Behavioral Activity and Hypoxia Tolerance of African Weakly Electric Fish

Dissertation

zur Erlangung des akademischen Grades
Doctor rerum naturalium (Dr. rer. nat.)

Eingereicht an der Lebenswissenschaftlichen Fakultät
der Humboldt-Universität zu Berlin
von

M.Sc. STEFAN MUCHA

Präsidentin der Humboldt-Universität zu Berlin: Prof. Dr. Julia von Blumenthal
Dekan der Lebenswissenschaftlichen Fakultät: Prof. Dr. Dr. Christian Ulrichs

Gutachter/innen: 1. PD. Dr. Mirjam Knörnschild
2. Prof. Dr. Rüdiger Krahe
3. Prof. Dr. Ana Silva

Tag der mündlichen Prüfung: 02.12.2022

Stefan Mucha

Behavioral Activity and Hypoxia Tolerance of African Weakly Electric Fish

PhD-Thesis

Humboldt-Universität zu Berlin, 2022

Abstract

Fish have evolved to be the most species-rich group of all vertebrates and occupy nearly all aquatic habitats on this planet. Their physiology and behavior are shaped by ecological interactions with their environment. Daily and seasonal fluctuations in environmental conditions, such as ambient light intensity and oxygen availability, are common in aquatic habitats. How fish respond to environmental fluctuations, through behavioral, morphological and/or physiological adjustments, remains in many cases poorly understood. One limiting factor for our understanding of the adaptive capacity of fish is the lack of field data, which are necessary to inspire laboratory studies and validate their findings.

In this thesis, I investigated the morpho-physiology and behavior of two species of African mormyrid weakly electric fish, *Marcusenius victoriae* and *Petrocephalus degeni*, in the laboratory and in one of their natural habitats, the Lwamunda Swamp in Uganda. The two main objectives of this work were to (i) observe behavioral rhythms and habitat use patterns of both species under natural and laboratory conditions, and (ii) assess expression and plasticity of morpho-physiological traits that might enable *P. degeni* to survive naturally occurring low oxygen conditions (hypoxia).

To investigate behavioral rhythms, I recorded swimming activity and temporal patterns of electric organ discharges (EODs) of both species in the laboratory over 42 hours. Additionally, I distributed eight self-developed EOD-logging devices in a lagoon at Lwamunda Swamp and recorded EOD activity on six days between July and September 2019. In the laboratory, I found that both species spent close to 100% of the time in their shelter during the day and actively explore their environment at night. In the swamp lagoon, fish were most often encountered in structurally complex habitats under floating vegetation and ventured into open and unsheltered areas of the lagoon at night, presumably to forage and interact. Under the floating vegetation, where light conditions change less drastically over the diel cycle, fish remained active during the day. This suggests that ambient light is an important environmental cue for mormyrid behavior. Concomitant in-situ oxygen measurements revealed that these fish were present, and presumably most active during periods of extreme nocturnal hypoxia in their swamp habitat.

To investigate respiratory traits of swamp-dwelling *P. degeni*, I conducted respirometry experiments and measured blood lactate and hemoglobin and gill morphometrics on the first two gill arches. In agreement with previous studies, these fish had low routine oxygen consumption rates (0.23 ± 0.06 mg O_2 h^{-1}), which they maintained

until a very low ambient oxygen partial pressure was reached (1.56 ± 0.20 kPa). Additionally, they had high concentrations of hemoglobin (10.30 ± 0.72 g dl^{-1}) and lactate (6.83 ± 1.85 mM) in their blood. These adaptations indicate a high oxygen uptake and -carrying capacity and low metabolic energy demand. The appreciable concentration of blood lactate could imply the utilization of lactate as energy substrate, although further studies are needed to clarify the metabolic role of lactate for *P. degeni*. To assess the plasticity of respiratory traits, I repeated the measurements while subjecting fish to high oxygen conditions (normoxia) for up to 75 days. Long-term normoxia exposure significantly decreased hemoglobin concentration (-17%) and gill filament length (-14%). However, respirometry performance and blood lactate concentration remained unaffected, which might suggest that these fish are hypoxia specialists with only a limited capacity for phenotypic plasticity in adult fish.

Overall, the findings of this thesis paint a picture of the life of the study species that is coined by a preference for dark and structurally complex habitats, nocturnal bouts of exploration and foraging activity, and a remarkable capacity to cope with hypoxic conditions. The results of the detailed investigation of *P. degeni* from Lwamunda Swamp suggest that these fish are well adapted, and maybe even irreversibly specialized, for a life under chronically hypoxic conditions.

Zusammenfassung

Fische bilden die artenreichste taxonomische Gruppe unter den Wirbeltieren und bewohnen fast alle aquatischen Lebensräume auf diesem Planeten. Physiologie und Verhalten von Fischen sind bestimmt durch ökologische Wechselwirkungen mit ihrer Umwelt. Es kommt häufig vor, dass Umweltbedingungen, wie zum Beispiel Lichtintensität und Sauerstoffverfügbarkeit, täglichen und saisonalen Schwankungen unterliegen. Es ist nicht viel darüber bekannt, wie Fische auf fluktuierende Umweltbedingungen reagieren, beispielsweise durch Anpassungen von Verhalten, Physiologie und Morphologie. Ein limitierender Faktor für unser Verständnis dieser Anpassungsfähigkeit ist das Fehlen von Felddaten, die notwendig sind um Laborversuche zu inspirieren und/oder deren Ergebnisse zu validieren.

In dieser Arbeit habe ich die Morpho-Physiologie und das Verhalten zweier Arten Afrikanischer schwach elektrischer Fische, *Marcusenius victoriae* und *Petrocephalus degeni*, im Labor und in einem ihrer natürlichen Habitate im Lwamunda Sumpf in Uganda untersucht. Die zwei Hauptziele dieser Arbeit waren (i) tageszeitabhängige Verhaltensrhythmen (Aktivität, Habitatnutzung) im Labor und im Freiland zu untersuchen und (ii) die Ausprägung und Plastizität der morpho-physiologischen Merkmale von *P. degeni* zu untersuchen, die ihnen erlauben bei natürlich vorkommender, geringer Sauerstoffverfügbarkeit (Hypoxie) zu überleben.

Zur Untersuchung von tageszeitabhängigen Verhaltensrhythmen habe ich die Schwimmaktivität und die zeitlichen Muster der elektrischen Entladungen beider Arten im Labor über 42 Stunden erfasst. Zusätzlich habe ich acht selbstentwickelte Datenlogger, die die elektrischen Entladungen dieser Fische aufnehmen, in einer Lagune im Lwamunda Sumpf ausgebracht und an sechs Tagen zwischen Juli und September 2019 elektrische Aktivität gemessen. In den Laborversuchen verbrachten beide Arten tagsüber annähernd 100% der Zeit in einem bereitgestellten Versteck und schwammen nachts heraus um aktiv ihre Umwelt zu erkunden. In der Sumpflagune wurden die meisten Fische in strukturell komplexen Habitaten unter schwimmenden Pflanzen detektiert. Nachts schwammen die Fische aktiv in die offenen und ungeschützten Bereiche der Lagune, vermutlich um nach Futter zu suchen und zu interagieren. Unter den schwimmenden Pflanzen, wo die Lichtbedingungen weniger stark variieren zwischen Tag und Nacht, blieben die Fische auch während des Tages aktiv. Das deutet darauf hin, dass Licht ein wichtiger Umweltfaktor für das Verhalten von Mormyriden ist. Die Begleitende in-situ Messung der Sauerstoffverfügbarkeit zeigte, dass beide Arten präsent und vermutlich sogar am aktivsten waren während Phasen extremer nächtlicher Hypoxie.

Zur Untersuchung der respiratorischen Merkmale von *P. degeni*, die im Lwamunda Sumpf leben, habe ich Respirometrieversuche durchgeführt, Hämoglobin- und Laktatkonzentration im Blut gemessen, und morphologische Parameter an den ersten beiden Kiemenbögen erfasst. In Übereinstimmung mit vorherigen Studien zeigten die Fische niedrige Sauerstoffverbrauchsraten (0.23 ± 0.06 mg O_2 h^{-1}), welche sie bis zu einem sehr niedrigem äußeren Sauerstoffpartialdruck aufrechterhielten (1.56 ± 0.20 kPa). Zusätzlich zeigten sie hohe Hämoglobinkonzentrationen (10.30 ± 0.72 g dl^{-1}) und Laktatkonzentrationen (6.83 ± 1.85 mM) im Blut. Diese Ergebnisse deuten auf eine gut ausgebildete Fähigkeit dieser Fische zur Aufnahme und Speicherung von Sauerstoff im Blut, sowie einen niedrigen metabolischen Sauerstoffverbrauch hin. Die auffällig hohe Laktatkonzentration könnte mit der Nutzung von Laktat als metabolischer Energieträger einhergehen, dies muss jedoch durch weitere Untersuchungen geklärt werden.

Um die Plastizität der respiratorischen Merkmale zu untersuchen, habe ich die Messungen mit Fischen wiederholt, die für bis zu 75 Tagen bei hoher Sauerstoffverfügbarkeit (Normoxie) gehalten wurden. Langzeit-Normoxie verringerte die Hämoglobinkonzentration (-17%) und Länge der Kiemenfilamente (-14%) signifikant. Andere Merkmale wie Sauerstoffverbrauch und Blut-Laktatkonzentration blieben unbeeinflusst, was darauf hindeutet, dass diese Fische Hypoxie-Spezialisten mit einer begrenzten Kapazität für phänotypische Veränderungen sind.

Insgesamt zeichnen die Ergebnisse dieser Arbeit ein Bild vom Leben der beiden Fischarten, das geprägt ist von einer Präferenz für dunkle und strukturell komplexe Habitate, nächtlichen Phasen der Exploration und Futtersuche, und einer bemerkenswerten Hypoxie-Toleranz. Die Ergebnisse der detaillierten Untersuchung von *P. degeni* zeigen, dass diese Fische gut, und eventuell sogar irreversibel, angepasst sind an ein Leben unter chronisch hypoxischen Bedingungen.

Table of Contents

List of Figures

List of Tables

Abbreviations and Units

ASR	Aquatic surface respiration
ATP	Adenosine triphosphate
BL	Body length
BSR	Broken-stick regression
BW	Bodyweight
°C	Degree Celsius
ca.	Circa
cm	Centimeter
CV	Coefficient of variation
dl	Deciliter
DF	Degrees of freedom
DMA	Direct memory access
DO	Dissolved oxygen
e.g.	For example
EOD	Electric organ discharge
et al.	And others
FL	Gill filament length
FN	Gill filament number
FPS	Frames per second
h	Hour
HA	Gill hemibranch area
Hb	Hemoglobin
HIF	Hypoxia-inducible factor
Hz	Hertz
i.e.	That is
IPI	Inter-pulse interval
MDA	Mixture discriminant analysis
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
MMR	Maximum metabolic rate
$\dot{M}O_2$	Oxygen consumption rate
ms	Millisecond
n	Number of samples

NLR	Non-linear regression
nm	Nanometer
P	Probability
P1-dur	Duration of the first peak of a single EOD pulse at 60% relative amplitude
P2-dur	Duration of the second peak of a single EOD pulse at 60% relative amplitude
P_{crit}	Critical oxygen tension
peak-FFT	Frequency of the highest power of the fast Fourier transformed EOD pulse
PO_2	Oxygen partial pressure
PP-amp	Peak-to-peak amplitude of a single EOD pulse
PP-dur	Peak-to-peak duration of a single EOD pulse
PP-ratio	Peak 1/peak 2 amplitude ratio of a single EOD pulse
RI	Regulation index
RMR	Routine metabolic rate
RTC	Real-time clock
s	Second
SD	Standard deviation
SL	Standard body length
SMR	Standard metabolic rate
T	Temperature
TTFL	Transcription-translation feedback loop
V	Volt
ZC-slope	Slope of voltage change at zero-crossing of a single EOD pulse
µl	Microliter
µS	Microsiemens
µs	Microsecond

1 Introduction

1.1 Preface

Fresh water covers less than 1% of the earth's surface and makes up only 0.01% of the global water resources (Allen and Pavelsky, 2018; Dudgeon et al., 2006). Yet, freshwater ecosystems foster approximately 6% of all extant species, and their significance as global biodiversity hotspots is undisputed (Dudgeon et al., 2006). The current data situation regarding these precious, and increasingly threatened (Hamilton, 2010; Jenny et al., 2016a; Jenny et al., 2016b; Su et al., 2021), ecosystems is fragmentary at best, and it is reasonable to assume that a substantial part of freshwater biodiversity still lies unknown and hidden under the water surface.

Aquatic animals live in an environment that is fundamentally different from the world of terrestrial species. Although some parallels might be drawn between swimming and flying organisms - considering, for example, the resemblance of swarms of fish and birds - the physical properties of water impose different rules on the behavior and physiology of aquatic animals. Water as a medium is much more viscous than air, which makes it easier to move and interact in three dimensions under water than on land. This introduces depth as an important spatial dimension along which stratified aquatic ecosystems are organized. The low oxygen solubility in water makes it remarkably difficult to breathe underwater. This is a critical factor for most aquatic organisms, which depend on oxygen for their long-term survival (Graham, 1990; Kramer, 1984; Richards, 2009). Due to the slow diffusion speed of oxygen in water, dissolved oxygen (DO) concentration can vary considerably within just a few meters. Low oxygen availability (hypoxia), a condition that occurs only in few extreme terrestrial environments, is a common underwater phenomenon (see section 1.3.1).

In this demanding environment, fish have evolved to become the most species-rich group of all vertebrates[1], occupying nearly all aquatic habitats, from mountain streams to the deep sea, and from salt lakes to ion-poor tropical waters. Like all animals, fish are dependent on, and shaped by, their environment, and their wide geographical distribution is reflected in an astounding variety of phenotypes and behaviors. The fact that freshwater fish account for approximately half of all known fish species (Fricke et al., 2021) illustrates the immense significance of freshwater ecosystems for global biodiversity.

To date, many aspects of freshwater fish ecology are poorly understood, largely because they are difficult to assess in nature (see section 1.2.2). This thesis focuses on tropical freshwater fish and their interaction with, and adaptations to, their environment. More specifically, it explores behavioral rhythms and habitat use patterns of mormyrid

[1] According to the IUCN red list, fish constitute almost half of the 73,577 vertebrate species that have been described (Table 1a from https://www.iucnredlist.org/resources/summary-statistics, accessed November 2021).

weakly electric fish in the field and in the laboratory, and assesses the physiological and morphological traits that these fish have evolved to adapt to their DO environment.

The study system in which these issues are investigated is an excellent example of a challenging environment that has been impacted by human activity: The Lake Nabugabo region in Uganda. Here, fish, whose survival in the main lake has been imperiled by the introduction of the predatory Nile perch (*Lates niloticus*), persist in a severely hypoxic wetland area called Lwamunda Swamp (see section 1.5.1). Among them are the study species, two mormyrid weakly electric fish. Their special ability to generate and sense electric fields enables them to perceive their environment and communicate among each other in a completely different fashion than non-electric fish (see section 1.4) and offers unique opportunities for researchers to observe their behavior. As fish that are commonly described to be nocturnal, they represent animals that are difficult to observe in the wild.

The following sections will introduce the main research topics of this thesis (behavioral rhythms and aquatic hypoxia), provide background information on electric fish, and present the study system and research objectives.

1.2 Rhythms in Aquatic Ecosystems

From the morning songs of birds to the seasonal migrations of salmon, rhythmic patterns of behavior are ubiquitous in nature (Häfker and Tessmar-Raible, 2020; Wulund and Reddy, 2015). Rhythmic behavior is driven by cyclical (abiotic) shifts in environmental conditions, such as the diel (24 h) cycle, the lunar cycle, and seasonal changes, e.g., in temperature and rainfall. The predictable nature of these cycles has made it possible for organisms to adapt to, and anticipate, environmental change in order to optimize the use of resources and environmental conditions (Häfker and Tessmar-Raible, 2020).

1.2.1 Environmental and Endogenous Rhythms in Aquatic Ecosystems

Many aquatic habitats are characterized by rhythmic changes in environmental conditions, although these may differ from terrestrial cycles. Light and temperature, reliable terrestrial diel time cues (Hut et al., 2013), can be less predictable underwater. In deep and/or turbid water bodies, ambient light levels may reach values where day and night are indistinguishable. The large heat capacity of water and other factors, such as rainfall or vertical mixing, can affect daily temperature cycles (Häfker and Tessmar-Raible, 2020; Hut et al., 2013). Fluctuations of DO concentration on the other hand, are mostly negligible on land but can be subject to pronounced rhythms in water bodies. Reduced input of photosynthetically produced oxygen during the night affects diel shifts in DO concentration. Seasonal effects, such as ice cover and rainfall, can shift DO concentration over a longer timescale (e.g., Greenbank, 1945; Kannan and Job, 1980; Talling, 1957; Townsend, 1999). Alongside DO levels, pH and CO_2 concentration can fluctuate substantially on a diel and seasonal timescale (Baumann et al., 2015; Semesi et al., 2009). In tropical ecosystems, increased rainfall during the wet season can change inundation levels, flush nutrients and pollutants into water bodies and thereby change their hydrochemistry drastically (Adebisi, 1981; Hamilton, 2010).

All of these environmental cycles can shape complex behavioral patterns through direct physiological effects (e.g., change in the rates of temperature-dependent processes, change in energy availability) and by entraining endogenous clock systems that control various aspects of behavior and physiology, such as the cell-cycle, hormonal rhythms, and sleep and activity (Aschoff, 1960, reviewed in Frøland Steindal and Whitmore, 2019; Häfker and Tessmar-Raible, 2020). A major endogenous circadian clock mechanism consists of specific clock genes that are organized in transcription-translation feedback loops (TTFL), which produce oscillations in gene transcription and cellular regulation with periods of approximately 24 h (Reppert and Weaver, 2002). In zebrafish (*Danio*

rerio), these clock components are found in all tissues and show robust circadian oscillations on the transcriptional level (Whitmore et al., 1998). Interestingly, these peripheral clocks are light sensitive without the need for specialized light sensing organs such as eyes or the pineal gland (Frøland Steindal and Whitmore, 2019; Whitmore et al., 2000). In addition to genetic oscillations, cellular metabolism and redox state can interact with the molecular clock and serve as a circadian pacemaker in the absence of the TTFL mechanism (Sandbichler et al., 2018).

Given that (i) environmental cycles are widely present in aquatic habitats and (ii) aquatic organisms possess endogenous clocks, it is no surprise that adaptive behavioral rhythms are just as commonly found in marine and freshwater habitats as on land (Häfker and Tessmar-Raible, 2020; Naylor, 2010). Many aspects of fish behavior follow diel rhythms: for example, shelter use (Vanderpham et al., 2012), swimming activity (Hurd et al., 1998; Tabata et al., 1989), food intake (Boujard and Leatherland, 1992; Sánchez-Vázquez et al., 1995), foraging behavior and migration (Hobson, 1973; Ibbotson et al., 2006; Neilson and Perry, 1990), and the formation of fish assemblages (Arrington and Winemiller, 2003; Rooker and Dennis, 1991). On a longer time scale, migration and reproductive behavior have been found in many species to be linked to lunar, seasonal, and annual rhythms (reviewed in Volpato and Trajano, 2005). To what extent these rhythmically occurring, complex behaviors are direct responses to environmental cues and/or are driven by endogenous clocks, however, remains in many cases unknown.

1.2.2 Tracking Animals in the Field

The study of activity rhythms in the field is challenging, and this is particularly true for the study of aquatic animals, due to the logistical challenges and limitations of underwater sampling methods. Advances in aquatic telemetry, including acoustic, visual and GPS-based methods, have led to a marked increase of ecological tracking data in recent times (Hussey et al., 2015; Nathan et al., 2022). However, this development has not yet found its way into tropical aquatic ecosystems, where most studies on fish activity rhythms are carried out using direct observation (Frehse et al., 2020; Frehse et al., 2021; Volpato and Trajano, 2005). This method is subject to a sampling bias that favors species that are diurnal and live in clear water. Unsurprisingly, natural behavioral patterns of more elusive fish, such as nocturnal species, remain largely unknown. In this respect, weakly electric fish offer unique opportunities for in-situ behavioral observations. They generate an electric field around their body, which can be recorded non-invasively with electrodes placed in the water (see section 1.4.1.1). Thus, the presence, activity state, number, and (if the characteristics of the electric signal are known) species of weakly electric fish can be detected in a water body without the need for visual or acoustic sampling. Previous studies have demonstrated the potential of electric recordings for opening a window into

tropical underwater worlds (Henninger et al., 2018; Henninger et al., 2020; Lissmann and Schwassmann, 1965; Madhav et al., 2018; Migliaro et al., 2018; Moller et al., 1979). The detailed observations of movement patterns, communication, and social activity that have been derived from these recordings are nearly impossible to obtain with non-electric fish. Yet, these observations cover only a small fraction of the >450 species of weakly electric fish (Crampton, 2019), and there is still much untapped potential in the application of electric recordings in the wild (see section 1.4.3).

1.3 Fish Respiration and Dissolved Oxygen

1.3.1 Aquatic Hypoxia

Due to the low water solubility of oxygen, water contains 20-40 times less oxygen than air at a given temperature and oxygen partial pressure (PO_2, Brauner and Val, 2006; Graham, 1990). Diel and seasonal DO fluctuations (see section 1.2.1), conditions of low mixing of the water column, and/or oxygen-consuming biological processes, can result in low oxygen availability that negatively affects aquatic organisms, a condition called hypoxia (Diaz, 2001; Farrell and Richards, 2009; Greenbank, 1945; Kramer, 1984; Pollock et al., 2007). Hypoxia is a widespread environmental stressor for aquatic organisms, which is becoming more prevalent in recent times, as anthropogenic environmental degradation and global warming continue to impact aquatic ecosystems. Large oxygen minimum zones spread in the oceans, and coastal waters experience increasing deoxygenation due to warmer temperatures and eutrophication (Breitburg et al., 2018; Diaz and Rosenberg, 2008; Goldberg, 1995). In comparison to marine ecosystems, the data situation regarding hypoxia in tropical freshwater ecosystems is rather patchy. However, available data suggest that tropical freshwater systems frequently experience diel, seasonal, and/or chronic hypoxia due to high average water temperatures and high rates of oxygen-consuming decomposition processes and respiration (e.g., Crampton, 1998; Reardon and Chapman, 2008; Townsend, 1999). Hypoxia can have far-reaching negative effects on fish health, from reduced growth rates and higher disease susceptibility to large-scale fish kills (Abdel-Tawwab et al., 2019; Diaz and Rosenberg, 2008; Greenbank, 1945; Wang et al., 2009).

1.3.2 Respiration in Fish

All fish that have been investigated so far are obligate aerobes, i.e., they need oxygen for their long-term survival (Kramer, 1984; Nelson, 2016; Richards et al., 2009). Fish use a variety of structures to exchange gas with water and/or air, such as gills, vascularized swim bladders, their skin surface, and/or vascularized intestines (Brauner and Val, 2006). Air breathing has evolved multiple times in fish, presumably as a response to aquatic hypoxia (Brauner and Val, 2006; Kramer, 1984). In general, air-breathing fish sequester an air bubble in contact with their respiratory organ through which gas is exchanged with the blood (Johansen, 1970). Water-breathing fish, on the contrary, rely on the oxygen content of water for respiration and exchange gas over the gills and/or the body surface (Brauner and Val, 2006). Many species are bimodal breathers that can

breathe both water and air, and are able to compensate for low aquatic DO concentration by switching to air breathing (Kramer, 1984).

The primary structures for gas exchange in water-breathing fish are the gills (Brauner and Val, 2006). In general, teleost gills consist of four bony gill arches with two sets of filaments per arch. Fine lamellae on the filaments increase the surface for gas exchange and form a comb-like structure through which the water flows in close contact with the lamellae. Blood flows through the lamellae in a countercurrent direction to the water flow, which increases the oxygen extraction efficiency in the gills (Brauner and Val, 2006; Hughes and Morgan, 1973).

The transport chain of oxygen from the external medium to the mitochondria is often referred to as the oxygen transport cascade (Biro, 2013). After diffusion from the medium through the respiratory epithelium, oxygen is delivered from the respiratory structures to the mitochondria, primarily bound to hemoglobin (Hb, Brauner and Val, 2006). Deoxygenation of Hb in the tissue is facilitated by low tissue PO_2 and low pH, which reduces the oxygen-binding affinity of Hb (Bohr effect, Riggs, 1988). Oxygen diffuses through the cell membrane to the mitochondria, where more than 95% of the consumed oxygen is used as electron acceptor for synthesis of adenosine triphosphate (ATP) via oxidative phosphorylation (Richards, 2009).

If the oxygen availability at the mitochondria is insufficient to meet the cellular energy demand, ATP is produced anaerobically (anaerobiosis), primarily through lactate-yielding glycolysis and substrate phosphorylation (Richards, 2009). Anaerobiosis is considered disadvantageous for fish health because it generates 15-30 times less ATP per mol glucose than oxidative phosphorylation, and can lead to the accumulation of harmful amounts of lactate and protons, which eventually lead to necrosis and death (Richards, 2009).

Because central steps in the oxygen transport cascade depend on diffusive particle motion, which itself depends on partial pressure differences (Fick, 1855), the physiologically most commonly used unit for reporting oxygen levels is oxygen partial pressure (PO_2, e.g., in kPa). Aquatic ecologists and limnologists, on the other hand, tend to report DO concentration (e.g., in mg/L, Ultsch and Nordlie, 2019). This thesis overlaps with both fields, ecology and physiology, and I report oxygen levels as PO_2 (kPa), when they are associated with physiological and morphological functions, and as saturation (% air saturation), when they appear in an ecological context, such as diel DO fluctuations.

1.3.3 Responses to Aquatic Hypoxia

Fish have evolved a tremendous variety of adaptive responses to satisfy their metabolic oxygen demand and mitigate hypoxic stress, ranging from molecular to behavioral adjustments (Table 1, reviewed in Abdel-Tawwab et al., 2019; Kramer, 1984

Mandic et al., 2009; McBryan et al., 2013; Richards, 2009; 2011; Soares et al., 2006). Hypoxia responses can differ greatly between species and individuals (Virani and Rees, 2000; Wannamaker and Rice, 2000), and often reflect the ecological background (Borowiec et al., 2020; Fu et al., 2014) and acclimation state of an animal (Borowiec et al., 2015; Cook et al., 2013). The specific combination of adaptive responses that an animal employs to cope with hypoxia can be described as its respiratory strategy (Borowiec et al., 2015; van Raaij et al., 1996).

Hypoxia adaptations can manifest in constitutive traits, such as specialized air breathing organs, and in plastic responses, which are expressed during hypoxia exposure. Some plastic responses serve to increase oxygen uptake capacity, such as gill remodeling[2], or increase the storage and supply of oxygen to the tissue, such as a change of blood Hb concentration and function (Wells, 2009). Other responses reduce metabolic oxygen demand; metabolic rate depression (Storey and Storey, 1990), or upregulation of anaerobic ATP production (Shoubridge and Hochachka, 1980) are examples.

The timescales on which hypoxia responses act can vary considerably (reviewed in Porteus et al., 2011). Acute, behavioral responses to hypoxia, including aquatic surface respiration (ASR)[3], can take effect immediately to reconcile metabolic demand and supply of oxygen (Lewis, 1970). Physiological and morphological responses, such as gill remodeling, can occur within minutes to days (Nilsson, 2007). Longer-term adjustments, found in seasonal variation in the sensitivity of acute plastic responses, for instance, can enable a more extended homeostasis (Love and Rees, 2002).

Phenotypic plasticity of respiratory traits can also be beneficial when DO is not a limiting factor. For example, gill remodeling can shift the osmorespiratory compromise[4] towards lower costs for osmoregulation during normoxia (Sollid and Nilsson, 2006), and metabolic rate plasticity enables individuals to optimize growth rate and use of resources (Norin and Metcalfe, 2019). By alleviating energetic trade-offs, plastic responses help species to cope with variation in environmental conditions (Borowiec et al., 2020; Collins et al., 2015; McBryan et al., 2013; Seebacher et al., 2015) and are likely a substrate on which natural selection acts to produce more constitutive hypoxia adaptations.

[2] Gill remodeling: in some species, the space between gill lamellae is filled with a cell mass during normoxic or low temperature conditions, which can be reduced during hypoxia or high temperature conditions (Nilsson, 2007; Sollid and Nilsson, 2006). Thus, effective respiratory surface area of the gills is adjusted in response to respiratory requirements and environmental DO concentration.

[3] During ASR, fish swim close to the water surface and skim the well-oxygenated layer of water at the surface, thus increasing their oxygen uptake (Lewis, 1970).

[4] Osmorespiratory compromise: a large gill surface increases not only oxygen uptake but also the flux of ions from or into the body, which has to be counteracted by energetically expensive ion pumps, a trade-off termed osmorespiratory compromise (Gonzalez and McDonald, 1992; Nilsson, 1986).

Table 1 - Hypoxia adaptations, their respiratory effects, and their approximate temporal dynamics.

Trait/Response	Effect	Expression dynamic	Exemplary references
Avoidance	Decouples respiration from local hypoxia	Plastic - acute	Burleson et al., 2001; Mucha et al., 2021
Behavioral inactivity	Reduces whole-animal energy demand	Plastic - acute	Nilsson et al., 1993; Speers-Roesch et al., 2018
Bimodal/air breathing	Decouples respiration from aquatic DO	Constitutive	Graham, 1997; Johansen, 1970; Kramer, 1984
Aquatic surface respiration	Decouples respiration from aquatic DO	Plastic - acute	Abdallah et al., 2015; Kramer and McClure, 1982; Lewis, 1970
Large gill surface area	Increases oxygen extraction capacity	Constitutive (e.g., filament length, filament number, lamellar area) and plastic (e.g., gill remodeling) - hours to days	Chapman and Hulen, 2001; Crampton et al., 2008; Mandic et al., 2009; Nilsson, 2007; Nilsson et al., 2012; Sollid and Nilsson, 2006
High gill ventilation rate	Increases oxygen extraction rate	Plastic - acute	Jones, 1952; Timmerman and Chapman, 2004
High Hb concentration	Increases oxygen-carrying capacity	Constitutive and plastic - hours to days	Timmerman and Chapman, 2004; Wells, 2009
High Hb-oxygen affinity	Increases oxygen extraction capacity	Constitutive and plastic - hours to days	Mandic et al., 2009; Pan et al., 2017; Wood and Johansen, 1972
Metabolic rate depression and anaerobiosis	Reduces tissue oxygen demand	Constitutive and plastic - minutes to hours	Martinez et al., 2004; Richards, 2010; Shoubridge and Hochachka, 1980; Storey and Storey, 1990; Virani and Rees, 2000

1.3.4 Concepts for Measuring Metabolic Activity in Fish

A common method for assessing physiological responses to hypoxia is to measure metabolic activity under different DO conditions. The large heat capacity of water makes caloric measurements (i.e., measuring the heat that is produced by an animal, a standard technique to measure metabolic activity in terrestrial animals) extremely difficult to conduct with fish (Nelson, 2016, but see Regan et al., 2017, for a recent example). Thus, metabolic activity is most commonly measured indirectly by quantifying the rate of oxygen consumption ($\dot{M}O_2$, Nelson, 2016). The underlying assumption is that $\dot{M}O_2$ relates directly to metabolic activity, as most metabolic processes are fueled by aerobic respiration[5]. Oxygen consumption can be measured comparatively easily in a respirometer. In the simplest case, a respirometer consists of a sealed, water-filled chamber with an oxygen probe and a fish in it (closed respirometry, e.g., Beamish and Mookherjii, 1964; Fry and Hart, 1948). By measuring the decline of DO inside the respirometer that is due to the respiration of the fish, $\dot{M}O_2$ can be calculated, and thus metabolic activity of the fish can be approximated.

The $\dot{M}O_2$ of fish depends on a variety of factors, such as water temperature, locomotor activity, food intake, and agitation state of the animal, and efforts have been undertaken to standardize respirometer designs, experimental protocols, and statistical procedures to make results comparable among studies (Chabot et al., 2016a; Rosewarne et al., 2016, but see Clark et al., 2013; Farrell, 2013; Regan et al., 2019; Wood, 2018 for a lively debate about proper procedures and use of metrics). Different concepts for metabolic rate measures have emerged from this (Figure 1; Chabot et al., 2016a; Rosewarne et al., 2016). Standard metabolic rate (SMR) represents the minimum $\dot{M}O_2$ that an animal needs to maintain a steady state, and it has to be measured in an unstressed, immobile, and postabsorptive (i.e., empty stomach and intestines) animal. Routine metabolic rate (RMR) allows for minimal movement of the animal in an unstressed and postabsorptive state (Chabot et al., 2016b). Maximum metabolic rate (MMR) represents the upper limit of the aerobic metabolism of an animal, and has to be measured in an animal that operates at its highest level of metabolic activity (e.g., directly after being chased for 5 minutes, Norin and Clark, 2016; Rosewarne et al., 2016). The difference between MMR and SMR is called the aerobic scope, and it represents the range of metabolic rate that is available for processes other than routine operation at a given temperature (Farrell, 2016; Fry, 1971).

[5] However, as Nelson (2016) points out, oxygen consumption and metabolic rate can differ from each other, e.g., when metabolic processes are fueled by anaerobiosis.

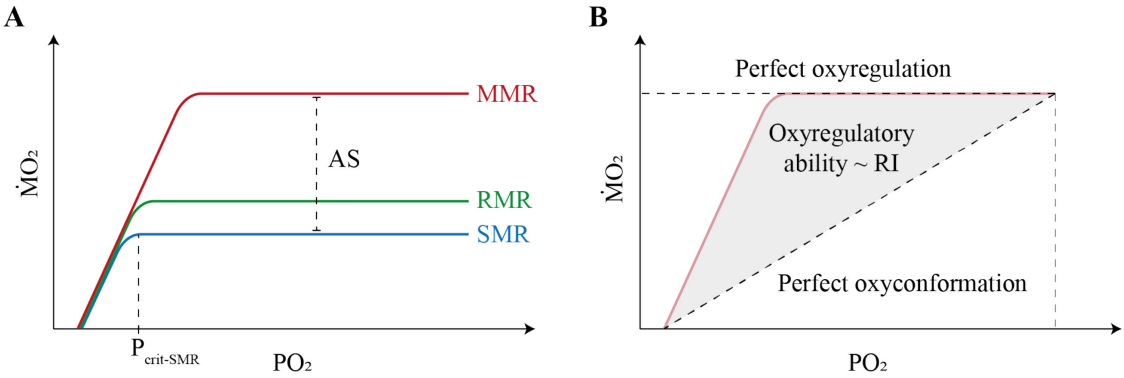


Figure 1 - Schematic representations of metabolic rates as functions of PO_2. (A) Maximum metabolic rate (MMR, red), routine metabolic rate (RMR, green), and standard metabolic rate (SMR, blue). Most fish maintain a stable $\dot{M}O_2$ over a gradient of PO_2 values (oxyregulation). The point at which oxyregulatory ability is lost is called critical oxygen tension (P_{crit}, here shown as $P_{crit-SMR}$). The difference between MMR and SMR is the aerobic scope (AS), which represents the oxygen that is available for processes other than maintenance. (B) To assess the oxyregulatory ability of an animal, the $\dot{M}O_2$ vs. PO_2 curve can be compared with the predicted trajectories for a perfect oxyregulator (horizontal dashed line) and a perfect oxyconformer (diagonal dashed line). The regulation index (RI) is a quantitative measure for this relationship that is derived by dividing the area under the $\dot{M}O_2$ vs. PO_2 curve that lies above the trajectory for a perfect oxyconformer (grey area) by the total area between the trajectories for a perfect oxyconformer and a perfect oxyregulator. In this figure, the oxyregulatory ability is shown for a fish operating at MMR for illustrative purposes.

Hypoxia tolerance is usually extrapolated from the metabolic response of an animal to declining DO. Some fish maintain a stable $\dot{M}O_2$ over a wide range of partial pressures, and are called oxyregulators, whereas others conform in their $\dot{M}O_2$ to environmental PO_2 and thus are called oxyconformers (e.g., Pörtner and Grieshaber, 1993). The vast majority of species show intermediate responses, i.e., their $\dot{M}O_2$ decreases in progressive hypoxia, but at a lower rate than environmental PO_2 (Steffensen, 2006; van Winkle and Mangum, 1975). At some PO_2, all fish lose the ability to maintain $\dot{M}O_2$, either due to breakdown of the oxygen transport cascade or due to regulatory changes in the energy metabolism (e.g., upregulation of anaerobic energy production, Beamish, 1964; Pörtner and Grieshaber, 1993; Ultsch et al., 1978). This threshold is called critical oxygen tension (P_{crit}), and is widely used to assess and compare the hypoxia response of organisms (see meta-analysis in Rogers et al., 2016). Another measure that quantifies the oxyregulatory ability of an organism in declining PO_2 is the regulation index (RI, Figure 1 B, Mueller and Seymour, 2011). The RI is derived by dividing the area under the $\dot{M}O2$ vs. PO2 curve that lies above the predicted trajectory for a perfect oxyconformer by the total area between the trajectories for a perfect oxyconformer and a perfect oxyregulator (see section 2.3.2.3 for further details). A RI close to 1 indicates that the fish behaves mostly as an oxyregulator, whereas a value close to 0 indicates that $\dot{M}O_2$ mostly conforms to PO_2.

For this thesis, I have used intermittent-flow respirometry in which a fish is kept in a slow current in a water circuit that can be closed (to measure $\dot{M}O_2$) and opened (to flush the circuit with water and change DO levels, Svendsen et al., 2016) to measure RMR.

Following intermittent-flow respirometry I quantified P_{crit} and RI using closed respirometry in which the water circuit is closed until the fish loses the ability to maintain a stable $\dot{M}O_2$ (see section 2.3.2).

1.4 Electric Fish

The ability to generate electric fields with specialized electric organs has evolved multiple times in several groups of fish independently. Marine species, e.g., electric rays, skates and some stargazers, as well as freshwater fish, e.g., the gymnotiform knifefish from South- and Central America, African Mormyroidea and several siluriform catfish, possess electric organs (reviewed in Alves-Gomes, 2001; Moller, 1995). The electrogenic ability of some fish had been known long before the term 'electricity' was coined by William Gilbert in 1600 (Gilbert, 1600), and since then, the peculiar physiology of electric fish has sparked the interest of some of the most notable scientists. Probably the first written evidence for electricity generated by fish dates back to Greek antiquity, when Plato, Aristotle and Hippocrates experimented with the curative application of electric shocks from torpedo rays (Wu, 1984). In the 17th century, the electric properties of the torpedo were, again, subject to thorough study, and likely had influenced Luigi Galvani and Alessandro Volta in their foundational work on electrophysiology (Mauro, 1969; Walsh, 1773). Later, Charles Darwin prominently addressed the electric organ as an example of convergent evolution in his *Origin of Species* (Darwin, 1859). Since then, electric fish have emerged as popular research subjects in many fields of the natural sciences, ranging from neurophysiology and ethology to ecology and evolution, physics, computational biology and robotics (see reviews in Bullock et al., 2005; Carlson et al., 2019; Krahe and Fortune, 2013).

The subjects of this work are two species of African mormyrid weakly electric fish (Figure 2): *Marcusenius victoriae*, Worthington, 1929 (previously classified as *Gnathonemus victoriae*, Seegers et al., 2003), and *Petrocephalus degeni*, Boulenger, 1906 (previously classified as *Petrocephalus catostoma*, Kramer et al., 2012). In contrast to strongly electric fish, weakly electric fish use their electric discharges, not to shock their prey, but rather to sense their environment and communicate.

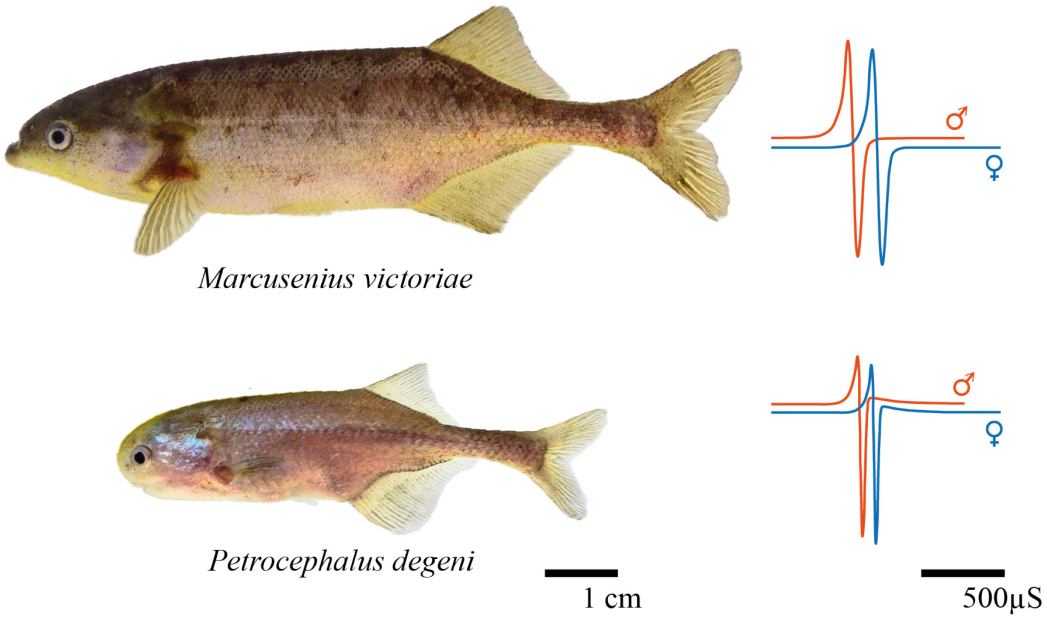


Figure 2 - Species used in this work. Normalized-amplitude head-to-tail waveforms of EOD pulses from male and female fish are shown on the right side. EODs for illustrations were low-pass filtered (50 kHz) and recorded at a sample rate of 250 kHz. Photos by Ina Seuffert and Stefan Mucha.

