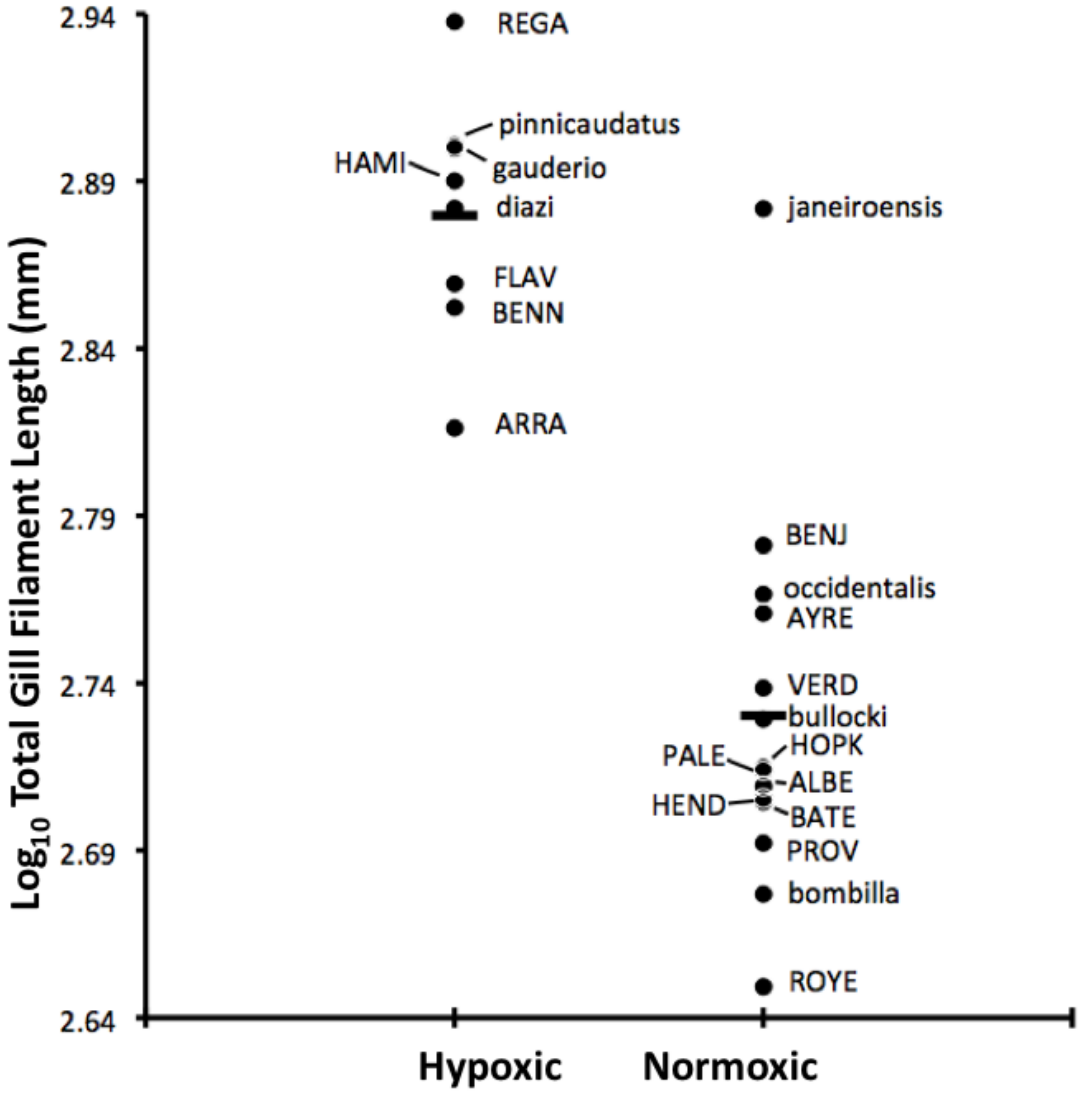

Figure 5. Biplot of total gill filament lengths for 22 stenotopic species categorized as restricted to hypoxic habitats (n = 8) and normoxic habitats (n = 14). The y-axis represents size adjusted total gill filament lengths (TGFL) calculated as summarized in Figure 3. Bold horizontal lines represent the means for each category.

2.4 Discussion

Mirroring the results of previous interspecific studies (Gibbs and Hurwitz 1967; Fernandes et al. 1994; Mazon et al. 1998; Crampton et al. 2008), the results presented here show that, when corrected for body size, stenotopic species restricted to hypoxic environments exhibit significantly larger gill surface areas than stenotopic species restricted to normoxic environments. This observation supports the hypothesis that large gill surface areas evolved as an adaptation for tolerating hypoxia – by permitting more oxygen to be extracted from the water (or air, during aerial breathing). With the exception of one species, *B. janeiroensis*, species restricted to hypoxic and normoxic systems exhibited completely non-overlapping ranges of TGFL. The TGFL of *B. janeiroensis* is within the range of species restricted to hypoxic systems and yet was classified as occurring in normoxic systems based on a single oxygen determination (of 4.1 mg/l) made by W. Crampton in 2006 during a collecting expedition to the type locality of this species – a small terra firme stream on the coastal floodplain of Rio de Janeiro State, Brazil. There are no published studies of seasonal variation in DO or other water quality for aquatic systems of the coastal floodplain of this region of Brazil, the original species description (Costa and Campos da Paz 1991) does not comment on DO, and museum records for the species give no records on water quality (W. Crampton pers. obs). The large gill size of this species is clearly within the range of a species from hypoxic environments – suggesting that the habitat classification may be premature. Further work is needed to characterize diurnal and seasonal variation in DO in the habitats of *B. janeiroensis*

The conclusion that large gill size has evolved as an adaptive response to hypoxia provides a clear explanation for why *Brachyhypopomus* with small gills are largely excluded from hypoxic habitats; however, it does not explain why species with large gills appear to be excluded from normoxic habitats, or why species do not evolve large gills in normoxic habitats. Below I consider three sets of potential explanations for why large gills may be disadvantageous in normoxic environments.

i. A large gill surface area is disadvantageous in low-conductivity, acidic blackwater systems

Most species of *Brachyhypopomus* that are categorized as occurring in normoxic systems are found in blackwater streams and floodplains (the only exceptions are the trans-Andean stream-dwelling *B. occidentalis* and *B.* n. sp. PALE, which occur in high-conductivity clearwater streams draining Andean erosion zones, with near neutral pH). Two chemical properties of blackwater systems, low pH (typically in the range 3.5 – 5.5) and low conductivity (typically 5- 30 μScm-1) (see Furch 1984; Goulding 1980; Val and Almeida-Val 1995; Walker and Henderson 1996) could, in principle, influence gill surface area in ways that are independent of dissolved oxygen. Fishes that tolerate low pH and low conductivity conditions face challenges in ion regulation, osmoregulation and acid-base regulation across the gill lamellae. In particular, they must be able to mitigate ion loss at the gill surface (Booth et al. 1988; Freda and McDonald 1988) and limit the tendency for low pH to inhibit Na^+ and Cl^- uptake mechanisms (McDonald 1983). Mangum et al. (1978) and Potts (1984) suggested that the larger the gill surface area, the more likely the hypotonic environment will come in contact with hypertonic blood plasma, leading to ion loss and

water influx. These considerations lead some authors (e.g. Sollid et al. 2003) to predict that a small gill surface area is expected to be favored over a large gill surface area in acidic, low conductivity waters. However, McDonald et al. (1991) noted for five species from environments of different pH levels, species from acidic environments showed no difference in gill lamellar surface area compared to species found in non-acidic environments, suggesting that acid-tolerance is not correlated to gill surface area. Nonetheless, the range of pH values over which this study was conducted did not embrace some of the extreme values typical of Amazonian blackwaters. Moreover, among the many studies of adaptations of fishes in low conductivity and low pH blackwater systems of the Amazon (e.g. Wilson et al. 1999; Gonzalez et al. 1997, 1998, 2002, 2006; Gonzalez and Preest 1999), none have noted a reduction in gill surface areas. Instead, a range of physiological adaptations to regulate ion flow have been well-described – including the evolution of unusual high-affinity and high-capacity ion transport systems (e.g. Gonzalez et al. 1997; Gonzalez and Preest 1999; Gonzalez and Wilson 2001; Gonzalez et al. 2002), low-affinity and low-capacity Na^+ uptake systems (Gonzalez et al. 2002), and increased branchial affinity for Ca^{2+} ions (e.g. Gonzalez et al. 1997, but see Gonzalez et al. 1998 for contrary views). These kinds of adaptations for life in extremely low conductivity, low pH waters are extremely common because a very large proportion of Neotropical freshwater fishes live in these conditions. For instance, in excess of 1,000 species are known from the giant blackwater Rio Negro basin alone (Val and Almeida-Val 1995; Gonzalez et al. 1998). It appears that there is, as yet, no compelling evidence that low pH and conductivity represent strong selective pressures against large gill surface areas in blackwaters. Nonetheless, no study has yet searched for gill variation size specifically correlated to pH alone, or to conductivity alone (or a combination of pH and

conductivity) across systems in which DO is not a confounding variable. For instance, a comparison of Amazonian blackwaters and clearwaters (both normoxic and low conductivity) would permit a ready analysis of the effects of pH, because blackwaters have low pH (<5.5) while clearwaters are typically near neutral (Goulding et al. 2003). Likewise, within the immense blackwater Rio Negro basin there is considerable variation in pH between tributaries, but all of these systems have similarly low pH and high DO. Further studies are clearly needed to explore whether pH and or conductivity alone may influence gill size in Amazonian systems.

ii. Trade-offs between gill size and adjacent morphological structures.

Large gill surface areas may perform well in normoxic environments, but come at the cost of reducing the space available for the development of surrounding morphological structures (Barel 1983; Cech and Massingill 1995). Large gill surface areas are invariably associated with an expansion of the volume of the cranial region occupied by the gills (e.g. Schaack and Chapman 2003; Chapman et al. 1999; 2000; 2008). Therefore, an expansion in gill surface area is only expected to evolve when the costs associated with reductions in adjacent skeletal elements are outweighed by the benefits of increased oxygen uptake. In other words, there may be trade-offs between gill surface area and the development of other morphological structures. Under normoxic conditions, it is possible that these trade-offs may act to reduce gill surface area – accounting for why species restricted to normoxic systems have smaller gill surface areas (even if selection against large gill size associated with the low pH and low conductivity of normoxic blackwater systems were absent).

Some recent studies have demonstrated that increases in gill size in fishes from hypoxic systems correlates with a reduction of key trophic muscles and/or skeletal elements in areas adjacent to the operculum, and also to feeding performance (review in Chapman 2007). For instance, morphological trade-offs between gill size and elements of the pharyngeal jaw musculature have been demonstrated convincingly in the African cyprinid *Barbus neumayeri*, with large gill sizes impeding feeding ability (Schaack and Chapman 2003). Likewise, based on split-brood rearing experiments, Chapman et al. (2000, 2008) demonstrated that large gill sizes in populations of the cichlid *Pseudocrenilabrus multicolor victoriae* from hypoxic systems are associated with a reduction in the width, length and depth of the pharyngeal jaws relative to populations in normoxic systems.

In principle, gill size might also be traded off with the size of the posterior portions of the neurocranium, which are close to the operculum, and thus indirectly result in a reduction in brain size. Brain size is especially critical in electric fishes, which have proportionally larger brains than most other teleosts – presumably because of the computationally intensive nature of the combined electrogenic and electrosensory system (Hopkins 1983). Two studies of African fishes have compared brain mass, and gill metrics between taxa from hypoxic and normoxic systems (for mormryiform species see Chapman and Hulen 2001; for populations of the cichlid *Pseudocrenilabrus multicolor victoriae* see Chapman and Hulen 2001). Both studies found that brain size was smaller in species and populations from hypoxic environments when compared to their counterparts in normoxic environments. In contrast, gill surface area varied in an inverse manner. One explanation for these results is that brain size and gill size are traded off, with a large gill surface area evolving at the cost of a reduction of neurocranum size (and thus indirectly brain size) in

hypoxic environments. However, Cripso and Chapman et al. (2010a) argue that the reduction of brain size in hypoxic environments is more likely due to the expensive metabolic costs associated with maintaining a large brain size in a hypoxic environment. Although outside the scope of this thesis, it would be profitable to explore the relationship between gill size, neurocranium and brain size, and the size of jaw elements in the *Brachyhypopomus* system.

iii. Adaptation to local conditions

A full explanation of the correlation between gill surface area and oxygen regime should also explore the possibility that species with large gills may be unable to disperse into normoxic habitats (thus accounting for their absence in normoxic systems although not necessarily accounting for why large gills do not evolve in normoxic systems in the first place) - not because large gill surface area is selected against *per se*, but instead because these species possess suites of non-gill traits that adapt them to life in the seasonally hypoxic floodplains environments of large, turbid-water rivers (e.g. high pH, high conductivity etc.). Here I focus on adaptations for electrical impedance matching and ion regulation that may act to restrict species to a narrow range of environmental conditions, such that the ecological distributions of *Brachyhypopomus* that we observe may be only partially explained by adaptations to hypoxia.

