Thermal tolerance of range-shifting fishes in Western Uganda

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January 2025

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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ABSTRACT

As ecological conditions change across the globe species are shifting their geographic ranges in order to track their required habitat. As ectotherms, fish are highly dependent on their external environment in regulating their physiological processes, and many studies have already shown that increased water temperatures can have a multitude of negative effects on fish physiology. Some species of fishes show thermal acclimation capacity, which may allow them to persist in their current habitats in the face of warming waters. Despite this, distributional shifts through range contractions and expansions have been reported in many species of fishes, whereby fish are shifting their distribution to cooler habitats to match their thermal requirements. These range-shifting species are likely to encounter other stressors, which might then require rapid changes to facilitate persistence. This thesis explores the relationship between thermal tolerance and range shifts and how range expansion along one environmental gradient is affected by response to a second strong environmental gradient.

In chapter 1, I explored the relationship between thermal tolerance and range shifts in different fish species of the cyprinid genus *Enteromius*. *Enteromius apleurogramma* has recently expanded its range into a northern upstream tributary of the Mpanga River in Kibale National Park, Uganda. This tributary system was previously only inhabited by the congener *Enteromius neumayeri* and the clariid catfish *Clarias liocephalus.* In addition, other congeneric species in the south of *E. apleurogramma's* distribution, *Enteromius alberti* and *Enteromius kerstenii*, have not experienced such range-shifts. We quantified both the lower and upper thermal limits of *E. apleurogramma* across its range in the Mpanga River, as well of those of the upstream and downstream congeners. CT_{max} did not differ significantly among *E. apleurogramma*, *E. alberti*, and *E. kerstenii*; and the CT_{max} of *E. apleurogramma* was significantly lower than that of *E. neumayeri*. For CT_{min} we found a significant level of interspecific variation with *E. apleurogramma* having the lowest CT_{min} followed by *E. kerstenii* and *E. alberti*. Our results suggest that CT_{min} may be an important thermal metric when it comes to predicting species' range contraction at the southern end of their distribution.

In chapter 2, I explored how range expansion along a thermal gradient is affected by response to a second strong environmental gradient – dissolved oxygen. To accomplish this I focused on a different species within the same river system, *Platypanchax modestus*, which is also shifting its distribution upstream to a higher altitude. Interestingly, the putative source population of this species inhabits normoxic waters in its home range, but it has expanded its range upstream into both high-oxygen streams and low-oxygen swamps. We used this system to test whether fishes that have shifted their range into upstream hypoxic habitats show evidence of increased oxygen uptake capacity relative to conspecifics that have shifted their range upstream into normoxic habitats and whether there is a relationship between CT_{max} and oxygen concentration and/or water temperature of the habitat. Gill analysis suggested differences in oxygen uptake capacity among *P. modestus* populations with the population from the original river habitat having the smallest total gill filament length. *P. modestus* that expanded its range into a hypoxic swamp had the largest gills suggesting greater oxygen uptake capacity in the new hypoxic range of this species. For thermal tolerance, we found a weak positive relationship between CT_{max} and environmental dissolved oxygen but no relationship between CT_{max} and water temperature. Our results support the hypothesis for the oxygen dependency of upper thermal tolerance in *P. modestus* and suggest that oxygen uptake capacity may be driven by environmental effects.

RÉSUMÉ

À mesure que les conditions écologiques changent à travers le monde, les espèces déplacent leurs aires de répartition géographique afin de suivre l'habitat dont elles ont besoin. En tant qu'ectothermes, les poissons dépendent fortement de leur environnement pour réguler leurs processus physiologiques, et de nombreuses études ont déjà montré que l'augmentation de la température de l'eau peut avoir plusieurs effets négatifs sur la physiologie des poissons. Certaines espèces de poissons présentent une capacité d'acclimatation thermique, ce qui peut leur permettre de persister dans leurs habitats actuels face au réchauffement des eaux. Malgré cela, des changements de répartition par contraction et expansion de l'aire de répartition ont été signalés chez de nombreuses espèces de poissons, dans lesquelles les poissons déplaçant leur répartition vers des habitats plus frais pour répondre à leurs besoins thermiques. Les espèces qui changent d'aire de répartition sont susceptibles de rencontrer d'autres facteurs de stress, qui pourraient alors nécessiter des changements rapides pour faciliter la persistance. Cette thèse explore la relation entre la tolérance thermique et les changements d'aire de répartition et comment l'expansion de l'aire de répartition le long d'un gradient environnemental est affectée par la réponse à un deuxième gradient environnemental.