1.4.1 The Sensory World of Weakly Electric Fish

Of all known sensory modalities, the active electrosense of electric fish is certainly amongst the most alien to us humans as it does not rely on any of the physical cues that play a role in our perception of the environment. Furthermore, the receptors that measure the electrical image are scattered over the body surface of the fish, giving them an omnidirectional sense of their environment (Snyder et al., 2007). Electroreception has evolved multiple times independently, and it is important to distinguish between active and passive electrosensation (Alves-Gomes, 2001). The active electrosense is a characteristic feature of electric fish that depends on sensing reafferent electrosensory information, which is generated by discharges of an animal's own electric organ (electric organ discharge, EOD). Passive electrosensory abilities, on the other hand, are found in all major vertebrate groups and depend on sensing weak electric fields that are generated by external sources, such as other animals (New, 1997).

1.4.1.1 Electric Organ Discharges

Weakly electric fish generate an electric field around their body by discharging their specialized electric organ (Lissmann, 1951), which in mormyrids is located in their tail stem (caudal peduncle). The electric organs of all weakly electric fish, with the exception

of the South American genus *Apteronotus*, are comprised of electrocytes, cells with an often disk-like shape that are derived from skeletal muscle cells (Bennett, 1961; Szabo, 1960, reviewed in Markham, 2013). Within the electric organ of mormyrids, electrocytes are stacked in four columns parallel to the spine with their flattened faces oriented perpendicular to the rostral-caudal axis. Synaptic input from spinal motor neurons elicits action potentials on the electrically excitable membranes on the flat faces of electrocytes, creating a directed current of ions, such as Na^+ and K^+, along the body axis (Markham, 2013). The coordinated firing of action potentials from a population of electrocytes during an EOD creates an electric field in the surrounding water with a field geometry, which, at some distance to the fish, resembles a dipole (Assad et al., 1999; Benda, 2020). Like all mormyrids, *M. victoriae* and *P. degeni* produce pulse-type EODs: discrete discharges that are emitted with varying inter-pulse intervals (IPIs). In the case of the study species, the EODs are very short (ca. 300-500 µs) with a bi- or triphasic waveform (Figure 2; Carlson, 2016). Depending on the behavioral context, the rate with which these EODs are emitted varies between less than 1 Hz to more than 100 Hz (e.g., shown in Carlson, 2002; pers. observation).

1.4.1.2 Active Electrolocation

Weakly electric fish use the electric field that they generate during an EOD to detect and localize objects (Lissmann, 1951; Lissmann and Machin, 1958). The electric field extends around the body of the discharging fish and creates transdermal potential differences across its body surface. Objects with different conductive properties from water (resistance and capacitance) perturb the electric field and modulate the patterns of transdermal potential differences (Assad et al., 1999; Caputi et al., 1998), creating an electric image that the fish perceives through specialized electroreceptors scattered across their body surface (Lissmann and Machin, 1958; Szabo, 1965). Inanimate objects, such as stones, mainly differ from water in their resistive properties and affect the local amplitude of the EOD. Living matter has resistive and capacitive properties, which affect both the local EOD amplitude and waveform (Emde, 1999). In mormyrids, the electroreceptors that provide sensory information for electrolocation are called mormyromasts (Bennett, 1965; Szabo, 1965). Type A and type B mormyromasts are sensitive to EOD amplitude, and type B receptors encode for EOD waveform distortions (Emde, 1999).

The short EODs of pulse-type fish produce only very brief electric images of their environment, comparable to the effect of a stroboscope in a dark room. A high rate of EOD production increases the temporal resolution of electrosensory information, and occurs commonly during behaviors that are associated with a higher level of activity, e.g., exploration, fast swimming, feeding or novelty responses (Bullock et al., 2005; Moller, 1995; Post and Emde, 1999).

1.4.1.3 Electrocommunication

The duration, number of phases, and overall waveform of EODs is determined by the physiology and morphology of the electric organ and its electrocytes. These EOD features are generally species-specific (Carlson et al., 2011; Hopkins, 1980; 1981) but can vary between different populations of the same species (Gallant et al., 2011), and between the sexes, especially during the breeding season (Bass and Hopkins, 1983; Crawford, 1992). Within individual fish, EOD features are highly consistent across time (Carlson, 2002; Crawford, 1992; Friedman and Hopkins, 1996). Thus, EOD features have the potential to convey useful information about the identity of the sender. In mormyrids, information about the temporal structure of the EOD waveform is provided by electroreceptors called knollenorgans (Franz, 1920). Knollenorgans respond to positive voltage changes (i.e., caused by an incoming EOD) with a phase-locked spike. The timing of spike responses of knollenorgans is dependent on the waveform of the EOD (Hopkins and Bass, 1981). By comparing the responses of knollenorgans from different parts of the body in a midbrain region called the exterolateral nucleus, temporal features of the EOD waveform are extracted (Xu-Friedman and Hopkins, 1999).

In addition to EOD waveform features that are consistent across time, the temporal variability with which pulse-type fish emit their EODs constitutes a flexible component of this communication system that is well suited to signal the behavioral state. Stereotypical patterns of sequences of pulse intervals have been linked to different social contexts: aggressive, submissive or courtship behavior, for example (reviewed in Carlson, 2002; Carlson and Hopkins, 2004).

Both components of the electrosensory communication system, EOD features and patterns of pulse intervals, have been found to play an important role in species recognition (Nagel et al., 2018b; Nagel et al., 2018a). The divergence of EOD signals between different populations of the same species, e.g., driven by sexual selection (Arnegard et al., 2010), adaptation to ecological niches (Hopkins, 1981), and genetic drift in some cases[6], contributes to reproductive isolation, and is a possible cause of the high species diversity of weakly electric fish (Crampton and Albert, 2006).

1.4.2 Mormyrid Weakly Electric Fish

Mormyrids are native to tropical and subtropical African freshwater systems (Nelson, 1994), where they inhabit a great variety of aquatic environments from the

[6] Genetic drift seems sufficient to explain signal divergence between populations of the gymnotiform *Brachyhypopomus occidentalis* (Picq et al., 2016), but not in the mormyrid *Paramormyrops kingsleyae* (Gallant et al., 2011; Picq et al., 2020).

Congo River rapids (Poll et al., 1982) to the stagnant wetland habitats where the subjects of this study are found. With more than 230 species, they constitute the largest family of freshwater fish that are endemic to Africa (Fricke et al., 2021; Kramer and Wink, 2013). The earliest accounts of mormyrids can be found in reliefs in Egyptian tombs near Saqqara, which date back as far as 2750 BCE (Gaillard, 1923; Kellaway, 1946; Paugy, 2010). These depictions and other findings, such as mummified specimens and ancient figurines of *Mormyrus* sp., illustrate the ecological and cultural significance that mormyrids bear as part of the native fauna and food resources for humans in this region.

The morphology of mormyrids varies considerably across species. Their size ranges between ca. 5 cm and 1.5 m, with most species measuring between 9 cm and 50 cm[7]. Their primary food source are benthic invertebrates, such as insect larvae (Blake, 1977; Boussou et al., 2019; Corbet, 1961; Ikomi, 1996). One of the most striking morphological features of mormyrids is their great variety of snout and chin shapes, which appears to be linked to the adaptation to different feeding niches (Marrero and Winemiller, 1993). For example, in the genus *Campylomormyrus*, different snout lengths have been linked to preferences for different substrates in which fish forage for their prey (Amen et al., 2020). The elephant-nose fish, *Gnathonemus petersii,* has an elongated chin appendage with a high density of electroreceptors, which it uses to sense prey buried in substrate (Bacelo et al., 2008; Pusch et al., 2008). With their special electrosensory abilities, weakly electric fish have access to food resources that are inaccessible to other fish. Therefore, they take an important place in the food web of tropical freshwater systems by extending it to small planktonic and benthic invertebrates (Crampton, 1996; Lundberg et al., 1987).

In comparison to other taxa, mormyrids have unusually large brains with an enlarged cerebellum (Nieuwenbuys and Nicholson, 1969; Sukhum et al., 2016; Sukhum et al., 2018). The formation of these peculiar neuroanatomical features has been associated with the evolution of active electrosensing (Sukhum et al., 2018). In *G. petersii*, a species with extreme encephalization, the brain constitutes about 3% of the body mass, and has been reported to account for an impressive 60% of whole body oxygen consumption[8] (Nilsson, 1996), easily outcompeting human brains, which account for 2-2.5% of body mass and approximately 20% of whole body oxygen consumption.

[7] Mormyrid body length information: https://www.fishbase.se/summary/FamilySummary.php?ID=40 (accessed November 2021)

[8] Some details, such as acclimation time and behavioral activity during measurement, have not been disclosed by the author of this study. Thus, it should be assumed that brain oxygen consumption accounts for 60% of RMR (Chabot et al., 2016a; Nilsson, 1996). The proportion of SMR that is accounted for by the brain presumably lies even above 60%.

1.4.3 Activity Patterns of Weakly Electric Fish

Besides traditional knowledge about the natural behavior of mormyrids, which is rooted in artisanal fisheries, not much is known about their activity patterns. It is generally assumed that weakly electric fish are nocturnal animals, and this has been documented in some species that show nocturnal increases of locomotor and/or EOD activity in the laboratory (Bässler et al., 1979; Cobert, 1984; Franchina and Stoddard, 1998; Harder et al., 1964; Markham et al., 2009; Migliaro and Silva, 2016). Evidence from field studies generally supports the notion of nocturnality in weakly electric fish (Henninger et al., 2018; Henninger et al., 2020; Kruger, 1973; Lissmann, 1961; Lissmann and Schwassmann, 1965; Madhav et al., 2018; Migliaro et al., 2018; Moller et al., 1979). However, a close examination of this body of research shows that there are examples of weakly electric fish that remain active during the day (e.g., mormyrids that live in dark habitats, Hopkins, 1980; Kruger, 1973), and that nocturnal activity patterns have often been observed in shallow and/or clear water habitats, where light conditions change drastically from day to night (Henninger et al., 2018; Henninger et al., 2020; Lissmann, 1961; Lissmann and Schwassmann, 1965). Therefore, it appears that activity patterns of weakly electric fish depend primarily on light conditions among other exogenous and endogenous factors, and it seems likely that among the >450 species of weakly electric fish (Crampton, 2019) there are some that do not exhibit a nocturnal lifestyle (e.g., species that live under constant dark conditions). Previous studies have shown that fish can display extraordinary plasticity in their diel and circadian activity rhythms (reviewed in Reebs, 2002). Other factors than illumination, such as social stimuli (Franchina et al., 2001; Silva et al., 2007) and temperature (Ardanaz et al., 2001; Dunlap et al., 2000), can affect activity, and the interplay of these and other, hitherto unknown environmental influences, might shape natural behavioral patterns that differ from those observed in the laboratory. An environmental factor whose influence on diel behavior has been studied little in the field is ambient DO (Pollock et al., 2007). Many weakly electric fish experience seasonal, diel, or chronic hypoxia (Chapman et al., 1996b; Crampton, 1998). The degree to which weakly electric fish can tolerate hypoxia might be limited by the energetic costs for generation and sensing of EODs (see section 1.4.4). Thus, although weakly electric fish appear to be generally active under dark conditions, little is known about the influence of other environmental factors on their activity, and it remains unclear how representative laboratory studies are of natural activity patterns of weakly electric fish.

1.4.4 Energetics of Electric Signaling

With the large ionic currents underlying EOD generation (Markham, 2013; McAnelly and Zakon, 2000), electric organs are considered to be energetically expensive tissue. In this, they are similar to brains, but make up a larger proportion of body mass than brain tissue (Markham et al., 2016). This suggests that electric signaling is metabolically expensive for electric fish. However, it remains surprisingly unclear what ecological and evolutionary constraints the metabolic cost of EOD production imposes on weakly electric fish.

One the one hand, there is convincing evidence that EOD generation does indeed consume a considerable proportion of metabolic energy: Salazar and Stoddard (2008) calculated that, in the pulse-type gymnotiform *Brachyhypopomus pinnicaudatus*, males use up to 22% of their SMR on electric signaling whereas females only use ca. 3% of theirs. In their study, costs for electric signaling peaked at night due to nocturnal increases in EOD rate and amplitude. Salazar and colleagues (2013) found that the wave-type gymnotiform *Eigenmannia virescens* might use up to 28% of their RMR for EODs at a discharge rate of 400 Hz. This was corroborated by Markham and colleagues (2013), who reached similar results for *E. virescens* using a computational model, and by Lewis and colleagues (2014), using respirometry and finding EOD costs amounting to more than 30% of RMR.

On the other hand, it appears that the high cost of electrosensation at the organ level does not necessarily translate to a high whole-body energy demand. Julian and colleagues (2003) found that oxygen consumption rates of gymnotiforms do not seem to depend on EOD type (wave or pulse), and that they are much lower than what would be predicted for temperate teleost species at neotropical temperatures. This suggests that adaptations in physiological and/or morphological traits might buffer the high costs of electrosensation.

Given the great diversity of weakly electric fish, it does not seem sensible to seek a general answer concerning the cost of active electrosensation and its ecological and evolutionary implications. Some electric fish, such as the study species, can occupy severely hypoxic habitats, and show a high degree of physiological hypoxia tolerance (Chapman and Chapman, 1998; Clarke et al., 2020; Moulton et al., 2020; Nilsson, 1996), whereas others are restricted to high-DO environments (Crampton, 1998). It is most likely that the cost of electrosensation varies flexibly, as has been shown for some species (e.g., pulse-type species that can adapt their discharge rate, or wave-type species that modulate their EOD amplitude, Reardon et al., 2011; Salazar and Stoddard, 2008; Stoddard et al., 2007), and that ecological patterns (e.g., in fish distribution) reflect this flexibility.

1.5 Study System

1.5.1 The Lake Nabugabo Region

Lake Nabugabo is a small satellite lake of Lake Victoria in southern Uganda with a surface area of 33 km² and a mean depth of 3.13 m (Figure 3 A; Nyboer and Chapman, 2013). It separated from the main lake about 5000 years ago (Stager et al., 2005). The Lake Nabugabo system is home to a unique community of fish species with a small number of haplochromine cichlid species that are endemic to Lake Nabugabo and other nearby satellite lakes (Greenwood, 1965; Kaufman and Ochumba, 1993; Trewavas, 1933) and four mormyrid species (Chapman et al., 2002; Ogutu-Ohwayo, 1993). Under British colonial rule, the Nile perch was introduced into the Lake Nabugabo region in the 1960s to bolster commercial fishing (Ogutu-Ohwayo, 1993; Pringle, 2005). Like many African freshwater systems, this ecosystem was heavily impacted by the introduction of this large predator, which coincided with a dramatic decline or loss of several native species from the waters of the main lake (Chapman et al., 2003; Ogutu-Ohwayo, 1993).

The lake is surrounded to the north, east and south by the Lwamunda Swamp, an extensive wetland area, which is dominated by the emergent grass *Miscanthidium violaceum* that transitions to water lilies (*Nymphaea lotus* and *N. carulea*) or hippo grass (*Vossia cuspidata*) at the lake edge (Figure 3 B; Chapman et al., 1996a; Chrétien and Chapman, 2016). Various fish species occur deep within the wetland (see species list in Chapman et al., 1996b), including *P. degeni* and *M. victoriae* (Chapman et al., 1996b; Chapman and Hulen, 2001; Chapman et al., 2002; Moulton, 2016). The hypoxic conditions and great structural complexity in the wetland likely provide protection for some endemic species from the relatively hypoxia-intolerant Nile perch (Chapman et al., 1996b; Chapman et al., 1996a; Chapman et al., 2002; Schofield and Chapman, 2000).

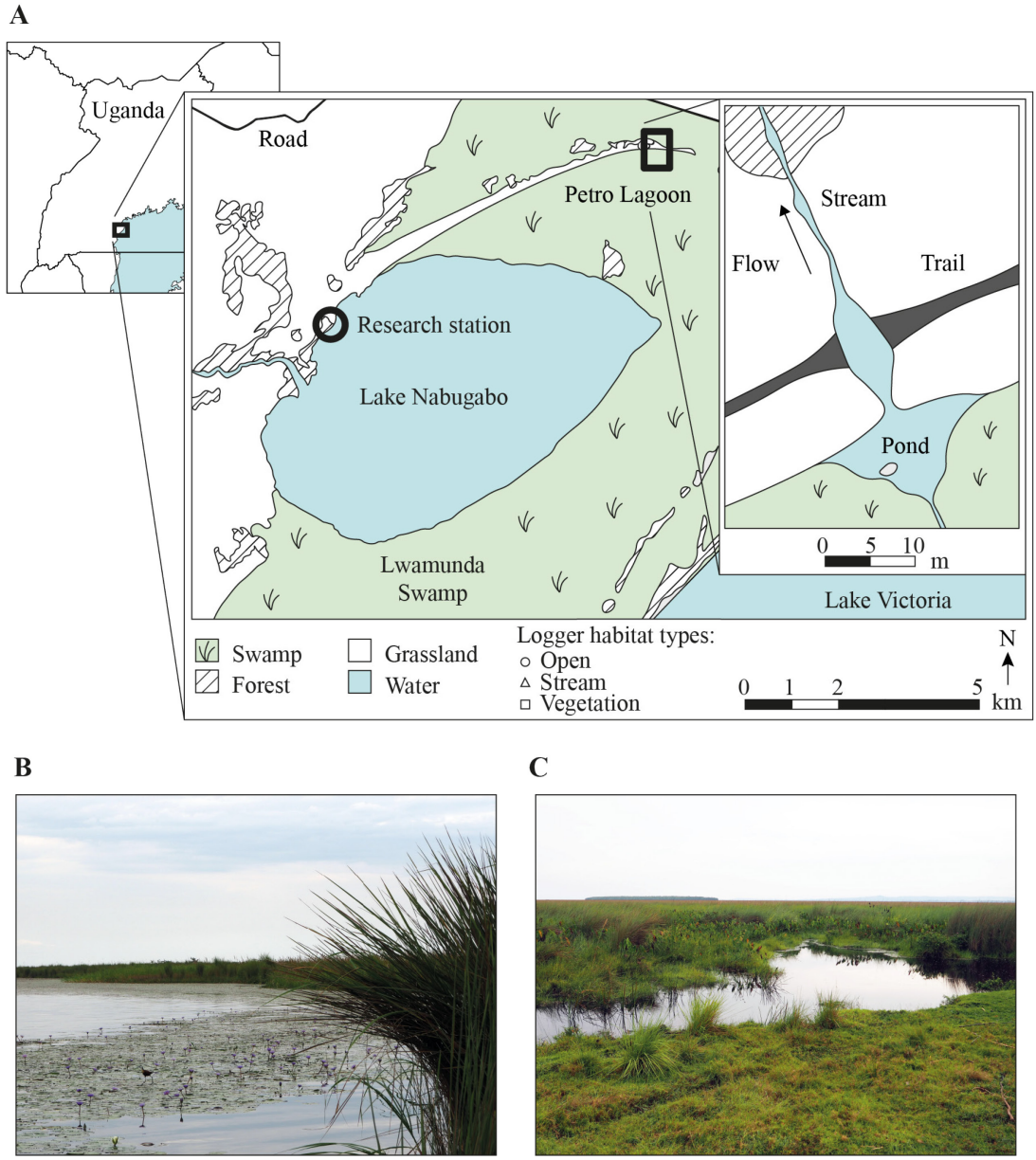


Figure 3 - The Lake Nabugabo system and Petro Lagoon. (A) Map of Lake Nabugabo. The position of the Nabugabo Research station on the shore of Lake Nabugabo is marked by a black circle. Details of Petro Lagoon are shown in the enlarged map section in the top right corner. Map data was obtained from satellite images (Google Earth, https://earth.google.com/web/, accessed November 2021) and own measurements made at Petro Lagoon. (B) Transitional vegetation of hippo grass and water lilies on the edge of Lake Nabugabo. (C) Open pond area in Petro Lagoon.

1.5.2 Research Sites

The inner parts of the wetland are difficult for researchers to access due to the dense growth of floating vegetation. A system of small lagoons on the land edge of the wetland, where the swimming vegetation clears, offers good opportunities to sample and capture fish from the wetland – a circumstance that is exploited by researchers and piscivorous birds alike (pers. observation). Most of the field data for this thesis was collected in one such lagoon (Petro Lagoon) in the northern parts of Lwamunda Swamp where water from the swamp transitions to a pond-like area and flows outwards from the swamp to seasonally inundated grassland (0°19'07" south, 31°56'48" east; inlay in Figure 3). Towards the wetland, the open area of the lagoon is surrounded by floating mats of vegetation with dense root masses where fish can often be found during the day. Inundation levels, water temperature and DO in the wetland show seasonal and diel variation, though DO remains generally low (previously reported diurnal ranges: 11.6 – 28.9% air saturation, Reardon and Chapman, 2008; 7.6 – 9% air saturation, Moulton et al., 2020), and water conductivity is very low (10-20 µS cm^{-1}, this study). Nocturnal temperature and DO measurements from this system have not been published prior to this study.

1.5.3 Study Species

Marcusenius victoriae and *P. degeni* occupy habitats with greatly differing environmental conditions within the Lake Victoria basin of East Africa, including Lake Victoria, Katonga River, and Lake Nabugabo (Chapman et al., 1996b; Chapman and Chapman, 1998; Chapman and Hulen, 2001; Chapman et al., 2002; Clarke et al., 2020; Kramer et al., 2012; Moulton et al., 2020; Ogutu-Ohwayo, 1993). They belong to different subfamilies of the Mormyridae, Mormyrinae and Petrocephalinae, respectively, and differ in their electrosensory anatomy, overall morphology, and social behavior (Figure 2; Carlson, 2016).

Marcusenius victoriae are small to medium-sized fish (usually 7 – 15 cm standard length) with a small fleshy protuberance on their chin. Their knollenorgan receptors are distributed evenly across their body surface and their exterolateral nucleus is differentiated into two subdivisions – a feature that has been associated with enhanced EOD waveform recognition ability (Carlson et al., 2011; Carlson, 2016). They have been found predominantly alone in nature and show a high degree of intraspecific competition for shelters (Carlson, 2016).

Petrocephalus degeni are shorter (usually 5-9 cm standard length) with a blunt snout and a subterminal mouth (Chapman and Chapman, 1998). Their knollenorgan electroreceptors are clustered in three rosettes on each side of the head, and their

exterolateral nucleus shows no subdivisions. They are often found in groups and show pronounced social affiliation behavior (Carlson, 2016).

Both species produce very short (ca. 300 – 500 µs), bi- or triphasic EOD pulses with a species-specific waveform (Figure 2). *Marcusenius victoriae* shows some inter-individual variability of EOD waveform whereas the waveform of EODs from *P. degeni* is highly similar across individuals (Carlson, 2016), which might be associated with the ability for waveform discrimination in *M. victoriae* and the lack thereof in *P. degeni*.

Despite their history as research subjects (Ackerly et al., 2017; Ackerly et al., 2018; Carlson, 2016; Chapman and Chapman, 1998; Clarke, 2019; Clarke et al., 2020; Moulton, 2016; Moulton et al., 2020), no systematic description of the diel habitat use patterns and activity rhythms of the study species exists to date.

1.5.3.1 Hypoxia Tolerance of *M. victoriae* and *P. degeni*

Marcusenius victoriae and *P. degeni* have consistently been found in severely hypoxic habitats at Lwamunda Swamp. Investigations of swamp-dwelling specimens have revealed a suite of hypoxia adaptations that indicate a high degree of hypoxia tolerance in these fish, such as a low RMR (0.08 and 0.05-0.13 mg O_2 h^{-1} g^{-1}, respectively), a low P_{crit} (1.91 and 1.21-1.46 kPa O_2, respectively), and a large total gill surface area (5.89 and 4.15 cm^2 g^{-1}, respectively; Chapman and Chapman, 1998; Chapman and Hulen, 2001; Clarke et al., 2020; Moulton et al., 2020). Other physiological parameters, such as blood Hb and lactate concentration, might play an important role in the persistence of these fish as well, but have received little attention so far. A previous study has found high blood Hb concentration in two *P. degeni* specimens from the swamp (Chapman et al., 2002), however, given the small sample size, these measurements are preliminary.

In their recent study, Clarke and colleagues (2020) exposed *P. degeni* from the hypoxic swamp to long-term normoxia and found that their P_{crit} increased and RI decreased. This implies that adult *P. degeni* express some plasticity of their oxygen extraction and supply capacity. However, it remains unclear which morpho-physiological traits underlie the plastic respiratory response. A comparison of *P. degeni* from the swamp habitat to conspecifics from the well-oxygenated open waters of nearby Lake Kayanja by Chapman and Hulen (2001) showed larger gill surface area and, possibly, smaller brain size in *P. degeni* from the hypoxic swamp. It remains an open question whether these interdemic differences are due to heritable differences between the two populations of *P. degeni*, or whether they are caused by phenotypic plasticity. It is one goal of this thesis to replicate the acclimation study conducted by Clarke and colleagues to investigate in greater detail the morpho-physiological changes that accompany the respiratory response of swamp-dwelling *P. degeni* to long-term normoxia exposure.

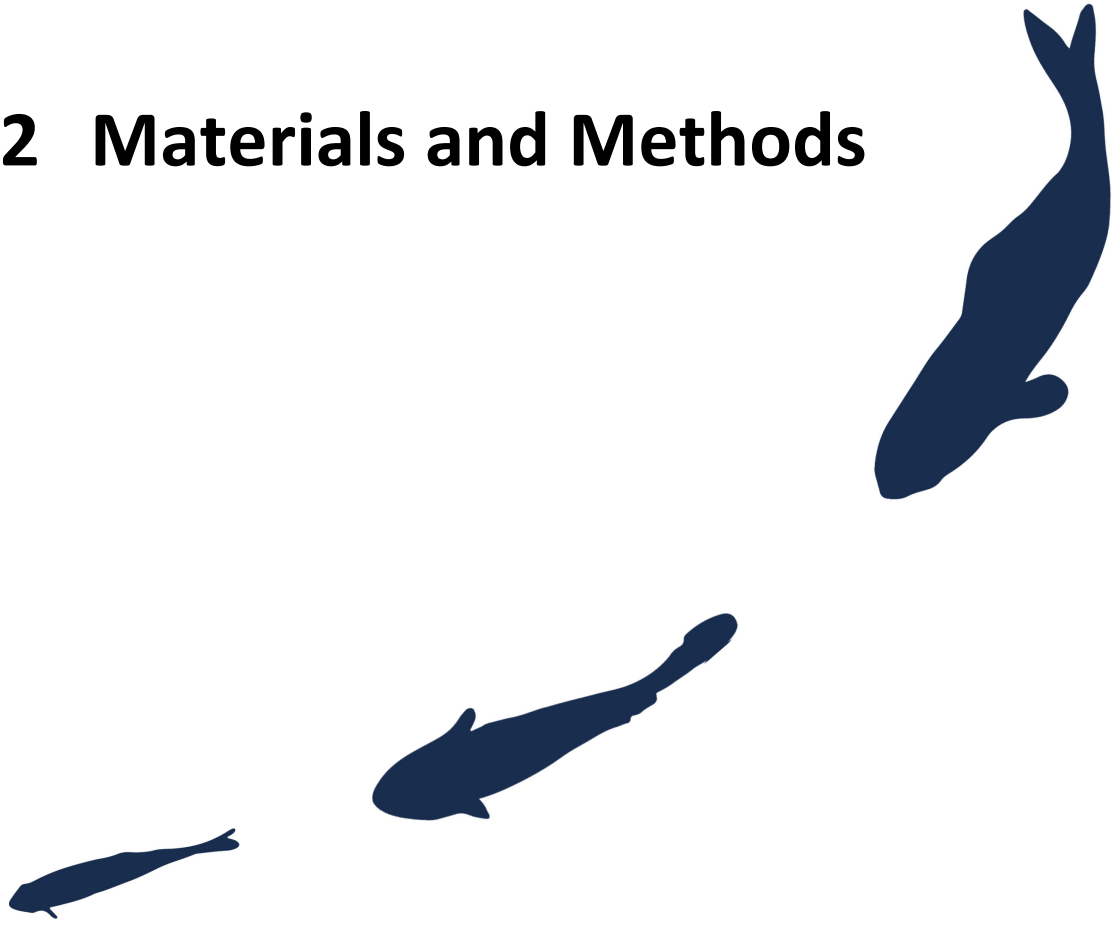
1.6 Objectives and Research Questions

The central objectives of this work were to i) observe behavioral rhythms and habitat use patterns of *M. victoriae* and *P. degeni* under natural and laboratory conditions, and ii) assess morpho-physiological traits of swamp-dwelling *P. degeni* that allow these fish to cope with hypoxia, and their plasticity during long-term normoxia exposure.

In particular, this thesis aims to answer the following research questions:

- Do *M. victoriae* and *P. degeni* show the nocturnal activity patterns that are expected of weakly electric fish?
- How do behavioral patterns of *M. victoriae* and *P. degeni* observed in the laboratory compare to their natural behavioral patterns observed in-situ?
- How do diel variations in behavior manifest in habitat use, EOD activity and swimming activity?
- How do environmental fluctuations of temperature and DO correlate with behavioral patterns?
- Can previously published values of RMR, RI, and P_{crit} of *P. degeni* be reproduced?
- How do P_{crit} values of *P. degeni* relate to diel variation in environmental DO concentration?
- How do metabolic traits, such as RMR and P_{crit} relate to blood Hb, blood lactate and gill size?
- How does long-term acclimation to normoxia affect the quantified hypoxia-related traits and their possible interaction?

2 Materials and Methods



2.1 Laboratory Activity Patterns

2.1.1 Measurement of Activity under Controlled Conditions

Activity of laboratory-housed *M. victoriae* and *P. degeni* was quantified in a shuttle-box system (Loligo Systems Inc., Viborg, Denmark, Figure 4 A). The fish were caught in the Lwamunda Swamp in Uganda and kept under controlled conditions for at least one year in the animal housing facility of the Institut für Biologie of Humboldt-Universität zu Berlin, Germany, prior to experiments. *Marcusenius victoriae* were housed individually in 70 L tanks, and *P. degeni* were kept in groups of up to 3 fish in 70 L tanks. The light regime was set to a 12:12 h light:dark cycle with a 30-minute dusk/dawn period during which ambient light levels gradually transitioned between light and dark phase.

The shuttle-box system consisted of two circular compartments (diameter of 50 cm each), that were connected by a central passage (8.5 cm wide and 14 cm long). One opaque PVC tube (5.5 cm inner diameter, 16 cm length) was placed in each compartment to serve as shelter for the fish during the experiments. A piece of plexiglass was glued to the bottom of the shelter tubes to secure their position against water currents. Water temperature was maintained by a thermostat (ITC-308, Shenzhen Inkbird Technology Co. LTD, Shenzhen, China) that controlled the power supply to two silicone rubber heating mats on either side of the shuttle-box system and one heat radiator that was placed near the setup. The temperature sensor of the thermostat was submerged in the passage between the two compartments of the shuttle-box system. Two pumps (Universal Pumpe 1048, EHEIM GmbH &Co.KG, Deizisau, Germany) created a circular current in both compartments to homogenize temperature gradients in the system. The water current was regulated to a slow speed using screw clamps on the tubing of the shuttle-box which reduced water flow. The shuttle-box system rested on a self-constructed LED panel (12V DC dimmable SMD3528-300 LEDs, LEDLightsWorld, China), which illuminated the system from below with white light during the day (4000-4500 Kelvin neutral white spectrum) and infrared light during the night (940 nm wavelength). The LED panel was controlled by a programmable LED timer (TC420, Shenzhen Leynew Technology Co. LTD, Shenzhen, China), and the light:dark cycle was synchronized with the light regime in the animal housing facility. A camera (Grasshopper3 GS3-U3-41S4C-C 1, Teledyne FLIR LLC., Wilsonville, OR, USA) was mounted above the shuttle-box to record swimming activity of the fish. Videos were recorded at sample rates of 25-40 frames per second (FPS) using Matlab R2018 (TheMathWorks Inc., Natick, MA, USA). To minimize disturbance, the setup was visually isolated from the surrounding area with lightproof fabric that was mounted on an aluminum frame around the shuttle-box system,

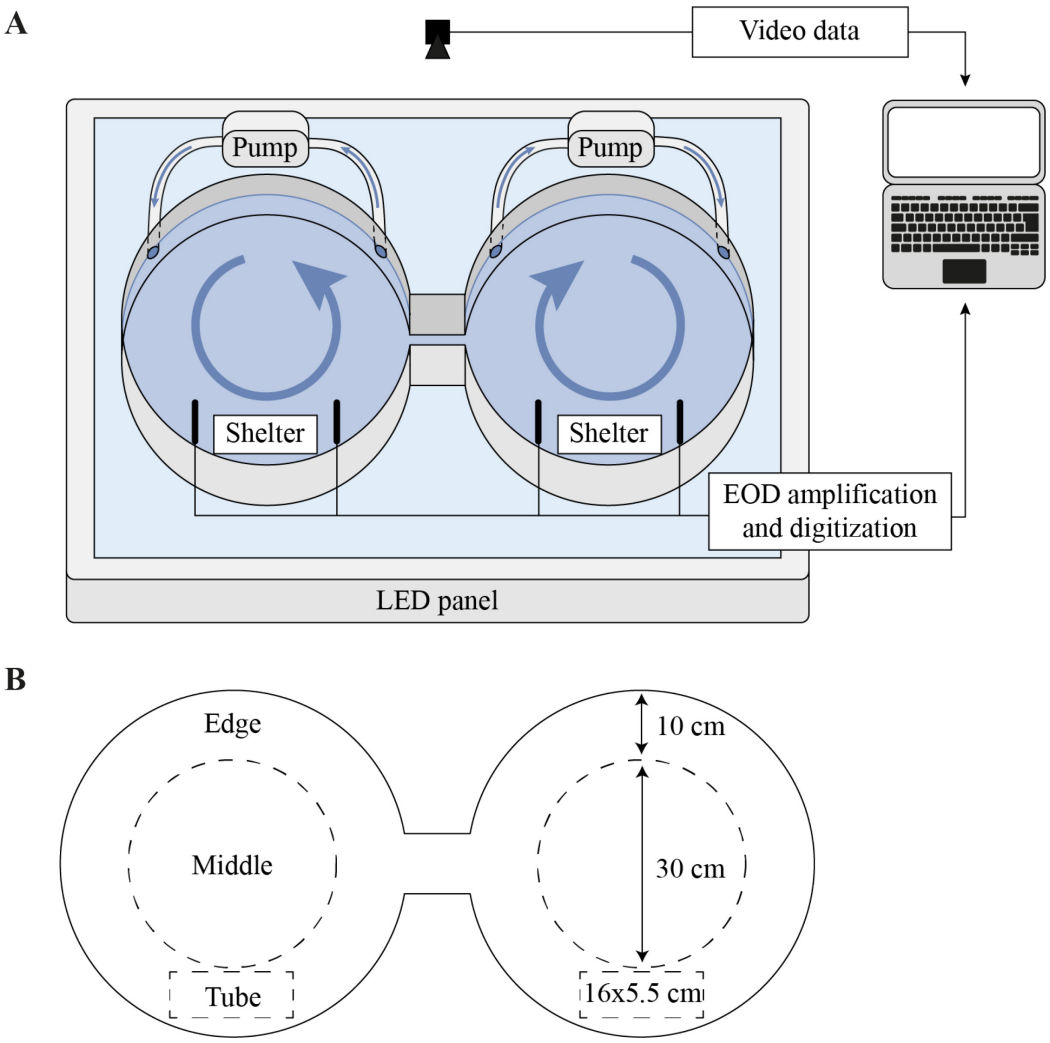


Figure 4 - Schematic of shuttle-box setup for behavioral activity measurements. (A) Experimental setup. The setup was illuminated from below by a LED panel and videos were recorded from above. EODs were measured with carbon rod electrodes in both compartments that were arranged to both sides of each PVC shelter tube and, in experiments with *P. degeni*, with an additional pair of electrodes per compartment that was arranged on a perpendicular axis. Pumps created a slow circular current in both compartments. Heating system is not shown here. (B) Definition of zones in shuttle-box tank. Edge: within 10 cm of shuttle-box wall; middle: more than 10 cm away from shuttle-box wall; tube: in PVC tube.

LED panel, and camera. A Faraday cage made of steel mesh was mounted on the same aluminum frame and connected to an electric ground to reduce electric noise.

EODs were recorded with carbon rod electrodes that were submerged in both compartments of the shuttle-box. During experiments with *M. victoriae*, one pair of electrodes was used in each compartment. Due to the smaller peak-to-peak amplitude of EODs of *P. degeni*, two pairs of electrodes in each compartment were necessary to reliably record their EODs during activity measurements. EODs were band-pass filtered (100 Hz - 20 kHz), amplified (10 x gain, DPA-2FS, NPI, Tamm, Germany), digitized at a sample rate of 50 kHz (NI-PCI 6259, National Instruments, Austin, TX, USA), and stored on a computer using Matlab R2017.

For each trial, one fish was introduced in a randomly chosen compartment of the shuttle-box in the late afternoon, before the onset of the dark phase, and left undisturbed for 42 h. Over the duration of the trial, movements and EOD activity were recorded continuously. After the conclusion of the trial, the fish was removed from the shuttle-box system, weighed and measured, and returned to its home tank. Between every two trials, the shuttle-box was cleaned with salt water and rinsed to remove residues from the previous trial.

2.1.2 Video and Electric Organ Discharge Analysis

Videos and EODs of each trial were saved in separate files in intervals of 2 h. The onset of EOD recording and video recording was offset by approximately 20-30 seconds, probably due to a time lag during initiation of data acquisition and storage of the large amounts of data (up to > 16 gigabyte per file). To precisely synchronize video and EOD tracks for analysis, a LED light on the side of the LED panel signaled the beginning of each new EOD recording.

Videos were overlaid with a tracking mask using FFMPEG ver. 4.4[9] to obscure the parts of the image that did not show the shuttle-box system and increase tracking speed. Fish position was then tracked using the BioTracker 3 software (Mönck et al., 2018). The resulting dataset was further processed using R ver. 4.1.0[10]. Tracking data were aligned with the corresponding EOD recording using the timestamp of the synchronization-LED signal. High-velocity jumps of the track position (> 400 cm/s) that occurred (e.g., when fish traversed their opaque shelter tubes or when tracking position was transiently lost) were corrected by connecting the two positions before and after the jump with a series of equally interspaced steps. Small jitters of the track position were then smoothed using a second-order Savitzky-Golay filter (Savitzky and Golay, 1964). To control for differences in body size, swim speed was converted to body lengths per second (BL s^{-1}). Tracking data were further annotated with categorical information about the position of the fish based on the x and y coordinates of the track position. For this, three zones in the shuttle-box tank were defined: shelter tube (tube), within 10 cm of the edge (edge), more than 10 cm away from the edge (middle, Figure 4 B). To quantify swimming behavior, the mean swimming speed and the percentage of time spent in the tube or in the middle/edge of the shuttle-box system were summarized for every 10 minutes of the trial.

EODs were extracted offline from electrical recordings using Python ver. 3.8.8[11] with a custom-written Python script and peak-finding functions from the Thunderfish

[9] FFMPEG: https://ffmpeg.org/ (accessed December 2021)

[10] R: https://www.r-project.org/ (accessed December 2021)

[11] Python: https://www.python.org/ (accessed December 2021)

library[12]. Signals were identified using a peak-finding algorithm, which detected the positive and negative peak of each EOD pulse above a preset peak-to-peak amplitude threshold of 0.1 V. The EOD timestamp was extracted from the zero crossing between positive and negative peaks. EOD rate and IPI coefficient of variation (IPI CV) were calculated from the timestamps of EOD-zero crossing for time bins of 1 second and 10 minutes using R. For analysis of location preference, resting EOD rate, and the correlation of EOD rate and velocity, only data from the last 24 h of each trial were used to reduce the effect of acclimation to the shuttle-box system.

2.1.3 Location Preference

The percentage of total time that each fish spent at different locations of the shuttle-box was computed for the light and dark phase of the trial. Percentages of time spent were not normally distributed (Shapiro-Wilk test: $P < 0.05$), and thus were tested for location differences using the Friedman test followed by a post-hoc paired Wilcoxon rank-sum test with Bonferroni correction in case of a significant outcome[13].

2.1.4 Electric Organ Discharge Rates

To control for individual differences in baseline EOD rate, normalized EOD rates were computed by dividing the EOD rate of each fish at each time point through its overall average EOD rate (see Appendix, Figure A1).

2.1.4.1 EOD Rates at Different Locations of the Shuttle-box

All 1-second segments that a fish spent completely in one location of the shuttle-box (middle, edge and tube) were extracted and normalized EOD rate was averaged per fish, location, and phase of the light cycle. Location-specific EOD rates were normally distributed but not homoscedastic (Levene test: $P < 0.05$). EOD rates from the dark phase of the trial were tested for location differences using the Friedman test followed by a post-hoc paired Wilcoxon rank-sum test with Bonferroni correction in case of a significant outcome. During the light phase, not all fish moved through all locations of the shuttle-box. Thus, light-phase EOD rates did not follow a complete block design and were tested

[12] Thunderfish: https://github.com/bendalab/thunderfish (accessed December 2021)

[13] I tested three methods to control for the increase of family-wise error rate in procedures that involved multiple hypotheses testing: Bonferroni, Bonferroni-Holm and Sidak. There were only marginal differences in the outcomes of these different methods; thus, I chose Bonferroni correction as it represents the most conservative approach.

for location differences using the Kruskal-Wallis test followed by a Conover post-hoc test with Bonferroni correction in case of a significant outcome.

2.1.4.2 Relationship Between EOD Rate and Swim Speed

Average swim speed and normalized EOD rate was extracted per species and phase of the light cycle from all 10-minute segments during which fish spent at least 10% of the time swimming outside of the shelter tube. The relationship between EOD rate and swim speed was assessed using linear regression.