1. Electrical impedance matching

The electric organ morphology of *Brachyhypopomus* is correlated to ambient conductivity (Crampton 1998b; Hopkins 1999). Species in high-conductivity (i.e. low electrical resistance) habitats typically possess electric organs where individual electroctyes in the caudal portion of the body are organized in parallel-configuration – which optimizes power output of the communication component of the electric signal under conditions of low resistance. This parallel arrangement of electrocytes is expressed as short, vertically enlarged tails. In contrast, species in low-conductivity environments typically possess thin, long tails where electrocytes are organized in a serial arrangement. These configurations of electrocytes are thought to be adaptations to maximize the power output of the signal (Hopkins 1999). This 'impedance matching' of electric organ morphology to ambient conductivity is thought to be an important determinant of ecological distributions in *Brachyhypopomus* (Crampton 1998b; Hopkins 1999) and suggests that species adapted to high conductivity, hypoxic floodplains may be excluded from nearby low-conductivity normoxic systems, not necessarily because of any disadvantages associated with possessing large gills, but because their electric organs are poorly impedance-matched to low conductivity (or for a combination of these reasons).

2. Ion regulation

Tolerance of low pH and conductivity in fishes involves a suite of biochemical and physiological adaptations for efficient ion regulation (Wilson et al. 1999; Gonzalez et al. 1998, 2002; Gonzalez and Preest 1999). These adaptations may be detrimental to fitness in the less acidic, higher conductivity conditions of floodplains. Likewise, floodplain fishes may

be poorly matched in terms of ion regulation to low-conductivity, acidic systems. This was demonstrated in a floodplain species, the armored catfish, tamoatá (*Hoplosternum littorale),* whose intolerance to low pH may reflect the differential mechanisms of ion regulation between blackwater species and floodplain species (Wilson et al. 1999).

2.5 Conclusions

The results of this study provide strong support, based on a comparison of multiple species, for the hypothesis that gill surface area is correlated to oxygen regime, with large gill surface area favored in habitats subject to hypoxia or anoxia. This coincides with previous research on African mormyrids and cichlids (Chapman and Hulen 2001; Chapman et al. 2002), and two Amazonian riverine species (a species of Sciaenidae and a species of Prochilodontidae) (Mazon et al. 1998).

Large gill surface areas are an important factor influencing the distribution of several *Brachyhypopomus* species into hypoxic environments. Five of the eight species of *Brachyhypopomus* that are restricted to hypoxic environments have demonstrated respiratory efficiency in hypoxic conditions (Crampton 1998a), and several species are able to utilize their gills as an air-breathing organ when DO concentrations are very low (< ca. 0.2 mg l^{-1}). Crampton's (1998a) observations suggest that water-breathing is sufficient in meeting metabolic demands of the fish until very low DO exposure, at which point it becomes more efficient to switch to branchial air breathing. The low DO thresholds for the switch from water to air-breathing may be due to the costs that arise when individuals are exposed to predators at or near the surface (Kramer et al 1983; Randle and Chapman 2004).

In sum, whether used for the extraction of oxygen from water or from air, or both, a large gill surface area is clearly critical for life in hypoxic environments. This hypothesis explains the exclusion of species with small gill surface areas from hypoxic environments, but does not explain why many species with large gill surface areas are not found in normoxic environments. I reviewed three potential explanations for the absence of large gill surface areas in species from normoxic environments.

There is ample evidence from the *Brachyhypopomus* system that variations in dissolved oxygen can drive phenotypic divergence in gill morphology across species. Quantifying the significance of aquatic oxygen in promoting interspecific variation of respiratory traits improves our understanding of hypoxia as an impetus for divergent selection.

CHAPTER 3: DISSOLVED OXYGEN AND GILL SURFACE AREA IN THREE EURYTOPIC SPECIES OF *BRACHYHYPOPOMUS* (TELEOSTEI, GYMNOTIFORMES, HYPOPOMIDAE): INTRASPECIFIC AND INTERSPECIFIC COMPARISONS

3.1 Introduction

Many studies have suggested that phenotypic variation among populations of the same species can be caused by either developmental plasticity in response to habitat-specific selective pressures (Chapman et al. 2000, 2007, 2008; Sultan and Spencer 2002; Rutjes 2006) or by genetic differences between populations that are adapted to local conditions (Nagy and Rice 1997; Sultan and Spencer 2002). When migration between populations of distinct environments is common and genetic exchange between populations is unconstrained (i.e. genes are homogenized among populations), population-level variation is invariably a consequence of developmental plasticity, and not local adaptation (Sultan and Spencer 2002, Chapman et al 1999; Storfer et al. 1999; Lenormand 2002; Kingsolver et al. 2002; Griswold 2006; Crispo and Chapman 2010a).

Many studies have noted population-level variation in gill morphology across gradients of oxygen availability. For example, in the African electric fishes *Gnathonemus victoriae* and *Petrocephalus castostoma* (Chapman and Hulen 2001), and the African cyprinid *Barbus neumayeri* (Chapman et al. 1999; Schaack and Chapman 2003), larger total gill surface area has been noted in populations that occur in hypoxic environments than in normoxic ones. Similar patterns have been noted in the North American sailfin mollie, *Poecilia latipinna*, where populations not only exhibit variation in gill surface area, but also

in a variety of physiological traits (e.g. metabolic rate, critical oxygen tension, and respiratory behavior) (Timmerman and Chapman 2004).

Studies of gill morphology across environmental gradients of oxygen availability have proven to be a good model for exploring the evolution of phenotypic plasticity. For example, studies of African fishes have also questioned whether intraspecific variation in gill morphology is a consequence of developmental plasticity or local adaptation. Chapman et al. (2000, 2008) conducted a rearing experiment using the offspring of a single individual of the sub-species *Pseudocrenilabrus multicolor victoriae,* collected from a fluctuating oxygen environment. Half of the brood was reared under stable hypoxic conditions and the other half under stable normoxic conditions. Total gill surface area was then compared between populations after development, and was found to be 18% greater in the group reared under hypoxic conditions. These results show unequivocally that variation in gill size among populations can arise as a consequence of developmental plasticity alone (i.e. in the absence of local adaptation). Developmental plasticity in gill morphology is not limited to fish species – it has been documented in decapods (Burd 1988), larval anura (Bond 1960), and larval salamanders, (Burggren and Mwalukoma 1983), among other groups.

None of these studies considered whether multiple closely related species exhibit similarity in population-level trait variation. For example, within a single genus do all species with eurytopic ecological distributions exhibit phenotypic plasticity, or only some? If only some, what causes such variation? The *Brachyhypopomus* system offers the opportunity to examine population-level variation of gill morphology in three eurytopic species (*B. beebei*, *B. brevirostris*, and *B.* n. sp. WALT). All three are common throughout the Amazon and Orinoco basins, and in most major drainages of the Guianas. My study is also the first to

explore branchial morphological adaptations to hypoxia at the intraspecific level in a Neotropical aquatic animal.

Finally, previous studies of morphological or physiological traits among populations of eurytopic species that are found in more than one oxygen habitat (i.e. hypoxic and normoxic habitats) have failed to consider how traits compare between eurytopic species and closely related species that are stenotopic, that is, restricted to either hypoxic or normoxic habitats. For example, in the case of branchial morphological adaptations to hypoxia, consider a case of eurytopic species in which populations from hypoxic environments exhibit larger gill surface areas than populations from normoxic environments. Do hypoxic populations of this eurytopic species (henceforth abbreviated "Eurytopic-Hypoxic") exhibit gill surface areas similar to stenotopic species that only occur in hypoxic habitats (henceforth abbreviated "Stenotopic-Hypoxic")? And, likewise, do normoxic populations of this eurytopic species (henceforth abbreviated "Eurytopic-Normoxic") exhibit gill surface areas similar to stenotopic species that only occur in normoxic habitats (henceforth abbreviated "Stenotopic-Normoxic")? Or are intraspecific trait disparities between hypoxic and normoxic populations of a eurytopic species exaggerated (or perhaps even reduced) in comparison to interspecific trait disparities between stenotopic species specialized to hypoxic and normoxic conditions respectively? The *Brachyhypopomus* system provides an excellent model to explore these novel questions.

3.2 Methods

Methods for collection, assessing ecological distributions, and dissections and measurements follow those in Chapter 2.

3.3 Statistical analyses

ii. Intraspecific variation of gill surface area in three eurytopic species

1. Analysis of covariance

For each of the three eurytopic species, analysis of covariance (ANCOVA) was used to compare total gill filament length (TGFL) against the covariate body mass, with alternative habitats (i.e. populations in hypoxic habitats versus populations in normoxic habitats) as treatment groups. ANCOVA offers the advantage of allowing both the intercept (TGFL for a given body size) and slope (the rate at which TGFL increases with body size) to be discriminated between populations. Unlike in Chapter 2, where ANCOVA was used only to yield single species-specific TGFL values for a subsequent t-test, here I employ ANCOVA as my primary statistical tool for exploring population-level disparities in TGFL among populations of the three eurytopic species. ANCOVA was implemented using a custom script in R.

The sample size of *B. brevirostris* (n = 46) was larger than *B. beebei* (n = 17) and *B.* n. sp. WALT (n = 25). To permit a more equitable comparison among species, the sample size for *B. brevirostris* was therefore reduced as follows: 11 individuals where randomly drawn from

each DO category and ANCOVA was run on those individuals. This procedure was then repeated 20 times and the intercept, slope, and their associated *F*-value and *P*-values were averaged among all 20 repeats.

ii. Comparison of gill surface area in eurytopic species with stenotopic species

1. One-way analysis of variance

Here I extend the interspecific comparison in Chapter 2 by including the three eurytopic species, represented by populations from normoxic and hypoxic habitats (i.e. six populations), with approximately equal sample sizes. These six populations, along with the 22 stenotopic species restricted to either hypoxic or normoxic habitats, were individually categorized as separate treatments, yielding a total of 28 taxa (species or populations). Taxa were categorized into four categories: 1) Stenotopic-Hypoxic; 2) Stenotopic-Normoxic; 3) Eurytopic-Hypoxic; 4) Eurytopic- Normoxic. Here I adopt the same approach as in Chapter 2, but rather than using a one-way t-test comparing two groups (i.e. species found exclusively in hypoxic environments and species found exclusively in normoxic environments), I applied one-way analysis of variance (ANOVA) to the four aforementioned groups. Again, as described in Chapter 2, the ANOVA was based on a single measure of TGFL derived for each taxon (i.e. the size adjusted mean) using a multi-taxon ANCOVA (the procedure being contingent on homogeneity of slopes among all taxa) (see Figure 3). The threshold for significance (i.e. *P*-value) was adjusted using the Bonferroni correction factor to 0.017 (calculated from 0.05 / k, where k equals the number of comparisons) for the three multiple

comparisons of the one-way ANOVA. In summary, this one-way ANOVA is intended to reveal significant disparities in gill surface area involving one or more of these four categories of *Brachyhypopomus*.

2. Two-way analysis of variance

I ran an additional two-way ANOVA to specifically address the question of whether there is a significant disparity between eurytopic species and stenotopic species, when controlled for habitat type (i.e. hypoxic versus normoxic). This ANOVA was structured as follows: the predictor variable was the habitat type, and the response variable was the adjusted mean TGFL. The two treatments were eurytopic species and stenotopic species. The first factor in this test is habitat type (hypoxic verses normoxic) and the second factor is "habitat specialization" (eurytopic versus stenotopic). A result in which the two factors are significant, but the interaction term between them is non-significant would permit us to deduce that eurytopic species differ from stenotopic species in TGFL in a manner that is independent of the disparity in TGFL associated with habitat type (i.e. normoxic versus hypoxic). The two-way ANOVA was executed in JMP Pro 9.0.0 64-bit Edition (SAS Institute Inc. Cary, NC) using the settings for unequal unbalanced sizes, with some samples exhibiting low sample size (i.e $n < ca. 6$).

3. Principal component analysis

Taxon was divided into the four categories summarized above. I used multivariate Principal Component Analysis (PCA) to ordinate all individual fishes from the 28 taxa in a multivariate space charactering gill morphology. PCA was computed using a custom R script from a correlation-matrix where rows represented individual fishes, and columns represented metrics of gill morphology. Here I included not only TGFL, but also the additional measured gill metrics described in Chapter 2 (iii. Dissection and measurements) (i.e. THA, TNF, GFL, TP, and AGL). Also, I included not only the specimens within the restricted 0.5 – 3.5 g range used for the ANOVA approach (as outlined in Chapter 2. iv. Statistical analyses), but also specimens outside this range.

PCA provides a graphical means to visualize relationships between a suite of measured gill morphology metrics and DO regime. An additional useful feature of PCA is the assignment of Eigen values to each principal component (PC) – providing an estimate of the total amount of variance in the dataset explained by each PC (Gotteli and Ellison 2004). Also, factor loadings can be calculated from PCA to determine the relative contribution of each gill metric to the variance along each principal component (axis) – i.e. a measure of the relative importance of each gill metric in accounting for between-group differences.