Dans le premier chapitre, j’ai exploré la relation entre la tolérance thermique et les changements d’aire de répartition chez différentes espèces de poissons du genre cyprinidé *Enteromius*. *Enteromius apleurogramma* a récemment étendu son aire de répartition à un affluent nord en amont de la rivière Mpanga dans le parc national de Kibale, en Ouganda. Ce système d’affluents n’était auparavant habité que par le congénère *Enteromius neumayeri* et le poisson-chat *Clarias liocephalus*. De plus, d’autres espèces congénères du sud de l’aire de répartition d’*E. apleurogramma, Enteromius alberti* et *Enteromius kerstenii*, n’ont pas connu de tels changements d’aire de répartition. Nous avons quantifié les limites thermiques inférieures et supérieures d’*E. apleurogramma* dans son aire de répartition, ainsi que celles des congénères en amont et en aval. Le $\mathrm{CT}_{\mathrm{max}}$ ne différait pas significativement entre *E. apleurogramma*, *E. alberti* et *E. kerstenii* ; et le $\mathrm{CT}_{\mathrm{max}}$ d’*E. apleurogramma* était significativement inférieur à celui d’*E. neumayeri*. Pour $\mathrm{CT}_{\mathrm{min}}$, nous avons trouvé un niveau significatif de variation interspécifique, *E. apleurogramma* ayant le $\mathrm{CT}_{\mathrm{min}}$ le plus bas, suivi par *E. kerstenii* et *E. alberti*. Nos résultats

suggèrent que CT_{min} peut être une mesure thermique importante lorsqu'il s'agit de prédire la contraction de l'aire de répartition des espèces à l'extrémité sud de leur distribution.

Dans le deuxième chapitre, j’ai étudié comment l’expansion de l’aire de répartition le long d’un gradient thermique est affectée par la réponse à un deuxième gradient environnemental – l’oxygène dissous. Pour y parvenir, nous nous sommes concentrés sur une espèce différente au sein du même système fluvial, *Platypanchax modestus*, qui déplace également sa répartition en amont vers une altitude plus élevée. Il est intéressant de noter que la population source de cette espèce habite des eaux normoxiques dans son aire de répartition, mais elle a étendu son aire de répartition en amont dans des cours d’eau riches en oxygène et des marais pauvres en oxygène. Nous avons utilisé ce système pour vérifier si les poissons qui ont déplacé leur aire de répartition vers des habitats hypoxiques en amont montrent des signes d’une capacité d’absorption d’oxygène accrue par rapport aux congénères qui ont déplacé leur aire de répartition vers l’amont dans des habitats normoxiques et s’il existe une relation entre le CT_{max} et la concentration en oxygène et/ou la température de l’eau de l’habitat. L’analyse des branchies a suggéré des différences dans la capacité d’absorption d’oxygène parmi les populations de *P. modestus*, la population de l’habitat fluvial d’origine ayant la plus petite longueur totale de filaments branchiaux. *P. modestus* qui a étendu son aire de répartition dans le marais hypoxique avait les plus grandes branchies, ce qui suggère une plus grande capacité d'absorption d'oxygène dans la nouvelle aire hypoxique de cette espèce. Pour la tolérance thermique, nous avons trouvé une faible relation positive entre CT_{max} et l'oxygène dissous, mais aucune relation entre CT_{max} et la température de l'eau. Nos résultats soutiennent l'hypothèse de la dépendance à l'oxygène de la tolérance thermique supérieure chez *P. modestus* et suggèrent que la capacité d'absorption d'oxygène peut être déterminée par des effets environnementaux.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor Dr. Lauren Chapman – I am incredibly grateful for your support throughout my entire Master's Degree and without you none of this would be possible. Thank you for sharing your expertise and for all you have taught me, both within and outside of aquatic ecology. You have been one of my most influential role models, and I hope that someday I can follow in your footsteps.

I would also like to thank my committee members Dr. Andrew Hendry and Dr. Rowan Barrett – your guidance and feedback in designing my project were extremely valuable. I would like to thank Professor David Green for the time and effort that he dedicated to reading my thesis - I am incredibly grateful for the insightful comments and valuable feedback that you have given me.