2.1.4.3 Resting EOD Rate

All sequences of the recording during which fish spent at least 5 seconds in their shelter tube and EOD amplitude remained constant (amplitude CV < 0.12, suggesting low locomotor activity, Silva et al., 2007) were extracted, and resting EOD rate was calculated as average normalized EOD rate per fish and phase of the light cycle. Resting EOD rate was normally distributed and was tested for day-night differences using the paired t-test.

2.2 Environmental and Behavioral Rhythms

2.2.1 Electric Fish Loggers

I developed an autonomous data-logging device to record electric signals in the natural habitat of weakly electric fish (Figure 5). The electric fish logger consists of a Teensy 3.5 microcontroller board (PJRC.COM, LLC., Sherwood, OR, USA) that is connected to a DS3231 real-time clock module (AZ-Delivery Vertriebs GmbH, Deggendorf, Germany) for timekeeping functionality, and powered by four AA batteries. The electronic components of the logger are housed in a PVC tube with an inner diameter of 45 mm, which is sealed on one side with a glued-on lid and on the other side with a screw lid to allow replacement of batteries and the microSD card. Two small carbon discs (1 cm diameter, 4 mm long) serve as electrodes to measure EODs. The disks are glued onto the lids of the logger housing using aquarium silicone and connected to the analog-digital converters of the Teensy board via copper wire.

Voltage from the electrodes is recorded continuously in differential or single-ended mode, digitized and stored on a microSD card. The dynamic range for differential input lies between -3.3 and +3.3 V. Effective voltage resolution is scalable between 6-bit and 12-bit. At 12-bit resolution, the ADCs sample at rates of up to 500 kHz. For the field recordings in this thesis, data were recorded differentially and sampled at a rate of 100 kHz and with 12-bit resolution.

The program for the logger was written using the Arduino IDE[14] and the Teensyduino plugin[15]. To achieve sufficient sampling rates, the transfer of analog voltage data from the onboard ADC to a dynamic buffer in the program memory is routed via the system bus, using direct memory access. When the buffer section is full, the data is appended to a binary file on the microSD card and the buffer is re-filled. Every ten minutes, a new file is created and the timestamp of file creation is read from the DS3231 real-time clock and pasted into the new file name (Figure 5 B). The code for the logger program has been archived and is maintained online on GitHub, alongside a more detailed documentation[16].

[14] Arduino GUI: https://www.arduino.cc/ (accessed December 2021)

[15] Teensyduino: https://www.pjrc.com/ (accessed December 2021)

[16] Electric fish logger code: https://github.com/muchaste/EOD-Logger (accessed December 2021)

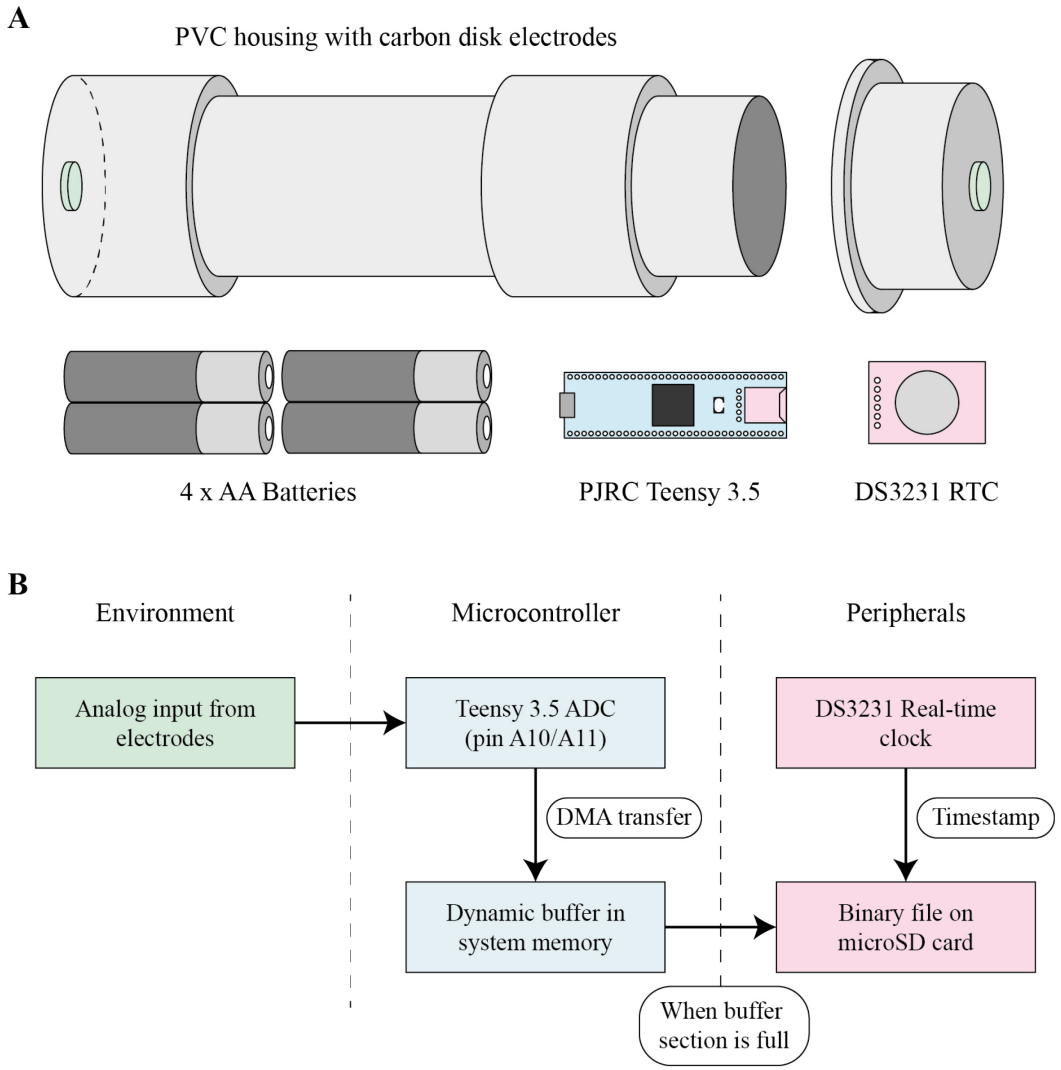


Figure 5 - Electric fish logger schematic. (A) Core components of the electric fish logger. RTC: real-time clock. (B) Flow chart of simplified logger operating principle. DMA: direct memory access.

2.2.1.1 Assessment of Detection Range

The detection range of the electric fish logger depends on the amplitude of the signal that is generated by the fish. Due to the dipole character of the electric field, the recorded EOD amplitude depends on the distance and the position of the fish relative to the electrodes (see Benda, 2020 for a comprehensive review of electric field geometry). As the two poles of the electric field are located on the head-to-tail axis of the fish, EOD amplitude is largest when the measurement electrodes align with this axis, and practically reaches zero when the fish is perpendicular to the line connecting the two measurement electrodes.

I measured the detection range of the electric fish loggers in a rectangular tank (200 cm x 100 cm) with freely swimming specimens of each species (n = 4 per species). A camera (Grasshopper3 GS3-U3-41S4C-C 1, Teledyne FLIR LLC., Wilsonville, OR,

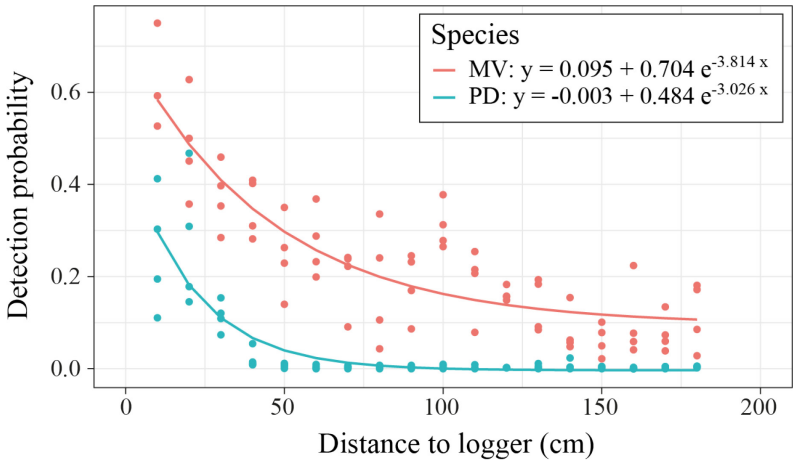


Figure 6 - Detection probability of EOD pulses emitted by freely swimming *M. victoriae* (MV) and *P. degeni* (PD). Probability was calculated for distance bins of 10 cm. Probability was calculated as the number of EOD pulses that were detected by the electric fish logger divided by the total number of EOD pulses as recorded by control electrodes. Solid lines indicate exponential regressions; regression functions are provided in the figure legend.

USA) was mounted above the tank and infrared lamps (940 nm wavelength) illuminated the tank from below. Videos were recorded at a rate of 25 FPS using Matlab R2018. Five pairs of carbon rod electrodes (1 cm diameter, ca. 7 cm long) were attached to the long walls of the tank to record EODs and serve as control for the logger recordings. Signals from the control electrodes were band-pass filtered (100 Hz - 20 kHz), and amplified (10 x gain, DPA-2FS, npi electronic GmbH, Tamm, Germany), digitized at a sample rate of 41.67 kHz (NI-PCI 6259, National Instruments, Austin, TX, USA), and stored on a computer using Matlab R2018. Prior to recordings, water conductivity in the home tanks of the fish was lowered over the course of several days to 20 µS cm^{-1} to match the conditions at Petro Lagoon. For each trial, the tank was filled with water, and temperature and conductivity were adjusted to home tank conditions. The electric fish logger was placed near one of the short walls of the tank, and one fish was introduced into the tank next to the logger. Lights were switched off and infrared light was turned on. The fish was left undisturbed to swim around in the tank for 20 minutes while video and EODs were recorded through the control electrodes.

EODs were extracted offline from logger and control recordings as described above (see section 0) with an amplitude threshold of 0.066 V for EOD detection (i.e., 1% of the dynamic range of the electric fish logger). The EOD timestamp was extracted from the zero crossing between the positive and negative peak. Timestamps of logger and control recordings were aligned using cross-correlation in R.

Fish position was tracked using the BioTracker 3 software (Mönck et al., 2018), and the distance from the fish to the midpoint of the electric fish logger was calculated for each frame. Distances were binned in 10 cm steps and the EOD detection probability was calculated for each distance bin using the following equation:

$$Detection\ probability = \frac{n_{EODs\ from\ logger\ recordings}}{n_{EODs\ from\ control\ recordings}} \quad (1)$$

Detection probability decreased with distance, following an exponential decay function (Figure 6), and was overall lower for *P. degeni*, approaching zero at 50 cm radial distance from the logger. Detection probability for EODs of *M. victoriae* reached about 10% at the maximum distance of 180 cm.

2.2.2 In-situ Recording of Electric Organ Discharge Activity

Eight electric fish loggers were deployed in a small lagoon (Petro Lagoon, 0°19'07" south, 31°56'48" east) in the Lwamunda Swamp in three different habitats: under floating vegetation with ca. 1 m distance to the edge of vegetation cover (vegetation, n = 3), under the open water surface of the lagoon with at least 1 m distance to the nearest vegetation (open, n = 2), and in the middle of the narrow stream-like area of the lagoon where water flows seasonally from the wetland to the inland (stream, n = 3). These habitats were chosen to capture a variety of environmental conditions representative of Petro Lagoon (Figure 7, Table 2). To secure the logger position against perturbations, each logger was attached to a stick that was driven firmly into the ground. Each logger was placed approximately in the middle of the water column (Figure 7 C).

In-situ recordings were made on six sampling sessions in 2019 (July: 23-24 and 29-30, August: 8-9 and 21-22, September: 2-3 and 19-20). Electric fish loggers were deployed in the morning and left to record continuously for at least 24 h.

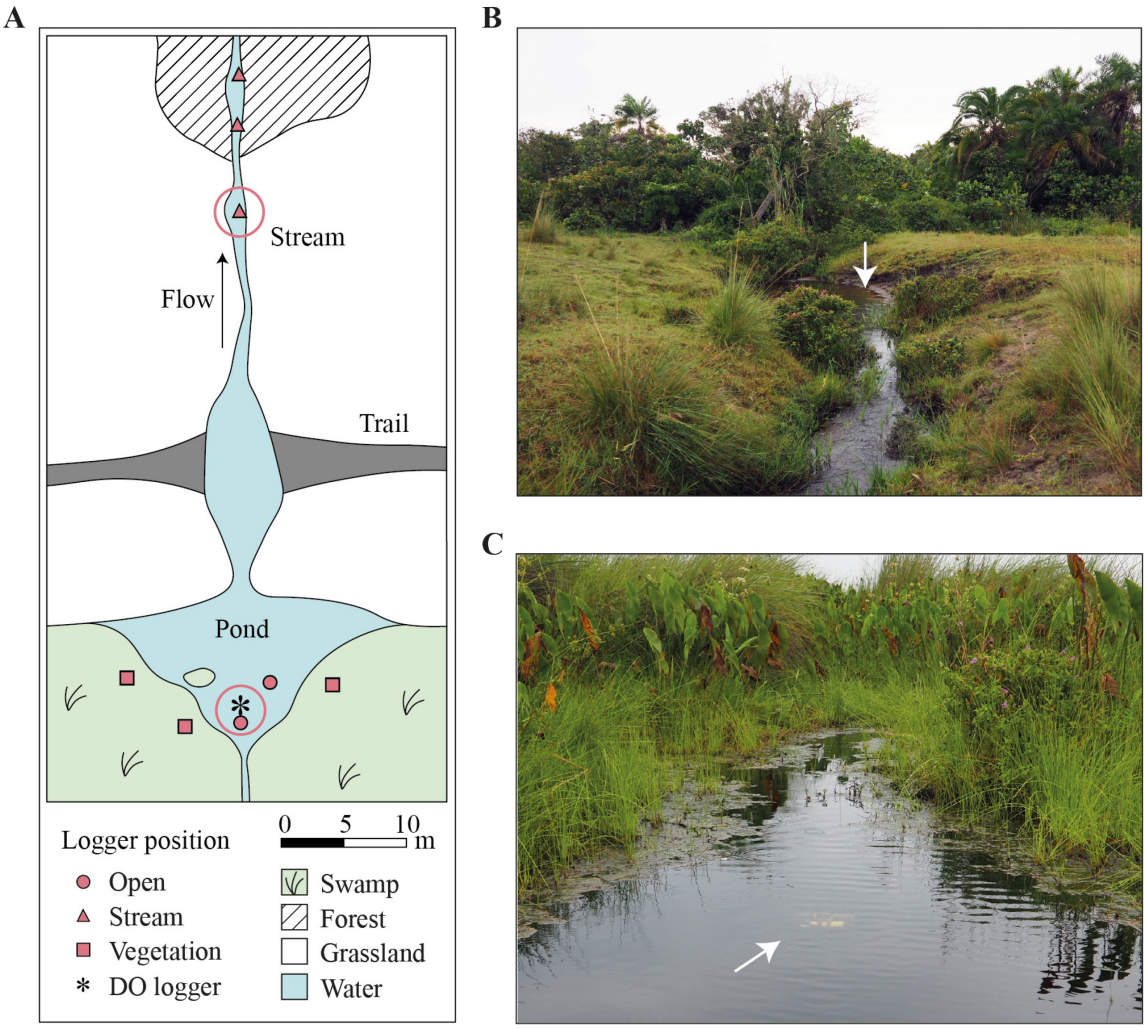


Figure 7 - Placement of data loggers at Petro Lagoon for in-situ recordings. (A) Map of Petro Lagoon. Electric fish logger positions are indicated with red symbols, the DO logger position is indicated with an asterisk. The positions of loggers that are marked with a red circle are indicated in panels B-C with white arrows. (B) The stream area of the lagoon where water flows seasonally towards inland. (C) Transition of floating vegetation to open water surface in the pond area of the lagoon.

Table 2 - Characteristics of different habitats in Petro Lagoon.

Habitat	Loggers (n)	DO	Water flow	Water Depth (cm)	Vegetation cover	Shelter options
Vegetation	3	Low	Very low	33 (22-45)	Full cover by swimming mats of vegetation	Structurally complex root masses
Open	2	Low	Very low	66 (56-77)	No cover	No shelter
Stream	3	Low	Continuous	33 (12-55)	Shaded by overhanging trees and shrubs	Vegetation and roots along the stream edges

DO, dissolved oxygen; Water depth is expressed as mean (range).

2.2.2.1 Fish Encounters

After retrieval of the loggers, EODs were extracted from electrical recordings as described above (see section 0) with a voltage threshold of 0.066 V for EOD detection. A short data fragment (1 ms) centered around the zero crossing of each EOD was extracted, and sample rate was interpolated to 200 kHz for waveform analysis. Several features were extracted from each interpolated EOD pulse (Figure 8): timestamp of zero-crossing, peak-to-peak duration (PP-dur), peak-to-peak amplitude (PP-amp), peak 1/peak 2 amplitude ratio (PP-ratio), slope at zero-crossing (ZC-slope), duration of the two main peaks at 60% relative amplitude (P1-dur and P2-dur), and the frequency of the highest power of the FFT-transformed pulse (peak-FFT).

EODs were grouped together into encounters based on their zero-crossing timestamp using R. An encounter was defined as a coherent EOD pulse train with at least 10 pulses and a maximum IPI of 5 seconds. These selection criteria served to eliminate very brief and/or erroneous fish detections that occurred due to electrical noise, e.g., through lightning discharges. EODs were then assigned to either *M. victoriae* or *P. degeni* based on their EOD waveform, using mixture discriminant analysis (MDA) and extracted EOD features (Figure 8). To compute a discriminant function for species prediction, laboratory recordings from freely swimming fish were used (*M. victoriae*: n = 20, *P. degeni*: n = 14). EODs were extracted as described above, and a subset of 200 pulses around the EOD with the highest peak-to-peak amplitude was selected from each fish's recording. The resulting dataset was split randomly into a training (80%) and a test set (20%). The training set was used to compute a discriminant function. Four different

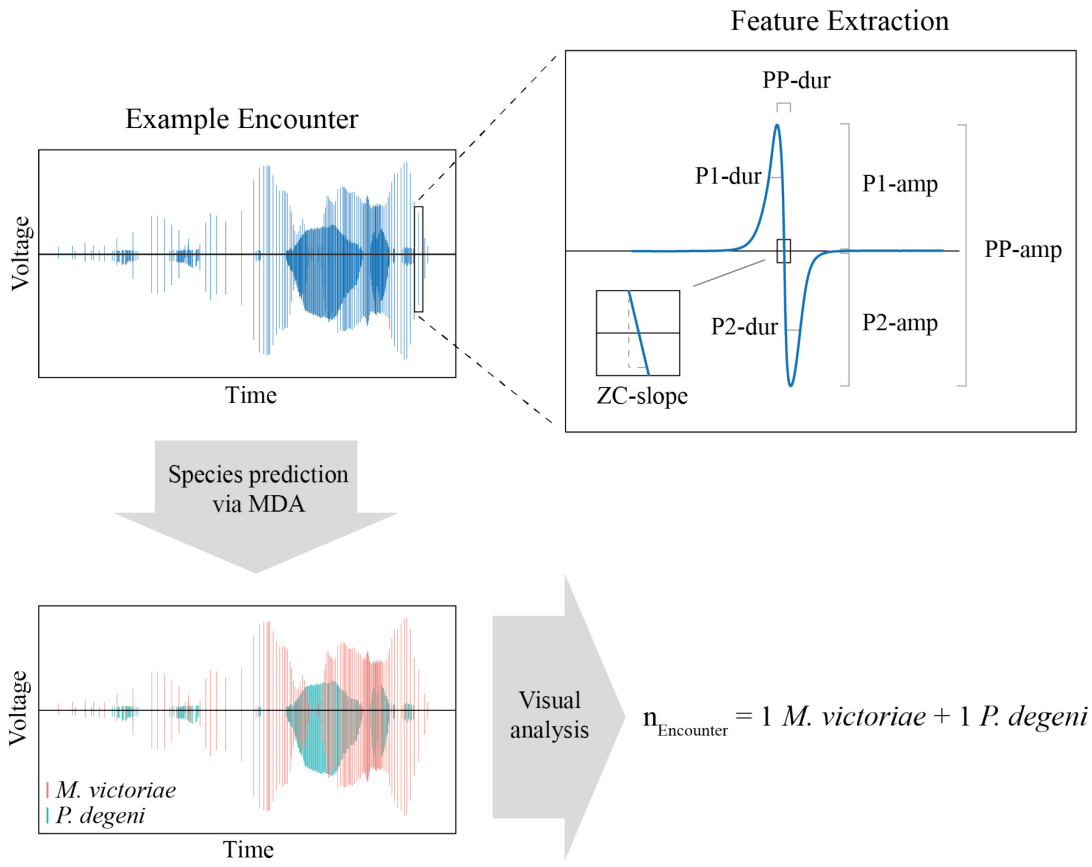


Figure 8 - Schematic diagram showing analysis of in-situ EOD recordings. EODs were grouped into encounters with at least 10 EODs with IPIs below 5 seconds. Several features were extracted from each pulse: amplitude of first and second peak (P1-amp/P2-amp), duration of first and second peak at 60 % of maximum peak amplitude (P1-dur/P2-dur), peak-to-peak amplitude (PP-amp), peak-to-peak duration (PP-dur), voltage slope at zero crossing (ZC-slope), and the frequency of the highest power of the FFT-transformed pulse (peak-FFT, not shown). Pulses were then assigned to either *M. victoriae* or *P. degeni* based on PP-dur, PP-amp and peak-FFT. The number of fish of each species in an encounter ($n_{Encounter}$) were determined visually.

models were tested using different EOD features (see Table A1 for a list of model features and discrimination performance). Three features, Peak-FFT, PP-dur and PP-ratio, were sufficient for robust species discrimination with a fidelity of 98.3% in the test set. The MDA model was then applied to predict the species for each EOD pulse in the field recordings. After species prediction, each encounter was inspected visually to determine and validate the number and species of the fish. Encounters with single fish that could not be assigned with confidence (i.e., less than 70% of pulses could be assigned to either species) were removed from analysis. This was the case in 14.5% of single-fish encounters.

2.2.2.2 Frequency of Encounters

As individual animals of a given species could not be identified unambiguously based on their EOD features, it is likely that some animals were encountered multiple times. This is more likely for *M. victoriae*, which have been described as territorial (Carlson, 2016), and can pause their EODs for extended durations, thus creating "new" encounters. Although, in theory, absolute animal densities might be estimated despite double counting of individuals (Campos-Candela et al., 2018; Follana-Berná et al., 2020), the data required to satisfy the assumptions underlying such methods are not available for this study system (e.g., fish must display home ranging behavior and home range centers must be homogeneously distributed, Campos-Candela et al., 2018). Thus, data on fish distribution are presented not as fish density estimates but as frequency of encounters, which depends on fish activity as well as fish density. The frequency of encounters was summarized as the total number of fish per species that were encountered per logger over intervals of 30 minutes. The frequency of encounters was not normally distributed; however, due to the large sample size (n = 1276 measurements during the day and n = 1080 measurements during the night), I tested for day-night differences using the t-test. Because counts of encounters varied greatly among the habitats (i.e., there were no encounters with fish of either species during the daytime in the open habitat), this comparison was conducted for each species without blocking for habitat type.

2.2.2.3 Co-occurrences

Multiple fish were deemed to have co-occurred when they were present simultaneously during one encounter (e.g., as shown in the example encounter in Figure 8). To assess species-specific patterns, co-occurrence was summarized as proportion of the total number of encounters per phase of the diel cycle where at least one fish of the respective species was present, using the following equation:

$$Co - occurrence_{Species\ A} = \frac{n_{\ Encounters}\ (> 1\ fish\ of\ species\ A)}{n_{Encounters}\ (\geq 1\ fish\ of\ species\ A)} \qquad (2)$$

The proportions of co-occurrences were not normally distributed and were tested for day-night and species differences using the U-Test. Similar to frequency of encounters, the habitat variable was not included in this comparison due to the strong variation of encounter numbers in the different habitats.

2.2.2.4 Encounter Characteristics

Duration of encounters (from first to last EOD) and EOD rate during encounters were extracted to compare encounter characteristics between habitat types and phase of

the diel cycle. For the comparison of encounter duration and EOD rate, only encounters with single fish were used, as they occurred most consistently across time and space. Encounter characteristics were not normally distributed; however, due to the large sample size (*M. victoriae*: n = 850, *P. degeni*: n = 1017), I used parametric tests to assess habitat differences. Characteristics of nocturnal encounters with *P. degeni* were tested for habitat differences using ANOVA, followed by a Tukey post-hoc test with Bonferroni correction of P-values in case of a significant result. Characteristics of nocturnal encounters with *M. victoriae* and diurnal encounters with *P. degeni* were tested for habitat differences using the t-test because they only occurred in two habitats.

2.2.3 In-situ Temperature and Dissolved Oxygen Measurement

One oxygen and temperature logger with an optical oxygen sensor (miniDOT, PME, Vista, CA, USA) was placed with the electric fish loggers in the open habitat of the lagoon to record water conditions during in-situ EOD recordings (Figure 7). Water temperature and DO were sampled at least every 30 minutes for the duration of EOD recordings. After each sampling day, data were collected from the logger, and the first and last 30 minutes of measurements were deleted to remove disturbances in the measurements that were caused by placing and retrieving the logger. Previous manual measurements that were taken during the daytime at Petro Lagoon between 2005 and 2018 showed that temperature varies on average by 0.2 ± 0.5°C and air saturation varies by 1.8 ± 7.9% between the water surface and at a water depth of 50 cm (n = 690 measurements). I placed the DO and temperature logger in the middle of the water column, expecting the measured values to be representative of large parts of the water column where the fish are located. In addition to continuous measurements, I recorded diurnal temperature and DO in all habitats twice per sample day using a handheld meter (Handy Polaris, OxyGuard, Farum, Denmark).

2.3 Hypoxia Tolerance of *P. degeni*

All experiments were conducted at the Lake Nabugabo field research site in Uganda. Respirometry experiments, blood sampling and gill measurements were conducted between July and September 2019, supplemental blood samples were taken between May and August 2018. All statistical analyses were conducted using R. Prior to analysis of gill morphometrics and respirometry data, trial data were anonymized and randomized to minimize experimenter bias[17].

2.3.1 Fish Collection and Normoxia Acclimation Treatment

Petrocephalus degeni were captured from hypoxic lagoons in the Lwamunda Swamp. Fish were located using a fish finder that detects EODs with two submerged electrodes and amplifies and converts them into acoustic signals via a handheld speaker (RadioShack Corp., USA). Fish were then captured using dip nets and transported to the nearby field research station for the normoxia acclimation treatment and experiments. Before and after each fish capture, I measured water pH (pHTestr 10, Oakton Instruments, Vernon Hills, IL, USA), conductivity (ecTestr 11, Oakton Instruments, Vernon Hills, IL, USA), temperature and DO (Handy Polaris, OxyGuard, Farum, Denmark) in the lagoons (Table 3).

For normoxia acclimation, fish were housed at the field research station in groups of 4-14 fish in plastic coolers ranging from 30 L to 90 L in volume at an approximately

Table 3 - Diurnal water conditions at lagoons in Lwamunda Swamp and during laboratory normoxia acclimation at the research station.

Environment	pH	Conductivity[a)] ($\mu S\ cm^{-1}$)	Temperature (°C)	DO (kPa)	DO ($mg\ L^{-1}$)
Lwamunda Swamp	5.2 (5.0-5.6)	10 (10-20)	23.0 (21.4-28.2)	2.43 (0.38-7.62)	0.99 (0.16-3.16)
Normoxia acclimation	6.6 (4.2-7.8)	40.5 (20-80)	22.6 (19.6-25.9)	16.95 (11.73-18.07)	7.02 (4.89-7.71)

DO, dissolved oxygen. Measurements from Lwamunda Swamp were taken between 8am and 1pm on 16 occasions between May - June 2018 (n = 4) and July - September 2019 (n = 12). All measurements were taken using handheld measurement devices. Values are expressed as mean and range. [a)] The resolution of the conductivity meter was 10 $\mu S\ cm^{-1}$

[17] As original fish IDs contained information about the normoxia acclimation duration, each fish was assigned a random number, which was used as an identifier and determined the order in which fish were analyzed. After conclusion of statistical analyses, the random numbers were reverted to the original fish IDs using a dictionary in which the original ID and random number were listed.

equal density of ca. 1 fish per 7 L of water. Each cooler was connected to 1-2 air pumps that supplied a continuous airflow into the water to maintain normoxic conditions. Water was replenished regularly with water from Lake Nabugabo to maintain pH and conductivity at stable levels (Table 3).

All fish were held at ambient temperature and were fed daily a small number of earthworms and live mosquito larvae. Fish that were used for respirometry, blood sampling, and gill measurements in 2019 were split into three acclimation groups: short-term (2-6 days, n = 6), medium-term (30-36 days, n = 8), and long-term normoxia exposure (62-75 days, n = 7). Fish that were used for blood samples in 2018 were split into five normoxia acclimation groups covering a comparable range of acclimation duration: 1 day (n = 12,), 10 (n = 10), 30 (n = 4), 50 (n = 9) and 70 days (n = 11) of normoxia exposure. In addition to these, blood was sampled from a small subset of fish within 10 minutes after capture (n = 5) in 2018.

2.3.2 Respirometry

A combination of intermittent-flow and closed respirometry was used to measure $\dot{M}O_2$ and P_{crit} of *P. degeni* (Figure 9). I used a flow respirometer that creates a current within the respirometry circuit to ensure the mixing of water and the precise measurement of DO (Svendsen et al., 2016). As fish used small movements to maintain their position against the current in the respirometry chamber, I assume that they operated at RMR rather than SMR (see section 1.3.4), and the critical oxygen tension measured here represents $P_{crit\text{-}RMR}$. For the sake of readability, $P_{crit\text{-}RMR}$ is hereafter abbreviated as P_{crit}.

The respirometer consisted of a water circuit made of plastic tubing (12 mm inner diameter) connected to an acrylic glass tube (36 mm inner diameter) that served as respirometry chamber to hold the fish, and a pump (Universal 1005, EHEIM GmbH & Co., KG, Deizisau, Germany; Figure 9 A). Water flow was regulated with a shutoff valve (Absperrhahn, EHEIM GmbH & Co., KG, Deizisau, Germany) to a rate of ca. 760 ml per minute. A 3D-printed plastic baffle was inserted into the PVC fittings that connected the plastic tubing to the respirometry chamber to homogenize water flow inside the chamber. The complete setup was submerged in an aerated water bath inside a cooler that was covered with a dark cloth to prevent disturbance and minimize animal stress. A plastic hose connector was used to manually open and close the respirometry circuit to allow for intermittent exchange of water between the respirometry circuit and the water bath. Pressure fluctuations during the opening and closing of the circuit were compensated by a thin plastic tube (4 mm inner diameter, 2 m length and filled with water to prevent diffusion of oxygen into the respirometry circuit), which was connected to the respirometry circuit via a T-connector. Temperature and DO were measured with an optical oxygen meter (FireSting-O2, PyroScience, Aachen, Germany) connected to a

temperature sensor in the water bath and a fiberglass probe that was fixed to a DO sensor spot inside the respirometry chamber. Values were recorded at a sample interval of 1 second using the software Pyro Oxygen Logger ver. 3.315 (PyroScience, Aachen, Germany)[18]. Fish were not fed for 48 h prior to trials to ensure a postabsorptive state (Chabot et al., 2016b). The respirometry setup was filled with water from Lake Nabugabo in the afternoon before trials, and temperature, pH and conductivity were matched to the fish housing parameters. The respirometry circuit was closed without a fish and DO was measured for 30 minutes to quantify pre-trial background respiration caused by microbial activity. The circuit was then opened to reoxygenate the water, and a fish was inserted into the respirometry chamber. The chamber was connected to the plastic tubing, the respirometry circuit was opened to allow water exchange with the water bath, and the fish left to acclimate overnight to the respirometry chamber. Following 12 h of acclimation to the respirometry setup, RMR was measured the next day using intermittent-flow respirometry: the respirometry circuit was closed for 45 minutes, or until PO_2 dropped below 17.5 kPa (ca. 85% air saturation), whichever occurred first, and then opened to allow PO_2 to return to normoxic levels. This procedure was repeated twice. The circuit was then closed for a third time and PO_2 was allowed to drop until the P_{crit} was reached. Swimming behavior was observed in regular intervals throughout the respirometry experiment. After the P_{crit} was reached, which was visible in the DO measurements as a flattening of the curve of decreasing PO_2 values, the respirometry circuit was opened to allow PO_2 to return to a normoxic level. After reoxygenation, the fish were given a recovery time of 60 minutes during which all fish regained normal swimming behavior. Afterwards, fish were removed from the respirometry chamber and anesthetized for blood sampling, followed by preservation for gill metrics. The respirometry loop was closed for another 30 minutes to measure post-trial background respiration. Between trials, the setup was cleaned with bleach solution to prevent the build-up of microorganisms. Respirometry data were then analyzed using the respirometry package for R[19]. I analyzed 18 of the total 21 respirometry trials that were conducted covering a normoxia exposure period of 2-75 days. Two trials were aborted before the fish reached their P_{crit} due to extremely low oxygen consumption rates, and one trial was excluded from analysis due to erratic fish behavior and fluctuating $\dot{M}O_2$ values throughout the trial.

[18] https://www.pyroscience.com/en/downloads/legacy (accessed December 2021)

[19] https://cran.r-project.org/package=respirometry (accessed December 2021)

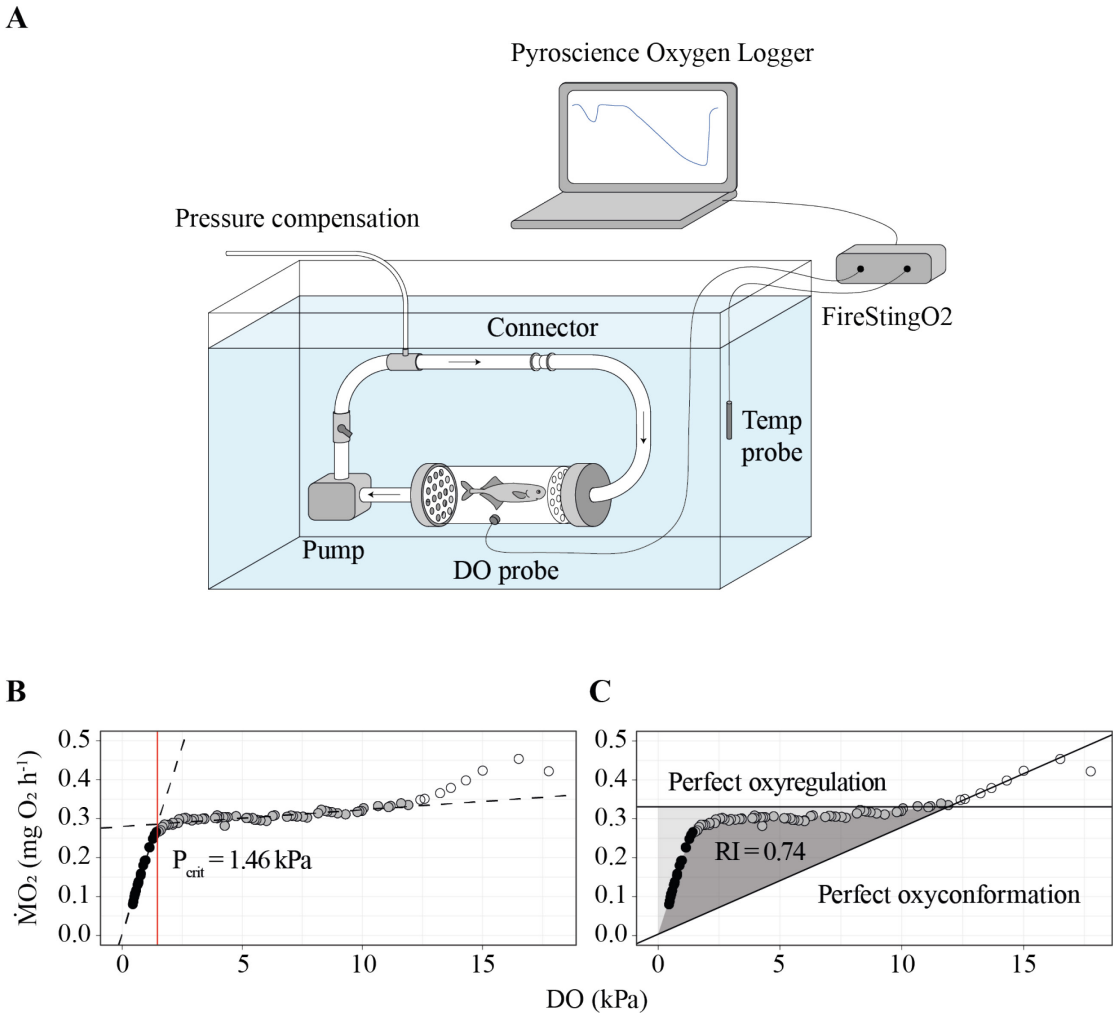


Figure 9 - Respirometry design. (A) Schematic of respirometry setup. Arrows indicate water flow. The connector was used to either close the respirometry circuit to measure fish respiration or to open it and allow water exchange with the aerated water bath. Pressure changes during opening and closing of the circuit were compensated with a water filled plastic tube. Oxygen partial pressure was measured with an optical sensor spot in the respirometry chamber. Temperature was measured in the surrounding water bath (Temp probe). Temperature and PO_2 were recorded on a PC using the software Pyro Oxygen Logger. (B-C) Example of critical oxygen tension (P_{crit}) and regulation index (RI) estimation from closed-respirometry data. White circles show data points that were excluded from analysis due to high overall respiration at PO_2 above 12 kPa. Grey circles show $\dot{M}O_2$ during oxyregulation (before P_{crit}), black circles show $\dot{M}O_2$ during oxyconformation. Dashed lines represent linear regressions that were fit to the oxyregulation and oxyconformation sections of the data. The red vertical line at their intersection represents the P_{crit}. Solid lines represent predicted trends for perfect oxyregulation (horizontal) and oxyconformation (diagonal). The RI is derived by dividing the area under the $\dot{M}O_2$-versus-PO_2 relationship of the fish that lies above the trend for oxyconformation (dark grey) by the area for perfect oxyregulation (light grey).

2.3.2.1 Routine Metabolic Rate

Oxygen consumption rate was calculated from DO measurements using the following equation:

$$\dot{M}O_2 = \frac{\Delta\, DO}{\Delta\, time * body\ mass} \quad (3)$$

All fish showed elevated $\dot{M}O_2$ with a high degree of oxyconformation at PO_2 above 12 kPa, a phenomenon that has also been observed in previous respirometry studies conducted at this site with *P. degeni* (Clarke et al., 2020) and *M. victoriae* (Moulton et al., 2020). I conducted long-term control measurements and found that $\dot{M}O_2$ followed a similar curve when there was no fish present in the respirometer, which suggests that this phenomenon is due to biological activity in the lake water that was used for respirometry (see Appendix, Figure A2). To follow established protocols (Chabot et al., 2016a; Rosewarne et al., 2016), I measured RMR at PO_2 above 17.5 kPa despite this phenomenon. I measured background respiration at similarly high PO_2 to control for this effect; however, it is possible that RMR values were affected by this phenomenon.

To calculate RMR, $\dot{M}O_2$ values were calculated from each intermittent respirometry loop and averaged per fish. Subsequently, RMR was adjusted for background respiration assuming a linear increase of background respiration between pre-trial and post-trial control measurements. Routine metabolic rate scaled with body mass and therefore was adjusted to a common mean mass before assessing the effect of normoxia exposure (Table 4). An allometric power scaling equation was adapted from Ihssen et al. (1981) for the adjustment:

$$Variable_{adj} = Variable_{obs} * (mass_{mean} * mass_{obs}^{-1})^{b} \quad (4)$$

The scaling exponent b is equal to the slope of the bilogarithmic linear regression between RMR and body mass. Prior to adjustment, the effect of normoxia acclimation on the slope of the bilogarithmic relationship between RMR and mass was tested using ANCOVA with log mass, acclimation group, and their interaction as effects. As this resulted in a non-significant outcome for the interaction term (i.e., normoxia acclimation did not affect the relationship between RMR and mass), the slope of the bilogarithmic linear regression was used without blocking for the acclimation group. The effect of normoxia exposure was tested on mass-adjusted RMR using ANOVA with hypoxia acclimation group as grouping variable followed by a Tukey post-hoc test in case of a significant result.

2.3.2.2 Critical Oxygen Tension

To determine the P_{crit}, $\dot{M}O_2$ values were calculated repeatedly over short intervals for the duration of the closed phase of the respirometry trial using equation (3) (Figure 9 B). The length of the time intervals over which $\dot{M}O_2$ is calculated is an important variable in this procedure as large intervals increase the reliability but decrease the temporal resolution of the results. Since trial duration varied based on fish size and overall $\dot{M}O_2$, the length of intervals over which $\dot{M}O_2$ was calculated was adjusted to one tenth of trial duration at the highest PO_2 (to compensate for lower precision of optical DO meters at high DO concentrations[20]), and decreased in ten equal steps to one 100th of trial duration at the lowest PO_2. I used only $\dot{M}O_2$ values that were measured at PO_2 below 12 kPa to eliminate the confounding effect that the high background respiration at high PO_2 had on the regressions that were used to determine P_{crit} (Figure 9 B, see section 2.3.2.1).

There has been considerable debate about the most appropriate statistical approach to quantify P_{crit}. I tested three different approaches that had been suggested by previous studies: broken-stick regression (BSR, Yeager and Ultsch, 1989), non-linear regression (NLR, Marshall et al., 2013), and the P_{crit} - Alpha method[21] (Seibel et al., 2021). Other approaches, such as the low-linear PO_2 method (Reemeyer and Rees, 2019) and the sub-prediction interval metric (Birk et al., 2019) were excluded because I did not quantify SMR, which is a prerequisite for reliable estimation of low-linear PO_2, and because the sub-prediction interval method results in systematic underestimation of P_{crit}. The three methods did not produce significantly different P_{crit} values (see Appendix, Figure A3, Table A2); however, non-linear regression tended to produce erroneous results when $\dot{M}O_2$ values increased towards P_{crit}. To provide comparability with previous studies, which have used the BSR method, I decided to focus on BSR, as well. For this approach, two linear regressions are fitted to the $\dot{M}O_2$ values corresponding to the phases of the trial when the fish behaved as oxyregulator or as oxyconformer (Figure 9 B). The intersection between the two linear regressions marks the P_{crit}, at which the $\dot{M}O_2$ becomes strongly dependent on external PO_2.