I plotted only the first two canonical axes, which were sufficient to explain most of the important variance in the gill morphology dataset (see Chapman et al. 2008; Crampton et al. 2008 for similar approaches). All gill measurements included in PCA were size-adjusted by dividing each by the body mass of the individual (in g) prior to analysis. For each of the first two principle components, factor loadings were obtained for the six gill metrics using custom scripts in R.

3.4 Results

i. Intraspecific variation of gill surface area in three eurytopic species of *Brachyhypopomus*

ANCOVA demonstrated that in two of the three eurytopic species (*B*. n. sp. WALT and *B. beebei*) the total gill filament lengths of populations found in hypoxic habitats were significantly greater than populations found in normoxic habitats (Figure 6). *B.* n. sp. WALT exhibited substantial differences in both the intercept and slope of these disparities, with the greatest disparities in TGFL occurring at larger sizes (n =25; intercept: $F = 31.405$, $P < 0.0001$; slope: $F = 21.870$, $P < 0.0001$; Table 2). The other, *B. beebei*, showed significant intercepts but not slopes (n =17; intercept: $F = 38.434$, $P < 0.0001$; slope: $F = 1.792$, $P = 0.203$; Table 2). The third species, *B. brevirostris*, exhibited no significant differences in the intercepts and slopes, suggesting no evidence for population difference between hypoxic and normoxic habitats (n =22; intercept: $F = 0.873$, $P = 0.461$; slope; $F = 2.348$, $P = 0.474$; Table 2).

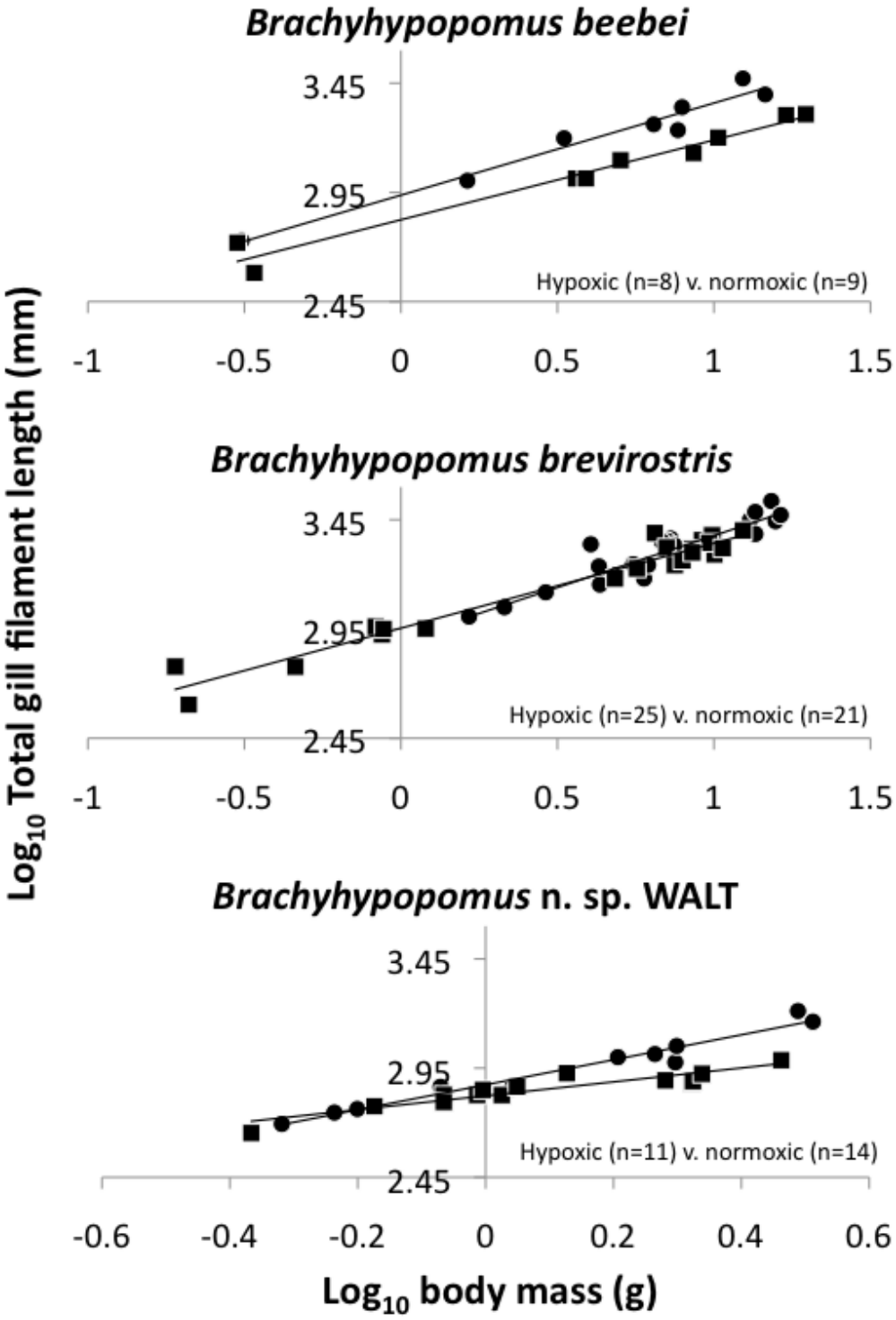

Figure 6. Bilogarithmic regressions comparing total gill filament length of populations from hypoxic habitats (circles) to populations from normoxic habitats (squares) for three eurytopic species of *Brachyhypopomus*.

Table 2. Summary of ANCOVA analyses examining population-level differences in total gill filament length (TGFL) for three eurytopic species of *Brachyhypopomus.* Values significant at a threshold of $p < 0.05$ are reported in bold.

Species	Habitat	n	Mean mass (g) (range)	TGFL (mm) (range)	Slope	Intercept	r^2	Slope P-value	Intercept P-value
B. beebei	hypoxic	8	6.77 (0.31-14.58)	1789.85 (536.23-2961.71)	0.42	2.97	0.962	0.203	< **0.0001**
	normoxic	9	7.64 (0.3-19.67)	1245.20 (381.72-2031.39)	0.36	2.82	0.967		
B. brevirostris	hypoxic	11	7.79 (1.65-16.33)	2055.99 (1015.91-3435.08)	0.47	2.90	0.842	0.474	0.461
	normoxic	11	5.85 (0.19-12.38)	1618.69 (401.40-2519.37)	0.38	2.95	0.954		
***B.* n. sp. WALT**	hypoxic	11	1.58 (0.48-3.25)	934.15 (494.70-1627.90)	0.57	2.78	0.962	< **0.0001**	< **0.0001**
	normoxic	14	1.39 (0.43-2.90)	721.69 (449.47-969.20)	0.31	2.82	0.846		

ii. Comparisons of gill surface area in eurytopic species and stenotopic species

1. One-way analysis of variance

The results of a one-way ANOVA comparing gill surface area among the following four categories is summarized in Table 3: 1) Stenotopic-Hypoxic; 2) Stenotopic-Normoxic; 3) Eurytopic-Hypoxic; 4) Eurytopic-Normoxic. The size adjusted total gill filament lengths for all 28 taxa, divided into these four groups, are plotted in Figure 7.

ANOVA revealed a marginally significant disparity between the Eurytopic-Hypoxic category and Eurytopic-Normoxic category in total gill filament lengths (n = 6, df = 3 and 24, t = -1.960, P = 0.0617). The fact that this test revealed only a marginally significant disparity is clearly a consequence of the lack of population-level differences in total gill filament lengths for *B. brevirostris* (see previous section i. Intraspecific variation of gill surface area in three eurytopic species). With *B. brevirostris* removed from this ANOVA, the disparity becomes strongly significant (n = 4, df = 3 and 22, t = -3.303, $P < 0.005$).In a separate comparison, the Stenotopic-Hypoxic category did not exhibit significantly larger total gill filament lengths than the Eurytopic-Normoxic category (n = 11, df = 3 and 24, t = 0.2040, P = 0.8403).

This ANOVA also demonstrated that the Eurytopic-Hypoxic category had significantly larger total gill filament lengths than the Stenotopic-Hypoxic category (n = 11, df = 3 and 24, t = 2.567, P = 0.0169). Likewise, the Eurytopic-Normoxic category had larger total gill filament lengths than the Stenotopic-Normoxic category (n = 17, df = 3 and 24, t = 4.418, $P < 0.005$).

Table 3. Summary of one-way ANOVA comparing size-adjusted total gill filament length (TGFL) among taxa of *Brachyhypopomus*, divided into four categories on the basis of the oxygen regime. Size adjusted TGFLs are presented with antilog values reported in brackets. Non-significant differences between groups is denoted by underlined group initials.

Category	**Size adjusted TGFL**	**P-value**	**Significant disparity**
Stenotopic species restricted to hypoxic habitats (S-H)	2.87 [758.1]	**<0.001**	E-H E-N S-H S-N
Stenotopic species restricted to normoxic habitats (S-N)	2.73[537.4]		
Populations of eurytopic species from hypoxic habitats (E-H)	2.97 [948.2]		
Populations of eurytopic species from normoxic habitats (E-N)	2.88 [771.8]		

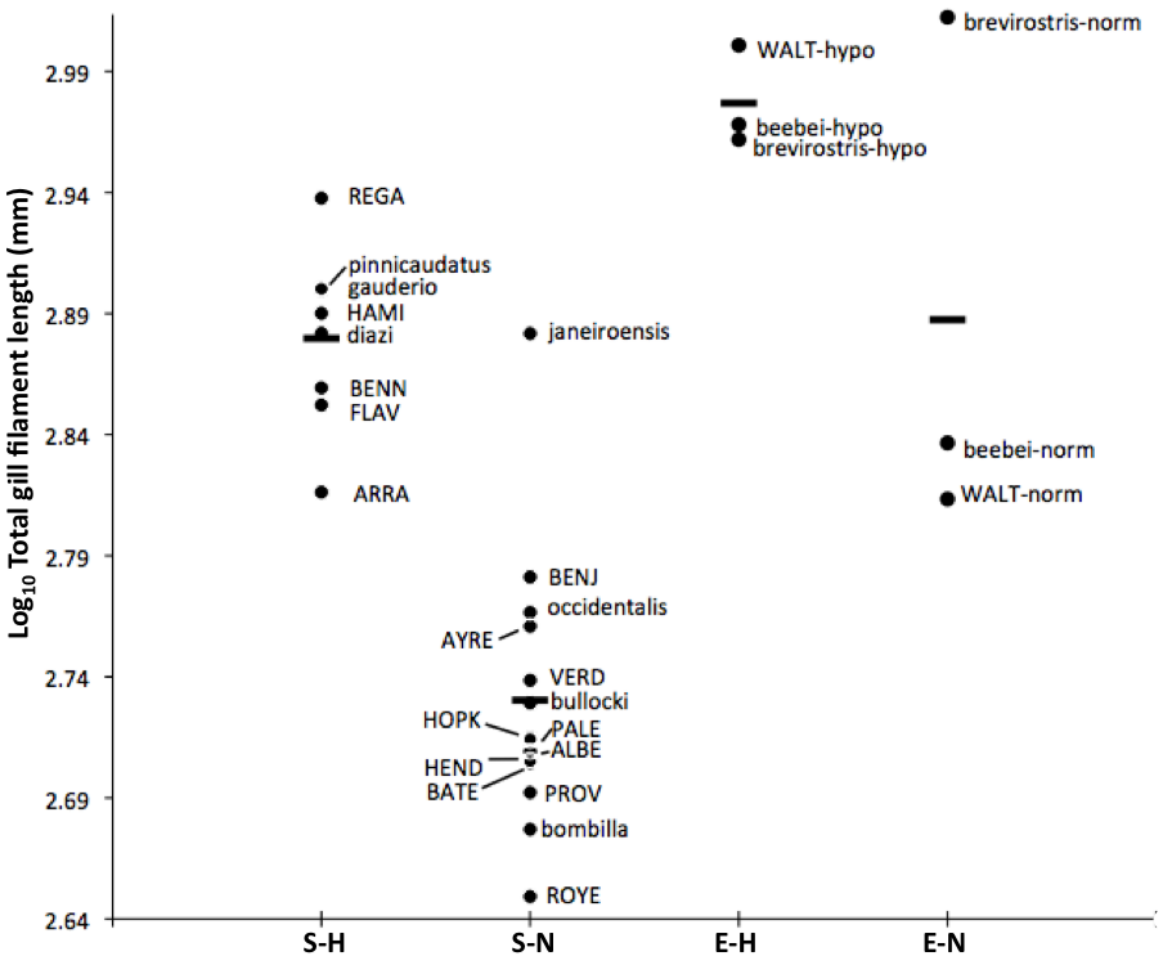

Figure 7. Biplot of total gill filament lengths for 28 taxa categorized among four dissolved oxygen categories: 1) Stenotopic-Hypoxic (S-H, n = 8); 2) Stenotopic-Normoxic (S-N, n = 14); 3) Eurytopic-Hypoxic (E-H, n = 3); and 4) Eurytopic-Normoxic (E-N, n =3). The y-axis represents size adjusted total gill filament lengths calculated as summarized in Figure 3. Bold horizontal lines represent the means for each category.