I owe an enormous debt of gratitude to the entire Kibale Fish and Monkey Project Team: Emmanuel, Nicolas, Mark, Robert, Clovis, Tusiime, and Peter – each of you have taught me something new and unique which gave me new perspectives. A special thanks to Emmanuel, Nicolas, and Mark, for sharing your knowledge with me, for being patient with me, teaching me, telling me stories, answering all my questions, and for keeping me safe every day. I will always cherish the silly moments that I have had in and out of the field with you. I also need to thank our amazing team of ladies at the Makerere University Biological Field Station: Goretti Adyeeri, Rebecca, and Grace – thank you for taking care of us, for teaching me how to cook, and for making the chapati we used as fish bait. It was always a pleasure to sit in the kitchen with you and chat over a cup of tea or help in preparing meals. I admire the three of you very much. I would also like to thank the rest of the staff at the Makerere University Biological Field Station including Dorothy who handled all of our UWA/UNCST related permits and planning as well as nurse Dennis who was available round the clock in case of emergency. I would like to especially thank Emmanuel Opito – for all your help with field work, for all the time you spent with me in and out of the lab, and for always having such positive energy. I am incredibly grateful to have made such an amazing friend and will forever cherish all the moments of laughter I have shared with you. And of course, last but not least I am grateful to Dr. Patrick Omeja – words cannot describe how much I appreciate your support. Thank you for being there through the highs and the lows, no matter the time of day. I would not have been able to do it without you there. It was always a pleasure to be your “passenger buddy” as we ate maize and chatted during long car rides. Each and every one of you will forever have a special place in my heart, thank you for making Kibale feel like home.

The members of the Chapman lab also deserve appreciation for their help and support throughout my Master’s Degree. Thank you all for your continuous input and feedback, especially in presentations and conferences. Special thanks to Igor Mitrofanov for his work on gill dissections. David- thank you for teaching me everything I need to know about R studio and for the countless trouble shooting zoom calls when things did not work out. Veronica, Liana, and David – thank your for taking the time to read over my thesis, your comments and feedback were extremely valuable and words cannot describe how much I appreciate your support. Most importantly, I need to thank my colleague Liana – for all your support during our three months in Kibale, I am so grateful that this adventure brought me such an amazing friendship. There is no one else I would have rather shared this experience with, and I will forever cherish the memories I made here with you.

Thank you to all my friends and family – for your love and encouragement during my degree and for supporting me through the highs and the lows. For listening to me talk endlessly about fish to calming me down in moments of doubt and everything in between. And a special thank you to my grandparents, Leo and Patsy, who try so hard to understand my project only to retain “she studies fish”.

Finally, thank you to every adorable *Platypanchax modestus, Enteromius apleurogramma*, *Enteromius neumayeri*, *Enteromius alberti* and *Enteromius kerstenii*. Without them this study would not be possible.

PREFACE

Thesis Format

This thesis is written as a collection of two separate manuscripts following the guidelines provided by McGill University for manuscript based theses. It includes a general abstract both in French and in English, a general introduction, two manuscripts linked by a statement, a general discussion, and a conclusion. All references are formatted in the style of Ecology and listed at the end of each respective manuscript.

The titles of the manuscripts presented in this thesis are as follow:

1. Thermal tolerance of congeneric fishes in Kibale National Park, Uganda: Implications for range shifts.
2. Thermal tolerance of a range-shifting killifish in the face of hypoxia.

Author contributions

This thesis is the product of my own independent research and field work conducted under the supervision of Dr. Lauren J. Chapman of the Department of Biology at McGill University. For the first chapter of my thesis, Dr. Chapman proposed the study system and species, which was part of a larger FRQNT research proposal in collaboration with my committee members Dr. Andrew P. Hendry and Dr. Rowan D.H. Barrett (Department of Biology, McGill University). All of the experimental design, field work, and laboratory experiments were designed in consultation with Dr. Chapman and carried out at the Makerere University Biological Field Station in Kibale National Park, Uganda under the permission of the Ugandan Wildlife Authority and under clearance from the Ugandan National Council for Science and Technology. During field work, I was assisted by field assistants, Amooti Emmanuel Aliganyira and Amooti Nicholas Bahati who helped with field work, water quality maintenance, and collection of limnological data. I compiled and analyzed all the data from this study to report the results in a written manuscript that was co-authored by Dr. Chapman who provided feedback for the entirety of the writing and analyses.