Critical oxygen tension scaled with body mass and was adjusted to a common mean body mass as described above using equation (4). Acclimation group had no effect on the slope of the bilogarithmic relationship between mass and P_{crit} (Table 4); thus, P_{crit} was adjusted without blocking for the acclimation group. The effect of normoxia exposure on

[20] According to the specifications of the oxygen sensor spot, measurement accuracy decreases from ± 0.02% to ± 0.2% oxygen saturation, and resolution decreases from 0.01% to 0.05% oxygen saturation when DO increases from 1% to 20% oxygen saturation (retrieved from the data sheet on the product page, https://www.pyroscience.com/en/products/all-sensors/oxsp5#Downloads, accessed April 2022).

[21] P_{crit} - Alpha: the physiological oxygen supply α_0 is estimated as ($\dot{M}O_2$ / PO_2). The P_{crit} - Alpha is defined as the PO_2 at which physiological oxygen supply reaches its peak value (e.g. during progressing hypoxia, Seibel et al., 2021).

mass-adjusted P_{crit} was tested using ANOVA with hypoxia acclimation group as grouping variable followed by a Tukey post-hoc test in case of a significant result.

2.3.2.3 Regulation Index

The RI is a measure of the oxyregulatory ability of an organism in declining PO_2 (Mueller and Seymour, 2011). To calculate RI, the area under the $\dot{M}O_2$ versus PO_2 curve (A_{trial}) is estimated, as well as the area for perfect oxyregulation (i.e., under the horizontal line intersecting $\dot{M}O_2$ at the highest PO_2, A_{reg}), and the area for perfect oxyconformation (i.e., under a diagonal line through the origin and $\dot{M}O_2$ at the highest PO_2, A_{conf}, Figure 9 C). The RI is then calculated using the following equation:

$$RI = \frac{A_{trial} - A_{conf}}{A_{reg} - A_{conf}} \quad (5)$$

A RI close to 1 indicates that the fish behaves mostly as an oxyregulator, whereas a value close to 0 indicates that $\dot{M}O_2$ mostly conforms to PO_2. Similarly to P_{crit} determination, I used only $\dot{M}O_2$ at PO_2 below 12 kPa for RI determination. Regulation index did not scale with body mass (Table 4), and the effect of normoxia exposure on RI was tested using ANOVA with hypoxia acclimation group as grouping variable followed by a Tukey post-hoc test in case of a significant result.

2.3.3 Gill Morphometrics

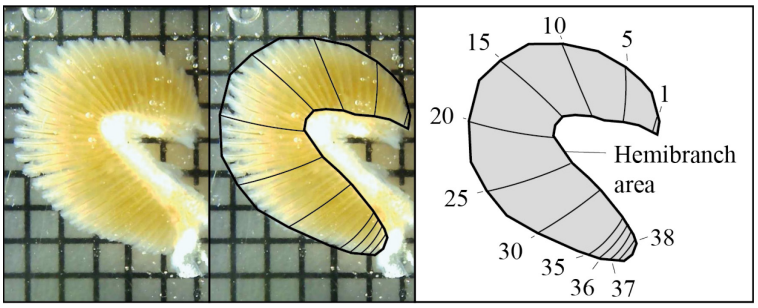


Figure 10 - Procedure for gill morphometric estimation on one hemibranch. Filament length was measured on every 5th filament from the dorsal end of the hemibranch onwards and approximated for filaments in between measurements as mean of the two closest measured filaments. Towards the ventral end, the final filaments (here: filaments 35-38) were measured individually. Hemibranch area was measured by fitting a polygon to the area of the hemibranch that was covered by filaments (grey area).

Gills were sampled from fish that were used in respirometry trials. Approximately 60 minutes after respirometry trials, fish were submerged in clove oil solution until deep

anesthesia was reached. This was marked by complete cessation of motor functions, which occurred at 3-7 minutes after submersion. Fish were then weighed and standard body length was measured with a slide gauge from the snout to the caudal end of the caudal peduncle. Fish were then euthanized by drawing blood from the caudal vein for blood parameter measurement (see below). Euthanized specimens were preserved in 4% formaldehyde for 24 h and soaked in distilled water for 24 h prior to gill morphology measurement. Afterwards, all four gill arches on the left body side were extracted using a needle scalpel and carefully rinsed with distilled water to remove residual tissue. Both sides of each arch (i.e., two hemibranchs) were photographed under magnification using a portable microscope camera (WiFi USB Mikroskop, ROTEK Inc., Aurora, OH, USA).

As some gill arches were damaged during the extraction process, only the first two gill arches (i.e., the four hemibranchs on the outer two gill arches) were used for analysis. I used ImageJ ver. 1.53c[22] and followed previously established procedures to measure a suite of traits on each of the four hemibranchs: number of filaments (FN), filament length (FL) and hemibranch area (HA; Figure 10; Chapman et al., 1999; Crampton et al., 2008; Hughes, 1984; Potts et al., 2021). Gill FL has previously been shown to correlate with gill surface area both within (Crispo and Chapman, 2010) and across species (Palzenberger and Pohla, 1992). Filament length was estimated by measuring the length of every 5th filament from the dorsal end of the hemibranch outwards. The length of intermediary filaments (i.e., between two measured filaments) was approximated as the average length of the two closest neighboring filaments that were measured. The last filaments at the ventral end of the arch were measured individually because their lengths varied greatly. Finally, the lengths of all filaments from the four measured hemibranchs were added together to represent FL. Hemibranch area was measured by fitting a polygon to the area of each measured hemibranch that was covered by gill filaments. The results of the four hemibranchs were added together to represent HA. The number of filaments were counted on all four measured hemibranchs and added together to represent FN. Due to the missing 3rd and 4th gill arch in my analysis, these measurements do not represent the total FN, FL and HA of the fish gills, but rather indices of these traits, which allow me to compare different acclimation treatments. Additionally, as the 1st and 2nd gill arch of *P. degeni* are larger than the 3rd and 4th, I assume to have captured a relevant portion of the gill filaments with these measurements.

Gill morphometrics scaled with body mass and were adjusted to a common mean body mass as described above using equation (4). Acclimation group had no effect on the slope of the bilogarithmic relationship between body mass and gill morphometrics (Table 4). The HA measurements violated the assumption of homogeneity of variance across groups (Levene test: $P < 0.01$). However, as there are no robust alternatives to ANCOVA

[22] https://imagej.nih.gov/ij/ (accessed December 2021)

with more than two groups available in R, the effect of the acclimation group on the slope of the bilogarithmic relationship between body mass and HA was tested with ANCOVA and found to be non-significant. For that reason, all gill morphometrics were adjusted without blocking for the acclimation group. The effect of normoxia exposure on FL and FN was estimated using ANOVA with hypoxia acclimation group as grouping variable followed by a Tukey post-hoc test in case of a significant result. The effect of normoxia exposure on HA was estimated using the Kruskal-Wallis test with hypoxia acclimation group as grouping variable followed by a Conover post-hoc test with Bonferroni correction of P-values in case of a significant result.

2.3.4 Blood Sample Collection

Hemoglobin and lactate concentrations were measured in full blood using handheld devices (Hb: DiaSpect Tm; lactate: Lactate Scout +; both: EKF Diagnostics, Cardiff, UK). Both devices have been evaluated and found to deliver sufficient accuracy and reliability, particularly for comparisons of repeated measurements (Bonaventura et al., 2015; Ranjan et al., 2020). In 2018, samples were collected within 10 minutes of capture and after 1, 10, 30, 50 and 70 days of normoxia exposure to evaluate the effect of long-term normoxia on blood parameters. Fish were submerged in clove oil solution until deep anesthesia was reached, as described above. Fish were then weighed and standard body length was measured with a slide gauge. The caudal peduncle was severed with a scalpel and blood was collected from the caudal vein with heparinized capillary tubes. Hemoglobin and lactate concentrations were measured in full blood immediately after extraction according to the instructions of the measurement devices.

I additionally sampled blood in 2019 from the fish that were used in respirometry trials to correlate respirometry data, gill morphometrics, and blood parameters. Blood was sampled ca. 60 minutes after the respirometry trial using the same anesthesia and measurement protocol as described above, but a different extraction technique. In samples collected in 2018, severance of the caudal peduncle had in some cases yielded insufficient amounts of blood for measurement. Thus, blood was extracted directly from the caudal vein using syringes that were flushed with a heparin-PBS solution. However, this technique did not increase sampling success, but caused a systematic dilution of measured lactate and Hb concentrations due to residual heparin-PBS solution in the syringe and the small volume of blood that was collected (in many cases less than 20µl).

The results from these two cohorts of fish were analyzed separately to account for the different extraction protocols that were used and the possible effect of acute hypoxic stress during respirometry trials on blood hemoglobin and lactate. Neither parameter scaled with body mass (Table 4). The effect of normoxia exposure on full blood hemoglobin and lactate was estimated using linear regression for data from undisturbed

fish and ANOVA for data from fish that were used in respirometry trials, followed by a Tukey post-hoc test in case of a significant result.

2.3.5 Correlations Between Traits

All traits that were measured in the same fish (RMR, P_{crit}, RI, FL, HA, FN, Hb, lactate) were tested for correlations using Pearson correlation analysis. As the number of individuals on which all metrics were successfully measured was small (n = 10), I repeated Pearson correlation analysis with respiratory (RMR, P_{crit}, RI) and gill metrics (FL, HA, FN), which were measured successfully on 16 fish, to increase the statistical power of the correlation analysis.

Table 4 - Results of ANCOVA on allometric relationships of log-transformed variables and slopes of linear regressions.

	Effect: log M			**Effect: acclimation**			**Interaction**			**Slope**
	F(1,12)	**P**	η_g^2	**F(1,12)**	**P**	η_g^2	**F(1,12)**	**P**	η_g^2	**b**
log RMR	**31.647**	**<0.001**	**0.725**	1.302	0.308	0.178	1.306	0.307	0.179	**1.70**
log P_{crit}	**7.825**	**0.016**	**0.395**	2.016	0.176	0.251	0.683	0.524	0.102	**0.38**
log RI	0.461	0.510	0.037	1.018	0.391	0.145	0.033	0.967	0.005	-0.20
	F(1,14)			**F(2,14)**			**F(2,14)**			
log FL	**33.396**	**<0.001**	**0.705**	**6.204**	**0.012**	**0.470**	0.083	0.921	0.012	**0.48**
log HA	**34.018**	**<0.001**	**0.708**	**6.067**	**0.013**	**0.464**	0.027	0.974	0.004	**0.62**
log FN	**18.273**	**<0.001**	**0.566**	1.202	0.330	0.147	0.011	0.989	0.002	**0.20**
	F(1,26)			**F(2,26)**			**F(2,26)**			
log Hb[a)]	1.951	0.174	0.070	**11.905**	**<0.001**	**0.478**	1.794	0.186	0.121	0.16
	F(1,8)			**F(2,8)**			**F(2,8)**			
log Hb[b)]	0.527	0.488	0.062	2.275	0.165	0.363	0.883	0.450	0.181	-0.27
	F(1,43)			**F(2,43)**			**F(2,43)**			
log Lac[a)]	1.584	0.215	0.036	0.812	0.450	0.036	0.924	0.404	0.041	0.15
	F(1,7)			**F(2,7)**			**F(2,7)**			
log Lac[b)]	0.001	0.980	0.000	1.994	0.206	0.363	2.477	0.154	0.414	-0.20

b, slope of bilogarithmic regression between trait and body mass; M, body mass; F, F statistic; FL, gill filament length; FN, gill filament number; HA, gill hemibranch area; Hb, blood hemoglobin concentration; Lac, blood lactate concentration; P_{crit}, critical oxygen tension; RMR, routine metabolic rate; η^2_g, generalized eta squared for effect size. Numbers in brackets indicate degrees of freedom in the numerator and denominator. Bold numbers indicate statistically significant outcomes. [a)] blood samples from 2018; [b)] blood samples from 2019.

2.4 Reproducibility and Data Availability

All scripts for R and Python, datasets and figures that were created for this thesis are publicly available on the publication server of the Humboldt-Universität zu Berlin under the following link: https://doi.org/10.18452/25018

All statistical procedures and figures shown can be reproduced using the supplied R scripts. The figures for this thesis were formatted using Adobe Illustrator CC 2018 (Adobe Inc., San Jose, CA, USA).

3 Results

If not otherwise stated, all values are represented as mean ± SD.

3.1 Activity Patterns in the Laboratory

To assess behavioral patterns under controlled conditions, I recorded EOD and swimming activity of *M. victoriae* (n = 11) and *P. degeni* (n = 10) over diel cycles in a shuttle-box system while maintaining constant temperature and DO.

The activity patterns of *M. victoriae* and *P. degeni* in the lab corresponded closely to ambient light levels, with long phases of inactivity and strong shelter preference during the light phase and increased activity and exploration behavior during the dark phase of the trial (Figure 11, Table 5, Table 6). During the light phase, fish spent on average more than 90% of the time in a shelter tube, with seven *M. victoriae* and eight *P. degeni* spending more than 99% of the time sheltered. During the dark phase, all fish spent more time outside their shelters. *Marcusenius victoriae* showed no preference for either shelter tube or unsheltered areas of the shuttle-box (edge and middle). However, when fish were located outside of the shelter tube, they spent significantly more time close to the edge of the shuttle-box than in the middle. *Petrocephalus degeni* spent significantly more time in the unsheltered areas of the shuttle-box during the dark phase without a preference for the edge or the middle of the shuttle-box tank (Figure 11 B, Table 5, Table 6). *Marcusenius victoriae* emitted EODs at significantly lower rates in the shelter tube than in the unsheltered areas of the shuttle-box throughout the diel cycle. *Petrocephalus degeni* emitted EODs at lower rates in the shelter tube during the light phase. There was a small but significant difference in EOD rates of *P. degeni* between tank areas during the dark phase (Figure 11 B, Table 5, Table 6).

Swim speed and EOD rate of both species increased sharply during the 30-minute dusk period, peaked at the onset of complete darkness, and remained at elevated levels for the entire duration of the dark phase (Figure 12 A, Table 5). The dark phase proportion of the total distance that fish swam within 24 h was 96.3 ± 7.8% for *M. victoriae* and 97.7 ± 4.6% for *P. degeni* (data not shown). There was no evidence of anticipation of the dark phase (i.e., no increase of EOD rate or swim activity before the dusk period). During the light phase, EOD rate decreased steadily in contrast to swim speed, which dropped quickly close to 0 BL s^{-1} at the onset of the light phase and remained there until the dusk period. Despite this difference, EOD rate was overall positively correlated with swim speed throughout the light and dark phases for both species (Figure 12 B, Table 6).

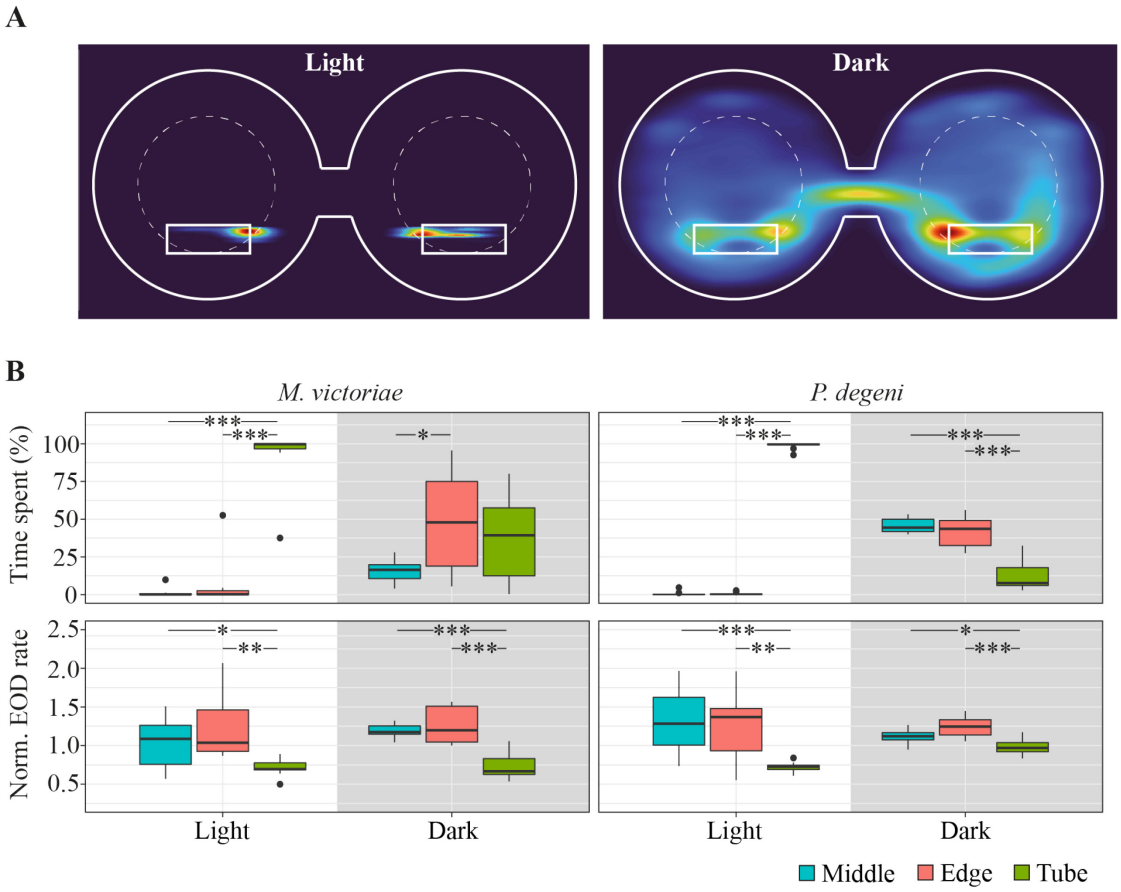


Figure 11 - Example heatmap and location preference of *M. victoriae* (n = 11) and *P. degeni* (n = 10) in the shuttle-box. (A) Heatmap of swim activity of one *P. degeni* during the light and the dark phase. Warmer colors indicate that the fish spent more time at this location. The margins of the shuttle-box tank and the two shelter tubes are indicated with solid white lines, the area boundaries between edge and middle are indicated with dashed white lines. Two-dimensional kernel density estimation was used to convert X-Y coordinates from tracked video data (downscaled to 1 frame per second) into a probability density function for the location of the fish. (B) Top row: proportion of time fish spent in different locations in the shuttle-box as percentage of total time. Bottom row: normalized EOD rates, averaged per fish, period of the light cycle, and area of the shuttle-box. See Table 5 for absolute EOD rates and Table 6 for statistical test outcomes (P < 0.05*, P < 0.01**, P < 0.001***).

There were some differences between species in swim and EOD activity. Swim activity of *P. degeni* was significantly higher than that of *M. victoriae* during the dark phase (Table 5, Wilcoxon test: P < 0.001), and *P. degeni* increased swim speed and EOD rate at the end of the dark phase, an effect that was not as prominent in *M. victoriae*. Inter-pulse interval coefficient of variation (IPI CV) of *P. degeni* showed a clear diel pattern indicating that EOD rate was more variable during their active phase. By contrast, IPI CV of *M. victoriae* did not differ between light and dark phase, and some individuals showed greatly irregular IPIs during the inactive phase, mostly due to longer cessations of EOD generation. The resting EOD rate of *P. degeni* was significantly higher at night than during the day. This effect was not observed in *M. victoriae* (Figure 12 C, Table 6).

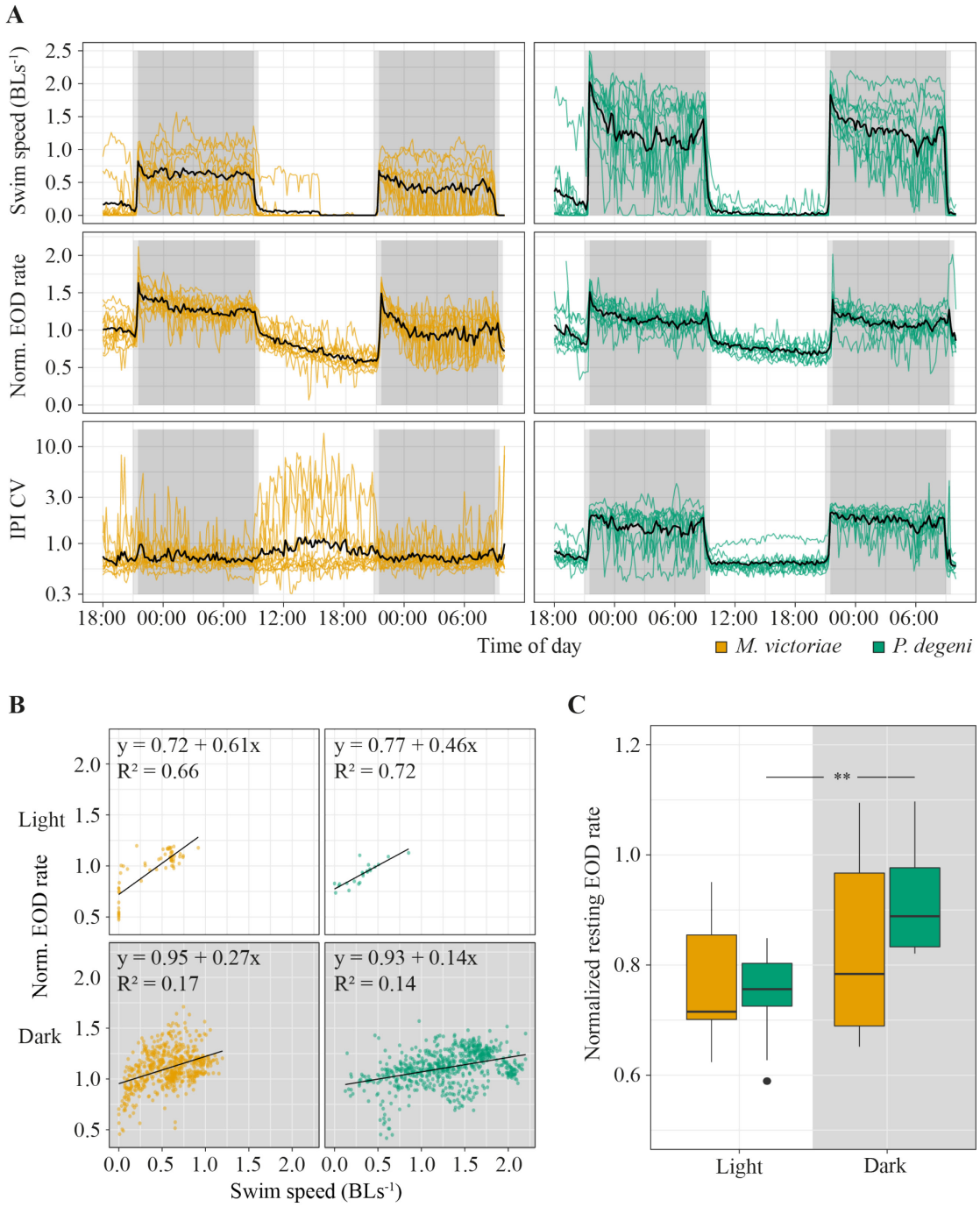


Figure 12 - Behavioral activity of *M. victoriae* (n = 11) and *P. degeni* (n = 10) under laboratory conditions. (A) Swim speed in body lengths per second (BL s^{-1}, top row), normalized EOD rate (middle row), and coefficient of variation of inter-pulse intervals (IPI CV, bottom row) of *M. victoriae* (left) and *P. degeni* (right) in the shuttle-box. All variables were averaged per fish over 10-min intervals. Colored lines represent individual fish, black lines represent the average over all fish, grey shaded areas represent periods of dusk/dawn (light grey) and complete darkness (dark grey). Notice that IPI CV is shown on a logarithmic y-axis. (B) Linear regressions of swim speed and normalized EOD rate. Each point represents a measurement from an actively swimming fish that was averaged over a 10-min interval. Points from different fish are not distinguished. (C) Box plot of normalized EOD rate of fish that rested in their shelter tubes during the day and night. Resting EOD rate was averaged per fish (t-test: $P < 0.01$ **, Table 6).

Table 5 - Summary of behavioral traits measured in the shuttle-box, grouped by species, location and phase of the light cycle.

Phase	Species	Location			Overall
		Edge	Middle	Tube	
		Time spent (%)			
Light	MV	5.7 ± 15.6	1.2 ± 2.9	93.1 ± 18.5	-
	PD	0.6 ± 1.0	0.6 ± 1.5	98.7 ± 2.4	-
Dark	MV	48.1 ± 32.7	15.5 ±7.0	36.4 ± 27.5	-
	PD	41.7 ± 10.2	45.8 ± 5.0	12.5 ± 10.0	-
		EOD rate (Hz)			
Light	MV	15.0 ± 7.3	11.5 ± 4.2	8.0 ± 1.7	8.4 ± 1.6
	PD	15.9 ± 8.3	14.8 ± 6.3	7.9 ± 2.7	8.0 ± 2.7
Dark	MV	14.0 ± 2.9	13.4 ± 2.3	8.5 ± 2.7	11.2 ± 2.2
	PD	13.8 ± 5.4	12.7 ± 5.4	11.0 ± 4.5	13.0 ± 5.4
		Swim speed (BL s^{-1})			
Light	MV	0.57 ± 0.54	0.20 ± 0.19	0.00 ± 0.00	0.03 ± 0.09
	PD	4.51 ± 2.44	0.94 ± 0.92	0.00 ± 0.00	0.03 ± 0.06
Dark	MV	0.64 ± 0.14	0.53 ± 0.18	0.01 ± 0.01	0.44 ± 0.28
	PD	1.46 ± 0.27	1.19 ± 0.37	0.02 ± 0.01	1.29 ± 0.40

BL s^{-1}, body lengths per second; Hz, hertz; MV, *M. victoriae*; PD, *P. degeni*. Values are represented as mean ± SD.

Table 6 - Outcomes of statistical tests on behavioral traits measured in the shuttle-box.

Friedman test	**Phase**	**Effect**	**Species**	**n**	**χ^2**	**DF**	**P**
Time spent	Light	Location	MV	11	20.2	2	**< 0.001**
			PD	10	18.2	2	**< 0.001**
	Dark	Location	MV	11	3.5	2	0.178
			PD	10	12.6	2	**0.002**
Normalized EOD rate	Dark	Location	MV	11	13.8	2	**< 0.001**
			PD	10	14.6	2	**< 0.001**
Paired Wilcoxon	**Phase**	**Comparison**	**Species**	**n**	**U**		**P**
Time spent	Light	Middle - Tube	MV	11	0		**<0.001**
			PD	10	0		**<0.001**
		Edge - Tube	MV	11	1		**<0.001**
			PD	10	0		**<0.001**
	Dark	Middle - Edge	MV	11	21		**0.025**
		Middle - Tube	PD	10	100		**<0.001**
		Edge - Tube	PD	10	97		**<0.001**
Normalized EOD rate	Dark	Middle - Tube	MV	11	119		**<0.001**
			PD	10	83		**0.023**
		Edge - Tube	MV	11	115		**<0.001**
			PD	10	95		**<0.001**
Kruskal Wallis	**Phase**	**Effect**	**Species**	**n**	**χ^2**	**DF**	**P**
Normalized EOD rate	Light	Location	MV	28	11.0	2	**0.004**
			PD	26	11.9	2	**0.003**
Conover test	**Phase**	**Comparison**	**Species**	**n_1**	**n_2**	**t**	**P**
Normalized EOD rate	Light	Middle - Tube	MV	11	11	3.0	**0.010**
			PD	9	10	4.2	**<0.001**
		Edge - Tube	MV	6	11	3.8	**0.001**
			PD	7	10	3.4	**0.004**
Lin. regression	**Phase**	**Ind. variable**	**Species**	**F**	**DF**		**P**
Normalized EOD rate	Light	Swim speed	MV	**112**	**1, 57**		**< 0.001**
			PD	51	1,20		**< 0.001**
	Dark		MV	116	1,590		**< 0.001**
			PD	115	1,684		**< 0.001**
Paired t-test		**Comparison**	**Species**	**n_1**	**n_2**	**t**	**P**
Normalized resting EOD rate		Light - Dark	MV	10	10	-0.9	0.392
			PD	10	10	-3.6	**0.006**

DF, degrees of freedom; MV, *M. victoriae*; n, sample size; PD, *P. degeni*; χ^2, chi-square. Significant outcomes of the Friedman test were followed by a paired wilcoxon test with Bonferroni correction of P-values; significant outcomes of the Kruskal Wallis test were followed by a Dunn post-hoc test with Bonferroni correction of P-values. All results of the Friedman and Kruskal Wallis test are reported, whereas only significant outcomes of post-hoc tests are shown here. Significant outcomes are marked with bold P-values.

3.2 Environmental and Behavioral Patterns at Petro Lagoon

I measured environmental temperature and DO, and recorded EODs of *M. victoriae* and *P. degeni* in three habitats in a swamp lagoon on six sampling days between July and September 2019 to record diel patterns of activity and habitat preference, and abiotic environmental cycles.

3.2.1 Temperature and Dissolved Oxygen at Petro Lagoon

Temperature and DO at Petro Lagoon fluctuated over the diel cycle (Figure 13 A). Dissolved oxygen reached minimum levels at night (5.1 ± 5.4% air saturation at 11.5 h after sunset) and increased in the morning. Rainfall and/or disturbance of the water surface led to transient peaks in DO values and contributed to the increase of mean DO in the morning. Water temperature followed a more balanced oscillation than DO, reaching its maximum in the late afternoon (24.9 ± 1.1°C at 3.5 h before sunset) and minimum in the early morning (21.6 ± 0.7°C at 0.5 h after sunset). Day length remained constant throughout the sampling period (12:06-12:07 h, Sunrise: 06:47-06:47 am, Sunset: 06:50-07:03 pm)[23]. Across sampling days, DO was highest on August 21st, due to continuous rainfall during that day, but remained low on average (9.2 ± 7.3% air saturation) and was extremely low on August 8th (2.7 ± 1.4%, Figure 13 B). Daily mean water temperature remained at a relatively constant value of 23.0 ± 0.6°C despite diel fluctuations. Water depth also remained relatively constant in all habitats and was highest in the open habitat. Manual measurements that were taken during the day showed little difference in temperature and DO between the open and vegetation habitat. However, DO and temperature tended to be slightly but systematically higher in the stream habitat than in other areas of the lagoon (by 6.4 ± 2.4% air saturation and 0.4 ± 0.7 °C, Figure 13 B).

[23] Sunrise and sunset times were retrieved from https://www.worldweatheronline.com/masaka-weather-history/masaka/ug.aspx (accessed January 2022)

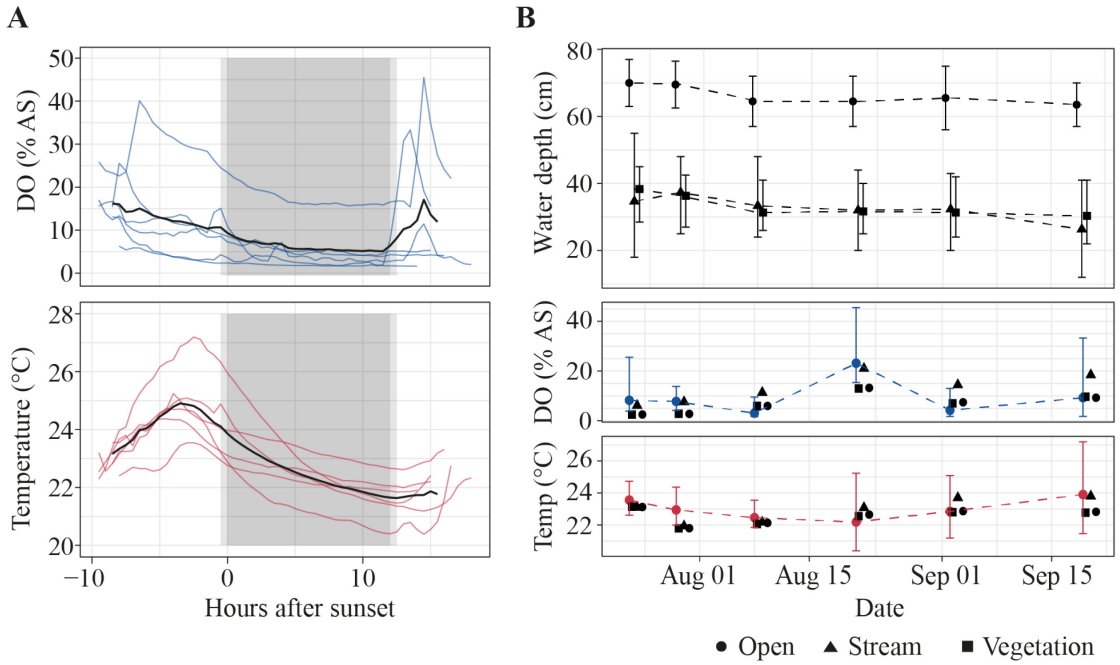


Figure 13 - Environmental conditions at Petro Lagoon. (A) Daily changes in air saturation (top panel) and water temperature (bottom panel). Thin lines indicate individual sampling days, the thick black line shows the mean over all sampling days (n = 6). Light grey boxes indicate low light conditions during dusk and dawn, dark grey boxes indicate dark light conditions during the night. (B) Variation of water level, air saturation (AS) and water temperature (°C) across sampling days. Black symbols represent manual measurements, colored symbols represent mean values of logger measurements, error bars represent the range of values. The symbols have been offset on the x-axis to improve their readability.

3.2.2 Presence of Electric Fish in the Different Habitats

Electric fish loggers were placed in three different habitats: (i) under floating vegetation where light levels remained low during the day and there was a high abundance of shelter structures; (ii) under the open water surface of the lagoon with at least 1 m distance to the next floating shelter structure; (iii) in the narrow stream that was shaded by overhanging vegetation, where fish found shelters along the edge of the stream (Table 2). I collected more than 1,122 h of electric recordings between July 23rd and September 20th 2019. I registered 2849 encounters with at least one *M. victoriae* and/or *P. degeni*, most of which happened during the night (2218). Both species were encountered equally often (*M. victoriae*: 1433, *P. degeni*: 1416). By far the highest number of encounters was recorded in the vegetation habitat (1137 and 852, respectively), followed by the open habitat (581 and 78, respectively), and the stream (0 and 201, respectively).

The number of daily fish encounters varied considerably across sampling days (Figure 14 A). Encounters with *M. victoriae* increased in the open habitat and vegetation habitat from 84 to 526 encounters. Numbers of encounters with *P. degeni* remained

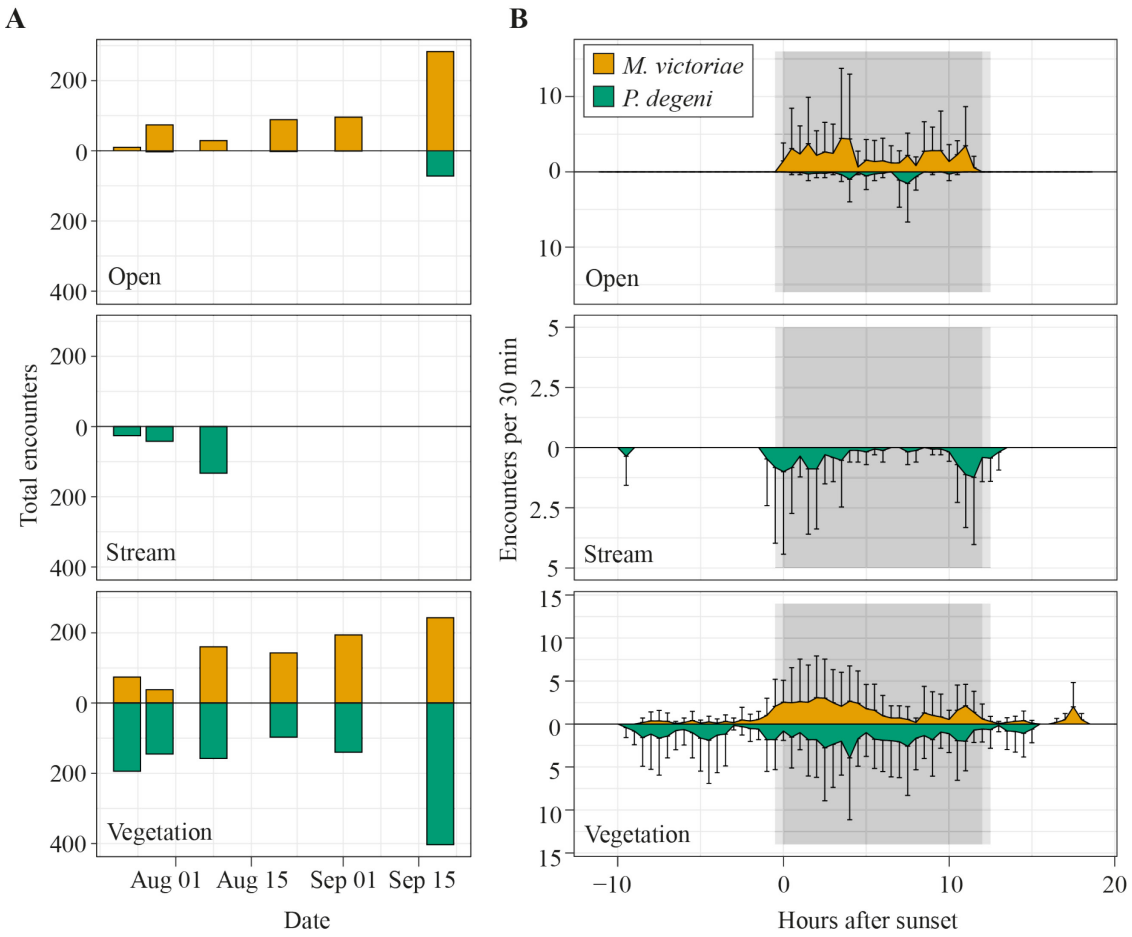


Figure 14 - Diel patterns of EOD activity in Petro Lagoon. (A) Total number of fish that were encountered per sample day in the open (top), stream (middle) and vegetation (bottom) habitat. (B) Average number of fish encounters per 30 minutes in the open (top), stream (middle) and vegetation (bottom) habitat. To calculate the average number of fish encounters per 30 minutes, the number of fish was extracted from each encounter, summed up for each logger over intervals of 30 minutes and averaged over the number of loggers per habitat (open: 2, stream: 3, vegetation: 3) and all sampling days (n = 6). Error bars representing SD extend only into one direction for better readability. Notice that the *y*-axis scaling differs between the different panels. Data for *M. victoriae* are shown above the x-axis, those for *P. degeni* below.

relatively constant under the floating vegetation except for a peak of 403 encounters on the last sampling day. In the stream habitat, *P. degeni* was encountered only during the first three sampling days, and in the open habitat only on the last sampling day. Overall, 35% of all fish encounters were made on the last sampling day.

The frequency with which encounters occurred in the different habitats varied between species and periods of the diel cycle (Table 7, Table 8, Figure 14 B). In the open habitat, both *M. victoriae* and *P. degeni* were encountered only at night, and *M. victoriae* accounted for the majority of the nocturnal encounters. Under the floating vegetation, both species were encountered throughout the diel cycle. However, the frequency of encounters with *M. victoriae* increased more than fivefold from daytime to nighttime, whereas *P. degeni* were encountered more continuously during the day and the night

under the floating vegetation (Table 7). In the stream habitat, *P. degeni* were encountered primarily at night.

3.2.3 Co-occurrence of Fish

In 18.7% of encounters, more than one fish was detected (co-occurrence; Figure 15 A). On average, the proportion of encounters during which multiple *M. victoriae* were detected simultaneously was higher at night than during the day. *Petrocephalus degeni,* likewise, co-occurred more often at night than during the day (Table 7, Table 8). There was no difference between the two species in the percentage of encounters that included more than one conspecific. In 50.9% of co-occurrences, fish from both species were present simultaneously.

3.2.4 Encounter Duration and Electric Organ Discharge Rate

Encounter duration and EOD rate differed among habitats and periods of the diel cycle, suggesting that fish displayed higher activity in the open and stream habitat and around sunset and sunrise (Figure 15 B-C, Table 7, Table 8).

In the vegetation habitat, where most encounters were detected throughout the diel cycle, EOD rates of *M. victoriae* peaked within one hour of sunset and again shortly before sunrise. EOD rates of *P. degeni* were highest during the day but showed a small peak before sunrise (Figure 15 B). These changes were transient and did not lead to elevated nocturnal EOD rates, as were observed in the laboratory. Both species emitted EODs at a significantly lower rate under the vegetation than in the other habitats, both during the day and at night, and encounters lasted significantly longer under the vegetation than in the other habitats (Figure 15 C, Table 7, Table 8). Overall, the EOD rates of *M. victoriae* in the field were comparable to those measured in the laboratory during the day (10.3 ± 4.3 vs. 8.4 ± 1.6 Hz) and at night (11.1 ± 6.0 vs. 11.2 ± 2.2 Hz, Table 5, Table 7). EOD rates of *P. degeni* were significantly higher in the field than in the laboratory during the day (13.2 ± 6.9 vs. 8.0 ± 2.7 Hz, U-test: $U = 3189$, $P < 0.01$), but not during the night (12.9 ± 5.1 vs. 13.0 ± 5.4 Hz, Table 5, Table 7). There were no significant day-night differences within habitats.