2. Two-way analysis of variance

The two-way ANOVA showed a significant disparity between eurytopic and stenotopic species, with eurytopic species exhibiting significantly larger gill surface areas (n = 6, mean = 2.93, SE = 0.023) than stenotopic species (n = 22, mean = 2.78, SE = 0.0124) (t ratio = 4.90, $P < 0.0001$; Table 4). Likewise, this ANOVA also repeated the earlier observation) (see preceding section 1. One-way analysis of variance), that taxa from hypoxic systems (n = 11, mean = 2.91, SE = 0.0189) have larger gills than those in normoxic environments (n = 17, mean = 2.758, SE = 0.0178) (t ratio = 4.6, $P = 0.0001$; Table 4). Importantly, the two-way ANOVA also showed an insignificant interaction term between the two factors (i.e. 1: habitat type [normoxic vs. hypoxic] and 2. habitat specialization [stenotopic vs. eurytopic]) (t ratio = -1.15, $P = 0.2596$; Table 4). This indicates that the disparity observed here between stenotopic and eurytopic species occurs independently of the disparity between species that occur in hypoxic and normoxic systems. Thus, eurytopic species have larger gills than stenotopic species, even when corrected for the confounding effect of them exhibiting populations in both kinds of habitats (normoxic and hypoxic).

Table 4. Summary of two-way ANOVA. Differences in total gill filament length (TGFL) among 28 species or populations of *Brachyhypopomus* were analyzed using habitat type (hypoxic versus normoxic) and habitat specialization (eurytopic versus stenotopic) as the first and second factors, respectively. Significant values are reported in bold.

Source	DF	Sum of squares	F-value	P-value
Habitat type	1	0.066	21.15	**0.0001**
Habitat specialization	1	0.074	23.97	**<0.0001**
Interaction	1	0.004	1.33	0.2596

3. PCA

Principal component analysis (PCA) generated six principal components (PCs), corresponding to the six gill metrics, with the largest proportion of variance (0.9654) contained in the first two PCs (0.9010 and 0.0644 respectively). The biplot of the first two PCs (Figure 8) shows that the second PC mostly separates the Stenotopic-Hypoxic category (strongly positive values) from the Stenotopic-Normoxic category (strongly negative values), with only marginal overlap. The second PC also partially separates the Eurytopic-Hypoxic category from the Eurytopic-Normoxic category. In contrast the first PC axis does not separate the four groups. Instead, variance on the first PC probably reflects gill-shape related variation in gill metrics unrelated to TGFL (body size related variation in gill metrics is unlikely to explain this variance because all gill metrics were size-adjusted, see Methodology).

For the first two PCs, squared factor loadings (r) were calculated for each gill metric (Table 5). Factor loadings explain the relationship between gill metrics and each principle component, where high values indicate that a given gill metric explains a larger amount of the variance on the component (Cohen 1988; Rodgers and Nicewander 1988). The gill metric weighing most heavily on the second PC (which partially separates individuals by four DO categories) is total hemibranch area (r = 0.51). All gill parameters display factor loadings that weigh heavily on the first PC, especially total gill filament length and average hemibranch length - again likely reflecting overall gill-shape related variation (Table 5).

Mirroring the results of the one-way ANOVA, populations of eurytopic species from hypoxic environments occupied a different, but overlapping, area of multivariate space – corresponding to larger gills (higher values on PC 2).

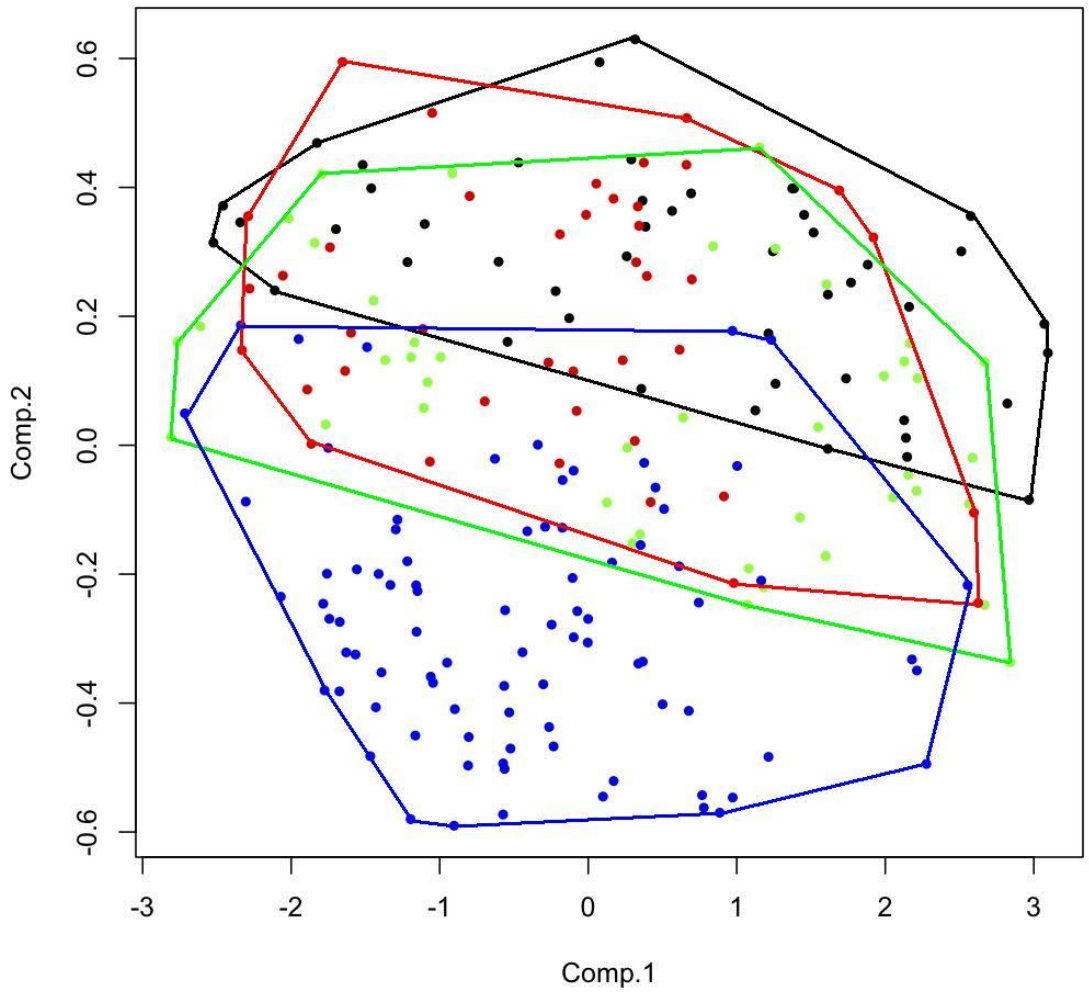

Figure 8. Biplot of the first two principal components from a principal component analysis of multiple gill measures from individuals of 25 species of *Brachyhypopomus.* Individuals are color-coded according to four dissolved oxygen categories: 1) Stenotopic-Hypoxic (red, n = 47), 2) Stenotopic-Normoxic (blue, n = 88), 3) Eurytopic-Hypoxic (black, n = 50), and 4) Eurytopic-Normoxic (green, n = 44). Boundaries are drawn around furthest outliers for each category to visualize overlap in multivariate space. Gill measurements included in were: total filament length (TGFL), total hemibranch area (THA), total gill filament number (TNF), grand average filament length (GFL), total hemibranch perimeter (TP), and average gill hemibranch length (AGL).

Table 5. Squared factor loadings (*r*) based upon correlation matrix for six gill metrics (total gill filament length [TGFL], total hemibranch area [THA], total gill filament number [TNF], grand average filament length [GFL], total hemibranch perimeter [TP], and average gill hemibranch length [AGL]). Numbers in bold represent variables that loaded heavily on each component (>0.40) and therefore are considered to contribute substantively to between-species variation.

Gill Metric	PC1	PC2
TGFL	**0.98**	0.13
THA	**0.86**	**0.51**
TNF	**0.96**	-0.24
GFL	**0.96**	-0.07
TP	**0.96**	-0.1
AGL	**0.97**	-0.17

3.5 Discussion

i. Intraspecific variation: local adaptation or plasticity?

Two processes, either alone or acting together, may be responsible for the salient correlations between gill surface area and oxygen regime that I have observed in the eurytopic species *B. beebei* and *B.* n. sp. WALT (but not *B. brevirostris*): The first is phenotypic plasticity (sensu West-Eberhard 1989, 2003; Stearns 1989; Price et al. 2003), where one genotype can produce alternate phenotypes depending on the environment that acts on the organism during development. The second is local adaptation (e.g. Nagy and Rice 1997; Sultan and Spencer 2002), where genetically distinct populations have adapted to local environmental conditions.

Phenotypic plasticity: Numerous studies have shown that species that are widely dispersed over heterogeneous environments are more plastic than species restricted to one habitat type (Daehler 2003; Richards et al. 2006; Crispo 2008). Several rearing studies have noted phenotypic plasticity in gill surface area of fishes, with a larger gill surface area found in adult individuals from populations exposed to hypoxic conditions during development relative to those raised in normoxic conditions (Chapman et al. 2000, 2007, 2008; Saroglia et al. 2002).

Local Adaptation: Population-level variation in gill surface area has not been attributed to localized adaptation in fishes. Nonetheless, evidence of divergent selection driving phenotypic diversity between populations of the same species occurring in different habitats

has been documented in many other taxa (e.g. considering fishes: *Gasterosterus* spp., Schluter 1993, 1995; *Coregonus* spp. Lu and Bernatchez 1999; *Poecilia vivipara,* Gomes and Monterio 2008; *Gambusia caymanensis*, Langerhans and Gifford 2009).

Several authors have noted that gene flow between populations exposed to different environmental conditions is contrary to an outcome of localized adaptation, and instead should favor the evolution of phenotypic plasticity (Haldane 1948; Slatkin 1973; Felsenstein 1976; Sultan and Spencer 2002, Chapman et al 1999). Under these circumstances, plasticity is expected to evolve if the benefits it confers outweigh any cost of maintaining it (e.g. maintaining the genetic machinery to permit differential developmental trajectories under differing environmental conditions), and as long as the environmental cues leading to an alternate morphology are reliable (see Dewitt et al. 1998; Sultan 2000 for a discussion of the conditions under which plasticity is expected to evolve). For instance, Crispo and Chapman (2010a) noted population-level variation in gill surface areas for an African cichlid (*Pseudocrenilabrus multicolor victoriae*) dispersed across a heterogeneous dissolved oxygen landscape. Previous studies had demonstrated low divergence of microsatellite markers across this landscape, suggesting that either gene flow is high, or dispersal recent (Crispo and Chapman 2008, 2010b). Based on this suggestion of ongoing or recent genetic exchange among these populations, they speculated that variation is likely a manifestation of phenotypic plasticity. A hypothesis of phenotypic plasticity has also been independently confirmed in this species by rearing experiments (see Chapman et al. 2000, 2008).

In the *Brachyhypopomus* system, there are several reasons to suspect that localized adaptation is unlikely and that cases of population-level variation in eurytopic species may instead be the result of phenotypic plasticity. All three of the eurytopic species considered

here have a very large geographical distribution. Throughout this range, normoxic blackwater habitats usually are in close proximity to hypoxic whitewater floodplain environments, and are linked hydrologically – with no barriers to dispersal. Moreover, there is a broad ecotone between these systems along most of the length of whitewater rivers, and the three eurytopic species considered here are especially common in these ecotone systems. In Amazonian habitats, gene flow is also enhanced by the passive transport of both juvenile and adult fishes in rafts of floating macrophytes, which break free and drift downstream (Henderson and Hamilton 1995; Schiesari et al. 2003). Finally, geomorphological studies indicate that the boundaries between floodplain and terra firme systems have been blurred, throughout the Quaternary, by eustatic oscillations known in the Amazon as "Irion cycles" (Irion 1984; Colinvaux 2007) – providing repeated opportunities for genetic exchange. Crispo (2008) and Crispo and Chapman (2010a) noted that in the absence of barriers to dispersal and movement across environmental gradients (as we document here for eurytopic *Brachyhypopomus* species), migration load limits the opportunity for local genetic adaptations to arise by divergent selection, but instead promotes the evolution of phenotypic plasticity.