For the second chapter of my thesis, Dr. Chapman and I designed a study that would complement the first chapter of my thesis by focusing on a different species within the same river system undergoing the same type of response as the one studied in my first chapter. Again, all of the experimental design, field work, and laboratory experiments were designed in consultation with Dr. Chapman and carried out at the Makerere University Biological Field Station in Kibale National Park, Uganda under the permission of the Ugandan Wildlife Authority and under clearance from the Ugandan National Council for Science and Technology. During field work, I was assisted by two field assistants, Amooti Emmanuel Aliganyira and Akiiki Mark who helped with field work, water quality maintenance and the collection of limnological data. I was also assisted by Dr. Patrick Omeja and Emmanuel Opito, who played a key role in fish sampling for field sites located at the park boundary. Igor Mitrofanov, a research assistant in the Chapman lab worked on the gill dissections. All of the data collected during this study were compiled and analyzed and the results reported in a manuscript that was co-authored by Dr. Chapman, who provided feedback and revisions for both writing and analyses.

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LIST OF ABBREVIATIONS

BIH- Bihehe
CT_{max}- Critical Thermal Maximum
CT_{min}- Critical Thermal Minimum
EA- *Enteromius apleurogramma*
EC- *Enteromius alberti (synonym E. cercops)*
EK- *Enteromius kerstenii*
EN- *Enteromius neumayeri*
ISE- Inlet Stream East
ISW- Inlet Stream West
IUCN- International Union of Conservation of Nature
MIK- Mikana Stream Up
PM- *Platypanchax modestus*
RSWL- Rwembaita Swamp Low
RSWM- Rwembaita Swamp Middle
RSWU- Rwembaita Swamp Up
SEB- Sebitoli
SEM- Standard Error of the Mean
T_{ag}- Agitation Temperature
T_{env}- Environmental Temperature
T_{opt}- Optimal Temperature
TFN- Total Filament Number
TGFL- Total Gill Filament Length
TSM_{max}- Thermal Safety Margin for Upper Thermal Limits
TSM_{min}- Thermal Safety Margin for Lower Thermal Limits
TSM_{tag}- Thermal Safety Margin for Agitation Temperature
UNCST- Uganda National Council for Science and Technology
UWA- Uganda Wildlife Authority

GENERAL INTRODUCTION

Range shifts

Shifts in species distributions are a common response to changing environmental and ecological conditions (Diamond 2018; Lawlor et al. 2024). Many environmental factors contribute to species' range shifts including habitat loss, invasive species, changing climatic conditions such as precipitation patterns and temperature, and changes in water chemistry including acidification and salinization, amongst many others (Coristine and Kerr 2011; Segurado et al. 2016; Yesuf et al. 2021; Mitchell et al. 2023; da Silva and Diamond 2024; Wesselmann et al. 2024). Of these, climate change (e.g., global temperature rise) is often hypothesized as one of the main drivers of contemporary range-shifts, and many studies have already shown that species' across many taxa are shifting their distribution towards cooler habitats, be it towards higher altitudes, latitudes, or greater depth to meet their thermal requirements (Moreno-Rueda et al. 2011; Alofs et al. 2014; Comte et al. 2014; Pecl et al. 2017; Williams and Blois 2018; Gomez-Ruiz and Lacher 2019; Hu et al. 2020; Pinsky et al. 2020; Champion et al. 2021; Couet et al. 2022; Rubenstein et al. 2023; Womersley et al. 2024). Based on the BioShifts database on range shift observations, an estimated 59% of documented range shifts are directionally consistent with climate change (Lawlor et al. 2024). Range shifts can have both community and ecosystem level impacts by reshaping ecological communities, altering ecosystem functions, and altering the provision of ecosystem services, which can have major impacts on human health (Pecl et al. 2017; Guo et al. 2018; Rubenstein et al. 2023). As many environmental stressors are expected to increase in frequency and intensity, the frequency of range shifts is expected to increase as well (Lonhart et al. 2019; Belleau et al. 2021).

Freshwaters and Freshwater Fishes

Freshwater systems have suffered from multiple anthropogenic perturbations over the past century including impacts from deforestation, agriculture, input of contaminants and fertilizers, habitat alterations (e.g., dams), invasive species, and overexploitation (Kremsner and Schnug 2002; Ricciardi and MacIsaac 2011; Brown et al. 2015; Barbarossa et al. 2020; Lo et al.