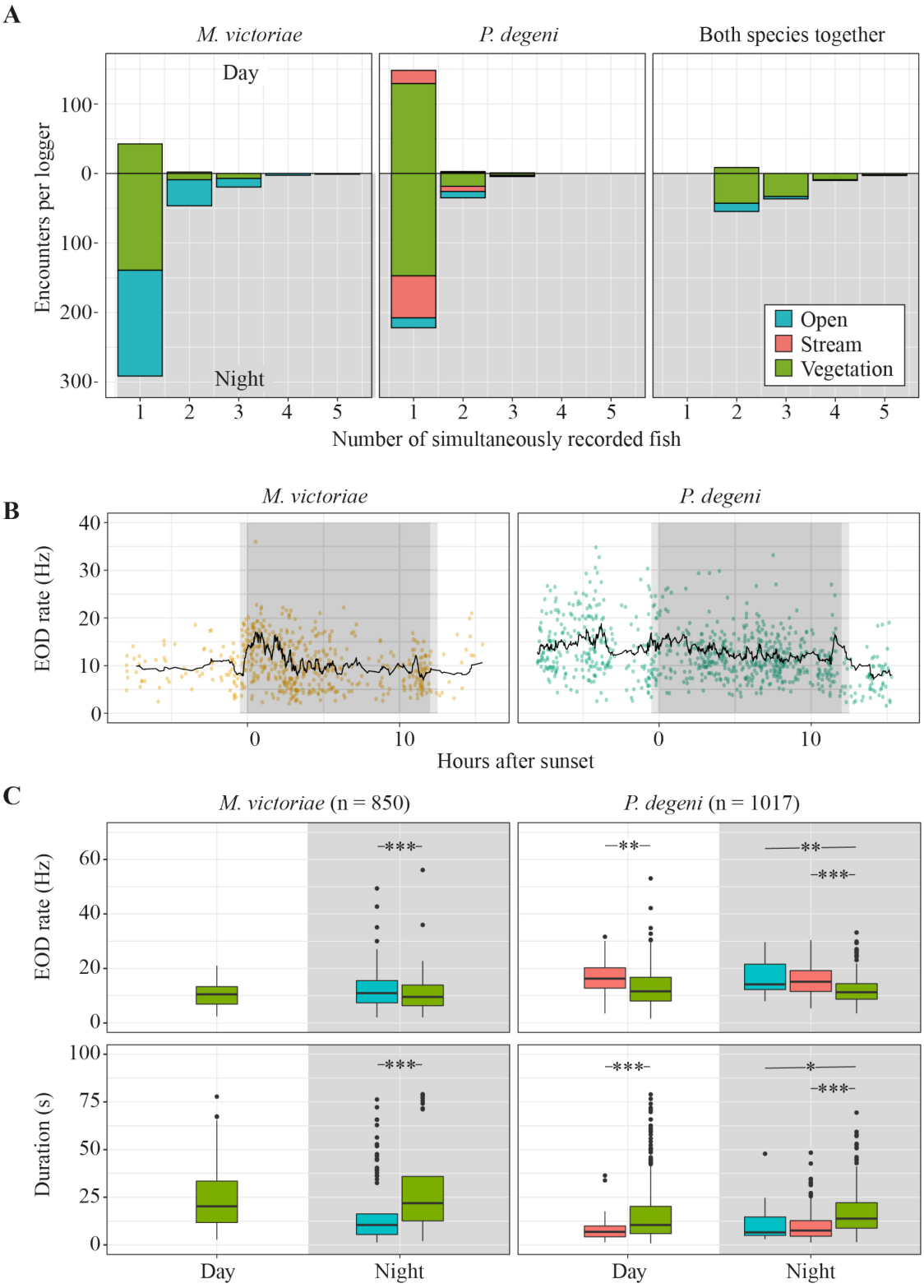


Figure 15 - Diel differences in fish encounters. (A) Total number of encounters by species, habitat, daytime and number of fish that were present during encounter. (B) Average EOD rate (top row) and duration (bottom row) of encounters by species, habitat and phase of the diel cycle (t-test: $P < 0.05$ *; $P < 0.001$ ***, see Table 8).

Table 7 - Summary of encounter frequency, co-occurrences, EOD rates, and encounter durations, grouped by species, habitat and phase of the diel cycle.

Phase	Species	Habitat			Overall
		Open	Stream	Vegetation	
Encounters per 30 minutes					
Day	MV	0	0	0.4 ± 1.1	0.1 ± 0.7
	PD	0	0.1 ± 0.8	0.9 ± 2.7	0.4 ± 1.8
Night	MV	2.2 ± 4.2	0	1.8 ± 3.4	1.2 ± 3.1
	PD	0.3 ± 1.6	0.4 ± 1.5	1.8 ± 3.9	0.9 ± 2.8
Co-occurrence (% of encounters)[a)]					
Day	MV	-	-	2.0 ± 2.8	2.0 ± 2.8
	PD	-	9.3 ± 8.5	0.5 ± 0.6	2.4 ± 4.1
Night	MV	25.9 ± 14.5	-	9.9 ± 11.6	17.4 ± 10.9
	PD	7.4 ± 14.8	12.8 ± 9.2	9.5 ± 10.9	10.8 ± 8.4
EOD rate (Hz)					
Day	MV	-	-	10.3 ± 4.3	10.3 ± 4.3
	PD	-	16.3 ± 6.3	12.9 ± 6.9	13.2 ± 6.9
Night	MV	12.1 ± 6.6	-	10.4 ± 5.4	11.1 ± 6.0
	PD	16.4 ± 6.1	15.6 ± 4.9	12.0 ± 4.7	12.9 ± 5.1
Encounter duration (s)					
Day	MV	-	-	25.6 ± 17.6	25.6 ± 17.6
	PD	-	8.7 ± 7.6	16.5 ± 15.5	15.8 ± 15.1
Night	MV	13.5 ± 12.1	-	25.7 ± 16.9	20.4 ± 16.2
	PD	10.5 ± 9.3	10.5 ± 8.9	16.8 ± 10.9	15.2 ± 10.8

Hz, hertz; MV, *M. victoriae*; PD, *P. degeni*; s, seconds. EOD rates and durations were extracted from encounters with single fish. Values are represented as mean ± SD. [a)] Co-occurrences were defined as encounters during which more than one conspecific was present. The proportion of co-occurrences is shown as percentage of those encounters where at least one fish of the respective species was present.

Table 8 - Outcomes of statistical tests on encounter frequency and encounter characteristics.

ANOVA	**Phase**	**Effect**	**Species**	**F**	**DF**	**η^2_g**	**P**
EOD rate	Night	Habitat	PD	34.9	2, 588	0.11	**< 0.001**
Encounter duration	Night	Habitat	PD	16.1	2, 588	0.05	**< 0.001**
Tukey	**Phase**	**Comparison**	**Species**		**Diff.**		**P**
EOD rate	Night	Open - Stream	PD		-0.8		0.713
		Open - Vegetation	PD		-4.4		**< 0.001**
		Stream - Vegetation	PD		-3.7		**< 0.001**
Encounter duration	Night	Open - Stream	PD		0.0		1
		Open - Vegetation	PD		7.5		**0.015**
		Stream - Vegetation	PD		6.0		**< 0.001**
t-test	**Phase**	**Comparison**	**Species**	**t**	**DF**		**P**
EOD rate	Day	Stream - Vegetation	PD	3.2	45.9		**0.003**
	Night	Open - Vegetation	MV	3.7	578.6		**< 0.001**
Encounter duration	Day	Stream - Vegetation	PD	-3.9	59.2		**< 0.001**
	Night	Open - Vegetation	MV	-10.2	714.0		**< 0.001**
Encounters $30min^{-1}$	-	Day - Night	MV	-11.2	1184.3		**< 0.001**
	-	Day - Night	PD	-5.1	1808.3		**< 0.001**
U-test	**Phase**	**Comparison**	**Species**	**U**	**Diff.**		**P**
Co-occurrence	Day	MV - PD	-	13	0.000		0.774
	Night	MV - PD	-	23	0.059		0.485
	-	Day - Night	MV	2	-0.123		**0.021**
	-	Day - Night	PD	3	-0.053		**0.020**

DF, degrees of freedom; Diff., estimated difference between groups; min, minutes; MV, *M. victoriae*; PD, *P. degeni*; η^2_g, generalized eta squared for effect size. Significant outcomes of ANOVA was followed by Tukey post-hoc test with Bonferroni correction of P-values to identify significant differences between groups. Bold P-values indicate statistically significant outcomes.

3.3 Hypoxia Tolerance of *P. degeni*

To assess hypoxia-related morpho-physiological traits in *P. degeni*, I replicated and extended the normoxia acclimation study conducted by Clarke et al. (2020). I captured fish from their hypoxic habitats in Lwamunda Swamp and subjected them to long-term normoxia at the Nabugabo Research Site. I conducted intermittent-flow respirometry to measure RMR and closed respirometry to measure P_{crit} and RI, and quantified gill morphometrics and full-blood lactate and Hb concentrations at various time points of normoxia exposure ranging from 2 to 75 days. Additionally, I sampled blood from an independent group of fish that were exposed to normoxia without undergoing respirometry to control for an effect of respirometry experiments on blood Hb and lactate levels.

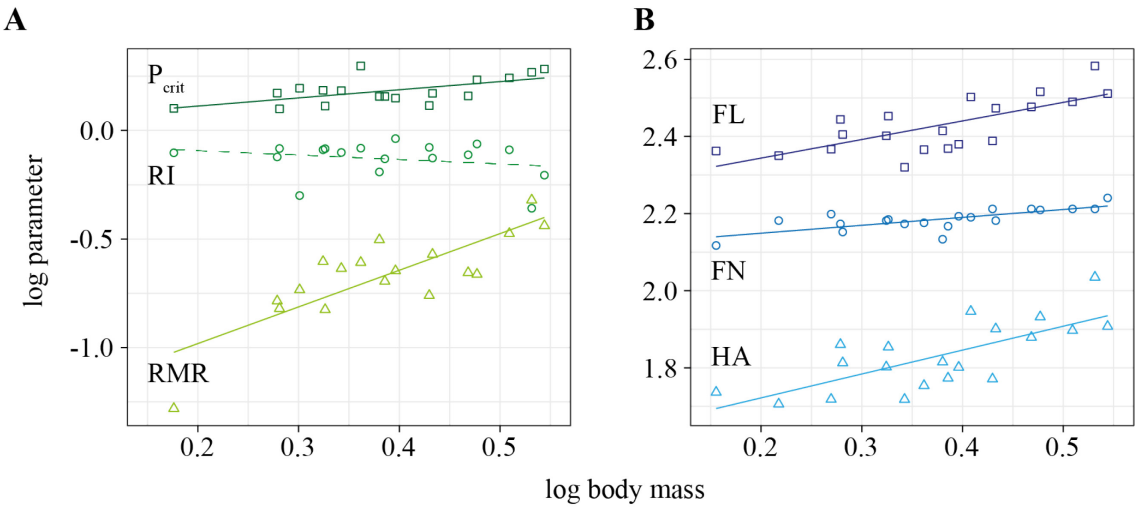


Figure 16 - Allometric relationships of respirometric traits and gill morphometrics. (A) Routine metabolic rate (RMR), critical oxygen tension (P_{crit}), and regulation index (RI) as function of body mass. (B) Gill filament length (FL), filament number (FN) and hemibranch area (HA) as function of body mass. All values have been log-transformed, solid lines indicate significant linear regressions ($P < 0.05$).

3.3.1 Respirometry

A total of 18 respirometry trials were analyzed. Across all three acclimation groups, RMR and P_{crit} scaled with body mass and were mass-adjusted to a mean body mass of 2.49 g. RI did not correlate with body mass (Table 4, Figure 16 A).

Normoxia acclimation had no significant effect on RMR, P_{crit}, and RI of *P. degeni* (Figure 17, Figure 18 A - C , Table 9, Table 10). Across all acclimation groups, mass-

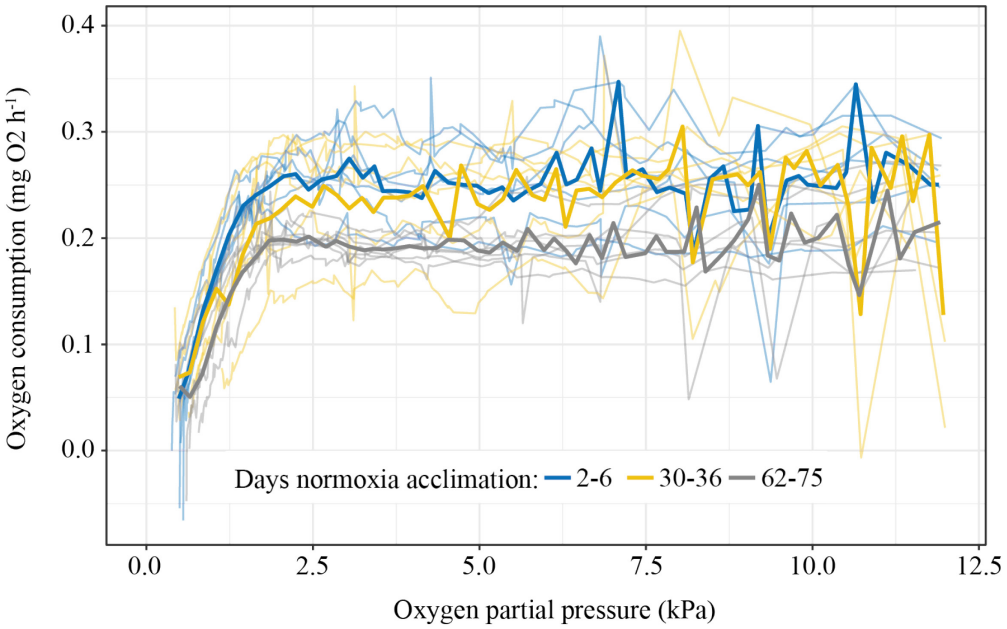


Figure 17 - Oxygen consumption rates during closed respirometry trials. Thin lines represent oxygen consumption of individual fish (n = 18), bold lines represent average oxygen consumption rates per acclimation group, averaged over intervals of 0.1 kPa. Extreme outliers with a distance of more than 3 times the interquartile range from the 1st and 3rd quartile were removed from this plot (n = 3 data points).

adjusted RMR averaged 0.23 ± 0.06 mg O_2 h^{-1}, mass-adjusted P_{crit} averaged 1.56 ± 0.20 kPa, and RI averaged 0.72 ± 0.12. Water temperature during trials ranged between 22.9°C and 24.9°C and averaged 24.0 ± 0.6°C.

3.3.2 Gill Morphometrics

Gill FL, HA, and FN of the first two arches of the left gill were measured on 20 fish. All metrics scaled with body mass across acclimation groups (Figure 16 B, Table 4) and were mass-adjusted to a mean body mass of 2.44 g. Mean mass-adjusted FL and HA were significantly reduced in fish after long-term (62-75 days) normoxia acclimation compared to fish in short-term (2-6 days) and mid-term (30-36 days) normoxia acclimation (Figure 18 D-E, Table 9, Table 10). Overall, FL decreased by 14% and HA decreased by 18% from short-term to long-term normoxia acclimation. The number of gill filaments remained unchanged during normoxia acclimation, with an adjusted mean of 154 filaments per fish (Figure 18 F).

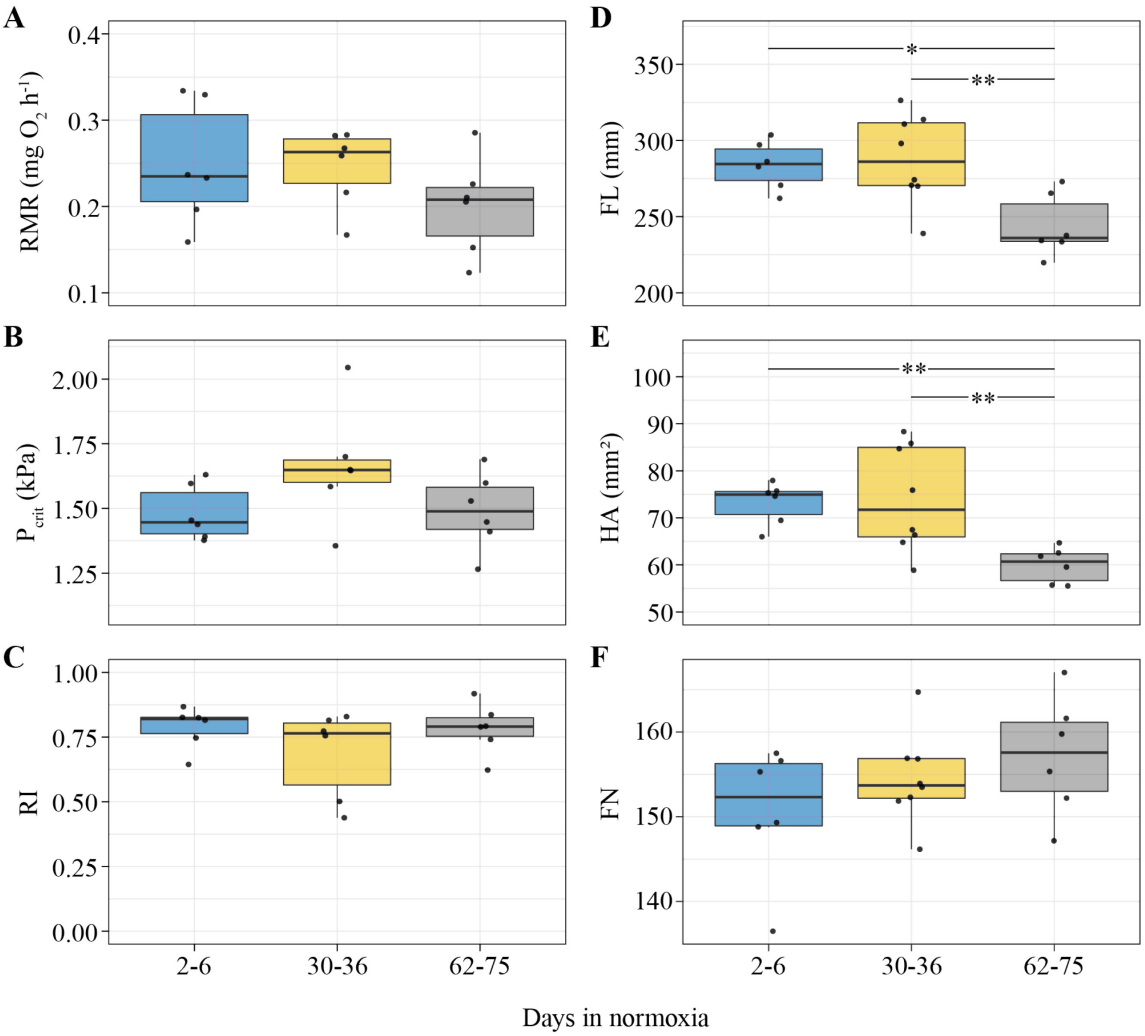


Figure 18 - Respirometry performance and gill morphometrics of *P. degeni* during normoxia acclimation. (A) Mass-adjusted RMR. (B) Mass-adjusted P_{crit}. (C) Regulation index. (D) Mass-adjusted gill FL. (E) Mass-adjusted gill HA. (F) Mass-adjusted FN. Gill morphometrics were measured on the first two arches of the left gill. Data were binned into three acclimation groups for comparison. See Table 10 for statistical test outcomes ($P < 0.05$*, $P < 0.01$**).

3.3.3 Blood Hemoglobin and Lactate Concentrations

Neither blood Hb nor lactate concentration scaled with body mass (Table 4). In fish that underwent undisturbed normoxia acclimation, mean Hb concentration was highest shortly after capture (10.30 ± 0.72 g dl^{-1}) and decreased significantly over the course of normoxia acclimation by 17%. In fish that were used in respirometry trials, Hb concentration showed a similar, but non-significant, trend and averaged 6.76 ± 2.25 g dl^{-1} across all acclimation groups (Figure 19 A, Table 9, Table 10).

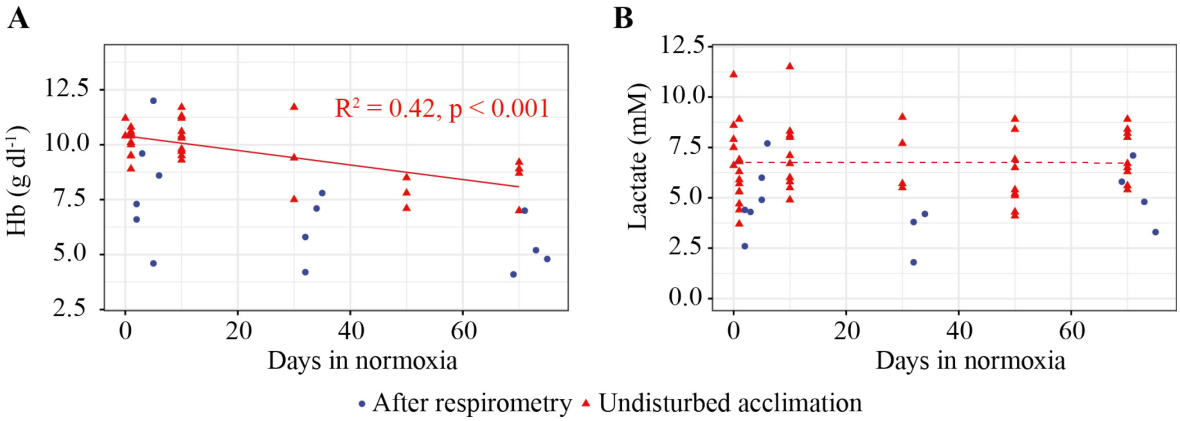


Figure 19 - Response of blood parameters to normoxia acclimation. (A) Full-blood hemoglobin (Hb) decreased significantly during normoxia exposure in fish that were left undisturbed. (B) Lactate concentration was not affected by normoxia exposure. Solid lines indicate significant linear regressions, dashed lines indicate non-significant linear regressions, red triangles indicate measurements from fish after undisturbed normoxia acclimation, blue circles indicate measurements from fish after respirometry.

Lactate was detectable in appreciable concentrations in all fish and showed no change over time (Figure 19 B, Table 9, Table 10). Lactate concentration averaged 6.58 ± 1.63 mM (undisturbed) and 4.67 ± 1.67 mM (after respirometry) across all acclimation groups.

3.3.4 Correlation Between Traits

All traits that were measured on the same individuals were tested for correlations using Pearson correlation test. There was a strong positive correlation between FL and HA ($r = 0.95$, $P < 0.001$), which was most likely due to the methodological relatedness of these two metrics, and a negative correlation between FN and RMR ($r = -0.64$, $P = 0.048$). The sample size of the complete set for all variables was small ($n = 10$), which was due to the poor success of blood sampling in some individuals. Thus, I re-ran the analysis on a subset of variables from respirometry and gill measurements (RMR, P_{crit}, RI, FL, HA and FN), which increased sample size ($n = 16$). However, re-analysis yielded the same correlations between FL and HA ($r = 0.97$, $P < 0.001$) and between FN and RMR ($r = -0.53$, $P = 0.034$).

Table 9 - Overview of biometric data and morpho-physiological traits of *P. degeni* measured in 2019.

	Acclimation group		
	Short-term	**Mid-term**	**Long-term**
Days acclimation	2-6	30-36	62-75
Fish n	6	9	7
Sex ratio	3:3	4:5	4:3
Mass (g)	2.4 (1.9-3.0)	2.4 (1.4-3.4)	2.4 (1.5-3.5)
Standard length (cm)	5.3 (4.9-5.9)	5.5 (4.7-6.2)	5.5 (4.8-6.4)
Respirometry			
RMR (mg O_2 h^{-1})	0.25 (0.16-0.33)	0.25 (0.17-0.28)	0.20 (0.12-0.29)
P_{crit} (kPa)	1.48 (1.38-1.63)	1.66 (1.36-2.04)	1.49 (1.27-1.69)
RI	0.79 (0.64-0.87)	0.69 (0.44-0.83)	0.78 (0.62-0.92)
Gills			
FL (mm)	284 (262-304)	288 (239-326)	244 (220-273)
HA (mm^2)	73 (66-78)	74 (59-88)	60 (56-65)
FN	151 (136-157)	155 (146-165)	157 (147-167)
Blood			
Hb (g dl^{-1})	8.1 (4.6-12.0)	6.2 (4.2-7.8)	5.3 (4.1-7.0)
Lactate (mM)	5.0 (2-6-7.7)	3.3 (1.8-4.2)	5.2 (3.3-7.1)

FL, gill filament length; FN, gill filament number; HA, hemibranch area; Hb, hemoglobin concentration; P_{crit}, critical oxygen tension; RI, regulation index; RMR, routine metabolic rate; Standard length, body length excluding caudal fin. Values are expressed as mean and range except for sample size n, which is expressed as absolute number, and sex ratio, which is expressed as female:male ratio. Note that sample sizes differ for each experiment: RMR, P_{crit} and RI were measured on 18 fish; FL, HA and FN on 20 fish; hemoglobin on 12 fish; and lactate on 11 fish.

Table 10 - Effect of normoxia acclimation on morpho-physiological traits.

ANOVA	Effect	Trait	F	DF	P	η^2_g
	Acclimation group	RMR	1.26	2, 15	0.313	0.144
		P_{crit}	2.29	2, 15	0.136	0.234
		RI	1.33	2, 15	0.295	0.150
		FL	**6.85**	**2, 17**	**0.007**	**0.446**
		FN	1.45	2, 17	0.262	0.146
		Hb[a)]	2.57	2, 11	0.121	0.318
		Lac[a)]	1.51	2, 10	0.267	0.232

Tukey	Comparison	Trait	n_1	n_2	Diff.	P
	short - medium	FL	6	8	4.1	0.944
	short - long	**FL**	**6**	**6**	**-39.8**	**0.024**
	medium - long	**FL**	**8**	**6**	**-43.9**	**0.008**

Kruskal-Wallis	Effect	Trait	H	DF	P
	Acclimation group	**HA**	**9.83**	**2**	**0.007**

Conover test	Comparison	Trait	n_1	n_2	t	P
	short - medium	HA	6	8	0.089	1
	short - long	**HA**	**6**	**6**	**3.654**	**0.003**
	medium - long	**HA**	**8**	**6**	**3.817**	**0.002**

Lin. regression	Independent variable	Trait	F	DF	P	R^2
	Days in normoxia	**Hb[b)]**	**21.77**	**1, 30**	**< 0.001**	**0.401**
		Lac[b)]	0.00	1, 47	0.961	-0.021

DF, degrees of freedom; FL, gill filament length; FN, gill filament number; HA, gill hemibranch area; Hb, hemoglobin concentration; Lac, lactate concentration; P_{crit}, critical oxygen tension; RI, regulation index; RMR, routine metabolic rate; η^2_g, generalized eta squared for effect size. Significant outcomes of ANOVA and Kruskal-Wallis were followed by Tukey's post-hoc test (ANOVA) or Wilcoxon test with Bonferroni correction of P-values (Kruskal-Wallis) to identify significant differences between groups. Bold numbers indicate statistically significant outcomes. [a)] blood measurements from fish after respirometry; [b)] blood measurements from fish after undisturbed normoxia acclimation.

4 Discussion

4.1 Summary

Tropical freshwater ecosystems are important resources for global biodiversity. Yet, comparably little is known about the behavior and ecology of tropical freshwater fish. In this study, I explore the activity patterns and habitat use of two species of mormyrid weakly electric fish, *M. victoriae* and *P. degeni*, and conduct a detailed investigation of the hypoxia adaptations of *P. degeni*.

My results show that the study species display the pronounced shifts in activity and habitat use in the laboratory that are stereotypical of nocturnal animals. In their swamp habitat, both species showed a clear nocturnal activity profile only in the open areas of Petro Lagoon, and remained active during the day in structurally complex, shaded habitats. This suggests that ambient light acts as an important environmental cue for mormyrid behavior, and that natural activity patterns of these fish might be more opportunistic than previously assumed. In-situ measurement of ambient DO revealed that the active phase of the fish coincided with extreme hypoxia in their swamp habitat. I characterized a variety of morpho-physiological hypoxia adaptations in *P. degeni*, which increase oxygen uptake and -carrying capacity and reduce metabolic energy demand, and likely enable the persistence of these fish in their habitat. Long-term normoxia exposure had only subtle effects on hypoxia-related traits of *P. degeni*, which might suggest that these fish are hypoxia specialists with only a limited capacity for phenotypic plasticity in adult fish.

4.2 Spatial and Temporal Patterns of Behavior

4.2.1 Fish Dispersal

In the laboratory, fish spent nearly 100% of the time in their shelters during the light phase and increased their behavioral activity with the onset of the dark phase, which resulted in higher swim speed and a change in their movement range from sheltering to active exploration of their environment. In the wild, fish likewise remained almost exclusively under floating vegetation during the day and moved into the open areas of the lagoon at night. Interestingly, *P. degeni* showed a stronger preference for open areas in the shuttle-box during the dark phase than *M. victoriae*, whereas in the wild, *M. victoriae* were encountered more often in the open area at night, and *P. degeni* seemed to prefer the vegetation habitat both during day and night. As the sheltered areas in the shuttle-box were limited to two tubes, it is possible that this was an effect of the overall higher swim activity of *P. degeni* compared to *M. victoriae*. The different activity levels of the two species could also help to explain the fish dispersion patterns at Petro Lagoon. Given that DO was overall low but highest in the stream habitat, it could have been expected that fish move into the stream at night to reduce hypoxic stress. Thus, it came as a surprise to find that only *P. degeni* were encountered here, and only on the first three sampling days. It is possible that their higher activity level makes *P. degeni* more vulnerable to hypoxia and/or better adapted to an environment with flowing water. The more sluggish *M. victoriae*, on the other hand, might be better adapted to stagnant environments. Another possible explanation for the lack of fish in the stream is that they had to cross a shallow section of the lagoon in order to reach it. Due to its low water depth, this section may have represented a movement barrier for the larger *M. victoriae* in the beginning of the sampling period. Over the sampling duration, water depth decreased slightly throughout the lagoon, which could have kept *P. degeni* from venturing into the stream on the later sampling days.

The temporal patterns of fish encounters and the transient increase of EOD rates under the vegetation during sunset and sunrise suggest that fish actively moved into the open and stream areas of the lagoon at night and returned to the floating vegetation around sunrise. This seems to align well with the activity peaks that were observed in the laboratory at the onset and towards the end of the dark phase. The question is, why is it that fish emerge at night and venture into unprotected areas? I suggest that fish use the open habitats to forage, interact, and carry out ASR at night at a lower risk of predation (e.g., Hammerschlag et al., 2010; Hobson, 1973; Nagelkerken et al., 2000; Saint-Paul and Soares, 1987; Sikkel et al., 2017). Although the low DO in the swamp protects fish from

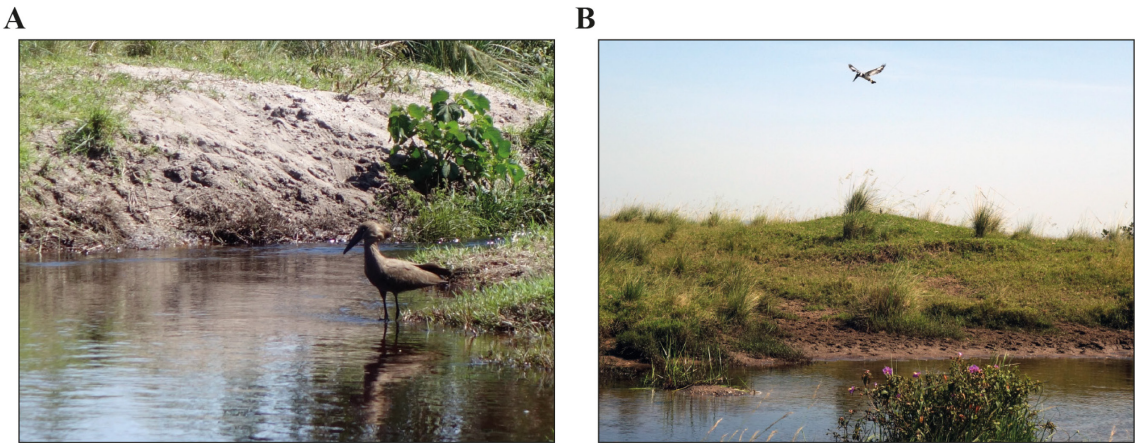


Figure 20 - Piscivorous birds that forage at Petro Lagoon during the day. (A) A hamerkop (S*cotus umbretta*) lurks for prey at a shallow area of the lagoon. (B) A pied kingfisher (*Ceryle rudis*) hovers over the open area of the lagoon.

large predators, such as the Nile perch (Chapman and Chapman, 1998; Chapman et al., 2002), there is some risk of predation during the daytime due to the presence of visually-oriented piscivorous birds, such as the hamerkop (*Scopus umbretta*) and the pied kingfisher (*Ceryle rudis*). These predators hunt predominantly in the open and shallow regions of the lagoon and turn them into dangerous territories for fish during the day (pers. observation, Figure 20). Open areas might be attractive feeding grounds, as the complete absence of mormyrids in the open and stream habitat during the day suggests that there is little diurnal foraging activity happening here. At night, fish can exploit the untapped resources at a lower risk of predation. The non-visual active electric sense of *M. victoriae* and *P. degeni* facilitates prey capture in the dark (Emde and Bleckmann, 1998; Emde, 1999), and gives them an edge over other visually-oriented nocturnal foraging species. Shorter encounter duration and higher EOD rates in the open and stream area suggest that the activity level of fish was higher in these habitats than in the vegetation, which aligns well with foraging behavior. Increased EOD rates increase the temporal acuity of electrosensory information, and can aid in the localization of prey and/or avoidance of predators. Similar nocturnal bouts of exploration and foraging activity has been described before in weakly electric fish (Friedman and Hopkins, 1996; Henninger et al., 2018; Henninger et al., 2020; Kruger, 1973; Lissmann and Schwassmann, 1965; Madhav et al., 2018; Moller et al., 1979). For example, Henninger and colleagues (2020) observed in a creek in Panama that three gymnotiform species, *Apteronotus rostratus*, *Eigenmannia humboldtii* and *Sternopygus dariensis*, move actively only during the night, traveling upstream after sunset and downstream in the second half of the night. In an early study in Brazil, Lissmann and Schwassmann (1965) demonstrated that sandfish (*Gymnorhamphychthys hypostomus*) rest, buried in the sand, during the day and emerge at night to forage and interact. In South Africa, Kruger (1973) found that *Marcusenius macrolepidotus* (formerly *Gnathonemus macrolepidotus*), a close relative to *M. victoriae*,

moves at night from bottom waters into the littoral zone, presumably to feed on chironomids. Moller and colleagues (1979) recorded mormyrids in the Swashi River in Nigeria, which hid during the day in a river inlet and moved actively in and out of the river at night. The behavior of *M. victoriae* and *P. degeni* described here matches these observations well.

There was an increase in total fish encounters towards the end of the sampling period, especially on the last sampling day. The last measurements were made just at the onset of the short rainy season, and there was a slight upwards trend in DO and temperature on the three last measurement days. The increase of encounters might be a result of higher behavioral activity driven by higher energy availability and temperature. Another intriguing possibility is that the increase of fish encounters was caused by seasonal breeding migrations. Some mormyrid species breed at the onset of the rainy season (Hopkins and Bass, 1981; Kirschbaum, 1995). Decreasing water conductivity, increasing water levels, and artificial rainfall on the surface all promote gonad growth in those species (e.g., Kirschbaum and Schugardt, 2002). Although not much is known about the natural breeding behavior of the study species, it is possible that the increase of encounters with *M. victoriae* and the high number of encounters with *P. degeni* on the last sampling day are a side-effect of breeding behavior. This would be an exciting finding, as it would mean that these fish reproduce, and possibly hatch and develop, under severely hypoxic conditions. A longer-term survey of the fish distribution in the Lake Nabugabo system during the rainy seasons, e.g., with electric fish loggers, should give better insights into this important aspect of the ecology of *P. degeni* and *M. victoriae*. I would expect to observe changes in territoriality patterns (Friedman and Hopkins, 1996; Zubizarreta et al., 2020) and increased sexual dimorphism in EOD shape and signaling behavior (e.g., Bass and Volman, 1987; Carlson, 2002; Hopkins and Bass, 1981) if fish were exhibiting breeding behavior.

4.2.2 Electric Activity

In the laboratory, EOD rate was closely correlated to swim activity in both species and showed clear diel rhythms with some species differences. *Petrocephalus degeni* emitted EODs at lower rates and more regular IPIs during their inactive phase and increased EOD rate at more irregular intervals during their active phase. *Marcusenius victoriae* also showed lower EOD rates during the inactive phase and higher EOD rates during the active phase. In contrast to *P. degeni*, however, IPIs did not become more regular during the inactive phase, and some *M. victoriae* showed greatly irregular IPIs during their inactive phase with pauses that exceeded 10 seconds and could reach up to >120 seconds. This finding demonstrates a fundamental difference in the electric signaling behavior of the two species: *P. degeni* generate bursts (high rate, low regularity)

whereas *M. victoriae* generate tonic SPI patterns (high rate, high regularity) when they are active, e.g., during exploration and foraging. Similar differences in EOD generation have been described by Carlson (2016) who found that *M. victoriae* respond to EOD playback with pauses of electric signaling whereas *P. degeni* tend to generate EOD bursts. Cessations of EOD generation have been associated with aggressive and submissive behavior in mormyrids (Carlson, 2002), traits that might be more typical of *M. victoriae* than *P. degeni* (Carlson, 2016). For solitary fish (as observed here), reducing EOD rate during their inactive phase likely reduces their detectability by electrosensory predators and/or aggressive conspecifics, while also reducing metabolic energy demand (Lewis et al., 2014).

Under the floating vegetation at Petro Lagoon, EOD rates of *M. victoriae* increased after sunset; however, this effect was much more transient than in the laboratory. The EOD rates of *P. degeni* were highest during the day and showed only a transient increase towards the end of the night. Overall, the clear pattern that was observed in the laboratory could not be reproduced in the field. One obvious difference between laboratory and field data was that I used normalized EOD rates in the analysis of laboratory data to control for inter-individual variability in baseline EOD rates. However, although this procedure increased the alignment of EOD rates on the y-axis, it did not affect the overall diel pattern of EOD rates (see Appendix, Figure A1). A variety of environmental factors may have influenced EOD activity patterns. First, the lower water temperatures likely reduced EOD rates during the night (e.g., Toerring and Serrier, 1978). Second, nocturnal hypoxia could have led to reduced EOD rates (Clarke et al., 2020; Moulton et al., 2020), if it was not compensated for, e.g., by ASR. Third, the availability of an extensive sheltered habitat might stimulate diurnal activity (e.g., exploration and foraging). This appears to be the case for *P. degeni,* which maintained a higher level of activity during the day under the floating vegetation than in the tubes of the shuttle-box. Another possible factor driving diurnal activity could be social behavior, with the group-living *P. degeni* exhibiting higher diurnal activity than the solitary *M. victoriae*, although this remains speculative.

4.2.3 Social Interaction

Unlike many other fish, which rely primarily on visual cues for social interaction (e.g., reviewed by Rowland, 1999), weakly electric fish can use their active electrosense to communicate and interact with conspecifics, and thus are well-equipped to do so at night (Carlson and Hopkins, 2004; Moller et al., 1989; Scheffel and Kramer, 1997).

The sampling design I used did not allow detailed observation of social interactions, and an in-depth analysis of EOD recordings from co-occurrences was beyond the scope of this study, which focused on the larger scale temporal and spatial dynamics of fish behavior. However, some conclusions can be drawn from the statistics of recorded co-

occurrences of fish. Here, it is important to consider the different detectability of the two species (Figure 6). While co-occurring *P. degeni* had to be within approximately 1 m of each other to be detected simultaneously in a logger recording, *M. victoriae* could be more than 3 m apart from each other and still be detected together. Thus, based on co-occurrences, the degree of social interaction is likely somewhat overestimated in *M. victoriae* (co-occurrence without interaction) and underestimated in *P. degeni* (detection of one fish out of a group). Notwithstanding these limitations, the fact that co-occurrences increased by 850% (*M. victoriae*) and 450% (*P. degeni*) strongly suggests that fish encountered and interacted with conspecifics more frequently at night.

Similar nocturnal bouts of social interaction have been found in phylogenetically distant gymnotiform weakly electric fish and could be a common characteristic of weakly electric fish (Henninger et al., 2018; Silva et al., 2007; Stoddard et al., 2007). *Marcusenius victoriae* has been described as a territorial species, which is solitary and displays high intraspecific competition for shelters (Carlson, 2016). Territoriality and competition for shelters might be most prominent during the daytime, when *M. victoriae* were exclusively located in the vegetation, and were alone in approximately 98% of those encounters. The increase of co-occurrences of *M. victoriae* during the night might indicate a higher degree of social affiliation and/or acceptance of conspecifics in their proximity. Interestingly, most co-occurrences of *M. victoriae* were recorded in the open habitat, which could suggest that they favor interacting in a more “neutral zone” than in the habitat where they occupy their shelters during the day. However, this could also be a side-effect of increased foraging and exploration activity in the open area. *Petrocephalus degeni* has been described as a gregarious species (Carlson, 2016) and can be kept in group tanks more easily than *M. victoriae* (pers. observation). There was no difference between the two species in the proportion of conspecific co-occurrences; however, given the considerable difference in detectability, this finding supports the notion that *P. degeni* occurs naturally in social groups.

During most encounters with multiple fish, both species were present. This implies that the two species occur commonly in close spatial proximity and likely are connected through interactions and/or competition for resources. More detailed behavioral observations, e.g., using an electrode grid (Henninger et al., 2018; Henninger et al., 2020; Madhav et al., 2018), could reveal interesting intra- and inter-specific interaction in these species.