The juxtaposition of hypoxic and normoxic habitats through the entire range of eurytopic *Brachyhypopomus* species, the complete modern and historical absence of barriers to dispersal between these habitats, and the known large-scale dispersal of gymnotiforms via rafts of floating meadows combine to disfavor the hypothesis of local adaptation. Instead, variations in gill surface area between populations of *Brachyhypopomus* from normoxic and hypoxic environments may likely be caused phenotypic plasticity. Nonetheless, although beyond the scope of this study, demonstrating phenotypic plasticity in the *Brachyhypopomus* system will ultimately require ruling out locally adapted genetic variation via rearing

experiments, in which fertilized eggs or small larvae from one fish are divided and exposed to experimentally controlled DO regimes (Chapman et al. 2000, 2008; Hall 2001).

ii. Gill surface area and body size

Of the two species which exhibited population-level differences in gill surface areas, in only one of these, *B.* n. sp. WALT, was the disparity between populations in hypoxic and normoxic systems exaggerated in magnitude at a larger body size (see significant slope as well as intercept disparities in ANCOVA, Table 2 Figure 6). The tendency for gill surface area to be exaggerated in populations from hypoxic environments relative to normoxic environments at larger body size may be related to interspecific variation in the importance of cutaneous respiration. It is well known that cutaneous respiration is the primary mode of respiration in the larvae of many fishes (de Silva 1974; see for review Feder and Burggren 1985; Rombough 1988). Later in development, when gills become more developed, the primary site of respiration moves from the skin to the gills (Rombough 1988; 1998). Although the skin is not the primary site of respiration for adults of most fishes, it has been reported that 10-30% of oxygen is typically obtained through cutaneous respiration (Feder and Burggren 1985; Kirsch and Nonnotte 1977; Nonnotte 1981). Julian et al. (2003) commented on the high levels of vascularization in the skin of some gymnotiforms and on their laterally-compressed knife-like shape, and speculated that cutaneous respiration may be especially important in this order (here it is also worth noting that gymnotiform scales are typically small and thin – with no protective function – and little impediment to the exchange of DO).

As ectotherms increase in body size, the surface area as a proportion of body volume decreases – and so cutaneous respiration is predicted to be less efficient in larger animals, independently of ontogenetic stage per se. (de Silva 1974; Hughes and Al-Kadhomiy 1988). Likewise, a decreased reliance on cutaneous respiration is expected to result in stronger selection for more efficient branchial respiration, explaining the exaggerated disparity in gill surface areas between hypoxic and normoxic populations of *B.* n. sp. WALT at larger body size. Nonetheless, for reasons that I am unable to explain, I did not observe this phenomenon in *B. beebei*. Also, previous studies of intraspecific variation in gill surface area in relation to oxygen have not revealed the patterns observed in *B.* n. sp. WALT (Chapman and Liem 1995; Chapman et al. 1999; Timmerman and Chapman 2004). These observations perhaps represent the first indication of species-level variation in the relative importance of cutaneous respiration at different stages of growth.

iii. Comparison of gill surface area in eurytopic species and stenotopic species

The observation of significantly larger gill surface areas in populations of eurytopic species from a given oxygen regime relative to stenotopic species restricted to that oxygen regime is surprising, and has no precedents. The observation is counterintuitive because where eurytopic species do exhibit population-level variation in gill surface area (as observed in *B. beebei* and *B.* n. sp. WALT) the baseline expectation is that the population in the hypoxic environment would approximately match that of stenotopic species restricted to hypoxic environments in terms of gill surface area – regardless of whether the population-level

variation involved local adaptation or phenotypic plasticity. Where eurytopic species *do not* exhibit population-level variation in gill surface area (as observed in *B. brevirostris*), and given trade-offs that may render large gills at a disadvantage where they are not needed, the baseline expectation is that gill surface area might exhibit an intermediate value between that of species that are restricted to hypoxic and normoxic environments.

An explanation of this paradoxical observation must necessarily not only consider why populations of eurytopic species in *hypoxic* environments exhibit larger gill surface areas than stenotopic species that occur only in hypoxic environments (rather than gill of approximately equal surface area), but also why populations of eurytopic species found in *normoxic* environments should exhibit larger gill surface areas than stenotopic species that occur only in normoxic environments – when one would not expect selection to drive the expression of this trait. Below I consider three potential explanations for this phenomenon.

One explanation is related to the abundance of eurytopic species in the broad ecotones that separate whitewater floodplain and blackwater ecosystems along the axis of all of the Amazon's major whitewater rivers. These ecotones receive influxes of water from adjacent systems with the amount of influx from each system depending upon local flooding conditions. Consequently the water quality in ecotones is constantly changing. Moreover, oxygen levels are prone to decline much more rapidly (with a sudden ingress of anoxic whitewater floodplain water) than occurs in whitewater floodplains, where oxygen levels decline steadily and slowly as the floodwaters gradually inundate the forests. The sudden flash deoxygenations of ecotone systems may favor gill surface areas that are even larger than are required to be successful in whitewater floodplains The rationale underlying this speculation is that floodplain species typically exhibit elevated blood hermatocrit levels and

hemoglobin concentrations (Val 1993; Val and Almeida-Val 1995), in addition to other physiological adaptations, but these typically elevate slowly in response to hypoxia (Val 1993) – requiring a relatively gradual acclimation. Interestingly, the diversity of electric fishes in ecotone systems of the central Amazon is generally low, but all of the three eurytopic species described here are especially abundant – constituting the top three most abundant species of *Brachyhypopomus* (Crampton pers obs).

An alternative explanation is that populations of the eurytopic species may only have invaded hypoxic habitats in the recent evolutionary past. Species that are restricted to whitewater floodplains and other hypoxic environments not only exhibit large gill surface areas, but likely also exhibit the typical array of physiological specializations found in fishes inhabiting these seasonally anoxic systems, e.g. modified hemoglobin structure, higher hermatocrit levels etc. (Val 1996, et al. 1998; see review in Kramer et al. 1987; Martinez et al. 2004). Recently invaded populations may not have had time to evolve very advanced physiological adaptations, and furthermore, physiological adaptations are generally not as quickly selected for as morphological adaptations (Falconer 1981; Mousseau and Roff 1987). These recently invaded populations may initially rely disproportionally on larger gills for extracting oxygen from the water, and from the air during aerial breathing. Consequently, via phenotypic plasticity, these populations may first develop especially large gills.

Third, I discuss an explanation specifically for the larger-than expected gills in normoxic populations of eurytopic species. The explanation centers on the reliability of environmental cues that determine plasticity. Not every year do major rivers of the Neotropics flood with the same amplitude. For instance, Henderson and Robertson (1999) illustrate variation in the flood amplitude of the Amazon river near Tefé from some 10 to 16

m. During a low-amplitude flood season or if the flood waters rise late, many larval *Brachyhypopomus* in floodplain systems will be exposed to normoxic conditions prolonged well into their development. Under these conditions, the genetic architecture of phenotypic plasticity may push these juveniles into a trajectory of gill development leading to relatively small gills. If the flood waters then rise later in the year, and oxygen levels decline rapidly, these individuals would have disadvantageously small gills, with consequent reduction in their fitness. The development of gill surface areas typical of species from hypoxic environments (note that the one-way ANOVA showed no disparity between the Eurytopic-Normoxic and Stenotopic-Normoxic categories; $P = 0.9726$), *even* in the absence of triggers during early development for additional expansion of gill size, would effectively constitute a 'fail safe' mechanism to facilitate survival in an extremely unpredictable environment. The cost of erring on the side of caution is that large gills develop where they are not required - in permanently normoxic systems. Under these conditions trade-offs with traits whose expression may be constrained by large gills may lower fitness. The fact that two eurytopic species have larger-than expected gills, and yet also show population-level variation, suggests that trade-offs may still be having some effect on limiting gill surface area in normoxic systems, that is, these costs do have consequences.

Finally, I discuss the case of *B. brevirostris*. This species not only is unique among the three eurytopic species in exhibiting little or no population-level variation in gill surface area, but in both populations from hypoxic and normoxic environments, body size-corrected gill surface area was exceptionally large (among the largest among all species). Why should this be the case? Crampton's (1998a) study of respiratory biology in floodplain gymnotiforms noted that of the seven species that occur in whitewater floodplains (and that

therefore must be able to tolerate protracted hypoxia), just two species *B. brevirostris* and *B.* n. sp. REGA did not exhibit air breathing. *B.* n. sp. REGA exhibited aquatic surface respiration, but not air breathing, and both species showed a drastic reduction in motor behavior and electric signal pulse rate well before other species during gradual, experimentally-induced hypoxia, and subsequent anoxia. *B. brevirostris* was unique in exhibiting a complete arrest of its movement and electric signal, until even branchial ventilation was reduced to very low amplitude and rate. Apparently, *B. brevirostris* relies heavily on physiological mechanisms to tolerate anoxic conditions, perhaps including a switch to anaerobic respiration. The inability for *B. brevirostris* to supplement water-breathing with air breathing is surprising, but may explain why the species exhibits unusually large gills – permitting it to extract as much oxygen as possible from hypoxic conditions, before switching to a state of metabolic quiescence during overt anoxia. As Crampton (1998a) demonstrates, *B. brevirostris* was the least active species when gradually exposed to hypoxia; the unusually large gill surface area of *B. brevirostris* may make performing ASR or aerial respiration unnecessary. However, under conditions of complete anoxia, it appears to be predominantly reliant on physiological mechanisms. Consistent with the notion that large gill surface areas may evolve in non-air breathing species which frequent hypoxic environments, *B.* n. sp. REGA (Crampton 1998a), exhibits the largest gill surface area among the species restricted to whitewater floodplains, and is also a non-air breather.

3.6 Conclusions

The data presented here indicate that gill surface area is likely a phenotypically plastic trait in two of three documented eurytopic species of *Brachyhypopomus* (*B. beebei* and *B.* n. sp. WALT). In contrast, gill surface area appears to be a fixed, non-plastic trait in one eurytopic species (*B. brevirostris*), and in all of the stenotopic species. There have been many studies that invoke plasticity in permitting some organisms to enhance their niche breadth, via the expression of a range of phenotypes, depending on developmental history (Bradshaw 1965; Van Valen 1965; Whitlock 1996; Richards et al. 2006). The patterns I have documented in *B. beebei* and *B.* n. sp. WALT are consistent with this rubric. Furthermore, these two eurytopic species comply with Baker's (1965) criterion for a plastic trait that may permit an organism to expand its distribution: 1) the trait should maintain fitness across heterogeneous environments and 2) the trait should be able to increase fitness in environments that are most favorable (Baker 1965; Sultan 2001). If the plastic trait maintains fitness at all environment types this represents a "jack-of-all-trades" scenario (sensu Richards et al. 2006). It appears as if *B. beebei* and *B.* n. sp. WALT qualify as jacks-of all-trades when it comes to gill surface area because: 1) eurytopic species are distributed throughout heterogeneous oxygen environments, and 2) the trait of large gill surface area is enhanced in hypoxic environments where this trait is most favored.

CONCLUDING REMARKS TO THE THESIS

The results presented in this thesis support a growing body of evidence that an enlarged gill surface area is an adaptive strategy for tolerating hypoxia in fishes (Hughes 1966; Hughes and Morgan 1973; Muir and Hughes 1969; Galis and Barel 1980; Pazenberger and Pohla 1992; Chapman and Liem 1995; Chapman et al 2000, 2008; Chapman and Hulen 2001; Crampton et al 2008; Paterson et al. 2010). In *Brachyhypopomus*, hypoxia is evidently a significant selective pressure that plays a role in the divergence of gill size at both the species- and population-level. Gill surface area in the eight species of *Brachyhypopomus* restricted to hypoxic habitats was, was substantially higher than in the 14 species restricted to normoxic habitats. This pattern was also documented at the intraspecific level in two eurytopic species (*B. beebei* and *B.* n. sp. WALT) in comparisons of populations from hypoxic and normoxic systems.

The observed patterns of intraspecific variation within two eurytopic species (*B. beebei* and *B.* n. sp. WALT) could, in principle, be the result of populations being either development plastic or, alternatively, genetically distinct and adapted to local environmental conditions. I concluded that the former is more likely because there are no barriers to gene flow between normoxic and hypoxic habitats throughout the distributional range of each species (all of which are found throughout the Amazon, Orinoco and coastal drainages of the Guianas). Under these conditions the chances for localized adaptation are unlikely.

This study was the first of its kind to compare eurytopic species to stenotopic species. Gill surface areas were found to be exceptionally large in eurytopic species when compared to either species exclusively found in hypoxic or normoxic environments. I provided three

possible explanations for this finding: first, eurytopic species are especially abundant in ecotone systems that are subject to fluctuations in oxygen levels over much shorter time frames than in whitewater floodplain systems. This may favor the evolution of extremely large gill surface areas. Second, eurytopic species could have recently invaded floodplain systems and have had time to evolve larger gills, but not a range of physiological adaptations for hypoxia, which are expected to take longer. Third, the unpredictability of the annual flood cycle may favor the evolution of especially large gills even in years where a pathway for gill enlargement in juveniles is not 'switched on' due to hypoxia in early development. Although advantageous in providing a large (though not largest) gill surface area in habitats where hypoxia sets in later in the year, populations in normoxic systems will always develop larger than necessary gills – with potential fitness disadvantages associated with osmotic regulation, or tradeoffs with other morphological structures.