2020). In addition, freshwater ecosystems are among the most vulnerable to global warming, which has resulted in warmer waters, environmental extremes, and changes to hydrological regimes (Capon et al. 2021; Piczak et al. 2023). Consequently, these ecosystems are expericing more severe biodiversity loss relative to other types of ecosystems (Piczak et al. 2023). For example, freshwater fishes make up 40% of all fish species and 25% of the vertebrates on Earth; however, it is estimated that one third of freshwater fishes are threatened with extinction (WWF 2021), making them one of the most threatened groups (Korkmaz et al. 2023). The sensitivity of freshwater ecosystems to environmental stressors is in part due to the facts that i) both water quality and flow regimes are impacted by environmental changes and anthropogenic activities including nutrient pollution, influx of contaminants, deforestation, hypoxia, and habitat modification, which can have both independent and interactive effects; and ii) many species within freshwater habitats have limited dispersal abilities due to fragmentation and ecological isolation which affects the ability of species' to shift their ranges (Woodword et al. 2010; Segurado et al. 2016; Jackson et al. 2017; Roman et al. 2019; Kong et al. 2022; Mitchell et al. 2023). If a species is not able to shift its distribution, it may not be able to avoid a given stressor.

The ecological impact of environmental stressors also depends on the physiological sensitivity of organisms (Deutsch et al. 2008). For example, ectothermic species, such as fishes, are extremely vulnerable to changes in temperature because they rely on their external environment in regulating their internal body temperature, and thus, many of their physiological processes (Huey and Stephenson 1979; McNab, 2002; Li et al. 2013; Christensen et al. 2021; Lindmark et al. 2022; Agarwal et al. 2024). The thermal window of an ectotherm is the temperature range between an organisms' upper and lower critical thermal limits and is defined as the range of temperatures that allow for the survival of a species (Huey and Stephenson 1979; Pörtner 2010; Schulte et al. 2015; Thyrring et al. 2019; Fernandes et al. 2023). As temperature begins to exceed thermal limits, species exhibit physiological disorganization and loss of equilibrium that can eventually lead to death (McDonnell and Chapman 2015). As such, increases in water temperature associated with deforestation, climate change and other natural or anthropogenically induced changes are thought to impact the physiology and behaviour of many ectotherms (Macusi et al. 2015; Neubauer and Andersen 2019; Brule et al 2022; Francispillai and Chapman 2024). Increases in water temperature can affect the reproductive success of fishes

(Servili et al. 2020; Brule et al. 2022; Pilakouta et al. 2022; Jones et al. 2023), reproductive phenology (Brule et al. 2022), gene expression, and morphological development of fishes (Politis et al. 2017). In addition, elevated water temperatures have also been shown to impact fish growth (Lema et al. 2019; Brule et al. 2022; Kraskura et al. 2023; Lindmark et al. 2023), morphology (Atkinson et al. 1996), and behaviour (Brodie and Russell 1999; Biro et al. 2010; Colchen et al. 2017) as well as many other phenotypic traits important to fitness. Among fishes and other ectotherms, tropical species may be even more vulnerable to climate change than temperate species as they experience small annual temperature fluctuations in their natural environment, show narrow thermal tolerance windows, and seem to live relatively close to their thermal maxima (Deutsch et al. 2008; Tewksbury et al. 2008; McDonnell and Chapman 2015).

It is likely that distributional shifts in fishes in the face of warming waters will depend on two factors 1) the ability of the species' to shift its distribution and 2) the ability and speed at which a species' can respond in situ through adaptive response be it through plasticity and/or heritable change (Diamond et al. 2018). There are a number of studies that have focused on measuring acclimation capacity of fishes by exposing fishes to a series of increasingly warmer water temperatures over a period of days to weeks and measuring their thermal tolerance (Comte and Olden 2017; Madeira et al 2017; Nyober and Chapman 2017; Morley et al. 2018; Campos et al. 2020; Chapman et al. 2022; Ruthsatz et al. 2024). These studies have found that fishes tend to have higher upper thermal limits (CT_{max}) when acclimated to warmer water temperatures and that a longer acclimation time improves thermal tolerance (Nyober and Chapman 2017; 2018). In addition, some studies have found that fish are able to adapt their thermal performance and metabolic rates to various temperatures through evolutionary changes (Clarke and Fraser 2004; Jutfeld 2022). Despite these *in situ* responses, range contractions and expansions associated with climate warming have been observed in many species of fishes, and studies have found that both temperate and tropical fishes are shifting their distributions poleward and in elevation to more thermally suitable cooler habitats in response warming waters (Comte et al. 2013; Alofs et al. 2014; Poesch et al. 2016; Sudo et al. 2022; Wu et al. 2022; Nuon et al. 2024).