4.2.4 Fish Detection with Electric Fish Loggers

Weakly electric fish occupy structurally complex habitats, thereby eluding traditional visual or acoustic underwater sampling methods. Previous studies have used electrode arrays (Henninger et al., 2018; Henninger et al., 2020; Madhav et al., 2018), or

single, handheld electrodes (Friedman and Hopkins, 1996; Lissmann and Schwassmann, 1965; Migliaro et al., 2018; Zubizarreta et al., 2020) to record activity of weakly electric fish in the wild. The methods that these studies employed necessitated the supervision of a researcher, which naturally limited the timespan over which observations were made. The present study introduces a new and unsupervised method to continuously record the EODs of weakly electric fish in the wild over longer time spans than previously possible. This approach is based on the deployment of autonomously operating logging devices. In comparison with the previously used electrode arrays, observations from individual loggers lack the spatial resolution that is necessary to track individual animals and document detailed behavioral interactions. Nevertheless, there are certain benefits to this approach. Distributing several individual loggers allowed me to sample from different habitats and cover a larger area than would be possible using an electrode array. The low price per unit (ca. 70 €) makes this technology affordable and reduces the financial risk of loss of equipment in the field. As the loggers are built using open-source technology, such as the Teensy and the Arduino programming language, they are accessible to a large community of scientists and nonscientists. These features make the electric fish logger a promising and open tool for studies, especially those that focus on longer-term temporal patterns (e.g., in fish dispersal), or are conducted in challenging environments that are difficult to access, such as swamps and lakes. The logger program can be adapted to a range of applications (e.g., for continuous or interval recordings at various sample rates and voltage resolutions; integration of environmental sensors, such as temperature and light sensors). Since conclusion of this thesis work, I developed a 2-channel differential amplifier for the electric fish logger, which makes it possible to record on up to eight channels per logger[24], and a first prototype of a logger-grid has been designed by Jan Benda and is currently being tested in the field[25].

[24] Teensy_Amp: https://github.com/muchaste/Teensy_Amp (accessed April 2022)

[25] TeeRec - A library for the Teensy for recording analog input data: https://github.com/janscience/TeeRec (accessed December 2021), and a prototype of a Teensy-based EOD grid: https://github.com/janscience/TeeGrid (accessed December 2021).

4.3 Environmental and Endogenous Rhythms

4.3.1 Influence of Light

In the laboratory, where temperature and DO were kept constant, behavioral activity of *M. victoriae* and *P. degeni* corresponded closely to ambient light levels: locomotor activity and EOD rate peaked precisely at the onset of darkness. Although activity level was not directly quantified in the field, the patterns of fish encounters corroborate this finding. In the open habitat where day-night changes in illumination are most pronounced, the number of fish encounters followed a clear diel pattern. In the vegetation habitat where light conditions remain mostly dark throughout the diel cycle, this pattern was less obvious, and *P. degeni* showed the same level of activity during the day and the night. Both observations suggest that light intensity is an important factor for behavioral activity; however, they are insufficient to disentangle the effects of endogenous clocks that are entrained by light and the direct effect of light as environmental cue on the behavior of *M. victoriae* and *P. degeni*.

There was no evidence that fish anticipated the dark phase, which could have served to suggest the existence of a circadian clock (Reebs, 1994). Two findings, however, could indicate the effect of an endogenous rhythm on behavioral activity in *P. degeni*. First, they showed an increase in swim speed and EOD rate towards the end of the dark phase, both in the laboratory and in the field. A similar phenomenon has been documented in *G. petersii* (Bässler et al., 1979). Second, their EOD rate at rest was significantly higher during the night than during the day. A similar increase in nocturnal EOD baseline rate has been found in the pulse-type gymnotiform *Gymnotus omarorum*, which lives under naturally constant darkness (Migliaro et al., 2018). Rhythms in baseline EOD rate have been hypothesized to be under endogenous control, although this has yet to be confirmed under laboratory conditions. In a study with the pulse-type gymnotiform *B. pinnicaudatus*, Stoddard and colleagues (2007) found circadian rhythms in electric signal parameters that are likely caused by melanocortin peptides, which act directly on electrocytes, and through serotonergic projections to neuronal pacemaker structures. However, for mormyrid species, nothing is known about their circadian clocks and the underlying neuronal and hormonal mechanisms. The presence of a circadian rhythm could be tested by subjecting fish to constant light and/or dark conditions for several days while assessing whether free-running behavioral rhythms can be detected (Aschoff, 1960).

4.3.2 Temperature and Dissolved Oxygen

Interestingly, the nocturnal increase of activity and movement range co-occurred with extreme nocturnal hypoxia at Petro Lagoon. In line with previous studies, which have consistently found both species in this site since the 1990s under hypoxic conditions (Carlson, 2016; Chapman et al., 1996b; Chapman and Chapman, 1998; Chapman et al., 2002; Clarke et al., 2020; Moulton et al., 2020), the continuous in-situ measurements of EODs, DO and temperature presented here show for the first time that these fish remain present, and likely are most active, during the most extreme nocturnal hypoxia periods. It is likely that, in addition to their morpho-physiological adaptations (e.g., see sections 1.3.31.5.3.1 and 3.3), fish compensate for the nighttime hypoxia by using ASR, during which they ventilate the well-oxygenated surface water layer (Chapman and Chapman, 1998). Since ASR requires that fish have free access to the water surface, fish have to emerge from the swimming vegetation to perform ASR. This might drive the nocturnal migration of both species into the open habitats. At night, ASR could be particularly well-suited to increase oxygen supply as the absence of diurnal predators reduces the otherwise high risk of predation (Kramer et al., 1983). A similar behavior has been identified in Amazonian fish that emerge from their macrophyte cover to perform ASR during periods of severe nocturnal hypoxia (Saint-Paul and Soares, 1987).

The diel fluctuation of DO and temperature could entrain endogenous circadian rhythms, and therefore indirectly affect behavioral activity. Temperature cycles are among the most common entraining cues in nature, and the effect of temperature as a synchronizer has been well established in vertebrates (Sweeney and Hastings, 1960). Many fish are particularly susceptible to temperature changes because they are poikilotherm, i.e., their body temperature is directly dependent on environmental temperature. Zebrafish have emerged as a model for studying the circadian clock in fish (Frøland Steindal and Whitmore, 2019; Idda et al., 2012; Vatine et al., 2011), and it has been shown that diel temperature variations, such as those measured at Petro Lagoon, would be sufficient to entrain the circadian clock in zebrafish (Lahiri et al., 2005; López-Olmeda and Sánchez-Vázquez, 2009). More recently, the interconnection between the circadian clock and the hypoxia-signaling pathway has been established in mammals (e.g., Adamovich et al., 2017) and zebrafish (Egg et al., 2013; Pelster and Egg, 2018; Sandbichler et al., 2018). Hypoxia-inducible factor 1α (HIF-1α) is a key regulator of the physiological hypoxia response. It dimerizes with HIF-1β under hypoxic conditions and regulates the transcription of genes by binding to hypoxia responsive elements (Schödel et al., 2011; Wenger et al., 2005). The expression of HIF-1α is tightly controlled by circadian clock genes and follows a circadian rhythm (Egg et al., 2013). On the other hand, hypoxic conditions lead to decreased expression levels of clock genes such as

period1, which play an important role in the TTFL[26] clock mechanism (Egg et al., 2013; Sandbichler et al., 2018). Adamovic and colleagues (2017) suggest that, through HIF-1α mediated suppression of the TTFL, hypoxia can serve as a resetting cue for circadian clocks. Oxygen availability and TTFL are also interconnected through cellular metabolism. In zebrafish cells, hypoxia shifts peak glycolytic activity to the night and aligns oscillations of various redox systems. This results in a highly oxidized cellular redox state at night, which reduces the binding affinity of the core clock transcription factor Clock/Bmal1 and reduces the expression of core clock genes (Sandbichler et al., 2018).

To what extent temperature and DO act as zeitgeber and/or directly influence the activity of the study species (e.g., through metabolic effects) remains to be elucidated. It is possible that the expression of clock genes is constantly dampened by hypoxia. Likewise, these fish may have evolved compensation mechanisms to protect the TTFL clock from hypoxia. The mormyrids of the Lake Nabugabo system could present intriguing opportunities to study these issues in the wild, as there are populations of fish that appear to live under constantly normoxic conditions in the lake and others that experience chronic hypoxia in the swamp.

[26] TTFL: Transcription-translation feedback loop, see section 1.2.1

4.4 Hypoxia Adaptations of Swamp-Dwelling *P. degeni*

The in-situ recordings of EODs and DO revealed that the mormyrids in Petro Lagoon experience chronic and severe hypoxic conditions, which they must compensate for in order to maintain their activity. This is an intriguing finding because, although both species express traits that are typical for hypoxia tolerant fish (such as a large gill surface, low oxygen consumption rates and low P_{crit}; Chapman and Chapman, 1998; Clarke et al., 2020; Moulton et al., 2020), the degree of nocturnal hypoxia found here far exceeded previously published P_{crit} thresholds of both species. In the study site, diel air saturation dropped as low as 2.7 ± 1.4% (ca. 0.6 ± 0.3 kPa, on August 8, 2019), which is well below any P_{crit} measured in swamp-dwelling *P. degeni* (this study: 1.6 ± 0.2 kPa; Chapman and Chapman, 1998: ca. 1.2 kPa; Clarke et al., 2020: ca. 1.5 kPa) and *M. victoriae* (Moulton et al., 2020: ca. 1.9 kPa). In fact, mean PO_2 at this site remained below the lowest published P_{crit} for more than 50% of the diel cycle on three of the six sampling days. Ackerly et al. (2018) showed that a PO_2 of ca. 3.6 kPa already negatively affected the critical swim speed and EOD rate of *M. victoriae*. Temperature decreased during the night at Petro Lagoon, which could reduce the rate of metabolic processes and somewhat buffer hypoxic stress. However, it seems unlikely that this effect would suffice to compensate for extremely low nocturnal DO, which suggests that environmental hypoxia significantly limited the performance of both species, particularly during their most active phase. Most likely, fish engage in ASR to increase their oxygen uptake during the night. However, during the day, fish were almost exclusively detected under the floating vegetation where they have no access to the water surface. This raises the question as to how these fish manage to maintain their levels of electrical and locomotor activity during extreme hypoxia.

To learn more about the hypoxia tolerance of *P. degeni* and the plasticity thereof, I replicated and extended a normoxia acclimation study conducted by Clarke and colleagues (2020). I measured respiratory traits (RMR, P_{crit}, RI), gill morphometrics (FL, FN, HA), and blood parameters (Hb and lactate concentration) of *P. degeni* while subjecting the fish to up to 75 days of normoxia acclimation upon capture in the hypoxic swamp.

4.4.1 Respiratory Traits of Swamp-Dwelling Fish

Blood Hb concentration was high in fish that were sampled within one day of capture (10.08 ± 0.61 g dl^{-1}). This concentration lies above 69 % of values reported from 69 teleost species across marine and freshwater habitats (Figure 21; Almeida et al., 2017

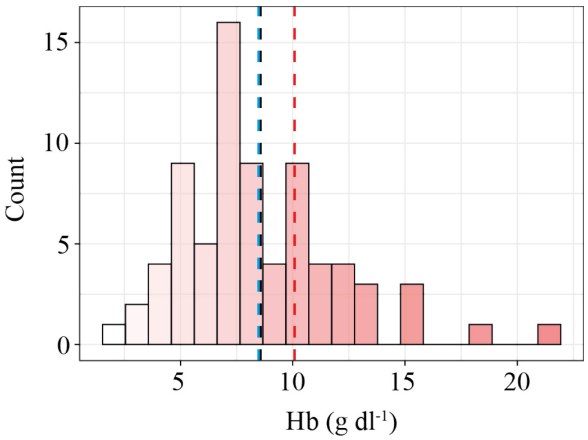


Figure 21 - Histogram of blood hemoglobin concentrations of 69 teleost species. Colored dashed lines represent average Hb values of *P. degeni* directly after capture (red) and after 70 days of normoxia acclimation (blue). The black dashed line represents the overall average Hb of all 69 species (data were compiled from Almeida et al., 2017; Chapman et al., 2002; Cook et al., 2011; Cook et al., 2013; Filho et al., 1992; Prosser et al., 1957; Timmerman and Chapman, 2004; Wells et al., 2005).

Chapman et al., 2002; Cook et al., 2011; Cook et al., 2013; Filho et al., 1992; Prosser et al., 1957; Timmerman and Chapman, 2004; Wells et al., 2005). This high Hb concentration supports aerobic metabolism by enhancing the oxygen-carrying capacity and affinity of blood (Brauner and Val, 2006). The value reported here is congruent with earlier measurements of blood Hb in swamp-dwelling *P. degeni*, which also yielded comparably high concentrations but were preliminary due to their low sample size (9.52 ± 0.27 g dl^{-1}, n = 2; Chapman et al., 2002).

On the oxygen-consumption side of their energy balance, *P. degeni* exhibited low RMR compared to the range of metabolic rates found in teleost species, which indicates a low tissue energy demand (Chapman and Chapman, 1998). Furthermore, fish exhibited a high oxyregulatory capacity and a P_{crit} that is amongst the lowest reported P_{crit} values for freshwater fish from a similar temperature range (the second lowest of 73 species, data from supplementary material of Rogers et al., 2016). Comparable values of RMR, P_{crit} and RI have been reported by earlier studies (Chapman and Chapman, 1998; Clarke et al., 2020). This extraordinary oxyregulatory capacity (i.e., a flat $\dot{M}O_2$ vs. PO_2 curve that saturates at low PO_2) in combination with the low RMR is not necessarily beneficial as it implies that swamp-dwelling *P. degeni* are relatively fixed to a low oxygen consumption, even when environmental PO_2 would allow for a higher oxygen consumption (see also section 4.4.3). However, in an environment where PO_2 is chronically low, such as Petro Lagoon, the benefits of low oxygen demand and high oxyregulatory capacity possibly outweigh this drawback. It is likely that the morpho-physiological adaptations characterized here are complemented by behavioral adaptations, such as increased gill ventilation rate and ASR, which serve to flexibly increase oxygen uptake and alleviate nocturnal hypoxia to some extent (Chapman and Chapman, 1998). The finding of high blood lactate concentration might suggest that anaerobic metabolism, likewise, plays a role in the hypoxia tolerance of *P. degeni*.

4.4.2 Blood Lactate

When tissue oxygen demand exceeds circulatory oxygen supply, ATP is increasingly supplied by anaerobic processes such as glycolysis yielding lactic acid, and substrate phosphorylation (anaerobiosis, Richards, 2009). I found high and sustained concentrations of lactate in the blood of *P. degeni* across all acclimation groups (6.6 ± 1.6 mM). Plasma lactate concentrations of the same order have been reported from fish that experience physiological stress as would occur under acute hypoxia exposure (e.g., Chippari-Gomes et al., 2005; Cook and Herbert, 2012; Maxime et al., 2000; Regan et al., 2017; Routley et al., 2002) or strenuous exercise and capture stress (e.g., Behrens and Steffensen, 2007; Frisch and Anderson, 2005), whereas values from normoxic or unstressed fish typically range between 0-2 mM (e.g., Chippari-Gomes et al., 2005; Regan et al., 2017; Virani and Rees, 2000; Wells and Baldwin, 2006). I took care to minimize handling stress prior to blood sampling, and to collect samples within 5-10 minutes of initial handling. In some fish, such as several trout species, this time span is already sufficient to increase plasma lactate concentration (Frisch and Anderson, 2005; Pankhurst and Dedualj, 1994), whereas in others, such as tropical labrids (*Hemigymnus melapterus*), this is not the case (Grutter and Pankhurst, 2000). The dynamics of the physiological stress response of *P. degeni* to capture and captivity have not yet been investigated. Thus, I cannot exclude that acute handling stress affected blood lactate levels. The comparably high blood lactate concentration that I measured in fish immediately after capture from their habitat (8.3 ± 1.8 mM) suggests either a rapid onset of the physiological lactate response to stress or that these fish indeed have elevated baseline lactate levels (or, possibly, both). It is conceivable that *P. degeni* produce lactate during severe hypoxia through anaerobiosis, and potentially even utilize it to mobilize metabolic energy. In mammals, lactate can act as a mobile fuel that is released from tissue that operates under hypoxic conditions (e.g., skeletal muscle) and is taken up by other cells to be oxidized (e.g., in heart and brain) or used as substrate for glycogen synthesis (Brooks, 1985; Gladden, 2004). Additionally, lactate production can stimulate glycolysis at low tissue PO_2 in mammals (Connett et al., 1990) and is involved in the regulation of different physiological processes (e.g., beta-oxidation of long-chain fatty acids, Baumgart et al., 1996; McClelland et al., 2003; upregulation of collagen deposition and angiogenesis during wound healing, Green and Goldberg, 1964; Trabold et al., 2003). In fish, lactate has been primarily associated with negative physiological states (e.g., stress, metabolic acidosis, low energy availability), however, there is some evidence for the utilization of lactate as an energy source in brains of rainbow trout (Polakof and Soengas, 2008b) and zebrafish that are exposed to acute hypothermia (Tseng et al., 2014). The finding of a static pool of lactate in the blood of *P. degeni* throughout normoxia acclimation says little about lactate production and utilization, and the confounding effect of handling and captivity stress might already suffice to explain this phenomenon.

However, given the environmental conditions under which these fish persist, it would be worthwhile to explore this topic further in *P. degeni*. A closer look into the dynamics of the physiological stress response and the turnover rates of lactate would be a reasonable first step to assess the role that lactate plays in the metabolism of these fish. Injections with a tracer, such as ^{14}C-labeled lactate, could reveal rates of appearance and disappearance in the circulation (e.g., Omlin and Weber, 2010; Omlin et al., 2014). However, this might be difficult to achieve due to the small body size of *P. degeni*, which makes cannulation with a catheter practically impossible. Other studies have measured lactate concentration and activity of relevant enzymes, such as lactate dehydrogenase and enzymes involved in glycolysis, to assess lactate metabolism *in vivo* and *in vitro* (e.g., Polakof and Soengas, 2008a; 2008b). Similar measurements could be conducted using energy-intense tissue, such as the brain and electric organ of *P. degeni*.

4.4.3 Plasticity of Respiratory Traits

Exposure to long-term normoxia only affected a subset of oxygen-related traits. Normoxia acclimation led to a significant decrease of blood Hb concentration (-17%), FL (-14%), and HA (-18%, as estimated from the first two gill arches) after 62-75 days, whereas RMR, P_{crit}, and RI appeared unaffected by normoxia acclimation, and lactate concentrations remained high throughout normoxia exposure. It is possible that captivity and laboratory housing had a confounding effect on the plastic response of the fish to normoxia acclimation. Due to the field background of this study, it was not possible to control for this effect, e.g., with a control group of fish housed under hypoxic conditions. Thus, the interpretation of these results is somewhat limited as to whether they were driven by normoxia exposure or by other effects of laboratory housing. The overall direction of the phenotypic shift, however, strongly suggests that it represented primarily a response to normoxic conditions. Reduced concentration of Hb reduces the oxygen-carrying capacity of blood and, if it coincides with a lower hematocrit and red blood cell count, can reduce the energetic cost for cardiac output (Gallaugher et al., 1995) and red blood cell maintenance (Wells and Baldwin, 2006). The reduction of FL and HA likely correlated with a decrease of respiratory gill surface area (Crispo and Chapman, 2010; Palzenberger and Pohla, 1992), which reduces the oxygen-extraction capacity and benefits the retention of ions in the body. Given that swamp-dwelling *P. degeni* live in an extremely ion-poor environment, the costs for osmoregulation are expected to be high for this species[27]. To my knowledge, only one other study has characterized phenotypic

[27] However, some fish that are exceptionally hypoxia tolerant or that live in ion poor water can actively decouple ion and water fluxes from oxygen uptake during hypoxia and thus somewhat alleviate the osmorespiratory compromise without large morphological changes (Somo et al., 2020; Wood and Eom,

plasticity of gill FL in adult fish to date. In a hypoxia acclimation study with killifish (*Fundulus heteroclitus*), Borowiec and colleagues (2015) noted a decrease of FL after 7-28 days of constant hypoxia (2 kPa O_2). This opposite effect of DO on gill morphology might serve to reduce osmoregulatory costs during hypoxia. This example illustrates again how different species may employ distinct respiratory strategies to cope with hypoxia. In summary, the phenotypic changes observed in *P. degeni* most likely result in reduced energetic cost for homeostasis in a high-DO environment at the expense of a reduced capacity for oxygen extraction and -carrying. To better evaluate the role of the osmorespiratory compromise in *P. degeni*, it would be interesting for future studies to investigate diffusive water fluxes, oxygen uptake rate, and changes in ion transporter density in the gills of *P. degeni* during normoxia acclimation.

An additional benefit of reduced gill surface area and Hb concentration might lie in the reduction of cellular PO_2, which in turn might dampen the generation of harmful reactive oxygen species. Especially fish that are hypoxia tolerant and have low oxygen consumption rates appear to exhibit low antioxidative capacities and might be vulnerable to reactive oxygen species at high ambient DO (Lushchak et al., 2001; Lushchak and Bagnyukova, 2006). Given that the reduction of gill surface occurred only after long-term normoxia acclimation, this effect did not protect *P. degeni* from acute oxidative stress during the first weeks of normoxia exposure, which might be buffered by the upregulation of antioxidant enzymes on the cellular level.

The finding that P_{crit} remained unaffected by normoxia exposure was unexpected, for two reasons in particular: (i) Clarke and colleagues (2020) found a small but significant increase of P_{crit} (from 1.46 ± 0.35 to 1.98 ± 0.30 kPa) after comparable long-term normoxia exposure of *P. degeni*, and (ii) because previous studies have linked P_{crit} to the oxygen-extraction and -carrying capacity: two parameters that were most likely affected by the observed shift in gill size and blood Hb (Childress and Seibel, 1998; Cook et al., 2011; Mandic et al., 2009). It is possible that I was unable to reproduce the results of Clarke and colleagues (2020) because I used a smaller sample size in respirometry trials (18 versus 28 fish). Moreover, the fish used by Clarke et al. (2020) were almost twice as heavy as the fish used here (4.6 g versus 2.5 g mean body mass). Although this did not seem to have affected overall P_{crit} estimates, it is possible that the effect of normoxia acclimation is more pronounced in larger fish. It is difficult to explain why neither reduced gill size nor reduced blood Hb concentration seemed to affect P_{crit}. Possibly, these changes were compensated for by concomitant changes in traits that were not quantified here, such as increased gill ventilation rate or increased Hb-O_2 binding affinity (Mandic et al., 2009).

2021). The mechanisms by which respiration and ion-exchange are decoupled involve dynamic changes in blood flow pathways and remodeling of the gill surface (Wood and Eom, 2021).

Altogether, the phenotypical shift observed here was surprisingly small, and was manifested only after long-term normoxia exposure. This shows that, on a baseline level, swamp-dwelling *P. degeni* retain a high degree of hypoxia tolerance, which appears to be a suitable adaptation to a chronically hypoxic habitat such as Lwamunda Swamp. On the other hand, being restricted to low oxygen consumption rates could be disadvantageous in high DO environments, as it might unnecessarily limit the amount of energy that is available for metabolic processes.

At the nearby lakes (Nabugabo, Kayanja), *P. degeni* live in normoxic conditions, and it has not been established yet to what extent the hypoxic wetland acts as a physiological barrier between these populations. An earlier study noted that the total gill FL of *P. degeni* from the normoxic waters of Lake Kayanja was approximately 21% shorter than that of conspecifics from the hypoxic Lwamunda Swamp (Chapman and Hulen, 2001). The findings presented here, show that this difference could lie within the capacity of *P. degeni* for phenotypic plasticity of their gill morphology. Further comparative analyses, e.g., of the respiratory traits of *P. degeni* from normoxic lakes and hypoxic swamp habitats in the Nabugabo system, could yield valuable insights into how fish adapt to these different environments, and which role phenotypic plasticity and heritable change play in this.

4.4.4 Comparison of Respirometry Studies

This thesis is the third study to conduct respirometry with *P. degeni* from Lwamunda Swamp. Chapman and Chapman (1998) used a closed respirometer to measure oxygen consumption and P_{crit}. Clarke and colleagues (2020) used an intermittent-flow and closed respirometry protocol similar to the one used in this thesis, however, with different components and a larger total volume. Metabolic rate estimates are low across studies, but they vary from the lowest to the highest value by a factor of >2 (Table 11). This variation might be due to differences in experimental setups and protocols. However, there is no clear pattern, as both the highest and the lowest metabolic rate were determined in a similar fashion from $\dot{M}O_2$ values measured during the closed part of respirometry trials (Chapman and Chapman, 1998; Clarke et al., 2020). Critical oxygen tension and RI estimates, on the other hand, are remarkably consistent across studies. All three studies used BSR for estimating P_{crit} (see methods section 2.3.2.2). Overall, this might indicate that P_{crit} and RI are less sensitive than RMR to variation in study design. All three studies are congruent in their general conclusion that swamp-dwelling *P. degeni* are remarkably hypoxia tolerant and show a high capacity for oxyregulation.

Despite the similarities in P_{crit} estimates, I was not able to reproduce the small shift of P_{crit} that Clarke and colleagues (2020) found. This effect might have been too subtle to surpass the inter-individual variation of P_{crit} values. Considering more significant changes

of P_{crit} that are found in other species (e.g., in killifish where 7 days of hypoxia exposure led to a reduction of P_{crit} by almost 50%, Borowiec et al., 2015), the P_{crit} of *P. degeni* seems relatively fixed to a low PO_2.

Table 11 - Overview of respirometry data from studies with *P. degeni*.

	Chapman & Chapman 1998	Clarke et al. 2020	This study
Fish n	9	28	18
Mean mass (g)	3.1	4.6	2.5
Metabolic rate (mg O_2 h^{-1} g^{-1})	0.13	0.05-0.06	0.09
P_{crit} (kPa)	1.21	1.46	1.56
RI	-	0.71	0.72

P_{crit}, critical oxygen tension; RI, regulation index. Only mean values are shown except for sample size n, which is expressed as absolute number. Metabolic rate was converted to mass-specific rate by dividing the mass-corrected rate that was reported for a fish of average body mass by the mean body mass to account for differences in fish weight across studies. Critical oxygen tension values from Clarke et al. were taken from short-term normoxia acclimated fish, as P_{crit} in the long-term normoxic fish was significantly elevated.

4.5 Conclusion and Outlook

Understanding how animals use their habitat, and how they respond to changes in their environment is crucial for explaining ecosystem dynamics and evolutionary patterns of biodiversity. This thesis establishes a new method for recording electric fish in the field and documents the behavioral patterns and respiratory adaptations of mormyrid fish that persist in a habitat with extreme nocturnal hypoxia. The key findings of this thesis are: (i) *M. victoriae* and *P. degeni* prefer structurally complex habitats during the day and increase their movement range, and possibly social interaction at night, despite severe nocturnal hypoxia; (ii) under conditions of constant DO and temperature, light is an important cue for the activity of the study species; the role that endogenous rhythms play for regulation of behavior remains to be determined; (iii) activity patterns in the wild can differ from the clear nocturnal patterns observed in the lab; fish showed higher levels of diurnal activity in a structurally complex and dark habitat; (iv) respiratory adaptations such as strong oxyregulation, low metabolic rate and high blood Hb concentration form the physiological basis for the hypoxia tolerance of *P. degeni*, which is likely further enhanced by behavioral and anaerobic hypoxia adaptations; (v) swamp-dwelling *P. degeni* show a plastic response to long-term normoxia exposure in some respiratory traits (blood Hb concentration and gill morphology), but not in others (RMR, P_{crit}, RI and blood lactate); whether *P. degeni* lack the capacity for a larger phenotypic change or whether the maintenance of some hypoxia adaptations is energetically advantageous, even under normoxic conditions, remains to be determined. Overall, these findings paint a picture of the life of these two species that is coined by diel and habitat specific patterns of behavior and a remarkable capacity to cope with hypoxic conditions (Figure 22). The investigation of morpho-physiological respiratory traits of *P. degeni* suggests that these fish from Lwamunda Swamp are well adapted, and perhaps even irreversibly specialized, for a life under chronically hypoxic conditions.

Major parts of this work were conducted either directly in the habitat of the study species or at a close-by field research station. This made it possible to directly relate the experimental findings to field observations (e.g., P_{crit} and environmental DO at Petro Lagoon), a gap that is not easily bridged in hypoxia-related studies (e.g., Pollock et al., 2007). The imbalance between the wealth of laboratory data and the lack of field data is a bottleneck for the integration of laboratory findings into ecological studies that needs to be addressed in the future. The electric fish logger introduced here is a valuable addition to the repertoire of methods for tracking animals in the wild and can contribute to reducing the data imbalance. Electric fish are widespread throughout tropical and neotropical freshwater systems, and the potential applications for electric fish loggers are manifold. From detailed observations in one habitat, such as the one conducted here, to large-scale surveys across habitats, they can be used to reveal behavioral patterns, habitat

preferences, and species distribution. In combination with on-site sampling of fish (e.g., through gill netting), detailed insights into assemblages of electric fish are possible. More surveys of hypoxic habitats could, for example, bring new impulses into the debate about whether weakly electric fish are more or less hypoxia tolerant than other fish (see section 1.4.4). Another obvious application would be to follow up on this thesis work with a more extensive deployment of electric fish loggers over the Lake Nabugabo region. It is known that mormyrids inhabit the major lakes of this region (Chapman et al., 2002; Ogutu-Ohwayo, 1993). However, their behavioral patterns and distribution are extremely difficult to assess with supervised methods, and so far only point measurements exist from these habitats (e.g., Moulton, 2016). The presence of mormyrids in many other, smaller water bodies has not been assessed at all, and it remains unknown whether the hypoxic wetland isolates normoxic lake fish from hypoxic swamp dwellers. A larger-scale survey of mormyrid activity in this region has great potential to produce a wealth of field data about the ecology of these elusive fish, which can also serve as inspiration for new research projects.

The field setting of the experimental work for thesis also introduced some limitations, and a number of findings are calling for further study. For example, the high blood lactate concentrations in *P. degeni*, even after long-term normoxia, are difficult to interpret due to the lack of a hypoxic control group. A follow-up study with laboratory-housed *P. degeni* would be relatively straightforward to conduct and would give a better insight into the role of lactate in the metabolism of these extraordinarily hypoxia tolerant fish. Another open question is how *M. victoriae* and *P. degeni* maintain their activity during extremely hypoxic conditions, and how these conditions might affect other crucial aspects of their ecology, such as growth rates and reproduction. This could be investigated in a follow-up field study conducted during the rainy season, when these species are expected to breed. Finally, the question of how endogenous and environmental cues affect behavioral patterns of mormyrids warrants more research. The common assumption that these fish are nocturnal seems overly simplistic given the great diversity of mormyrid fish, their behaviors, and the environments they inhabit. The results of this thesis show that, while some behavioral traits might follow nocturnal patterns (e.g., presence in unprotected habitats), others might be much more opportunistic (e.g., diurnal activity in dark habitats).

In the light of increasing human interferences in aquatic ecosystems, the research of natural behavioral patterns and phenotypic flexibility of fish will not lose its relevance in the future. Both phenomena are indicative of the interaction between animals and their environment, and of their resilience towards environmental shifts (McBryan et al., 2013). The increasing prevalence of hypoxia due to global warming and the ongoing eutrophication of water bodies will make aquatic habitats even more challenging environments for most fish in the future (Diaz and Rosenberg, 2008; 2008; Stramma et al., 2010; Vaquer-Sunyer and Duarte, 2008). An understanding of the biological

repertoire that animals have at their disposal to adjust to these changes is the necessary foundation on which ecological consequences of environmental degradation can be assessed.

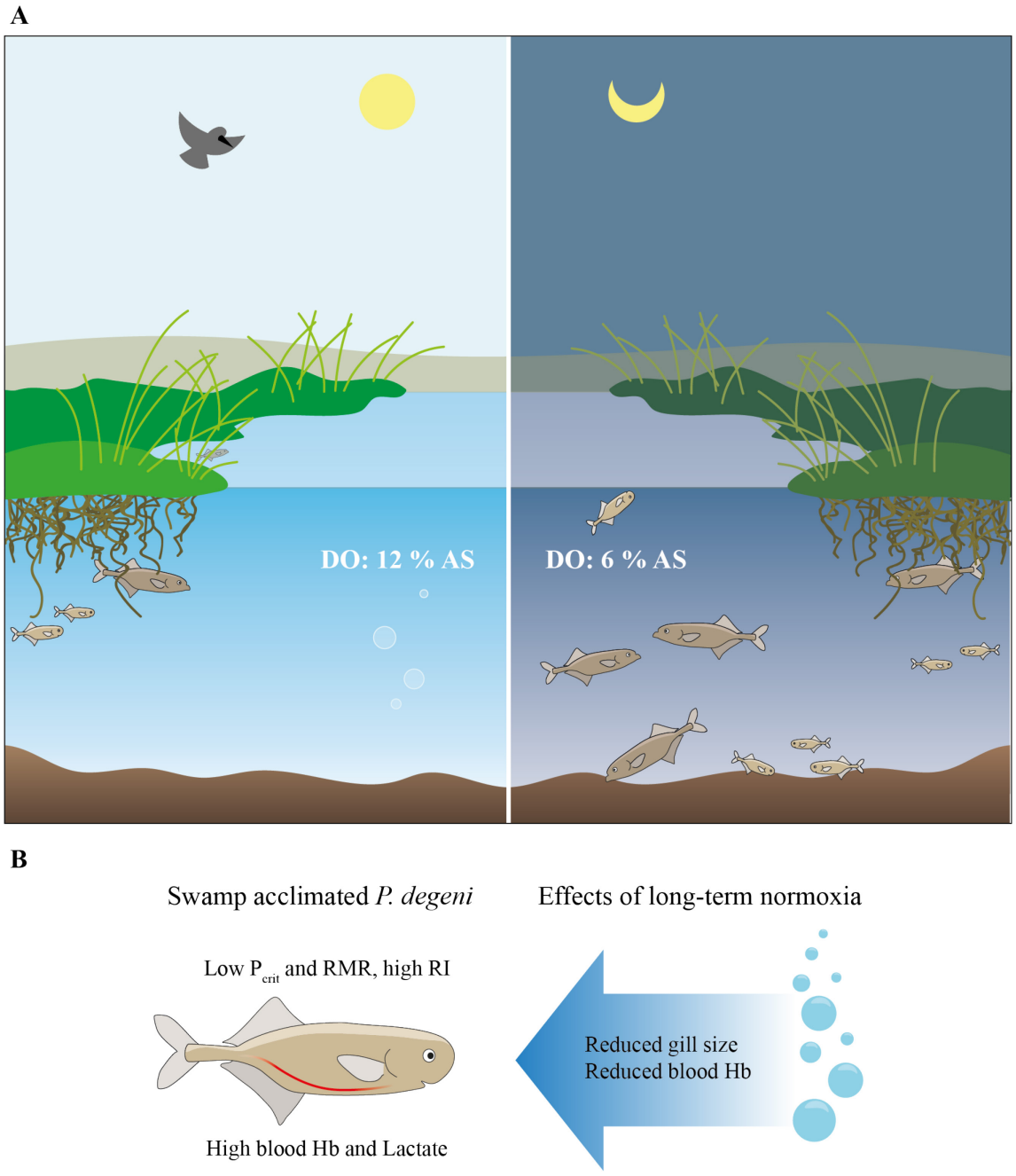


Figure 22 - Graphical summary of main findings. (A) Behavioral rhythms. Left: During the day, *M. victoriae* and *P. degeni* remain under floating vegetation in Petro Lagoon, which protects them from aerial predators. *Marcusenius victoriae* occur primarily alone, *P. degeni* are more often found in groups. Dissolved oxygen levels are low, but on average twice as high as during the night. Right: At night, both species venture into the open areas of the lagoon. Fish from both species are more often found in closer proximity to each other, and are more likely to interact. Possibly, fish move into the open area to forage for benthic invertebrates and to perform ASR at night. (B) Respiratory traits of *P. degeni*. Swamp acclimated fish showed a low P_{crit} and routine metabolic rate (RMR) and a high regulation index (RI); concentrations of hemoglobin (Hb) and lactate in their blood were high. Long-term normoxia exposure led to a significant decrease of gill filament length and hemibranch area and blood Hb concentration.

Ethics Statement

All research conducted in Uganda was approved by the Uganda National Council for Science and Technology (research clearance nr. 10601). Export of fish from Uganda to Germany was approved by the Ugandan Ministry of Agriculture, Animal Industry and Fisheries and the Senatsverwaltung für Justiz, Verbraucherschutz und Antidiskriminierung in Berlin. All experiments conducted at Humboldt-Universität zu Berlin followed approval by the Landesamt für Gesundheit und Soziales of Berlin, Germany (project number G0278/17). All experiments were designed following the 3R principles for animal testing (Reduce, Refine, Replace).

Contributions

All experiments and field sampling studies were designed by me, under the supervision of Prof. Rüdiger Krahe. I conducted all experiments, except for the laboratory activity measurement of *M. victoriae*, which was conducted by B.Sc. Franziska Oehlert under my supervision. All data analyses, creation of figures and statistical procedures were performed by me.

Prof. Lauren Chapman co-operates the Nabugabo Research Site, where the normoxia acclimation study with *P. degeni* was conducted, and assisted in the organization and logistics of the field work. The normoxia acclimation study is based on an earlier study by M.Sc. Shelby Clarke and Prof. Chapman. The respirometry setup was adapted from a design by M.Sc. Lisa Schilha.

The electric fish logger was developed by me; B.Sc. Leon Marquardt was involved in the early development of the Teensy program for the logger under my supervision. Dr. Patrick Omeja and Dr. Dennis Twinomugisha coordinated material transport, and field work schedules with the station's field assistants, Jackson Mutebbi, J. Kiberu, Fred Sseguya and Geoffrey Miiro, who assisted in the deployment of the electric fish loggers and fish capture.

Fish transport from Uganda to Germany was carried out by me; Prof. Chapman, Dr. Omeja and Dr. Twinomugisha assisted in the communication with the Ugandan Ministry of Agriculture, Animal Industry and Fisheries and Dr. Edward Rukuunya, who issued the export clearance. Prof. Krahe applied for the import permit from the Senatsverwaltung für Justiz, Verbraucherschutz und Antidiskriminierung, Berlin.

The fish illustrations that were used on the cover of this thesis and on the chapter pages were created by Rike Thoms.

Acknowledgments

Despite the prominent use of the first person singular in this thesis, absolutely nothing would have been achieved without the guidance and support by so many friends, family members, and colleagues. In a not particularly meaningful order, I want to thank:

My supervisor Rüdiger Krahe. Your patience and expertise have carried me through this work - especially the hard times and failed experiments - and helped me to develop my own identity as a researcher. You taught me that to be a great supervisor you don't only have to be an expert scientist but also an expert human. Thank you for everything!

My mentor and master's advisor Lauren J. Chapman. Your unmatched knowledge of Uganda's freshwater ecology has inspired many ideas in this thesis, and your incredible engagement in field work and teaching has provided me with the opportunity to travel to Uganda and conduct field work, which was a truly life changing experience for me.

My collaborators and former mentors that have educated and supported me. Stefan K. Hetz, your hands-on knowledge was a steady source of inspiration for my experiments, and your animal physiology lecture was the only thing that got me out of bed on a Monday at 6 am. My bachelor's supervisor, Jung-Won Youn, you taught me how to keep a clean workbench and provided me with knowledge about wet lab work on which I draw to this day. I also want to thank Jan Benda, Avner Wallach, Ana Silva, Sophie Picq, and Jörg Henninger for helpful discussions and encouragements. Special thanks to Isolde Dyson for rooting out all the misspellings in a completed deemed draft of this thesis.

The members of the Krahelab with whom I have shared the time, space and duct tape rolls, and who have always helped me with feedback or material support - Matthias Hennig, Claudia Reimann, Ina Seuffert, Livio Oboti, Lisa Schilha, Marie-Luise Vollbrecht, Dolunay Gönen, Sebastian Kraft, and all the lab alumni. Special thanks to Ina for being the heart and hands of the workgroup and to Livio for many hours of "extracurricular consultations". When I started my PhD work, there was not a single fish tank in our animal rooms. The establishment and upkeep of our fish housing facility was a huge challenge that would have been impossible to master without the amazing work of the Fish Care Team. Ina, Dolunay, Basti, Julia Kionka, Kay-Banu Lenz, thank you! I also want to thank the students whose work I supervised: Leon Marquardt, Anna-Lena Nitsche, Kay, Nina Schnabel, Franziska Oehlert, Dominik Bekaan, Dziana Shpakava, Theresa Thiele. I have learned so much working with you, and I wish you all the best for the future!

The colleagues and friends in Uganda. Dr. Dennis Twinomugisha and Dr. Patrick Omeja, your management of the research stations was flawless and without your logistical support, I would've been a fish on land. Jackson Mutebbi, Fred Sseguya, Geoffrey Miiro and J. Kiberu, thank you for sharing your skills, homes and friendship with me - I will never sit in a more cunningly captained boat - *Mwebale nyo, bassebo!* I had the great luck to share my time at the Nabugabo Research Station with numerous student researchers who I now may call friends: Sam, Eve, Megan, Kyle, Jai, Ming, Florence, Simon, Shelby, Will, Tristan, Tiffany, Bethany - thank you for being the best field companions one could wish for! I also want to thank the Uganda National Council for Science and Technology and Dr. Edward Rukuunya at the Ugandan Ministry of Agriculture, Animal Industry and Fisheries for the permission to carry out research at Lake Nabugabo and to export fish to Berlin. My employment at the Humboldt-Universität zu Berlin was partially funded by the Neurocure Cluster of Excellence by the Deutsche Forschungsgemeinschaft, for which I am grateful.

Am Ende möchte ich mich hier bei all meinen Freund*innen und meiner Familie bedanken. Ihr wart immer für mich da und habt mit einer wunderbaren Selbstverständlichkeit dafür gesorgt, dass ich die wichtigsten Dinge im Leben nie aus dem Blick verliere. Mama, Papa, Franzi, Martin, Bron und alle anderen (die Liste ist zu lang, ihr wisst, wer gemeint ist) - danke für die Liebe und die felsenfeste Unterstützung! Ihr habt dafür gesorgt, dass die Uni Arbeit immer in harter Konkurrenz stand, und doch konnte ich nur mit euch dieses Projekt abschließen. Danke für Alles, ihr seid die Besten!