I also noted that one species, *B. brevirostris*, was unusual in not only possessing the largest gill surface areas among all 25 studied species but was the only eurytopic species that did not have population-level variation. The exceptionally large gill surface area may be caused by the lack of aerial respiration under hypoxia stress as was demonstrated in an earlier study (Crampton 1998a). The lack of population-level variation may be caused by one or a combination of the three scenarios I mentioned above (the fast deoxygenation of ecotones associated with the rapid egress of deoxygenated water from adjacent floodplain systems, recent invasion in to floodplains, and unpredictability of floodplain regime) – all of which are expected to be exaggerated due to this species' unusual reliance on gills for aquatic respiration.

This thesis has brought about several unanswered questions that remain to be tested.

Although there have been studies that measure efficiency in respiratory performance as a metric of hypoxia tolerance, it would be valuable to measure actual oxygen uptake capacity among species of varying gill surface areas, as, surprisingly, large gill surface area has not yet been empirically linked to increased oxygen uptake capacity. With respect to gill surface area, the results of this thesis produced novel findings, and for the first time, eurytopic species were compared to stenotopic species. The question still remains, what causes the gill surface area of eurytopic species to be significantly larger than those of stenotopic species? Some of the explanations I raised may be tractable to experimental evaluation. For example, if eurytopic species have recently invaded hypoxic environments and thus not have time to evolve other adaptations, then a comparison of physiological adaptations between eurytopic and species that are exclusively found in floodplains would be expected to reveal a wider suite of physiological means of tolerating oxygen in the floodplain specialists (e.g. lower metabolic rate, higher hemoglobin and hermatocrit levels).

Although, as argued above, local adaptation is considered to be a less likely explanation for intraspecific variation in gill size for *B. beebei* and *B.* n. sp. WALT than phenotypic plasticity, further work is needed to confirm this hypothesis. A split-blood experiment of the kind conducted by (Chapman et al. 2000; 2008) would be the appropriate means to resolve the question. Evidence for developmental plasticity in gill surface area has been found in two Africa cichlid species *(Pseudocrenilabrus multicolor victoriae*) (Chapman et al. 2000; 2008) and (*Astatoreoshromis alluaudi*) (Chapman et al. 2007), the sea bass (*Dicentrarchus labrax*) (Saroglia et al 2002), and the North European cyprinid (*Carassius carassius*) (Sollid et al. 2003; 2005). Nonetheless, most gymnotiform fishes are difficult to breed in captivity and in any case the technology required to maintain DO level at controlled

levels is exceptionally expensive.

The results of this thesis will increase our general understanding of how morphological traits originate and are maintained by natural selection. The results of this study are likely to be of specific interest to evolutionary studies of electric fishes – including investigations of speciation, sensory biology, and the evolution of communication systems. Adaptations to hypoxia are of special interest to studies of the metabolic costs of electric signaling (e.g. Julian et al. 2003; Salazar and Stoddard 2008). One especially interesting line of investigation in *Brachyhypopomus* is the extent to which electric signals are plastic, and the way in which the endocrine system regulates this plasticity. Evidence is mounting for one model species, *B. gauderio*, that electric signaling is metabolically inexpensive in non-breeding males, and females (3% of basal metabolic rate, BMR), but comparatively expensive in breeding males (11-22% of BMR) (Salazar and Stoddard 2008). Because signal energetics is constrained by oxygen availability (Julian et al. 2003), a deeper understanding of the diversity of morphological adaptations to hypoxia is expected to add important new perspectives to this field.

LIST OF REFERENCES

Akaike H. 1992. Information theory and an extension of the maximum likelihood principle. *In:* Kotz S and Johnson (eds). *Breakthrough in Statistics.* Springer-Verlag, New York. pp. 610-624.

Albert JS and Crampton WGR. 2003. Family Hypopomidae. In: Reis RE, Kullander SO, Ferraris CJ Jr (eds). *Checklist of the freshwater fishes of South and Central America.* EDIPUCRS. Porto Alegre. pp. 500-502.

Albert JS and Reis RE (eds). 2011. *Historical Biogeography of Neotropical Freshwater Fishes*. University of California Press, Berkeley.

Almeida-Val VMF, Val AL, Hochachka, PW. 1993. Hypoxia tolerance in Amazon fishes: Status of an under-explored biological "goldmine". IPW Hochachka, PL Lutz, T Sick, M Rosenthal, and G Van der Thillart (eds.), *Surviving Hypoxia: Mechanism of Control and Adaptation.* CRC Press, Boca Raton. pp. 435-455.

Almeida-Val VF and Farias IP. 1996. Respiration in fish of the Amazon metabolic adjustments to chronic hypoxia. In: Val AL, Almeida-Val VMF, and Randall DJ (eds), *Physiology and Biochemistry of Fishes of the Amazon.* INPA, Manaus, Brazil. pp. 257-271.

Baker HG. 1965. Characteristics and modes of origin of weeds. IN: Baker HG and Stebbins GI (eds). *The Genetics of Colonizing Species*. Academic Press. New York. pp. 147-169.

Barel CDN. 1983. Towards a constructional morphology of cichlid fishes (Teleostei, Perciformes). *Netherlands Journal of Zoology.* 33: pp. 357-424.

Bennett MVL. 1971. Electric organs. In: Hoar W and Randall DJ (eds), *Fish Physiology*. Academic Press. New York. pp. 347-491.

Berg T and Steen JB. 1965. Physiological mechanisms for aerial respiration in the eel. *Comparative Biochemistry and Physiology.* 15(4): pp. 469-484.

Bickler PE and Buck LT. 2007. Hypoxia tolerance in reptiles, amphibians, and fishes: Life and variable oxygen availability. *Ann. Rev. Physiol.* 69: pp. 145-170.

Bond AN. 1960. An analysis of the response of salamander gills to changes in the oxygen concentration of the medium. *Developmental Biology.* 2: pp. 1-20.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics.* 13: pp. 115-155.

Bullock TH, Hopkins CD, Popper AN, Fay RR. (eds). 2005. Electroreception. Springer, New

York.

Burd BJ. 1988. Comparative gill characteristics of *Munida quadrispina* (Decapoda, Galatheidae) from differing habitat oxygen conditions. *Canadian Journal of Zoology*. 66: pp. 2320-2333.

Burggren WW and Mwalukoma A. 1983. Respiration during chronic hypoxia and hyperoxia in larval and adult bullfrogs (*Rana catebeiana*). I. Morphological responses of lungs, skin, and gills. *Journal of Experimental Biology.* 105: pp. 191-203.

Burnham KP and Anderson DR. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach.* Springer-Verlag, New York.

Caputi A and Budelli R. 1993. A realistic model of the electric organ discharge (EOD) of *Gymnotus carapo. Journal of Comparative Physiology A.* 173: pp. 751.

Carter GS. 1955. The papyrus swamps of Uganda- Heffer, Campbridge, 25

Carter GS and Beadle LC. 1930. The fauna of the swamps of the Paraguayan Chaco in relation to its environment II. *J. Linn. Soc. Zool. London.* 37: pp. 327-368.

Cech JJ and Massingill MJ. 1995. Tradeoffs between respiration and feeding in Sacramento blackfish, *Orthodon microlepidotus. Environmental Biology of Fishes.* 44: pp. 157-

163.

Chapman LJ. 2007. Morpho-physiological Divergence Across Aquatic Oxygen Gradients in Fishes. In: Fernandes MN, Rantin FT, Glass ML, and Kapoor BG (eds.), *Fish Respiration and Environment.* Science Publishers. Enfields, New Hampshire, pp. 13-39.

Chapman LJ, Kaufman LS, Chapman CA, and McKenzie FE. 1995. Hypoxia tolerance in twelve species of East African cichlids: Potential for low oxygen refugia in Lake Victoria. *Conservation Biology*. 9: pp. 1274-1288.

Chapman LJ and KF Liem. 1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. *Environmental Biology of Fishes*. 44: pp. 183-197.

Chapman LJ, Chapman CA, Brazeau D, McGlaughlin B, Jordon M. 1999. Papyrus swamps and faunal diversification: Geographical variation among populations of African cyprinid *Barbus neumayeri*. *Journal of Fish Biology.* 54: pp. 310-327.

Chapman LJ, Galis F, and Shinn J. 2000. Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters.* 3: pp. 387-393.

Chapman LJ and Hulen KG. 2001. Implications of hypoxia for the brain size and the gill morphometry of mormyrid fishes. *The Journal of Zoology*. 254: pp. 461-472.

Chapman LJ, Chapman CA, Nordlie FG, Rosenberger AE. 2002. Physiological refugia: swamps hypoxia tolerance, and maintenance of fish biodiversity in the Lake Victoria Region. *Comparative Biochemistry and Physiology- Part A: Molecular and Integrative Physiology.* 133: pp. 421-437.

Chapman LJ, DeWitt TJ, Tzenava V, and Paterson J. 2007. Interdemic variation in the gill morphology of a eurytopic African cichlid. In: Brauner CJ, Suvajdzic K, Nilsson G, and Randall (eds.). Proceedings of the 9th International Symposium on Fish Physiology, Toxicology, and Water Quality (EPA publication EPA/600/R-07/010). pp. 209-225.

Chapman LJ, Albert J, and Galis F. 2008. Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. *The Open Evolution Journal.* 2: pp. 75-88.

Chapman LJ and McKenzie DJ. 2009. Behavioral responses and ecological consequences. In: Richards JG, Farrell AP, and Brauner CJ (eds), *Hypoxia.* Academic Press, London. pp. 25-77.

Chippari-Gomes AR, Lopes NP, Paula-Silva MdN, Oliveria AR, and Almeida-Val MF.

2003. Hypoxia Tolerance and Adaptations in Fishes: The Case of Amazon Cichlids. In: Luis Val A, Kapoor BG (eds.), *Fish Adaptations.* Science Publishers. Enfields, New Hampshire, pp. 37-54.

Claireaux G and Lagardère J-P. 1999. Influence of temperature, oxygen and salinity on the metabolism of the *European* sea bass. *Journal of Sea Research.* 42: pp. 157-168.

Colinvaux PA. 2007. *Amazon expeditions: My quest for the Ice Age Equator. New Haven.* Yale University Press.

Costa WJEM and Campos-da-Paz R. 1999. Description d'une nouvelle espèce de poisson électrique du genre néotropical *Hypopomus* (Siluriformes: Gymnotoidei: Hypopomidae) du Sud-Est du Brésil. Revue Francaise d'Aquariologie. 18 (4): pp. 117-120.

Crampton WGR. 1996. *The electric fish of the Upper Amazon: Ecology and signal diversity*. Ph.D. dissertation, The University of Oxford, London.

Crampton WGR. 1998a. Effects of anoxia on the distribution, respiratory strategies and electric signal diversity of gymnotiform fishes. *Journal of Fish Biology.* 53: pp. 307-330.

Crampton WGR. 1998b. Electric signal design and habitat preferences in a species rich

assemblage of gymnotiform fishes from the upper Amazon basin. *An. Acad. Bras. Ci.* 70: pp. 805-847.

Crampton WGR 1999. Os peixes da Reserva Mamirauá: diversidade e história natural na planície alagável da Amazônia. In: Queiroz HL and Crampton WGR (eds). *Estratégias para Manejo de Recursos Pesqueiros em Mamirauá*. Sociedade Civil Mamirauá/CNPq, Brasília. pp. 10-36.

Crampton WGR. 2001. Diversity and conservation of the fishes of the Amazon Basin. In: Paitán SF (ed). *Amazonia: Directions for Sustainable Development.* Universidad Nacional de la Amazonía Peruana, Lima, Peru. pp. 121-140.

Crampton WGR. 2006. Evolution of electric signal diversity in gymnotiform fishes. II. Signal design. In: *Communication in Fishes*, F Ladich, S.P. Collin, P. Moller and B.G. Kapoor (eds.) Science Publishers, Enfield, 2: pp. 697-731.

Crampton WGR. 2007. Diversity and adaptation in deep channel Neotropical electric fishes. In: Sebert P, Onyango DW, and Kapoor BG (eds). *Fish life in special environments*. Science Publishers. Enfield NH. pp. 283-339.

Crampton WGR. 2008. Ecology and life history of an Amazon floodplain cichlid: the discus fish *Symphysodon* (Perciformes: Cichlidae). *Neotropical Ichthyology* 6:599-612.

Crampton WGR. 2011. An ecological perspective on diversity and distributions. In: Albert J and Reis R (eds). *Historical biogeography of Neotropical Freshwater Fishes.* University of California Press, Berkeley. pp. 165-189.

Crampton WGR and Albert JS. 2006. Evolution of electric signal diversity in gymnotiform fishes. In: Ladich F, Collin SP, Moller P, Kapoor BG (eds.), *Communication in Fishes.* Science Publishers, Enfield.