Multiple Stressors

Predicting distributional shifts in response to projected climate change scenarios may be an important tool in conservation management for freshwater biodiversity and ecosystems. However, it is not sufficient to only consider changes in temperature when predicting and understanding species range shifts, as freshwater ecosystems are impacted by several environmental stressors that can interact to have additive, synergistic, multiplicative, and/or antagonistic effects on freshwater fishes (Côté et al. 2016). Because of this, studies focusing on species distributions should consider the interactions between multiple environmental stressors and multiple ecological gradients. In some situations, range shifting species may encounter other environmental stressors or gradients that can limit their ability to change their distribution, unless the species is able to rapidly respond. Stressors that interact with elevated water temperatures in freshwater systems are many including low aquatic oxygen conditions.

Low-dissolved aquatic oxygen (termed hypoxia) is characterized by dissolved oxygen levels <2 mg O_2/l (Vaquer-Sunyer and Duarte 2008). Hypoxia occurs naturally in many systems characterized by low light and/or low mixing such as dense tropical swamps, the bottom waters of deep lakes, tidepools, and floodplain ponds (Chapman 2015). Hypoxia is also increasing in frequency and extent globally due to influx of nutrients and pollutants that can accelerate eutrophication and lead to hypoxia (Diaz and Brietburg 2009; Chapman et al. 2015). Hypoxia and elevated water temperatures are likely to co-occur as water temperature influences the solubility of gases whereby the solubility of oxygen decreases with increasing water temperatures (Chapman 2015; Walczynska and Sobczyk 2017; Woolway et al. 2022; Bonacina et al. 2023). Warmer waters may also drive eutrophication in freshwater systems that can be characterized by hypoxia events (Dupuis and Hann 2002; Chislock et al. 2013; Hale et al. 2016). These two environmental stressors are also likely to interact because 1) both elevated water temperature and hypoxia affect aerobic metabolism and 2) increases in water temperature can drive the rate of oxygen consumption of a fish, while hypoxia can limit oxygen supply (McBryan et al. 2013; Islam et al. 2020; Verbek et al. 2022; Volkoff and Ronnestad 2022). The functional association between hypoxia and thermal tolerance has often been explored in the context of oxygen- and capacity-limited thermal tolerance, which proposes that thermal performance curves are shaped by the capacity for oxygen delivery in relation to oxygen demand (Pörtner and Farrell 2010; McBryan et al. 2013; Verbek et al. 2016; Pörtner al. 2017; Islam et al. 2020; Jung et al. 2020). Under warming water temperatures, the maximum rate of oxygen consumption exceeds the capacity of the cardio-respiratory system to supply oxygen to tissues, manifested as a reduction in aerobic scope. Consequently, the upper thermal limits of fishes may be sensitive to hypoxia, with the corollary that changes that increase oxygen uptake (e.g., increased gill size) and delivery (e.g., improved cardiac output) may improve thermal tolerance.

This thesis explores the relationship between thermal tolerance and range shifts and how range expansion along a thermal gradient is affected by response to a second strong environmental gradient – dissolved oxygen.

Study system

Kibale National Park (0°13′–0°41′ N and 30°19′–30°32′E), is a mid-altitude moist evergreen tropical rainforest situated in Western Uganda (UWA 2024). Given that the park lies near the equator, there are two dry seasons that fall between May to August and December to February (Hunt et al. 2023). The Mpanga River is one of two main river systems that drains Kibale National Park. This river runs southwards down an elevational gradient of 250 m and is comprised of both well-oxygenated open stretches characterized by high-oxygen conditions (Chapman et al. 2004) and papyrus swamps characterized by naturally hypoxic water ranging from 1.82-3.15 mg/L (Chapman et al. 1999). Within the Mpanga River, at the northern boundary of the park at approximately 1440 m in elevation, lies the Rwembaita Swamp System (Fig. 1). The Rwembaita Swamp is fed by small high-oxygen streams (Mikana, Inlet