Let's grow old together!

References

Abdallah, S. J., Thomas, B. S. and Jonz, M. G. (2015). Aquatic surface respiration and swimming behaviour in adult and developing zebrafish exposed to hypoxia. *J. Exp. Biol.* **218**, 1777–1786. doi:10.1242/jeb.116343.

Abdel-Tawwab, M., Monier, M. N., Hoseinifar, S. H. and Faggio, C. (2019). Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. *Fish Physiol. Biochem.* **45**, 997–1013. doi:10.1007/s10695-019-00614-9.

Ackerly, K. L., Chapman, L. J. and Krahe, R. (2017). Hypoxia acclimation increases novelty response strength during fast-starts in the African mormyrid, *Marcusenius victoriae*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **213**, 36–45. doi:10.1016/j.cbpa.2017.08.008.

Ackerly, K. L., Krahe, R., Sanford, C. P. and Chapman, L. J. (2018). Effects of hypoxia on swimming and sensing in a weakly electric fish. *J. Exp. Biol.* **221**. doi:10.1242/jeb.172130.

Adamovich, Y., Ladeuix, B., Golik, M., Koeners, M. P. and Asher, G. (2017). Rhythmic oxygen levels reset circadian clocks through HIF1α. *Cell Metab.* **25**, 93–101. doi:10.1016/j.cmet.2016.09.014.

Adebisi, A. A. (1981). The physico-chemical hydrology of a tropical seasonal river - upper Ogun river. *Hydrobiologia*. **79**, 157–165. doi:10.1007/BF00006123.

Allen, G. H. and Pavelsky, T. M. (2018). Global extent of rivers and streams. *Science*. **361**, 585–588. doi:10.1126/science.aat0636.

Almeida, L. Z., Guffey, S. C., Sepúlveda, M. S. and Höök, T. O. (2017). Behavioral and physiological responses of yellow perch (*Perca flavescens*) to moderate hypoxia. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **209**, 47–55. doi:10.1016/j.cbpa.2017.04.009.

Alves-Gomes, J. A. (2001). The evolution of electroreception and bioelectrogenesis in teleost fish: A phylogenetic perspective. *J. Fish Biol.* **58**, 1489–1511. doi:10.1006/jfbi.2001.1625.

Amen, R., Nagel, R., Hedt, M., Kirschbaum, F. and Tiedemann, R. (2020). Morphological differentiation in African weakly electric fish (genus Campylomormyrus) relates to substrate preferences. *Evol. Ecol.* **34**, 427–437. doi:10.1007/s10682-020-10043-3.

Ardanaz, J. L., Silva, A. and Macadar, O. (2001). Temperature sensitivity of the electric organ discharge waveform in *Gymnotus carapo*. *J. Comp. Physiol. A*. **187**, 853–864. doi:10.1007/s00359-001-0256-8.

Arnegard, M. E., McIntyre, P. B., Harmon, L. J., Zelditch, M. L., Crampton, W. G. R., Davis, J. K., Sullivan, J. P., Lavoué, S. and Hopkins, C. D. (2010). Sexual signal evolution outpaces ecological divergence during electric fish species radiation. *Am. Nat.* **176**, 335–356. doi:10.1086/655221.

Arrington, D. A. and Winemiller, K. O. (2003). Diel changeover in sandbank fish assemblages in a neotropical floodplain river. *J. Fish Biol.* **63**, 442–459. doi:10.1046/j.1095-8649.2003.00167.x.

Aschoff, J. (1960). Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb. Symp. Quant. Biol.* **25**, 11–28. doi:10.1101/SQB.1960.025.01.004.

Assad, C., Rasnow, B. and Stoddard, P. K. (1999). Electric organ discharges and electric images during electrolocation. *J. Exp. Biol.* **202**.

Bacelo, J., Engelmann, J., Hollmann, M., Emde, G. von der and Grant, K. (2008). Functional foveae in an electrosensory system. *J. Comp. Neurol.* **511**, 342–359. doi:10.1002/cne.21843.

Bass, A. H. and Hopkins, C. D. (1983). Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science*. **220**, 971–974. doi:10.1126/science.6844924.

Bass, A. H. and Volman, S. F. (1987). From behavior to membranes: testosterone-induced changes in action potential duration in electric organs. *Proc. Natl. Acad. Sci. U. S. A.* **84**, 9295–9298. doi:10.1073/pnas.84.24.9295.

Bässler, G., Hilbig, R. and Rahmann, H. (1979). Untersuchungen zur circadianen Rhythmik der elektrischen und motorischen Aktivität von *Gnathonemus petersii* (Mormyridae, Pisces). *Z. Tierpsychol.* **49**, 156–163.

Baumann, H., Wallace, R. B., Tagliaferri, T. and Gobler, C. J. (2015). Large natural pH, CO_2 and O_2 fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. *Estuaries Coasts*. **38**, 220–231. doi:10.1007/s12237-014-9800-y.

Baumgart, E., Fahimi, H. D., Stich, A. and Völkl, A. (1996). L-lactate dehydrogenase A4- and A3B isoforms are bona fide peroxisomal enzymes in rat liver. Evidence for involvement in intraperoxisomal NADH reoxidation. *J. Biol. Chem.* **271**, 3846–3855. doi:10.1074/jbc.271.7.3846.

Beamish, F. W. H. (1964). Respiration of fishes with special emphasis on standard oxygen consumption: II. Influence of weight and temperature on respiration of several species. *Can. J. Zool.* **42**, 177–188. doi:10.1139/z64-016.

Beamish, F. W. H. and Mookherjii, P. S. (1964). Respiration of fishes with special emphasis on standard oxygen consumption: I. Influence of weight and temperature on respiration of goldfish, *Carassius auratus* L. *Can. J. Zool.* **42**, 161–175. doi:10.1139/z64-015.

Behrens, J. W. and Steffensen, J. F. (2007). The effect of hypoxia on behavioural and physiological aspects of lesser sandeel, *Ammodytes tobianus* (Linnaeus, 1785). *Mar. Biol.* **150**, 1365–1377. doi:10.1007/s00227-006-0456-4.

Benda, J. (2020). The physics of electrosensory worlds. In *The senses: a comprehensive reference* (ed. B. Fritzsch), pp. 228–254: Elsevier, Academic Press.

Bennett, M. V. (1965). Electroreceptors in mormyrids. *Cold Spring Harb. Symp. Quant. Biol.* **30**, 245–262. doi:10.1101/sqb.1965.030.01.027.

Bennett, M. V. L. (1961). Modes of operation of electric organs. *Ann. N. Y. Acad. Sci.* **94**, 458–509. doi:10.1111/j.1749-6632.1961.tb35555.x.

Birk, M. A., Mislan, K. A. S., Wishner, K. F. and Seibel, B. A. (2019). Metabolic adaptations of the pelagic octopod *Japetella diaphana* to oxygen minimum zones. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **148**, 123–131. doi:10.1016/j.dsr.2019.04.017.

Biro, G. P. (2013). From the atmosphere to the mitochondrion: the oxygen cascade. In *Hemoglobin-Based Oxygen Carriers as Red Cell Substitutes and Oxygen Therapeutics* (ed. H. W. Kim and A. G. Greenburg), pp. 27–53. Berlin, Heidelberg, s.l.: Springer Berlin Heidelberg.

Blake, B. F. (1977). Food and feeding of the mormyrid fishes of Lake Kainji, Nigeria, with special reference to seasonal variation and interspecific differences. *J. Fish Biol.* **11**, 315–328. doi:10.1111/j.1095-8649.1977.tb04125.x.

Bonaventura, J. M., Sharpe, K., Knight, E., Fuller, K. L., Tanner, R. K. and Gore, C. J. (2015). Reliability and accuracy of six hand-held blood lactate analysers. *J. Sports Sci. Med.* **14**, 203–214.

Borowiec, B. G., Darcy, K. L., Gillette, D. M. and Scott, G. R. (2015). Distinct physiological strategies are used to cope with constant hypoxia and intermittent hypoxia in killifish (*Fundulus heteroclitus*). *J. Exp. Biol.* **218**, 1198–1211. doi:10.1242/jeb.114579.

Borowiec, B. G., Hoffman, R. D., Hess, C. D., Galvez, F. and Scott, G. R. (2020). Interspecific variation in hypoxia tolerance and hypoxia acclimation responses in killifish from the family Fundulidae. *J. Exp. Biol.* **223**. doi:10.1242/jeb.209692.

Boujard, T. and Leatherland, J. F. (1992). Circadian rhythms and feeding time in fishes. *Environ. Biol. Fish.* **35**, 109–131. doi:10.1007/BF00002186.

Boussou, K. C., Yoboué, A. N., Djiriéoulou, K. C., Aliko, N. G. and Konan, K. F. (2019). Feeding patterns of the Mormyrid fish *Brienomyrus brachyistius* (Gill, 1862) in Kogon and Tinguilinta rivers (Guinea Republic). *Egypt. J. of Aquatic Biol. and Fish. (Egyptian Journal of Aquatic Biology and Fisheries)*. **23**, 239–248. doi:10.21608/ejabf.2019.54832.

Brauner, C. J. and Val, A. L. (2006). Oxygen transfer. In *The physiology of tropical fishes* (ed. A. L. Val, D. J. Randall and V. M. F. Almeida-Val), pp. 277–306. London: Elsevier Academic.

Breitburg, D. L., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D. and Isensee, K. et al. (2018). Declining oxygen in the global ocean and coastal waters. *Science*. **359**. doi:10.1126/science.aam7240.

Brooks, G. A. (1985). Lactate: glycolytic end product and oxidative substrate during sustained exercise in mammals - the "Lactate Shuttle". In *Circulation, respiration, and metabolism* (ed. R. Gilles), pp. 208–218. Berlin, Heidelberg: Springer Berlin Heidelberg.

Bullock, T. H., Hopkins, C. D., Popper, A. N. and Fay, R. R., eds. (2005). *Electroreception*. New York, NY: Springer.

Burleson, M., Wilhelm, D. R. and Smatresk, N. J. (2001). The influence of fish size on the avoidance of hypoxia and oxygen selection by largemouth bass. *J. Fish Biol.* **59**, 1336–1349. doi:10.1006/jfbi.2001.1745.

Campos-Candela, A., Palmer, M., Balle, S. and Alós, J. (2018). A camera-based method for estimating absolute density in animals displaying home range behaviour. *J. Anim. Ecol.* **87**, 825–837. doi:10.1111/1365-2656.12787.

Caputi, A. A., Budelli, R., Grant, K. and Bell, C. C. (1998). The electric image in weakly electric fish: physical images of resistive objects in *Gnathonemus petersii*. *J. Exp. Biol.* **201**, 2115–2128. doi:10.1242/jeb.201.14.2115.

Carlson, B. A. (2002). Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish. *J. Physiol. Paris*. **96**, 405–419. doi:10.1016/S0928-4257(03)00019-6.

Carlson, B. A. (2016). Differences in electrosensory anatomy and social behavior in an area of sympatry between two species of mormyrid electric fishes. *J. Exp. Biol.* **219**, 31–43. doi:10.1242/jeb.127720.

Carlson, B. A., Hasan, S. M., Hollmann, M., Miller, D. B., Harmon, L. J. and Arnegard, M. E. (2011). Brain evolution triggers increased diversification of electric fishes. *Science*. **332**, 583–586. doi:10.1126/science.1201524.

Carlson, B. A. and Hopkins, C. D. (2004). Stereotyped temporal patterns in electrical communication. *Anim. Behav.* **68**, 867–878. doi:10.1016/j.anbehav.2003.10.031.

Carlson, B. A., Sisneros, J. A., Popper, A. N. and Fay, R. R. (2019). *Electroreception: Fundamental insights from comparative approaches*. Cham, Switzerland: Springer; ASA press.

Chabot, D., Mckenzie, D. J. and Craig, J. F. (2016a). Metabolic rate in fishes: definitions, methods and significance for conservation physiology. *J. Fish Biol.* **88**, 1–9. doi:10.1111/jfb.12873.

Chabot, D., Steffensen, J. F. and Farrell, A. P. (2016b). The determination of standard metabolic rate in fishes. *J. Fish Biol.* **88**, 81–121. doi:10.1111/jfb.12845.

Chapman, L. J. and Chapman, C. A. (1998). Hypoxia tolerance of the mormyrid *Petrocephalus catostoma*: implications for persistence in swamp refugia. *Copeia*. **1998**, 762. doi:10.2307/1447812.

Chapman, L. J., Chapman, C. A., Brazeau, D. A., McLaughlin, B. and Jordan, M. (1999). Papyrus swamps, hypoxia, and faunal diversification: variation among populations of *Barbus neumayeri*. *J. Fish Biol.* **54**, 310–327. doi:10.1111/j.1095-8649.1999.tb00832.x.

Chapman, L. J., Chapman, C. A. and Chandler, M. (1996a). Wetland ecotones as refugia for endangered fishes. *Biol. Conserv.* **78**, 263–270. doi:10.1016/S0006-3207(96)00030-4.

Chapman, L. J., Chapman, C. A., Nordlie, F. G. and Rosenberger, A. E. (2002). Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **133**, 421–437. doi:10.1016/S1095-6433(02)00195-2.

Chapman, L. J., Chapman, C. A., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L. and Keiter, A. E. (1996b). Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. *Conserv. Biol.* **10**, 554–561. doi:10.1046/j.1523-1739.1996.10020554.x.

Chapman, L. J., Chapman, C. A., Schofield, P. J., Olowo, J. P., Kaufman, L., Seehausen, O. and Ogutu-Ohwayo, R. (2003). Fish faunal resurgence in Lake Nabugabo, East Africa. *Conserv. Biol.* **17**, 500–511. doi:10.1046/j.1523-1739.2003.01519.x.

Chapman, L. J. and Hulen, K. G. (2001). Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. *J. Zool.* **254**, 461–472. doi:10.1017/S0952836901000966.

Childress, J. J. and Seibel, B. A. (1998). Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J. Exp. Biol.* **201**, 1223–1232.

Chippari-Gomes, A. R., Gomes, L. C., Lopes, N. P., Val, A. L. and Almeida-Val, V. M. F. (2005). Metabolic adjustments in two Amazonian cichlids exposed to hypoxia and anoxia. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **141**, 347–355. doi:10.1016/j.cbpc.2005.04.006.

Chrétien, E. and Chapman, L. J. (2016). Habitat heterogeneity facilitates coexistence of native fishes with an introduced predator: the resilience of a fish community 5 decades after the introduction of Nile perch. *Biol. Invasions*. **18**, 3449–3464. doi:10.1007/s10530-016-1235-x.

Clark, T. D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771–2782. doi:10.1242/jeb.084251.

Clarke, S. B. (2019). Persistence of rare species in Lake Nabugabo, Uganda: a case study of low-oxygen tolerance in the weakly electric fish *Petrocephalus degeni*. *Master's Thesis,* McGill University, Montreal.

Clarke, S. B., Chapman, L. J. and Krahe, R. (2020). The effect of normoxia exposure on hypoxia tolerance and sensory sampling in a swamp-dwelling mormyrid fish. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **240**. doi:10.1016/j.cbpa.2019.110586.

Cobert, S. J. (1984). Environmental control of rhythmic behavior in the weak-electric fish, *Gnathonemus petersii* (Mormyridae). *PhD thesis,* The City University of New York, New York.

Collins, G. M., Clark, T. D. and Carton, A. G. (2015). Physiological plasticity v. inter-population variability: understanding drivers of hypoxia tolerance in a tropical estuarine fish. *Mar. Freshw. Res.* [Epub ahead of print]. doi:10.1071/MF15046.

Connett, R. J., Honig, C. R., Gayeski, T. E. and Brooks, G. A. (1990). Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. *J. Appl. Physiol.* **68**, 833–842. doi:10.1152/jappl.1990.68.3.833.

Cook, D. G. and Herbert, N. A. (2012). The physiological and behavioural response of juvenile kingfish (*Seriola lalandi*) differs between escapable and inescapable progressive hypoxia. *J. Exp. Mar. Biol. Ecol.* **413**, 138–144. doi:10.1016/j.jembe.2011.12.006.

Cook, D. G., Iftikar, F. I., Baker, D. W., Hickey, A. J. R. and Herbert, N. A. (2013). Low-O_2 acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. *J. Exp. Biol.* **216**, 369–378. doi:10.1242/jeb.073023.

Cook, D. G., Wells, R. M. G. and Herbert, N. A. (2011). Anaemia adjusts the aerobic physiology of snapper (*Pagrus auratus*) and modulates hypoxia avoidance behaviour during oxygen choice presentations. *J. Exp. Biol.* **214**, 2927–2934. doi:10.1242/jeb.057091.

Corbet, P. S. (1961). The food of non-cichlid fishes in the Lake Victoria basin, with remarks on their evolution and adaptation to lacustrine conditions. *Proc. Zool. Soc. Lond.* **136**, 1–101. doi:10.1111/j.1469-7998.1961.tb06080.x.

Crampton, W. G. R. (1996). Gymnotiform fish: An important component of Amazonian floodplain fish communities. *J. Fish Biol.* **48**, 298–301. doi:10.1111/j.1095-8649.1996.tb01122.x.

Crampton, W. G. R. (1998). Effects of anoxia on the distribution, respiratory strategies and electric signal diversity of gymnotiform fishes. *J. Fish Biol.* **53**, 307–330.

Crampton, W. G. R. (2019). Electroreception, electrogenesis and electric signal evolution. *J. Fish Biol.* **95**, 92–134. doi:10.1111/jfb.13922.

Crampton, W. G. R. and Albert, J. S. (2006). Evolution of electric signal diversity in gymnotiform fishes: Part A. Phylogenetic systematics, ecology, and biogeography. In *Communication in fishes,* pp. 647–731. Enfield, NH: Science Publ.

Crampton, W. G. R., Chapman, L. J. and Bell, J. (2008). Interspecific variation in gill size is correlated to ambient dissolved oxygen in the Amazonian electric fish *Brachyhypopomus* (Gymnotiformes: Hypopomidae). *Environ. Biol. Fish.* **83**, 223–235. doi:10.1007/s10641-007-9325-3.

Crawford, J. D. (1992). Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (*Pollimyrus isidori*). *J. Exp. Biol.* **164**, 79–102. doi:10.1242/jeb.164.1.79.

Crispo, E. and Chapman, L. J. (2010). Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *J. Evol. Biol.* **23**, 2091–2103. doi:10.1111/j.1420-9101.2010.02069.x.

Darwin, C. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London: John Murray.

Diaz, R. J. (2001). Overview of hypoxia around the world. *J. Environ. Qual.* **30**, 275–281. doi:10.2134/jeq2001.302275x.

Diaz, R. J. and Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science*. **321**, 926–929. doi:10.1126/science.1156401.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D. and Stiassny, M. L. J. et al. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev. Camb. Philos. Soc.* **81**, 163–182. doi:10.1017/S1464793105006950.

Dunlap, K. D., Smith, G. T. and Yekta, A. (2000). Temperature dependence of electrocommunication signals and their underlying neural rhythms in the weakly electric fish, *Apteronotus leptorhynchus*. *Brain Behav. Evol.* **55**, 152–162. doi:10.1159/000006649.

Egg, M., Köblitz, L., Hirayama, J., Schwerte, T., Folterbauer, C., Kurz, A., Fiechtner, B., Möst, M., Salvenmoser, W. and Sassone-Corsi, P. et al. (2013). Linking oxygen to time: the bidirectional interaction between the hypoxic signaling pathway and the circadian clock. *Chronobiol. Int.* **30**, 510–529. doi:10.3109/07420528.2012.754447.

Emde, G. von der (1999). Active electrolocation of objects in weakly electric fish. *J. Exp. Biol.* **202**, 1205–1215.

Emde, G. von der and Bleckmann, H. (1998). Finding food: senses involved in foraging for insect larvae in the electric fish *Gnathonemus petersii*. *J. Exp. Biol.* **201 (Pt 7)**, 969–980. doi:10.1242/jeb.201.7.969.

Farrell, A. P. (2013). Aerobic scope and its optimum temperature: clarifying their usefulness and limitations - correspondence on J. Exp. Biol. 216, 2771-2782. *J. Exp. Biol.* **216**, 4493–4494. doi:10.1242/jeb.095471.

Farrell, A. P. (2016). Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J. Fish Biol.* **88**, 322–343. doi:10.1111/jfb.12789.

Farrell, A. P. and Richards, J. G. (2009). Defining hypoxia. In *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 487–503. Amsterdam, Boston: Academic Press.

Fick, A. (1855). Ueber Diffusion. *Ann. Phys. Chem.* **170**, 59–86. doi:10.1002/andp.18551700105.

Filho, D. W., Eble, G. J., Kassner, G., Caprario, F. X., Dafré, A. L. and Ohira, M. (1992). Comparative hematology in marine fish. *Comp. Biochem. Physiol. A Physiol.* **102A**, 311–321.

Follana-Berná, G., Palmer, M., Lekanda-Guarrotxena, A., Grau, A. and Arechavala-Lopez, P. (2020). Fish density estimation using unbaited cameras: Accounting for environmental-dependent detectability. *J. Exp. Mar. Biol. Ecol.* **527**, 151376. doi:10.1016/j.jembe.2020.151376.

Franchina, C. R., Salazar, V. L., Volmar, C. H. and Stoddard, P. K. (2001). Plasticity of the electric organ discharge waveform of male *Brachyhypopomus pinnicaudatus*. II. Social effects. *J. Comp. Physiol. A*. **187**, 45–52. doi:10.1007/s003590000176.

Franchina, C. R. and Stoddard, P. K. (1998). Plasticity of the electric organ discharge waveform of the electric fish *Brachyhypopomus pinnicaudatus*. I. Quantification of day-night changes. *J. Comp. Physiol. A*. **183**, 759–768. doi:10.1007/s003590050299.

Franz, V. (1920). Zur mikroskopischen Anatomie der Mormyriden. *Zool. Jahrb., Abt. Anat.* **42**, 91–148.

Frehse, F. d. A., Weyl, O. L. F. and Vitule, J. R. S. (2021). Differential use of artificial habitats by native and non-native fish species in Neotropical reservoirs. *Hydrobiologia*. **848**, 2355–2367. doi:10.1007/s10750-021-04564-3.

Frehse, F. d. A., Weyl, O. L. F. and Vitule, J. R. S. (2020). Comparison of visual census and underwater video for fish sampling in Neotropical reservoirs. *Environ. Biol. Fish.* **103**, 1269–1277. doi:10.1007/s10641-020-01021-3.

Fricke, R., Eschmeyer, W. N. and Fong, J. D. (2021). Eschmeyer's Catalog of Fishes: Genera/Species by Family/Subfamily. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp. Accessed November 21, 2021.

Friedman, M. A. and Hopkins, C. D. (1996). Tracking individual mormyrid electric fish in the field using electric organ discharge waveforms. *Anim. Behav.* **51**, 391–407. doi:10.1006/anbe.1996.0037.

Frisch, A. and Anderson, T. (2005). Physiological stress responses of two species of coral trout (*Plectropomus leopardus* and *Plectropomus maculatus*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **140**, 317–327. doi:10.1016/j.cbpb.2005.01.014.

Frøland Steindal, I. A. and Whitmore, D. (2019). Circadian clocks in fish-what have we learned so far? *Biology*. **8**. doi:10.3390/biology8010017.

Fry, F. E. (1971). The effect of environmental factors on the physiology of fish. *Fish Physiol.* **6**, 1–98.

Fry, F. E. and Hart, J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. *Biol. Bull.* **94**, 66–77.

Fu, S.-J., Fu, C., Yan, G.-J., Cao, Z.-D., Zhang, A.-J. and Pang, X. (2014). Interspecific variation in hypoxia tolerance, swimming performance and plasticity in cyprinids that prefer different habitats. *J. Exp. Biol.* **217**, 590–597. doi:10.1242/jeb.089268.

Gaillard, M. C. (1923). Faune égyptienne antique. Recherches sur les poissons représentés dans quelques tombeaux égyptiens de l'Ancien Empire. In *Memoires de l'Institut Français d'Archéologie Orientale* (ed. V. Loret and C. Kuentz). Cairo: Imprimerie de l'Institut Français d'Archéologie Orientale.

Gallant, J. R., Arnegard, M. E., Sullivan, J. P., Carlson, B. A. and Hopkins, C. D. (2011). Signal variation and its morphological correlates in *Paramormyrops kingsleyae* provide insight into the evolution of electrogenic signal diversity in mormyrid electric fish. *J. Comp. Physiol. A*. **197**, 799–817. doi:10.1007/s00359-011-0643-8.

Gallaugher, P., Thorarensen, H. and Farrell, A. P. (1995). Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respir. Physiol.* **102**, 279–292. doi:10.1016/0034-5687(95)00065-8.

Gilbert, W. (1600). *De magnete, magneticisque corporibus, et de magno magnete tellure; Physiologia nova, plurimis & argumentis, & experimentis demonstrata.* London: Peter Short.

Gladden, L. B. (2004). Lactate metabolism: a new paradigm for the third millennium. *J. Physiol.* **558**, 5–30. doi:10.1113/jphysiol.2003.058701.

Goldberg, E. D. (1995). Emerging problems in the coastal zone for the twenty-first century. *Mar. Pollut. Bull.* **31**, 152–158. doi:10.1016/0025-326X(95)00102-S.

Gonzalez, R. J. and McDonald, D. G. (1992). The relationship between oxygen consumption and ion loss in a freshwater fish. *J. Exp. Biol.* **163**, 317–332.

Graham, J. B. (1990). Ecological, evolutionary, and physical factors influencing aquatic animal respiration. *Am. Zool.* **30**, 137–146. doi:10.1093/icb/30.1.137.

Graham, J. B. (1997). *Air-breathing fishes: Evolution, diversity, and adaptation*. San Diego: Academic Press.

Green, H. and Goldberg, B. (1964). Collagen and cell protein synthesis by an established mammalian fibroblast line. *Nature*. **204**, 347–349. doi:10.1038/204347a0.

Greenbank, J. (1945). Limnological conditions in ice-covered lakes, especially as related to winter-kill of fish. *Ecol. Monogr.* **15**, 343–392.

Greenwood, P. H. (1965). *The cichlid fishes of Lake Nabugabo, Uganda*. London: British Museum (Natural History).

Grutter, A. S. and Pankhurst, N. W. (2000). The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*. *J. Fish Biol.* **57**, 391–401. doi:10.1111/j.1095-8649.2000.tb02179.x.

Häfker, N. S. and Tessmar-Raible, K. (2020). Rhythms of behavior: are the times changin'? *Curr. Opin. Neurobiol.* **60**, 55–66. doi:10.1016/j.conb.2019.10.005.

Hamilton, S. K. (2010). Biogeochemical implications of climate change for tropical rivers and floodplains. *Hydrobiologia*. **657**, 19–35. doi:10.1007/s10750-009-0086-1.

Hammerschlag, N., Heithaus and Serafy, J. E. (2010). Influence of predation risk and food supply on nocturnal fish foraging distributions along a mangrove–seagrass ecotone. *Mar. Ecol. Prog. Ser.* **414**, 223–235. doi:10.3354/meps08731.

Harder, W., Schief, A. and Uhlemann, H. (1964). Zur Funktion des elektrischen Organs von *Gnathonemus petersii* (Gthr. 1862) (Mormyriformes, Teleostei). *Z. vergl. Physiologie*. **48**, 302–331. doi:10.1007/BF00339459.

Henninger, J., Krahe, R., Kirschbaum, F., Grewe, J. and Benda, J. (2018). Statistics of natural communication signals observed in the wild identify important yet neglected stimulus regimes in weakly electric fish. *J. Neurosci.* **38**, 5456–5465. doi:10.1523/JNEUROSCI.0350-18.2018.

Henninger, J., Krahe, R., Sinz, F. and Benda, J. (2020). Tracking activity patterns of a multispecies community of gymnotiform weakly electric fish in their Neotropical habitat without tagging. *J. Exp. Biol.* **223**. doi:10.1242/jeb.206342.

Hobson, E. S. (1973). Diel feeding migrations in tropical reef fishes. *Helgoländer wiss. Meeresunters.* **24**, 361–370.

Hopkins, C. D. and Bass, A. H. (1981). Temporal coding of species recognition signals in an electric fish. *Science*. **212**, 85–87. doi:10.1126/science.7209524.

Hopkins, C. D. (1980). Evolution of electric communication channels of mormyrids. *Behav. Ecol. Sociobiol.* **7**, 1–13. doi:10.1007/BF00302513.

Hopkins, C. D. (1981). On the diversity of electric signals in a community of mormyrid electric fish in West Africa. *Am. Zool.* **21**, 211–222. doi:10.1093/icb/21.1.211.

Hughes, G. M. (1984). Measurement of gill area in fishes: practices and problems. *J. Mar. Biol. Ass.* **64**, 637–655. doi:10.1017/S0025315400030319.

Hughes, G. M. and Morgan, M. (1973). The structure of fish gills in relation to their respiratory function. *Biol. Rev. Camb. Philos. Soc.* **48**, 419–475. doi:10.1111/j.1469-185X.1973.tb01009.x.

Hurd, M., DeBruyne, J., Straume, M. and Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiol. Behav.* **65**, 465–472. doi:10.1016/s0031-9384(98)00183-8.

Hussey, N. E., Kessel, S. T., Aarestrup, K., Cooke, S. J., Cowley, P. D., Fisk, A. T., Harcourt, R. G., Holland, K. N., Iverson, S. J. and Kocik, J. F. et al. (2015). Aquatic animal telemetry: A panoramic window into the underwater world. *Science*. **348**, 1255642. doi:10.1126/science.1255642.

Hut, R. A., Paolucci, S., Dor, R., Kyriacou, C. P. and Daan, S. (2013). Latitudinal clines: an evolutionary view on biological rhythms. *Proc. R. Soc. B*. **280**, 20130433. doi:10.1098/rspb.2013.0433.

Ibbotson, A. T., Beaumont, W. R. C., Pinder, A., Welton, S. and Ladle, M. (2006). Diel migration patterns of Atlantic salmon smolts with particular reference to the absence of crepuscular migration. *Ecol. Freshw. Fish*. **15**, 544–551. doi:10.1111/j.1600-0633.2006.00194.x.

Idda, M. L., Bertolucci, C., Vallone, D., Gothilf, Y., Sánchez-Vázquez, F. J. and Foulkes, N. S. (2012). Circadian clocks: lessons from fish. *Prog. Brain Res.* **199**, 41–57. doi:10.1016/B978-0-444-59427-3.00003-4.

Ihssen, P. E., Evans, D. O., Christie, W. J., Reckahn, J. A. and DesJardine, R. L. (1981). Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes Region. *Can. J. Fish. Aquat. Sci.* **38**, 1790–1807. doi:10.1139/f81-226.

Ikomi, R. B. (1996). Studies on the growth pattern, feeding habits and reproductive characteristics of the mormyrid *Brienomyrus longianalis* (Boulenger 1901) in the upper Warri River, Nigeria. *Fish. Res.* **26**, 187–198. doi:10.1016/0165-7836(95)00381-9.

Jenny, J.-P., Francus, P., Normandeau, A., Lapointe, F., Perga, M.-E., Ojala, A. E. K., Schimmelmann, A. and Zolitschka, B. (2016a). Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. *Glob. Chang. Biol.* **22**, 1481–1489. doi:10.1111/gcb.13193.

Jenny, J.-P., Normandeau, A., Francus, P., Taranu, Z. E., Gregory-Eaves, I., Lapointe, F., Jautzy, J., Ojala, A. E. K., Dorioz, J.-M. and Schimmelmann, A. et al. (2016b). Urban point sources of nutrients were the leading cause for the historical spread of hypoxia across European lakes. *PNAS*. **113**, 12655–12660. doi:10.1073/pnas.1605480113.

Johansen, K. (1970). Air breathing in fishes. In *The nervous system, circulation, and respiration* (ed. W. S. Hoar and D. J. Randall), pp. 361–411. New York: Academic Press.

Jones, J. R. E. (1952). The reactions of fish to water of low oxygen concentration. *J. Exp. Biol.* **29**, 403–415.

Julian, D., Crampton, W. G. R., Wohlgemuth, S. E. and Albert, J. S. (2003). Oxygen consumption in weakly electric Neotropical fishes. *Oecologia*. **137**, 502–511. doi:10.1007/s00442-003-1368-3.

Kannan, V. and Job, S. V. (1980). Diurnal depth-wise and seasonal changes of physico-chemical factors in Sathiar reservoir. *Hydrobiologia*. **70**, 103–117. doi:10.1007/BF00015496.

Kaufman, L. and Ochumba, P. (1993). Evolutionary and conservation biology of cichlid fishes as revealed by faunal remnants in northern Lake Victoria. *Conserv. Biol.* **7**, 719–730.

Kellaway, P. (1946). The part played by electric fish in the early history of bioelectricity and electrotherapy. *Bull. Hist. Med.* **20**, 112–137.

Kirschbaum, F. (1995). Reproduction and development in mormyriform and gymnotiform fishes. In *Electric fishes: history and behavior* (ed. P. Moller), pp. 267–301. London: Chapman & Hall.

Kirschbaum, F. and Schugardt, C. (2002). Reproductive strategies and developmental aspects in mormyrid and gymnotiform fishes. *J. Physiol. Paris*. **96**, 557–566. doi:10.1016/S0928-4257(03)00011-1.

Krahe, R. and Fortune, E. S., eds. (2013). Electric fishes: neural systems, behaviour and evolution [Special issue]. *J. Exp. Biol.* **216.**

Kramer, B., Bills, R., Skelton, P. and Wink, M. (2012). A critical revision of the churchill snoutfish, genus *Petrocephalus* Marcusen, 1854 (Actinopterygii: Teleostei: Mormyridae), from southern and eastern Africa, with the recognition of *Petrocephalus tanensis*, and the description of five new species. *J. Nat. Hist.* **46**, 2179–2258. doi:10.1080/00222933.2012.708452.

Kramer, B. and Wink, M. (2013). East–west differentiation in the *Marcusenius macrolepidotus* species complex in Southern Africa: the description of a new species for the lower Cunene River, Namibia (Teleostei: Mormyridae). *J. Nat. Hist.* **47**, 2327–2362. doi:10.1080/00222933.2013.798699.

Kramer, D. L. (1984). The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. In *Evolutionary ecology of Neotropical freshwater fishes* (ed. E. K. Balon and T. M. Zaret), pp. 67–80. Dordrecht: Springer Netherlands.

Kramer, D. L., Manley, D. and Bourgeois, R. (1983). The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. *Can. J. Zool.* **61**, 653–665. doi:10.1139/z83-087.

Kramer, D. L. and McClure, M. (1982). Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. *Environ. Biol. Fish.* **7**, 47–55. doi:10.1007/BF00011822.

Kruger, E. J. (1973). Autumn feeding cycle of the bull-dog fish, *Gnathonemus macrolepidotus* (Pisces, Mormyridae). *Afr. Zool.* **8**, 25–34.

Lahiri, K., Vallone, D., Gondi, S. B., Santoriello, C., Dickmeis, T. and Foulkes, N. S. (2005). Temperature regulates transcription in the zebrafish circadian clock. *PLoS biology*. **3**, e351. doi:10.1371/journal.pbio.0030351.

Lewis, J. E., Gilmour, K. M., Moorhead, M. J., Perry, S. F. and Markham, M. R. (2014). Action potential energetics at the organismal level reveal a trade-off in efficiency at high firing rates. *J. Neurosci.* **34**, 197–201. doi:10.1523/JNEUROSCI.3180-13.2014.

Lewis, W. M. (1970). Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. *Copeia*. **1970**, 319–326. doi:10.2307/1441653.

Lissmann, H. W. (1951). Continuous electrical signals from the tail of a fish, *Gymnarchus niloticus* Cuv. *Nature*. **167**, 201–202.

Lissmann, H. W. (1961). Ecological studies on gymnotids. In *Bioelectrogenesis: a comparative survey of its mechanisms with particular emphasis on electric fishes* (ed. C. Chagas and A. P. de Carvalho), pp. 215–226: Elsevier Amsterdam.

Lissmann, H. W. and Machin, K. E. (1958). The mechanism of object location in *Gymnarchus niloticus* and similar fish. *J. Exp. Biol.* **35**, 451–486.

Lissmann, H. W. and Schwassmann, H. O. (1965). Activity rhythm of an electric fish, *Gymnorhamphichthys hypostomus*, Ellis. *J. Comp. Physiol. A*. **51**, 153–171. doi:10.1007/BF00299291.

López-Olmeda, J. F. and Sánchez-Vázquez, F. J. (2009). Zebrafish temperature selection and synchronization of locomotor activity circadian rhythm to ahemeral cycles of light and temperature. *Chronobiol. Int.* **26**, 200–218. doi:10.1080/07420520902765928.

Love, J. W. and Rees, B. B. (2002). Seasonal differences in hypoxia tolerance in gulf killifish, *Fundulus grandis* (Fundulidae). *Environ. Biol. Fish.* **63**, 103–115. doi:10.1023/A:1013834803665.

Lundberg, J. G., Lewis, W. M., Saunders, J. F. and Mago-Leccia, F. (1987). A major food web component in the Orinoco River channel: evidence from planktivorous electric fishes. *Science*. **237**, 81–83. doi:10.1126/science.237.4810.81.

Lushchak, V. I. and Bagnyukova, T. V. (2006). Effects of different environmental oxygen levels on free radical processes in fish. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **144**, 283–289. doi:10.1016/j.cbpb.2006.02.014.

Lushchak, V. I., Lushchak, L. P., Mota, A. A. and Hermes-Lima, M. (2001). Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R100-R107. doi:10.1152/ajpregu.2001.280.1.R100.

Madhav, M. S., Jayakumar, R. P., Demir, A., Stamper, S. A., Fortune, E. S. and Cowan, N. J. (2018). High-resolution behavioral mapping of electric fishes in Amazonian habitats. *Sci. Rep.* **8**, 5830. doi:10.1038/s41598-018-24035-5.

Mandic, M., Todgham, A. E. and Richards, J. G. (2009). Mechanisms and evolution of hypoxia tolerance in fish. *Proc. R. Soc. B*. **276**, 735–744. doi:10.1098/rspb.2008.1235.

Markham, M. R. (2013). Electrocyte physiology: 50 years later. *J. Exp. Biol.* **216**, 2451–2458. doi:10.1242/jeb.082628.

Markham, M. R., Ban, Y., McCauley, A. G. and Maltby, R. (2016). Energetics of sensing and communication in electric fish: A blessing and a curse in the Anthropocene? *Integr. Comp. Biol.* **56**, 889–900. doi:10.1093/icb/icw104.

Markham, M. R., Kaczmarek, L. K. and Zakon, H. H. (2013). A sodium-activated potassium channel supports high-frequency firing and reduces energetic costs during rapid modulations of action potential amplitude. *J. Neurophysiol.* **109**, 1713–1723. doi:10.1152/jn.00875.2012.

Markham, M. R., McAnelly, M. L., Stoddard, P. K. and Zakon, H. H. (2009). Circadian and social cues regulate ion channel trafficking. *PLoS biology*. **7**, e1000203. doi:10.1371/journal.pbio.1000203.

Marrero, C. and Winemiller, K. O. (1993). Tube-snouted gymnotiform and mormyriform fishes: convergence of a specialized foraging mode in teleosts. *Environ. Biol. Fish.* **38**, 299–309. doi:10.1007/BF00007523.

Marshall, D. J., Bode, M. and White, C. R. (2013). Estimating physiological tolerances - a comparison of traditional approaches to nonlinear regression techniques. *J. Exp. Biol.* **216**, 2176–2182. doi:10.1242/jeb.085712.

Martinez, M. L., Chapman, L. J., Grady, J. M. and Rees, B. B. (2004). Interdemic variation in haematocrit and lactate dehydrogenase in the African cyprinid *Barbus neumayeri*. *J. Fish Biol.* **65**, 1056–1069.

Mauro, A. (1969). The role of the Voltaic pile in the Galvani-Volta controversy concerning animal vs. metallic electricity. *J. Hist. Med. Allied Sci.* **24**, 140–150. doi:10.1093/jhmas/xxiv.2.140.

Maxime, V., Pichavant, K., Boeuf, G. and Nonnotte, G. (2000). Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. *Fish Physiol. Biochem.* **22**, 51–59. doi:10.1023/A:1007829214826.

McAnelly, M. L. and Zakon, H. H. (2000). Coregulation of voltage-dependent kinetics of Na^+ and K^+ currents in electric organ. *J. Neurosci.* **20**, 3408–3414. doi:10.1523/JNEUROSCI.20-09-03408.2000.

McBryan, T. L., Anttila, K., Healy, T. M. and Schulte, P. M. (2013). Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integr. Comp. Biol.* **53**, 648–659. doi:10.1093/icb/ict066.

McClelland, G. B., Khanna, S., González, G. F., Eric Butz, C. and Brooks, G. A. (2003). Peroxisomal membrane monocarboxylate transporters: evidence for a redox shuttle system? *Biochem. Biophys. Res. Commun.* **304**, 130–135. doi:10.1016/S0006-291X(03)00550-3.

Migliaro, A., Moreno, V., Marchal, P. and Silva, A. C. (2018). Daily changes in the electric behavior of weakly electric fish naturally persist in constant darkness and are socially synchronized. *Biol. Open*. **7**. doi:10.1242/bio.036319.

Migliaro, A. and Silva, A. C. (2016). Melatonin regulates daily variations in electric behavior arousal in two species of weakly electric fish with different social structures. *Brain Behav. Evol.* **87**, 232–241. doi:10.1159/000445494.

Moller, P., ed. (1995). *Electric fishes: history and behavior*. London: Chapman & Hall.

Moller, P., Serrier, J., Belbenoit, P. and Push, S. (1979). Notes on ethology and ecology of the Swashi River mormyrids (Lake Kainji, Nigeria). *Behav. Ecol. Sociobiol.* **4**, 357–368. doi:10.1007/BF00303242.