Crampton WGR, Chapman LJ, and Bell J. 2008. Interspecific variation in gill size is correlated to ambient dissolved oxygen in the Amazonian electric fish *Brachyhypopomus* (Gymnotiformes: Hypopomidae). *Environmental Biology of Fishes*. 83: pp. 223-235.

Crawshaw LI. 1984. Low-temperature dormancy in fish *American Journal of Physiology.* 246: pp. R479-R486.

Crispo E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *Journal of Evolutionary Biology.* 21: pp. 1460-1469.

Crispo E and Chapman L. 2008. Population genetic structure across dissolved oxygen regimes in an African cichlid fish. *Molecular Ecology.* 17: pp. 2134-2148.

Crispo E and Chapman LJ. 2010a. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *Journal of Evolutionary Biology*. doi:10.1111/j.1420-9101.2010.02069. pp. 1-13.

Crispo E and Chapman LJ. 2010b. Temporal variation in population genetic structure of a riverine African cichlid fish. *Journal of Heredity*. 101(1): pp. 97-107

Daehler CC. 2003. Performance comparisons of co-occurring native and alien invasive plants for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics.* 34: pp. 183-211.

Dejours P. 1981. *Principles of comparative respiratory physiology.* Elsevier/Holland Biomedica, Amsterdam.

de Silva, C. 1974. Development of the respiratory system in herring and plaice larvae. In: The early life history of fish (J.H.S. Blaxter ed.). Springer-Verlag. p.465-485.

DeWitt TJ, Sih A, and Wilson DS.1998. Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution.* 13: pp. 77-81.

Diaz RJ and Breitburg DL. 2009. The hypoxic environment. In: *Hypoxia*, Richards JG, Farrell AP, and Brauner CJ (eds.) Academic Press, London. pp. 1-23.

Falconer DS. 1981. *Introduction to Quantitative Genetics.* Second edition. Longman, New York.

Feder ME and Burggren WW. 1985. Cutaneous gas exchange in vertebrates: design, patterns, control, and implications. *Biological Reviews.* 60(1): pp. 1-45.

Felsenstein J. 1976. The theoretical population genetics of variable selection and migration. *Annual Review of Genomics and Human Genetics.* 10: pp. 253-280.

Fernandes MN, Rantin FT, Kalinin AL, and Moron SE. 1994. Comparative study of gill dimensions of three erythrinid species in relation to their respiratory function. *Canadian Journal of Zoology.* 72: pp. 160-165.

Fernandez CC. 1997. Lateral migration of fishes in Amazon floodplains. *Ecology of Freshwater Fish.* 6: pp. 36-44.

Fernandes MN and de Mazon AdF. 2003. Environmental Pollution and Fish Gill Morphology. In: Luis Val A, Kapoor BG (eds.), *Fish Adaptations.* Science Publishers. Enfields, New Hampshire. pp. 203-231.

Freda J and McDonald DG. 1988. Physiological correlates of interspecific variation in acid tolerance in fish. *Journal of Experimental Biology.* 136: pp. 243-258.

Furch K. 1984. Water chemistry of the Amazon basin: the distribution of chemical elements among freshwaters. In: Sioli H (ed), *The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and Its Basin.* Junk, Dordrecht. pp. 167-199.

Galis F and Barel CDN. 1980. Comparative functional morphology of the gills of African lacustrine Cichlidae (Pisces, Teleostei): An ecomorphological approach. *Netherlands Journal of Zoology* 30: pp. 392-430.

Gayet M, Marshall LJ, Sempere T, Meunier FJ, Cappetta H, Rage JC. 2001. Middle Maastrichtian vertebrates (fishes, amphibians, dinosaurs and other reptiles, mammals) from Pajcha Patga (Bolivia). Biostatigraphical, palaeoecological and palaeobiogeographic implications. *Palaeogeography, Palaeoclimatology and Palaeoecology*. 169: pp. 39-68.

Gibbs RH and Hurwitz BA. 1967. Systematics and zoogeography of the stomiatoid fishes, *Chaulidus pammelas* and *C. sloani*, of the Indian Ocean. Copeia. pp. 798-805.

Gomes JL and Monteiro LR. 2008. Morphological divergence patterns among populations of *Poecilia vivapara* (Teleostei Poeciliidae): test of ecomorphological paradigm. *Biological Journal of the Linnean Society.* 93: pp. 799-812.

Gonzalez RJ, Dalton VM, and Patrick ML. 1997. Ion regulation in ion-poor, acidic water by

the blackskirt tetra (*Gynmnocorymbus ternetzi*), a fish native to the Amazon River. *Physiological Zoology*. 70: pp. 428-435.

Gonzalez RJ, Wood CM, Wilson RW, Patrick M, Bergman H, Narahara A, Val AL. 1998. Effects of water pH and calcium concentration on ion balance in fish of the Rio Negro, Amazon. *Physiological Zoology*. 71: pp. 15-22.

Gonzalez RJ and Preest MR. 1999. Ionoregulatory specializations for exceptional tolerance of ion-poor, acidic waters in the neon tetra (*Paracheirodon innesi*). *Physiological and Biochemical Zoology*. 72(2): pp. 156-163.

Gonzalez RJ and Wilson RW. 2001. Patterns of ion regulation in acidophilic fish native to the ion-poor, acidic Rio Negro. *Journal of Fish Biology*. 58: pp. 1680-1690.

Gonzalez RJ, Wilson RW, Wood CM, Patrick ML, Val AL. 2002. Diverse strategies for ion regulation in fish collected from the ion-poor, acidic Rio Negro. *Physiological and Biochemical Zoology*. 75(1): pp. 37-47.

Gonzalez, R. J., R. W. Wilson, and C. M. Wood. 2006. Ionoregulation in tropical fishes from ion-poor acidic blackwaters. Pp. 397-442 *in* A. L. Val, V. M. F. Almeida-Val, and D. J. Randall, eds. The Physiology of Tropical Fishes. Academic Press, London.

Goulding M. 1980. *The fishes and the forest*. University of California Press, Berkeley and

Los Angeles.

Goulding M, Barthem R, and Ferreira EG. 2003. *The Smithsonian Atlas of the Amazon.* Washington DC. Smithsonian Books.

Gotelli NJ and Ellison AM. 2004. The analysis of multivariate data. In. Gotelli NJ and Ellison AM (eds.), *Primer of Ecological Statistics.* Sinauer Associates. pp. 383-445.

Graham JB. 1990. Ecological, Evolutionary, and physical factors influencing aquatic animal respiration. *American Zoologists.* 30: pp. 137-146.

Graham JB. 1997. Air breathing fishes: Evolution, Diversity, and Adaptation. Academic Press, San Diego.

Gray IE. 1954. Comparative study of gill area of marine fishes. *Biology Bulletin Marine Biology Laboratory, Woods Hole.* 107: pp. 219-55.

Griswold CK. 2006. Gene flow's effect on the genetic architecture of a local adaptation and its consequences for QTL analyses. *Heredity.* 96: pp. 445-453.

Guppy M and Withers P. 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biological Reviews.* 74: pp. 1-40.

Haldane JBS. 1948. The theory of a cline. *Journal of Genetics.* 48: pp. 277-284.

Hall BK. 2001. Organic selection, proximate environmental effects on the evolution of morphology and behavior. *Biology and Philosophy.* 16: pp. 215-237.

Hamilton SK, Sippel SJ, and Melack JM. 1995. Oxygen depletion and carbon-dioxide and methane production in waters of the pantanal wetland of Brazil. *Biogeochemistry.* 30: pp. 115-141.

Hamilton SK, Sippel SJ, Calheiros DF, and Melack JM. 1997. An anoxic event and other biogeochemical effects of the Pantanal wetland on the Paraguay River. *Limnol. Oceanogr*. 42: pp. 257-272.

Hamilton SK, Sippel SJ, and Melack JM. 2004. Seasonal inundation patterns in two large savanna floodplains of South America: the Llanos de Moxos (Bolivia) and the Llanos del Orinoco (Venezuela and Colombia). *Hydrological Processes* .18: pp. 2103-2116.

Henderson PA and Hamilton HF. 1995. Standing crop and distribution of fish in drifting and attached floating meadow within an Upper Amazonian varzea lake. *Journal of Fish Biology.* 47: pp. 266-276.

Henderson PA and Robertson BA. 1999. On structural complexity and fish diversity in an Amazonian floodplain. In: Padoch C, Ayres JM, Pinedo-Vasquez, and Henderson A (eds.), *Varzea diversity, development, and conservation of Amazonia's whitewater*

floodplains. The New York Botanical Garden Press, New York. pp. 45-58

Hoorn C. 2006. The birth of the mighty Amazon. *Scientific American.* 295: pp. 52-59.

Hopkins CD. 1983. Functions and mechanisms in electroreception. In: Northcutt RG and Davis RE (eds.), *Fish Neurobiology*, vol 1. Ann Arbor: Michigan University Press.

Hopkins CD. 1991. *Hypopomus pinnicaudatus* (Hypopomidae), a new species of gymnotiform fish from French Guiana. *Copeia.* 1: pp. 151-161.

Hopkins CD. 1999. Design features for electric communication. *Journal of Experimental Biology.* 202(10): pp. 1217-1228.

Hughes GM. 1966. The dimensions of fish gills in relation to their function. *Journal of Experimental Biology.* 45: pp. 177-195.

Hughes GM and Al-Kadhomiy NK. 1988. Changes in scaling of respiratory systems during the development of fishes. *Journal of Marine Biological Association of the United Kingdom.* 68: pp. 489-498.

Hughes GM and Morgan M. 1973. The structure of fish gills in relation to their respiratory function. *Biological Reviews.* 48: pp. 419-475.

Irion G. 1984. Sedimentation and sediments of Amazonian rivers and evolution of the Amazonian landscape since pliocene times. In: Sioli J (ed). *The Amazon Limnology and landscape of a mighty tropical river and its basin.* Dr W. Junk, Dordrecht. pp. 201-204.

Johansen K. 1966. Air breathing in the teleost *Symbranchus marmoratus. Comparative Biochemistry and Physiology.* 18(2): pp. 383-395.

Johansen K. 1970. Air breathing in fishes. In: Hoar WS and Randall DJ (eds). *Fish Physiology. Volume 4.* Academic Press, New York. pp. 361-411.

Johnston IA and Dunn J. 1987. Temperature acclimation and metabolism in ectotherms and with particular reference to teleost fish. *Symposia of the Society for Experimental Biology.* 41: pp. 67-93.

Julian D, Crampton WGR, Wohlgemuth SE, and Albert JS. 2003. Oxygen consumption in weakly electric Neotropical fishes. *Oecologia.* 137: pp. 502-511.

Junk WJ. 1983. Aquatic habitats in Amazonia. *The Environmentalist* 3, pp. 24-35.

Junk WJ and Furch K. 1985. The physical and chemical properties of the Amazonian waters and their relationships with the biota. In: G. T. Prance and T. E. Lovejoy (eds), *Key environments: Amazonia*. Oxford, Pergamon/IUCN**:** 3-17.

Kingsolver JG, Pfenning DW, and Servedio MR. 2002. *Migration, local adaptation and the evolution of plasticity. Trends in Ecology and Evolution.* 17(12): pp. 540-541.

Kirsch R and Nonnotte G. 1977. Cutaneous respiration in three freshwater teleosts. *Respiration Physiology.* 29(3): pp. 339-354.

Kobza RM, Trexler JC, Loftus WF, Perry SA. 2004. Community structure of fishes inhabiting aquatic refuges in a threatened karst wetland and its implications for ecosystem management. *Biological Conservation.* 116: pp. 153-165.

Kramer DL. 1983. The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. *Environmental Biology of Fishes* 9: 145-158.

Kramer DL. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes.* 18: pp. 81-92.

Kramer DL, Lindsey CC, Moodie GE, and Stevens ED. 1978. The fishes and the aquatic environment of the Central Amazonian basin, with particular reference to respiratory patterns. *Canadian Journal of Zoology.* 58.

Kramer DL and McClure M. 1982. Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. *Env. Biol. Fish.* 7: pp. 47-55.

Kramer DL and Mehegan JP. 1981. Aquatic surface respiration, and adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). *Environmental Biology of Fishes.* 6: pp. 299-313.

Langerhans RB and Gifford ME. 2009. Divergent selection, not life-history plasticity via food limitation, drives morphological divergence between predator regimes in *Gambusia hubbsi. Evolution.* 63: pp. 561-567.

Lasso C, Rial AB, and Lasso-Alcalá O. 1997. Bioecological aspects of the taxocenosis of electric fishes (Ostariophysi: Gymnotiformes), in the Apure Llanos of Venezuela. *Acta Biológica Venezuélica.* 17: pp. 7-29.

Lemons DE and Crawshaw LI. 1985. Behavioral and metabolic adjustments to low temperatures in the largemouth bass (*Micropterus salmoides*). *Physiological Zoology*. 58. pp. 175-180.