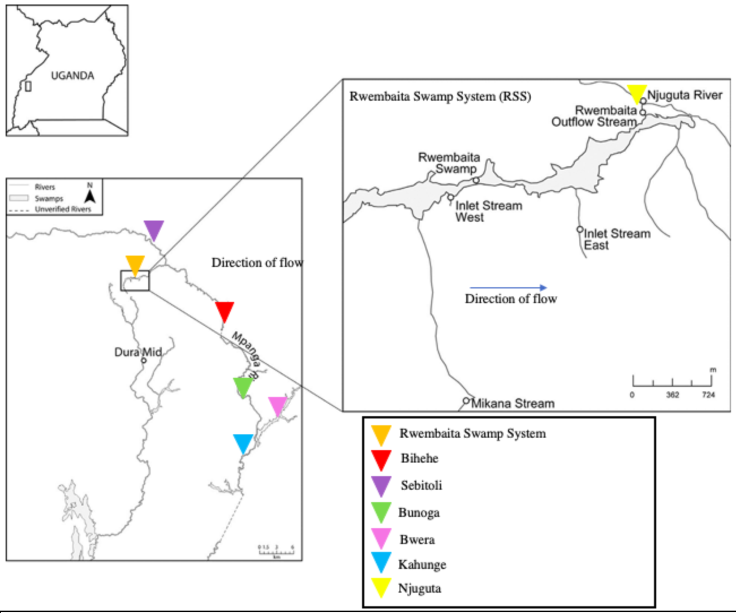

Figure 1: Map of the Mpanga River System and study sites used in Chapters 1 and 2 of this thesis.

Stream West, Inlet Stream East; Fig. 1) that are characterized by higher oxygen conditions than the Rwembaita Swamp ranging between 3.66 and 6.29 mg/L. The swamp itself drains into the Nujguta River, which feeds into the Mpanga River (Fig. 1). Given that the Mpanga River and its tributaries contain habitats that differ in both temperature and oxygen concentration, this makes it an ideal study system to explore species' tolerance to both of these stressors.

Average water temperature, averaged dissolved oxygen, and fish communities in the Rwembaita Swamp System have been sampled periodically as part of a long-term (34 year) monitoring program done by the Kibale Fish Project (Lauren Chapman, unpubl. data). Based on long-term data, there has been an increase in water temperature in the Rwembaita Swamp System of 1.41 °C between 1994 and 2016, which aligns with the local air temperature increase of 1.45 °C over the same time frame (L. Chapman, unpubl. data). Several studies have focused on comparing both the environmental features and fish faunal characteristics of the Rwembaita Swamp and its tributaries. Monthly sampling of fishes in the Rwembaita Swamp and its tributary streams between June 1990 and May 1991 (Chapman 1995) and between July 1993 and June 1997 (Chapman et al. 1999) captured only two fish species, the cyprinid *Enteromius neumayeri* (previously *Barbus neumayeri*; Fischer 1884) and the clariid catfish *Clarias liocephalus* (Boulenger 1898). Olowo and Chapman (2006) and Chapman (2005) also only reported *E. neumayeri* and *C. liocephalus* in sites within the Rwembaita Swamp System. In subsequent studies between 1999 and 2011 that focused on *E. neumayeri* (Schaack and Chapman 2003; 2004; Martinez et al. 2004, 2011; Harniman et al. 2013), *E. neumayeri* and *C. liocephalus* were the only species observed during field data collection for these studies (L. Chapman, personal observations). On the other hand, sampling in the downstream stretches of the Mpanga River at Kahunge, Bwera, and Bunoga (1230-1252 m in elevation; Fig 1.) reported a number of fish species including the cyprinids *E. neumayeri*, *Enteromius apleurogramma* (previously *Barbus apleurogramma*; Boulenger 1911), *Enteromius alberti* (previously *Enteromius cercops*; *Barbus cercops*; Whitehead 1960), and *Enteromius kerstenii* (previously *Barbus kerstenii*; Peters 1868); *C. liocephalus*; the cichlids *Pseudocrenilabrus multicolor* (Schöller 1903), *Astatoreochromis alluaudi* (Pellegrin 1904), and *Haplochromis* spp.; and the killifishes *Platypanchax modestus* (Pappenheim 1914) and *Nothobranchius* spp. (Raymond et al. 2006; Langerhans et al. 2007; Crispo and Chapman 2008; Binning et al. 2010; Joyner-Matos and Chapman, 2013). Given the long-term data on water quality and fish communities in this system, this makes it an ideal system to study the thermal tolerance of range shifting species.