Moller, P., Serrier, J. and Bowling, D. (1989). Electric organ discharge displays during social encounter in the weakly electric fish *Brienomyrus niger* L. (Mormyridae). *Ethology*. **82**, 177–191. doi:10.1111/j.1439-0310.1989.tb00498.x.

Mönck, H. J., Jörg, A., Falkenhausen, T. v., Tanke, J., Wild, B., Dormagen, D., Piotrowski, J., Winklmayr, C., Bierbach, D. and Landgraf, T. (2018). BioTracker: an open-source computer vision framework for visual animal tracking: arXiv.org [Preprint]. http://arxiv.org/pdf/1803.07985v1. Accessed January 22, 2022.

Moulton, T. L. (2016). Keeping a finger on the pulse of East African weakly electric fishes: Ecology and hypoxia tolerance of mormyrids in Lake Nabugabo, Uganda. *Master Thesis,* McGill University, Montreal.

Moulton, T. L., Chapman, L. J. and Krahe, R. (2020). Effects of hypoxia on aerobic metabolism and active electrosensory acquisition in the African weakly electric fish *Marcusenius victoriae*. *J. Fish Biol.* **96**, 496–505. doi:10.1111/jfb.14234.

Mucha, S., Chapman, L. J. and Krahe, R. (2021). The weakly electric fish, *Apteronotus albifrons*, actively avoids experimentally induced hypoxia. *J. Comp. Physiol. A* [Epub ahead of print]. doi:10.1007/s00359-021-01470-w.

Mueller, C. A. and Seymour, R. S. (2011). The regulation index: a new method for assessing the relationship between oxygen consumption and environmental oxygen. *Physiol. Biochem. Zool.* **84**, 522–532. doi:10.1086/661953.

Nagel, R., Kirschbaum, F., Engelmann, J., Hofmann, V., Pawelzik, F. and Tiedemann, R. (2018a). Male-mediated species recognition among African weakly electric fishes. *R. Soc. Open Sci.* **5**, 170443. doi:10.1098/rsos.170443.

Nagel, R., Kirschbaum, F., Hofmann, V., Engelmann, J. and Tiedemann, R. (2018b). Electric pulse characteristics can enable species recognition in African weakly electric fish species. *Sci. Rep.* **8**, 10799. doi:10.1038/s41598-018-29132-z.

Nagelkerken, I., Dorenbosch, M., Verberk, W., La Cocheret de Morinière, E. and van der Velde, G. (2000). Day-night shifts of fishes between shallow-water biotopes of a Caribbean bay, with emphasis on the nocturnal feeding of Haemulidae and Lutjanidae. *Mar. Ecol. Prog. Ser.* **194**, 55–64. doi:10.3354/meps194055.

Nathan, R., Monk, C. T., Arlinghaus, R., Adam, T., Alós, J., Assaf, M., Baktoft, H., Beardsworth, C. E., Bertram, M. G. and Bijleveld, A. I. et al. (2022). Big-data approaches lead to an increased understanding of the ecology of animal movement. *Science*. **375**. doi:10.1126/science.abg1780.

Naylor, E. (2010). *Chronobiology of Marine Organisms*: Cambridge University Press.

Neilson, J. D. and Perry, R. I. (1990). Diel vertical migrations of marine fishes: an obligate or facultative process? In *Advances in marine biology volume 26,* pp. 115–168: Elsevier.

Nelson, J. A. (2016). Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. *J. Fish Biol.* **88**, 10–25. doi:10.1111/jfb.12824.

Nelson, J. S. (1994). *Fishes of the World*. Chichester, New York, Brisbane…: Wiley.

New, J. G. (1997). The evolution of vertebrate electrosensory systems. *Brain Behav. Evol.* **50**, 244–252. doi:10.1159/000113338.

Nieuwenbuys, R. and Nicholson, C. (1969). A survey of the general morphology, the fiber connections, and the possible functional significance of the gigantocerebellum of mormyrid fishes. In *Neurobiology of cerebellar evolution and development* (ed. R. Llinas). Chicago: American Medical Association.

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

Nilsson, G. E. (2007). Gill remodeling in fish - a new fashion or an ancient secret? *J. Exp. Biol.* **210**, 2403–2409. doi:10.1242/jeb.000281.

Nilsson, G. E., Dymowska, A. and Stecyk, J. A. W. (2012). New insights into the plasticity of gill structure. *Respir. Physiol. Neurobiol.* **184**, 214–222. doi:10.1016/j.resp.2012.07.012.

Nilsson, G. E., Rosen, P. R. and Johansson, D. (1993). Anoxic depression of spontaneous locomotor activity in crucian carp quantified by a computerized imaging technique. *J. Exp. Biol.* **180**, 153–162.

Nilsson, S. (1986). Control of gill blood flow. In *Fish physiology: recent advances* (ed. S. Nilsson and S. Holmgren), pp. 86–101. Dordrecht: Springer Netherlands.

Norin, T. and Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *J. Fish Biol.* **88**, 122–151. doi:10.1111/jfb.12796.

Norin, T. and Metcalfe, N. B. (2019). Ecological and evolutionary consequences of metabolic rate plasticity in response to environmental change. *Philos. Trans. R. Soc. B*. **374**, 20180180. doi:10.1098/rstb.2018.0180.

Nyboer, E. A. and Chapman, L. J. (2013). Movement and home range of introduced Nile perch (*Lates niloticus*) in Lake Nabugabo, Uganda: implications for ecological divergence and fisheries management. *Fish. Res.* **137**, 18–29. doi:10.1016/j.fishres.2012.08.003.

Ogutu-Ohwayo, R. (1993). The effects of predation by Nile perch, *Lates niloticus* L., on the fish of Lake Nabugabo, with suggestions for conservation of endangered endemic cichlids. *Conserv. Biol.* **7**, 701–711.

Omlin, T., Langevin, K. and Weber, J.-M. (2014). Exogenous lactate supply affects lactate kinetics of rainbow trout, not swimming performance. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**, R1018-24. doi:10.1152/ajpregu.00200.2014.

Omlin, T. and Weber, J.-M. (2010). Hypoxia stimulates lactate disposal in rainbow trout. *J. Exp. Biol.* **213**, 3802–3809. doi:10.1242/jeb.048512.

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

Pan, Y. K., Ern, R., Morrison, P. R., Brauner, C. J. and Esbaugh, A. J. (2017). Acclimation to prolonged hypoxia alters hemoglobin isoform expression and increases hemoglobin oxygen affinity and aerobic performance in a marine fish. *Sci. Rep.* **7**, 7834. doi:10.1038/s41598-017-07696-6.

Pankhurst, N. W. and Dedualj, M. (1994). Effects of capture and recovery on plasma levels of cortisol, lactate and gonadal steroids in a natural population of rainbow trout. *J. Fish Biol.* **45**, 1013–1025. doi:10.1111/j.1095-8649.1994.tb01069.x.

Paugy, D. (2010). An historical review of African freshwater ichthyology. *Freshw. Rev.* **3**, 1–32. doi:10.1608/FRJ-3.1.1.

Pelster, B. and Egg, M. (2018). Hypoxia-inducible transcription factors in fish: expression, function and interconnection with the circadian clock. *J. Exp. Biol.* **221**. doi:10.1242/jeb.163709.

Picq, S., Alda, F., Bermingham, E. and Krahe, R. (2016). Drift-driven evolution of electric signals in a Neotropical knifefish. *Evolution*. **70**, 2134–2144. doi:10.1111/evo.13010.

Picq, S., Sperling, J., Cheng, C. J., Carlson, B. A. and Gallant, J. R. (2020). Genetic drift does not sufficiently explain patterns of electric signal variation among populations of the mormyrid electric fish *Paramormyrops kingsleyae*. *Evolution*. **74**, 911–935. doi:10.1111/evo.13953.

Polakof, S. and Soengas, J. L. (2008a). Differential effects of in vivo and in vitro lactate treatments on liver carbohydrate metabolism of rainbow trout. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **151**, 205–210. doi:10.1016/j.cbpa.2008.06.025.

Polakof, S. and Soengas, J. L. (2008b). Involvement of lactate in glucose metabolism and glucosensing function in selected tissues of rainbow trout. *J. Exp. Biol.* **211**, 1075–1086. doi:10.1242/jeb.014050.

Poll, M., Gosse, J.-P. and Orts, S. (1982). Le genre *Campylomormyrus* Bleeker, 1874, étude systématique et description d'une espèce nouvelle (Pisces, Mormyridae). *Med. K. Belg. Inst. Nat. Wet.*

Pollock, M. S., Clarke, L. M. J. and Dubé, M. G. (2007). The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environ. Rev.* **15**, 1–14. doi:10.1139/a06-006.

Porteus, C., Hedrick, M. S., Hicks, J. W., Wang, T. and Milsom, W. K. (2011). Time domains of the hypoxic ventilatory response in ectothermic vertebrates. *J. Comp. Physiol. B.* **181**, 311–333. doi:10.1007/s00360-011-0554-6.

Pörtner, H. O. and Grieshaber, M. K. (1993). Critical PO_2(s) in oxyconforming and oxyregulating animals: Gas exchange, metabolic rate and the mode of energy production. In *The vertebrate gas transport cascade: adaptations to environment and mode of life* (ed. Bicudo, J. Eduardo P. W). Boca Raton, London: CRC Press.

Post, N. and Emde, G. von der (1999). The "novelty response" in an electric fish: Response properties and habituation. *Physiol. Behav.* **68**, 115–128. doi:10.1016/S0031-9384(99)00153-5.

Potts, L. B., Mandrak, N. E. and Chapman, L. J. (2021). Coping with climate change: Phenotypic plasticity in an imperilled freshwater fish in response to elevated water temperature. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* [Epub ahead of print]. doi:10.1002/aqc.3620.

Pringle, R. M. (2005). The origins of the Nile perch in Lake Victoria. *Bioscience*. **55**, 780–787.

Prosser, C. L., Barr, L. M., Pinc, R. D. and Lauer, C. Y. (1957). Acclimation of goldfish to low concentrations of oxygen. *Physiol. Zool.* **30**, 137–141.

Pusch, R., Emde, G. von der, Hollmann, M., Bacelo, J., Nöbel, S., Grant, K. and Engelmann, J. (2008). Active sensing in a mormyrid fish: Electric images and peripheral modifications of the signal carrier give evidence of dual foveation. *J. Exp. Biol.* **211**, 921–934. doi:10.1242/jeb.014175.

Ranjan, V., Rana, R., Khillan, K. and Chauhan, K. (2020). A comparative quality evaluation of point-of-care methodology for testing hemoglobin in blood donors by two different technologies. *Curr. Med. Res. Pract.* **10**, 90–92. doi:10.1016/j.cmrp.2020.03.007.

Reardon, E. E. and Chapman, L. J. (2008). Reproductive seasonality in a swamp-locked African cichlid. *Ecol. Freshw. Fish*. **17**, 20–29. doi:10.1111/j.1600-0633.2007.00251.x.

Reardon, E. E., Parisi, A., Krahe, R. and Chapman, L. J. (2011). Energetic constraints on electric signalling in wave-type weakly electric fishes. *J. Exp. Biol.* **214**, 4141–4150. doi:10.1242/jeb.059444.

Reebs, S. G. (1994). The anticipation of night by fry-retrieving convict cichlids. *Anim. Behav.* **48**, 89–95. doi:10.1006/anbe.1994.1214.

Reebs, S. G. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Rev. Fish Biol. Fisheries*. **12**, 349–371. doi:10.1023/A:1025371804611.

Reemeyer, J. E. and Rees, B. B. (2019). Standardizing the determination and interpretation of P_{crit} in fishes. *J. Exp. Biol.* **222**. doi:10.1242/jeb.210633.

Regan, M. D., Gill, I. S. and Richards, J. G. (2017). Calorespirometry reveals that goldfish prioritize aerobic metabolism over metabolic rate depression in all but near-anoxic environments. *J. Exp. Biol.* **220**, 564–572. doi:10.1242/jeb.145169.

Regan, M. D., Mandic, M., Dhillon, R. S., Lau, G. Y., Farrell, A. P., Schulte, P. M., Seibel, B. A., Speers-Roesch, B., Ultsch, G. R. and Richards, J. G. (2019). Don't throw the fish out with the respirometry water. *J. Exp. Biol.* **222**. doi:10.1242/jeb.200253.

Reppert, S. M. and Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature*. **418**, 935–941. doi:10.1038/nature00965.

Richards, J. G. (2009). Metabolic and molecular responses of fish to hypoxia. In *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 443–485. Amsterdam, Boston: Academic Press.

Richards, J. G. (2010). Metabolic rate suppression as a mechanism for surviving environmental challenge in fish. In *Aestivation: molecular and physiological aspects* (ed. C. Arturo Navas and J. E. Carvalho), pp. 113–139. Berlin, Heidelberg: Springer.

Richards, J. G. (2011). Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.* **214**, 191–199. doi:10.1242/jeb.047951.

Richards, J. G., Farrell, A. P. and Brauner, C. J., eds. (2009). *Hypoxia*. Amsterdam, Boston: Academic Press.

Riggs, A. F. (1988). The Bohr effect. *Annu. Rev. Physiol.* **50**, 181–204. doi:10.1146/annurev.ph.50.030188.001145.

Rogers, N. J., Urbina, M. A., Reardon, E. E., Mckenzie, D. J. and Wilson, R. W. (2016). A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (P_{crit}). *Conserv. Physiol.* **4**, cow012. doi:10.1093/conphys/cow012.

Rooker, J. R. and Dennis, G. D. (1991). Diel, lunar and seasonal changes in a mangrove fish assemblage off southwestern Puerto Rico. *Bull. Mar. Sci.* **49**, 684–698.

Rosewarne, P. J., Wilson, J. M. and Svendsen, J. C. (2016). Measuring maximum and standard metabolic rates using intermittent-flow respirometry: a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. *J. Fish Biol.* **88**, 265–283. doi:10.1111/jfb.12795.

Routley, M. H., Nilsson, G. E. and Renshaw, G. M. (2002). Exposure to hypoxia primes the respiratory and metabolic responses of the epaulette shark to progressive hypoxia. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **131**, 313–321. doi:10.1016/S1095-6433(01)00484-6.

Rowland, W. J. (1999). Studying visual cues in fish behavior: a review of ethological techniques. *Environ. Biol. Fish.* **56**, 285–305. doi:10.1023/A:1007517720723.

Saint-Paul, U. and Soares, G. M. (1987). Diurnal distribution and behavioral responses of fishes to extreme hypoxia in an Amazon floodplain lake. *Environ. Biol. Fish.* **20**, 91–104. doi:10.1007/BF00005289.

Salazar, V. L., Krahe, R. and Lewis, J. E. (2013). The energetics of electric organ discharge generation in gymnotiform weakly electric fish. *J. Exp. Biol.* **216**, 2459–2468. doi:10.1242/jeb.082735.

Salazar, V. L. and Stoddard, P. K. (2008). Sex differences in energetic costs explain sexual dimorphism in the circadian rhythm modulation of the electrocommunication signal of the gymnotiform fish *Brachyhypopomus pinnicaudatus*. *J. Exp. Biol.* **211**, 1012–1020. doi:10.1242/jeb.014795.

Sánchez-Vázquez, F. J., Madrid, J. A. and Zamora, S. (1995). Circadian rhythms of feeding activity in sea bass, *Dicentrarchus labrax* L.: dual phasing capacity of diel demand-feeding pattern. *J. Biol. Rhythms*. **10**, 256–266. doi:10.1177/074873049501000308.

Sandbichler, A. M., Jansen, B., Peer, B. A., Paulitsch, M., Pelster, B. and Egg, M. (2018). Metabolic plasticity enables circadian adaptation to acute hypoxia in zebrafish cells. *Cell. Physiol. Biochem.* **46**, 1159–1174. doi:10.1159/000489058.

Savitzky, A. and Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* **36**, 1627–1639. doi:10.1021/ac60214a047.

Scheffel, A. and Kramer, B. (1997). Electrocommunication and social behaviour in *Marcusenius senegalensis* (Mormyridae, Teleostei). *Ethology*. **103**, 404–420. doi:10.1111/j.1439-0310.1997.tb00156.x.

Schödel, J., Oikonomopoulos, S., Ragoussis, J., Pugh, C. W., Ratcliffe, P. J. and Mole, D. R. (2011). High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood*. **117**, e207-17. doi:10.1182/blood-2010-10-314427.

Schofield, P. J. and Chapman, L. J. (2000). Hypoxia tolerance of introduced Nile perch: implications for survival of indigenous fishes in the Lake Victoria basin. *Afr. Zool.* **35**, 35–42. doi:10.1080/15627020.2000.11407189.

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change*. **5**, 61–66. doi:10.1038/nclimate2457.

Seegers, L., Vos, L. de and Okeyo, D. O. (2003). Annotated Checklist of the Freshwater Fishes of Kenya (excluding the lacustrine haplochromines from Lake Victoria). *J. East Afr. Nat. Hist.* **92**, 11–47. doi:10.2982/0012-8317(2003)92[11:ACOTFF]2.0.CO;2.

Seibel, B. A., Andres, A., Birk, M. A., Burns, A. L., Shaw, C. T., Timpe, A. W. and Welsh, C. J. (2021). Oxygen supply capacity breathes new life into critical oxygen partial pressure (P_{crit}). *J. Exp. Biol.* **224**. doi:10.1242/jeb.242210.

Semesi, I. S., Beer, S. and Björk, M. (2009). Seagrass photosynthesis controls rates of calcification and photosynthesis of calcareous macroalgae in a tropical seagrass meadow. *Mar. Ecol. Prog. Ser.* **382**, 41–47. doi:10.3354/meps07973.

Shoubridge, E. A. and Hochachka, P. W. (1980). Ethanol: novel end product of vertebrate anaerobic metabolism. *Science*. **209**, 308–309. doi:10.1126/science.7384807.

Sikkel, P. C., Welicky, R. L., Artim, J. M., McCammon, A. M., Sellers, J. C., Coile, A. M. and Jenkins, W. G. (2017). Nocturnal migration reduces exposure to micropredation in a coral reef fish. *Bull. Mar. Sci.* **93**, 475–489. doi:10.5343/bms.2016.1021.

Silva, A. C., Perrone, R. and Macadar, O. (2007). Environmental, seasonal, and social modulations of basal activity in a weakly electric fish. *Physiol. Behav.* **90**, 525–536. doi:10.1016/j.physbeh.2006.11.003.

Snyder, J. B., Nelson, M. E., Burdick, J. W. and MacIver, M. A. (2007). Omnidirectional sensory and motor volumes in electric fish. *PLoS biology*. **5**, e301. doi:10.1371/journal.pbio.0050301.

Soares, M. G. M., Menezes, N. A. and Junk, W. J. (2006). Adaptations of fish species to oxygen depletion in a central Amazonian floodplain lake. *Hydrobiologia*. **568**, 353–367. doi:10.1007/s10750-006-0207-z.

Sollid, J. and Nilsson, G. E. (2006). Plasticity of respiratory structures - adaptive remodeling of fish gills induced by ambient oxygen and temperature. *Respir. Physiol. Neurobiol.* **154**, 241–251. doi:10.1016/j.resp.2006.02.006.

Somo, D. A., Onukwufor, J. O., Wood, C. M. and Richards, J. G. (2020). Interactive effects of temperature and hypoxia on diffusive water flux and oxygen uptake rate in the tidepool sculpin, *Oligocottus maculosus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **250**, 110781. doi:10.1016/j.cbpa.2020.110781.

Speers-Roesch, B., Norin, T. and Driedzic, W. R. (2018). The benefit of being still: energy savings during winter dormancy in fish come from inactivity and the cold, not from metabolic rate depression. *Proc. R. Soc. B*. **285**. doi:10.1098/rspb.2018.1593.

Stager, J. C., Westwood, J., Grzesik, D. and Cumming, B. F. (2005). A 5500-year environmental history of Lake Nabugabo, Uganda. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **218**, 347–354. doi:10.1016/j.palaeo.2004.12.025.

Steffensen, J. F. (2006). Oxygen consumption of fish exposed to hypoxia: are they all oxyregulators or are any oxyconformers. In *Fish physiology, toxicology, and water quality.* Proceedings of the 9th International Symposium, Capri, Italy (ed. C. J. Brauner, K. Suvajdzic, G. E. Nilsson and D. J. Randall), pp. 239–244. Athens.

Stoddard, P. K., Markham, M. R., Salazar, V. L. and Allee, S. (2007). Circadian rhythms in electric waveform structure and rate in the electric fish *Brachyhypopomus pinnicaudatus*. *Physiol. Behav.* **90**, 11–20. doi:10.1016/j.physbeh.2006.08.013.

Storey, K. B. and Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Q. Rev.Biol.* **65**, 145–174. doi:10.1086/416717.

Stramma, L., Schmidtko, S., Levin, L. A. and Johnson, G. C. (2010). Ocean oxygen minima expansions and their biological impacts. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **57**, 587–595. doi:10.1016/j.dsr.2010.01.005.

Su, G., Logez, M., Xu, J., Tao, S., Villéger, S. and Brosse, S. (2021). Human impacts on global freshwater fish biodiversity. *Science*. **371**, 835–838. doi:10.1126/science.abd3369.

Sukhum, K. V., Freiler, M. K., Wang, R. and Carlson, B. A. (2016). The costs of a big brain: extreme encephalization results in higher energetic demand and reduced hypoxia tolerance in weakly electric African fishes. *Proc. R. Soc. B*. **283**. doi:10.1098/rspb.2016.2157.

Sukhum, K. V., Shen, J. and Carlson, B. A. (2018). Extreme enlargement of the cerebellum in a clade of teleost fishes that evolved a novel active sensory system. *Curr. Biol.* **28**, 3857-3863.e3. doi:10.1016/j.cub.2018.10.038.

Svendsen, M. B. S., Bushnell, P. G. and Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *J. Fish Biol.* **88**, 26–50. doi:10.1111/jfb.12797.

Sweeney, B. M. and Hastings, J. W. (1960). Effects of temperature upon diurnal rhythms. *Cold Spring Harb. Symp. Quant. Biol.* **25**, 87–104. doi:10.1101/sqb.1960.025.01.009.

Szabo, T. (1960). Development of the electric organ of Mormyridae. *Nature*. **188**, 760–762. doi:10.1038/188760b0.

Szabo, T. (1965). Sense organs of the lateral line system in some electric fish of the Gymnotidae, Mormyridae and Gymnarchidae. *J. Morphol.* **117**, 229–249. doi:10.1002/jmor.1051170208.

Tabata, M., Minh-Nyo, M., Niwa, H. and Oguri, M. (1989). Circadian rhythm of locomotor activity in a teleost, *Silurus asotus*. *Zool. Sci.* **6**, 367–375.

Talling, J. F. (1957). Diurnal changes of stratification and photosynthesis in some tropical African waters. *Proc. R. Soc. B*. **147**, 57–83. doi:10.1098/rspb.1957.0036.

Timmerman, C. M. and Chapman, L. J. (2004). Behavioral and physiological compensation for chronic hypoxia in the sailfin molly (*Poecilia latipinna*). *Physiol. Biochem. Zool.* **77**, 601–610. doi:10.1086/421754.

Toerring, M. J. and Serrier, J. (1978). Influence of water temperature on the electric organ discharge (EOD) of the weakly electric fish *Marcusenius cyprinoides* (Mormyridae). *J. Exp. Biol.* **74**, 133–150. doi:10.1242/jeb.74.1.133.

Townsend, S. A. (1999). The seasonal pattern of dissolved oxygen, and hypolimnetic deoxygenation, in two tropical Australian reservoirs. *Lakes Reserv.* **4**, 41–53. doi:10.1046/j.1440-1770.1999.00077.x.

Trabold, O., Wagner, S., Wicke, C., Scheuenstuhl, H., Hussain, M. Z., Rosen, N., Seremetiev, A., Becker, H. D. and Hunt, T. K. (2003). Lactate and oxygen constitute a fundamental regulatory mechanism in wound healing. *Wound Repair Regen.* **11**, 504–509. doi:10.1046/j.1524-475x.2003.11621.x.

Trewavas, E. (1933). Scientific results of the Cambridge Expedition to the East African Lakes, 1930-1.-11. The cichlid fishes. *Zool. J. Linn. Soc.* **38**, 309–341. doi:10.1111/j.1096-3642.1933.tb00062.x.

Tseng, Y.-C., Liu, S.-T., Hu, M. Y., Chen, R.-D., Lee, J.-R. and Hwang, P.-P. (2014). Brain functioning under acute hypothermic stress supported by dynamic monocarboxylate utilization and transport in ectothermic fish. *Front. Zool.* **11**. doi:10.1186/s12983-014-0053-1.

Ultsch, G. R., Boschung, H. and Ross, M. J. (1978). Metabolism, critical oxygen tension, and habitat selection in darters (*Etheostoma*). *Ecology*. **59**, 99–107.

Ultsch, G. R. and Nordlie, F. G. (2019). The case for reporting PO_2 (partial pressure of oxygen), in addition to DO (dissolved oxygen), in studies of aquatic systems. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **235**, 66–68. doi:10.1016/j.cbpa.2019.05.007.

van Raaij, M. T. M., Pit, D. S. S., Balm, P. H. M., Steffens, A. B. and van den Thillart, G. (1996). Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. *Horm. Behav.* **30**, 85–92.

van Winkle, W. and Mangum, C. (1975). Oxyconformers and oxyregulators: A quantitative index. *J. Exp. Mar. Biol. Ecol.* **17**, 103–110. doi:10.1016/0022-0981(75)90025-8.

Vanderpham, J. P., Nakagawa, S. and Closs, G. P. (2012). Diel variation in use of cover and feeding activity of a benthic freshwater fish in response to olfactory cues of a diurnal predator. *Environ. Biol. Fish.* **93**, 547–556. doi:10.1007/s10641-011-9949-1.

Vaquer-Sunyer, R. and Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *PNAS*. **105**, 15452–15457. doi:10.1073/pnas.0803833105.

Vatine, G., Vallone, D., Gothilf, Y. and Foulkes, N. S. (2011). It's time to swim! Zebrafish and the circadian clock. *FEBS letters*. **585**, 1485–1494. doi:10.1016/j.febslet.2011.04.007.

Virani, N. A. and Rees, B. B. (2000). Oxygen consumption, blood lactate and inter-individual variation in the gulf killifish, *Fundulus grandis*, during hypoxia and

recovery. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **126**, 397–405. doi:10.1016/S1095-6433(00)00219-1.

Volpato, G. L. and Trajano, E. (2005). Biological rhythms. In *The physiology of tropical fishes,* pp. 101–153: Academic Press.

Walsh, J. (1773). Of the electric property of the torpedo. *Philos. Trans. R. Soc.* **63**, 461–480. doi:10.1098/rstl.1773.0039.

Wang, T., Lefevre, S., Thanh Huong, D. T., van Cong, N. and Bayley, M. (2009). The effects of hypoxia on growth and digestion. In *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 361–396. Amsterdam, Boston: Academic Press.

Wannamaker, C. M. and Rice, J. A. (2000). Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. *J. Exp. Mar. Biol. Ecol.* **249**, 145–163. doi:10.1016/S0022-0981(00)00160-X.

Wells, R. M. G. (2009). Blood-gas transport and hemoglobin function. In *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 255–299. Amsterdam, Boston: Academic Press.

Wells, R. M. G. and Baldwin, J. (2006). Plasma lactate and glucose flushes following burst swimming in silver trevally (*Pseudocaranx dentex*: Carangidae) support the "releaser" hypothesis. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **143**, 347–352. doi:10.1016/j.cbpa.2005.12.015.

Wells, R. M. G., Baldwin, J., Seymour, R. S., Christian, K. and Brittain, T. (2005). Red blood cell function and haematology in two tropical freshwater fishes from Australia. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **141**, 87–93. doi:10.1016/j.cbpb.2005.04.005.

Wenger, R. H., Stiehl, D. P. and Camenisch, G. (2005). Integration of oxygen signaling at the consensus HRE. *Sci. STKE*. **2005**, re12. doi:10.1126/stke.3062005re12.

Whitmore, D., Foulkes, N. S., Strähle, U. and Sassone-Corsi, P. (1998). Zebrafish Clock rhythmic expression reveals independent peripheral circadian oscillators. *Nat. Neurosci.* **1**, 701–707. doi:10.1038/3703.

Whitmore, D., Foulkes, N. S. and Sassone-Corsi, P. (2000). Light acts directly on organs and cells in culture to set the vertebrate circadian clock. *Nature*. **404**, 87–91. doi:10.1038/35003589.

Wood, C. M. (2018). The fallacy of the P_{crit} - are there more useful alternatives? *J. Exp. Biol.* **221**. doi:10.1242/jeb.163717.

Wood, C. M. and Eom, J. (2021). The osmorespiratory compromise in the fish gill. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **254**, 110895. doi:10.1016/j.cbpa.2021.110895.

Wood, S. C. and Johansen, K. (1972). Adaptation to hypoxia by increased HbO_2 affinity and decreased red cell ATP concentration. *Nat. New Biol.* **237**, 278–279. doi:10.1038/newbio237278a0.

Wu, C. H. (1984). Electric fish and the discovery of animal electricity. *Am. Sci.* **72**, 598–607.

Wulund, L. and Reddy, A. B. (2015). A brief history of circadian time: The emergence of redox oscillations as a novel component of biological rhythms. *Perspect. Sci.* **6**, 27–37. doi:10.1016/j.pisc.2015.08.002.

Xu-Friedman, M. A. and Hopkins, C. D. (1999). Central mechanisms of temporal analysis in the knollenorgan pathway of mormyrid electric fish. *J. Exp. Biol.* **202**, 1311–1318. doi:10.1242/jeb.202.10.1311.

Yeager, D. P. and Ultsch, G. R. (1989). Physiological regulation and conformation: a BASIC program for the determination of critical points. *Physiol. Zool.* **62**, 888–907. doi:10.1086/physzool.62.4.30157935.

Zubizarreta, L., Quintana, L., Hernández, D., Teixeira de Mello, F., Meerhoff, M., Massaaki Honji, R., Guimarães Moreira, R. and Silva, A. (2020). Seasonal and social factors associated with spacing in a wild territorial electric fish. *PloS one*. **15**, e0228976. doi:10.1371/journal.pone.0228976.

Appendix

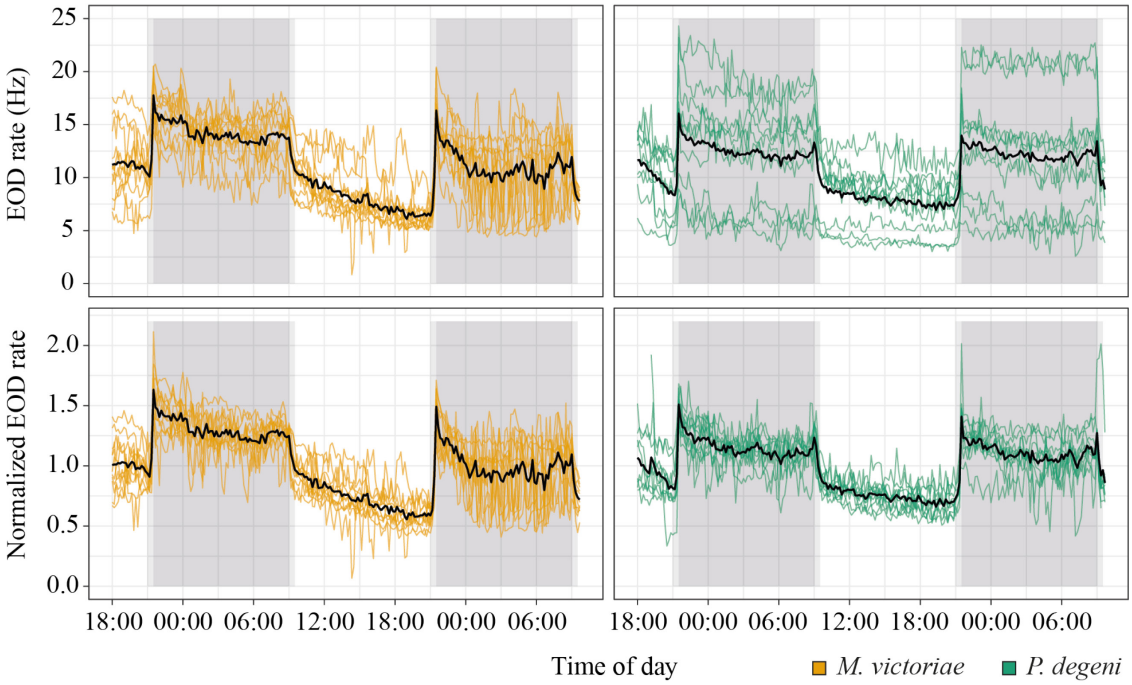


Figure A1 - Effect of normalization on EOD rates of *M. victoriae* (n = 11) and *P. degeni* (n = 10) in the shuttle-box. Top row: raw EOD rates. Bottom row: normalized EOD rates (divided by overall average EOD rate per fish). Colored lines represent individual fish, black lines represent the average over all fish, grey shaded areas represent periods of dusk/dawn (light grey) and complete darkness (dark grey).

Table A1 - Parameters and classification fidelity of different mixture discriminant analysis (MDA) models used for species discrimination in in-situ recordings.

MDA Model	Parameters	Classification Fidelity
Sparse	Peak-peak duration Peak-peak amplitude ratio Peak FFT Frequency	98.3 %
Alt. sparse	Peak-peak duration Peak FFT Frequency Slope at zero crossing	97.2 %
Medium	Peak-peak duration Peak-peak amplitude ratio Peak FFT Frequency Slope at zero crossing	98.1 %
Full	Peak-peak duration Peak-peak amplitude ratio Peak FFT Frequency Slope at zero crossing Peak 1 duration at 60% amplitude Peak 2 duration at 60% amplitude	97.9 %

FFT, fast Fourier transform

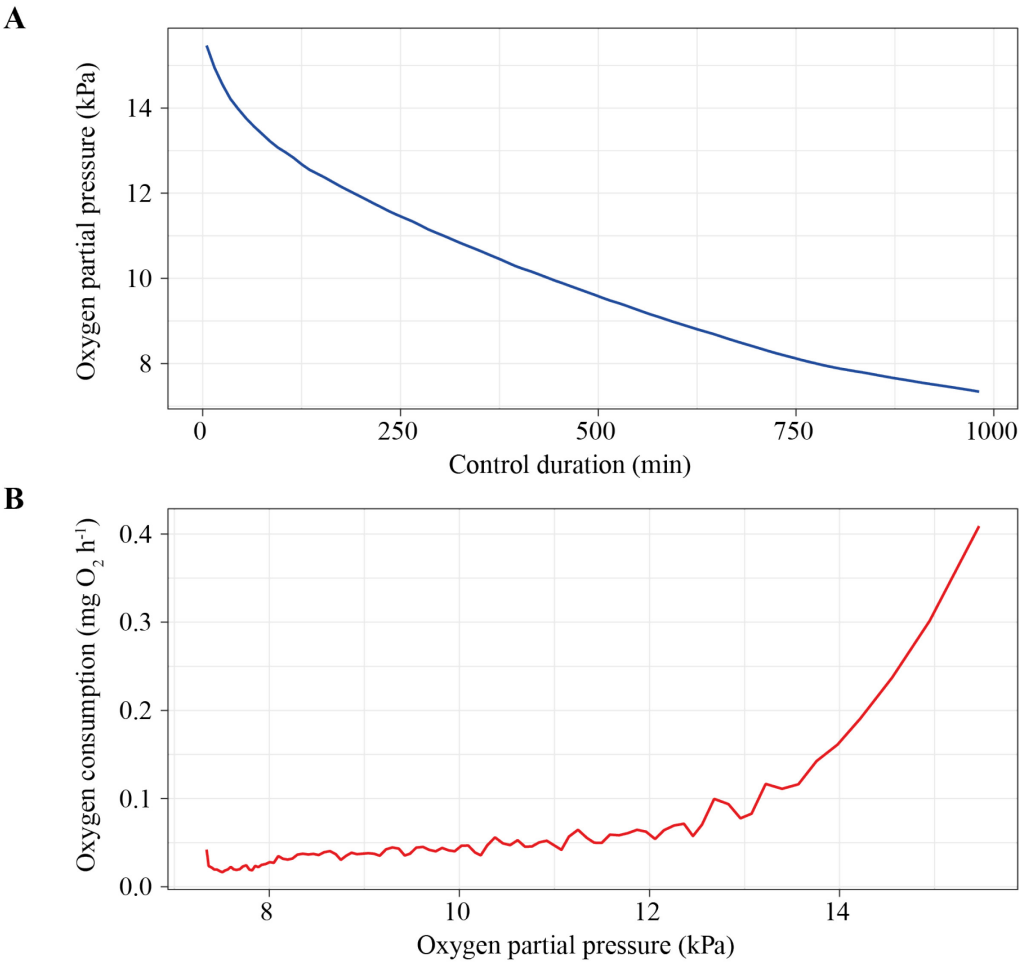


Figure A2 - Background respiration during long-term control measurement without fish. (A) Oxygen partial pressure as a function of time. (B) Oxygen consumption rate as a function of oxygen partial pressure. The oxygen consumption is due to biological activity in the water that was used for respirometry. Above a PO_2 of 12 kPa, background respiration was considerably elevated.

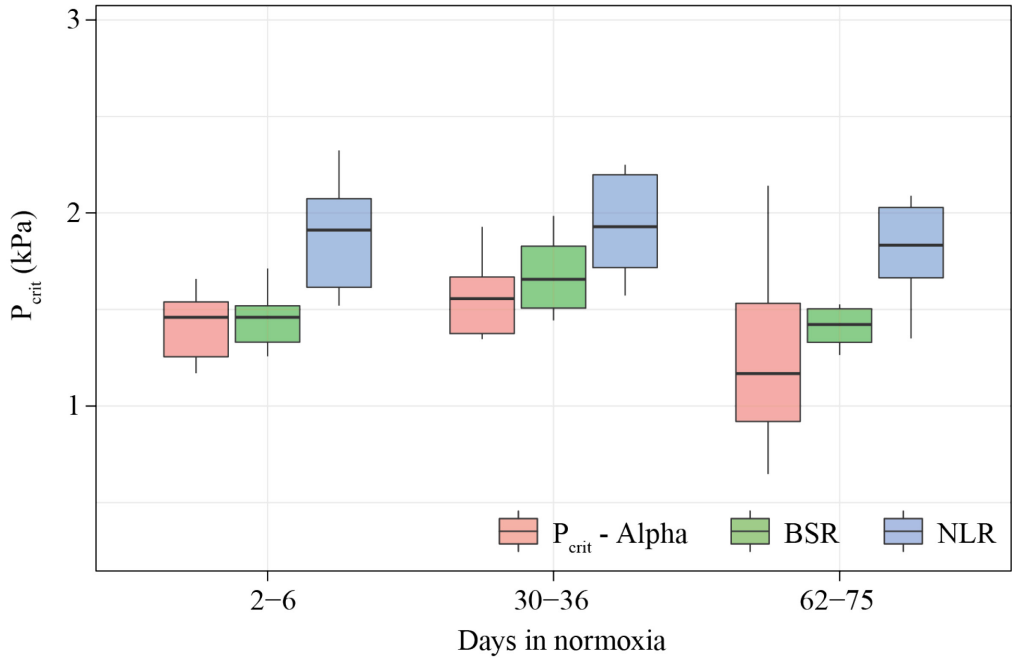


Figure A3 - Comparison of three different approaches for P_{crit} estimation. There were no statistically significant differences between approaches (ANCOVA with Tukey post-hoc test: P > 0.05). BSR: broken-stick regression, NLR: non-linear regression, see section 2.3.2.2 for reference.

Table A2 - Effect of statistical approach on P_{crit} estimates.

ANCOVA	Effect	F	DF	P	η^2_g
	Approach	6.308	2, 45	**0.004**	0.219
	Acclimation group	1.452	2, 45	0.061	0.061
	Approach x Acclimation group	0.342	2, 45	0.848	0.030

Tukey	Acclimation group	Comparison		P	Diff.
	2-10 days	P_{crit} - Alpha	BSR	0.989	0.036
		P_{crit} - Alpha	NLR	0.584	0.261
		BSR	NLR	0.668	0.225
	30-36 days	P_{crit} - Alpha	BSR	0.668	0.188
		P_{crit} - Alpha	NLR	0.052	0.558
		BSR	NLR	0.234	0.370
	62-75 days	P_{crit} - Alpha	BSR	0.705	0.207
		P_{crit} - Alpha	NLR	0.058	0.647
		BSR	NLR	0.232	0.441

DF, degrees of freedom; Diff., mean difference; P_{crit}, critical oxygen tension; η^2_g, generalized eta squared for effect size. BSR: broken-stick regression, NLR: non-linear regression. Bold numbers indicate statistically significant outcomes.

Eigenständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig verfasst habe und keine anderen Hilfsmittel und Quellen als die angegebenen verwendet habe. Sämtliche fremde Quellen wurden als solche markiert und ich habe gemäß den gängigen wissenschaftlichen Regeln zitiert. Es hat keine Zusammenarbeit mit gewerblichen Promotionsberatern stattgefunden. Die Dissertation oder Teile davon sind nicht bereits bei einer anderen wissenschaftlichen Einrichtung eingereicht, angenommen oder abgelehnt. Zugleich sei erwähnt, dass nach der Erstellung des Manuskripts für diese Dissertation Teile davon in folgendem Artikel veröffentlicht wurden:

Mucha, S., Oehlert, F., Chapman, L. J. and Krahe, R. (2022). A spark in the dark: uncovering natural activity patterns of mormyrid weakly electric fish. *Front. Ecol. Evol.* **10,** 870043. doi:10.3389/fevo.2022.870043.

Dazu gehören Textpassagen und Abbildungen aus den folgenden Abschnitten: 1.2, 1.5, 2.1, 2.2, 3.1, 3.2, 4.2, 4.3.

Ich habe mich nicht anderweitig um einen Doktorgrad beworben bzw. einen entsprechenden Doktorgrad erworben. Die Grundsätze der Humboldt-Universität zu Berlin zur Sicherung guter wissenschaftlicher Praxis wurden eingehalten.

Berlin,

_____

Stefan Mucha