Lenormand T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology and Evolution.* 17(4): 183-189.

Lorenzo D, Sierra F, Silva A, and Macadar O. 1990. Spinal mechanisms of electric organ discharge synchronization in *Gymnotus carapo. Jorunal of Comparative Physiology A.* 167: pp. 447-452.

Lowe-McConnell RH. 1964. The fishes of the Rupununi savanna district of British Guiana. Pt I. Groupings of fish species and effects of the seasonal cycles on the fish. *Zoological Journal of the Linnean Society (London).* 45: pp. 103-144.

Lowe-McConnell RH. 1975. Fish communities in tropical freshwaters: their distribution, ecology, and evolution. London: Longman Press.

Lowe-McConnell RH. 1987. *Ecological Studies in Tropical Fish Communities.* Cambridge: Cambridge University Press.

Lowe-McConnell RH. 1993. Fish faunas of the African Great Lakes: origins, diversity, and vulnerability. *Conservation Biology.* 7(3): pp. 634-643.

Lu G and Bernatchez L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution.* 53(5): pp. 1491-1505.

Lundberg JG. 1998. The temporal context for the diversification of neotropical fishes. In: *Phylogeny and Classification of Neotropical Fishes.* LR Malabarba, R.E. Reis, R.P. zvari, Z.M. Lucena and C.A. Lucena (eds.). Edipucrs, Porto Alegre, Brazil, pp. 49-68.

Lundberg JG, Kottelat M, Smith GR, Stiassny MLJ, Gill AC. 2000. So many fishes, so little time: An overview of recent ichthyological discovery in continental waters. *Annals Missouri Botanical Garden.* 87: pp. 26-62.

Machado-Allison A. 1990. Ecology of fishes of the floodplain areas of the Venezuelan Llanos. *Interciencia.* 15: pp. 411-423.

Mangum CP, Haswell MS, Johansen K, Towle DW. 1978. Inorganic ions and pH in the body fluids of Amazon animals. *Canadian Journal of Zoology.* 56: pp. 907-916.

Martinez MS, Chapman LJ, Grady JM, and Rees BB. 2004. Interdemic variation in hermatocrit and lactate dehydrogenase in the African cyprinid *Barbus neumayeri. Journal of Fish Biology* 65: pp. 1056-1069.

Mazon A de F, Fernandes MN, Nolasco MA, and Severi W. 1998. Functional morphology of gills and respiratory area of two active rheophilic fish species, *Plagioscion squamoisissimus* and *Prochilodus scrofa. Journal of Fish Biology.* 52: pp. 50-61.

McDonald DG. 1983. The interaction of calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri.* I. Branchial and renal net ion and H^+ fluxes. *Journal of Experimental Biology.* 102: 123-140.

McDonald DG, Walker RL, and Wilkes RLK. 1983. The interaction of calcium and low pH on the physiology of the rainbow trout *Salmo gaidneri.* II. Branchial ionoregulatory mechanisms. *Journal of Experimental Biology.* 102: pp. 141-155.

McDonald DG, Freda J, Cavdek V, Gonzalez R, and Zia S. 1991. Interspecific differences in gill morphology of freshwater fish in relation to tolerance of low-pH environments. *Physiological Zoology.* 64: pp. 124-144.

McKinsey DM and Chapman LJ. 1998. Dissolved oxygen and fish distribution in a Florida spring. *Environmental Biology of Fishes*. 53: pp. 211-233.

Mousseau TA and Roff DA. 1987. Natural selection and the heritability of fitness components. *Heredity.* 59(2): pp. 181-97.

Muir BS and Hughes GM. 1969. Gill dimensions for three species of tunny. *Journal of Experimental Biology.* 51: pp. 271-285.

Nagy ES and Rice KJ. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution.* 51: pp. 1079-1089.

Nilsson S and Sundin L. 1998. Gill blood flow control. *Comparative Biochemistry and Physiology.* 199A: pp. 137-147.

Nonnotte G. 1981. Cutaneous respiration in six freshwater teleosts. *Comparative Biochemistry and Physiology Part A: Physiology.* 70(4): pp. 541-543.

Nuttall CP. 1990. A review of the Tertiary non-marine molluscan faunas of the Pebasian and other inland basins of north-western South America. *Bulletin of the British Museum of Natural History – Geology*. 45: pp. 165-371.

Palzenberger M. and Pohla H. 1992. Gill surface area of water-breathing freshwater fish. *Reviews in Fish Biology and Fisheries* 2: pp. 187-216.

Paterson JA, Chapman LJ, and Schofield PJ. 2010. Intraspecific variation in gill morphology of juvenile Nile perch, *Lates niloticus,* in Lake Nabugabo, Uganda. *Environmental Biology of Fishes.* 88(2): pp. 97-104, DOI: 10.1007/s10641-010-9600-6.

Pazin VFV, Magnusson WE, Zuanon J, and Mendonca FP. 2006. Fish assemblages in temporary ponds adjacent to 'terra-firme' streams in Central Amazonia. *Freshwater Biology.* 51: pp. 1025-1037.

Perry S. 1997. The chloride cell: Structure and function in the gills of freshwater fishes. *Annual Review of Physiology.* 59: pp. 325-347.

Perry S. 1998. Relationship between branchial chloride cells and gas transfer in freshwater fish. *Comparative Biochemical Physiology.* 119A(1): pp. 9-16.

Potts WTW. 1984. Transepithelial potentials in fish gills. In: Hoar WE and Randall DJ (eds), *Fish Physiology, Volume 10B.* Academic Press, Orlando. pp. 105-128.

Price JW. 1931. Growth and gill development in the small-mouthed bass, *Micropterus dolomieu,* Lacépède. *Ohio State University Press.* 4: pp. 1-46.

Price TD, Qvarnstrom A, Irwin DE. 2003 The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society London B.* 270: 1433-1440.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISB

Reis RE. 1998. Systematics, biogeography, and the fossil record of the Callichthyidae: a review of the available data. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, and Lucena CAS (eds). *Phylogeny and Classification of Neotropical Fishes.* Edipucrs, Porto Alegre. pp. 351-362

Reis RE, Kullander SO, and Ferraris CJ. 2003. Introduction. In: Reis RE, Kullander SO, Ferraris CJ Jr. (eds), *Checklist of the Freshwater Fishes of South and Central America.* Edipucrs, Porto Alegre, pp. 1-3.

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, and Pigliucci M. 2006. Jack of all trades, masters of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters.* 9: pp. 981-993.

Rombough PJ. 1988. Respiratory gas exchange, anaerobic metabolism, and the effects of hypoxia during early life. In: Hoar WS and Randall DJ (eds). *Fish Physiology: volume XI: The physiology of developing fish: Part A: Eggs and Larvae.* Academic Press, Inc. San Diego, California. pp. 59-144.

Rombough PJ. 1998. Partitioning of oxygen uptake between the gills and skin in fish larvae: a novel method for estimating cutaneous oxygen uptake. *The Journal of Experimental Biology.* 201. pp. 1763-1769.

Rutjes JA. 2006. Phenotypic responses to lifelong hypoxia in cichlids. PhD dissertation. Leiden University.

Saint-Paul U and Soares MGM. 1987. Diurnal distribution and behavioral responses of fishes to extreme hypoxia in an Amazonian floodplain lake. *Environmental Biology of Fishes.* 20: pp. 91-104.

Saul WG. 1975. An ecological study of fishes at a site in upper Amazonian Ecuador. *Proc. Acad. Nat. Sci. Philadelphia.* 127: pp. 93-134.

Salazar VL and Stoddard PK. 2008. Sex differences in energetic costs explain sexual dimorphism in the circadian rhythm modulation of the electrocommunication signal of gymnotiform fish *Brachyhypopomus pinnicaudatus.* Journal of Experimental Biology. 211: pp. 1012:1020.

Saroglia M, Terova G, De Stradis A, and Caputo A. 2002. Morphometric adaptations of sea bass gills to different dissolved oxygen partial pressures. *Journal of Fish Biology.* 60: pp. 1423-1430.

Schaack S and Chapman LJ. 2003. Interdemic variation in the African cyprinid *Barbus neumayeri:* correlations among hypoxia, morphology, and feeding performance. *Canadian Journal of Zoology.* 81: pp. 430-440.

Schiesari L, Zuanon J, Azevedo-Ramos C, Garcia M, Gordo M, Messias M, and Vieira EM. 2003. Macrophyte rafts as dispersal vectors for fishes and amphibians in the Lower Solimões River, Central Amazon. *Journal of Tropical Ecology.* 19: pp. 333-336.

Slatkin M. 1973. Gene flow and selection in a cline. *Genetics.* 75: pp. 733-756.

Sollid J, De Angelis PM, Gundersen K , and Nilsson GE. 2003. Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *Journal of Experimental Biology.* 206: pp. 3667-3673.

Sollid J, Weber RE, and Nilsson GE. 2005. Temperature alters the respiratory surface area of crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*). *Journal of Experimental Biology.* 208: pp. 1109-1116.

Solid J and Nilsson G. 2006. Plasticity of respiratory structures – Adaptive remodeling of fish gill induced by ambient oxygen and temperature. *Respiratory Physiology and Neurobiology.* 154: pp. 241-251.

Steinbach AB. 1970. Diurnal movements and discharge characteristics of electric gymnotid fishes in the Rio Negro. Brazil. *Biology Bulletin.* 138: pp. 200-210.

Storfer A, Cross J, Rush V, and Caruso J. 1999. Adaptative coloration and gene flor as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri. Evolution.* 53: pp. 889-898.

Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science.* 5(12): pp. 537-542.

Sultan SE. 2001. Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. *Ecology.* 82: pp. 328-343.

Sultan SE and Spencer HG. 2002. Metapopulation structure favors plasticity over local adaptation. *American Naturalist.* 160(2): pp. 271-283.

Timmerman CM and Chapman LJ. 2004. Hypoxia and interdemic variation in *Poecilia latipinna. Journal of Fish Biology.* 65: pp. 635-650.

Townsend SA and Edwards CA. 2003. A fish kill event, hypoxia and other limnological impacts associated with early wet season flow into a lake on the Mary River floodplain, tropical northern Australia. *Lake Reservoirs and Reservoirs: Res. Manag.* 8: pp. 169-176.

Val AL. 1993. Adaptations of fishes to extreme conditions in freshwaters. In: Bicudo JEPW (eds), *The vertebrate gas cascade. Adaptations to environment and mode of life.* CRC Press. Boca Raton.

Val AL. 1996. Surviving low oxygen levels: Lessons from fishes of the Amazon. In: A.L. Val, V.M.F. Almeida-Val and D.J. Randall (eds.), *Physiology and Biochemistry of Fishes of the Amazon.* INPA, Manuas, Brazil, pp. 59-73.

Val AL. and Almeida-Val, V.M.F. 1995. *Fishes of the Amazon and Their Environments. Physiological and Biochemical Features.* Springer-Verlag, Heidelberg.

Val AL, Silva MNP, Almeida-Val VMF. 1998. Hypoxia adaptation in fish of the Amazon: a never-ending task. *South African Journal of Zoology.* 33: pp. 107-114.

Val V. 2000. Evolutionary features of hypoxia tolerance in fish of the Amazon: from molecular to behavioral aspects: In: Val V, Gonzalez R, and MacKinley D. (eds), *Evolution of Physiological and Biochemical Traits in Fish. Symposium Proceedings. International Congress on the Biology of Fish.* American Fisheries Society

Van Valen L. 1965. Morphological variation and width of ecological niche. *American Naturalist.* 99: pp. 377-390.

Vari RP and Malabarba LR. 1998. Neotropical Ichthyology: An overview. In: Malabarba L, Reis RE, Vari RP, deLucena CAS, and DeLucena ZMS. (eds), *Phylogeny and Classification of Neotropical Fishes.* Museu de Ciências e Tecnologia, Porto Alegre. pp. 1-11.

Walker I and Henderson PA. 1996. Ecophysiological aspects of Amazonian blackwater litterbank fish communities. In: Val AL, de Almeida-Val VMF, and Randall DJ. (eds), *Physiology and Biochemistry of the Fishes of the Amazon.* National Institute for Amazon Research, Manaus. pp. 7-22.

Wesselingh FP, Kaandorp RJG, Vonhof HB, Räsänen ME, and Renema W. 2006. The nature of aquatic landscapes in the Miocene of western Amazonia: an integrated palaeontological and geochemical approach. *Scripta Geologica.* 133: pp. 363-393.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics.* 20: pp. 249-278.

West-Eberhard MJ (ed). 2003. *Developmental Plasticity and Evolution.* Oxford University Press, Oxford.

Whitlock MC. 1996. The red queen beats the Jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *American Naturalists.* 148: pp. S65-S77.

Wilson RW, Wood CM, Gonzalez RJ, Patrick ML, Bergman HL, Narahara A, and Val AL. 1999. Ion and acid-base balance in three species of Amazonian fish during gradual acidification of extremely soft water. *Physiological and Biochemical Zoology.* 72(3): pp. 277-285.