Long-term sampling of fish communities has captured the range shift of three native fish species into the Rwembaita Swamp System, including the cyprinodontid *P. modestus* (first observed in 2012; L. Chapman, unpubl. data; Hunt et al. 2023; previously *Hypsopanchax deprimozi*), the cyprinid *E. apleurogramma* (first observed in 2015; Hunt et al. 2023) and the cichlid *P. multicolor* (first observed in 2022; L. Chapman, unpubl. data). These species have formed established populations, and have continued to be captured in both the wet and dry seasons up until the time of this study in 2024. As Kibale National Park has been a protected area since 1993, and given that these species are not the target of artisanal fisheries, it is unlikely that these species have moved by means of human assistance. Given that the water temperature in the Rwembaita Swamp System has increased co-incident with the range expansion of these species, it is possible that the range-shift is climate driven, though we cannot rule out other drivers.

Thesis objectives

The first chapter of my thesis focuses on exploring the relationship between thermal tolerance in the range shifting *E. apleurogramma* in comparison to its upstream (northern) and downstream (southern) congeners, which inhabit the same river system but have not experienced the same range shifts. To accomplish this, I sampled from multiple populations and multiple *Enteromius* species across their distribution in the Mpanga River. CT_{max} and CT_{min} trials were conducted to measure upper and lower thermal limits, respectively; and this was done within five days of capture to minimize potential acclimation effects. Thermal tolerance was compared across upstream populations of *E. apleurogramma* to those of its upstream and downstream congeners to gain a better understanding of the link between thermal tolerance and range shifts being observed in this system.

In the second chapter of my thesis I studied a different range shifting species in the Mpanga River System, *P. modestus*. The putative source population is hypothesized to be a normoxic stream population, however, this species has expanded its range upstream into both high-oxygen stream habitats and low-oxygen swamp habitats. In this section of my thesis, I focused on exploring how response to one environmental gradient (temperature) may elicit the response to a second environmental gradient (oxygen). To accomplish this, we measured CT_{max} in seven populations across both thermal and oxygen gradients. In addition, we measured the gill size of fish in the original downstream population as well as conspecifics that had shifted their range upstream into a high- and a low-dissolved oxygen site. We used gill data to ask whether there are any signs of divergence in oxygen uptake capacity between populations in the home habitat and the expanded range. In addition, we used long-term limnological data to ask whether average dissolved oxygen and/or water temperature is a significant predictor of CT_{max} across populations.

Each chapter of this thesis focuses on a different species of fish, both of which are expanding their range upstream in the same river system. Both projects explore the link between thermal tolerance and range shifts along a thermal gradient. We first focus on thermal tolerance across closely related species considering only one environmental factor. We then focus on thermal tolerance across a single species while considering multiple environmental stressors. With freshwater ecosystems and fishes being among the most threatened group in the face of environmental change, this allows us to gain a better understanding of how species may respond under future climate scenarios.

CHAPTER 1

Title: Thermal tolerance of congeneric fishes in Kibale National Park, Uganda: Implications for range shifts

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Abstract

Variation in thermal tolerance among species may be an important factor in understanding species response to changing climatic conditions. Here, we explore the relationship between thermal tolerance and range shifts in cyprinid fishes of the genus *Enteromius* in the Mpanga River System of Western Uganda. In this system, *Enteromius apleurogramma* has expanded its range northwards into upstream tributaries previously only inhabited by the congener *Enteromius neumayeri* and a clariid catfish *Clarias liocephalus.* Other congeneric species in the south of the river system *Enteromius alberti* and *Enteromius kerstenii* have not undertaken such range shifts. In this study, we quantified both the lower and upper thermal limits of *E. apleurogramma* across its range, as well of those of the upstream and downstream congeners to gain a better understanding of how thermal tolerance relates to the distribution of species that are closely related. CT_{max} did not differ significantly among *E. apleurogramma, E. alberti,* and *E. kerstenii*; and the CT_{max} of *E. apleurogramma* was significantly lower than that of *E. neumayeri*. For CT_{min} we found a significant level of interspecific variation with *E. apleurogramma* having the lowest CT_{min} followed by *E. kerstenii* and *E. alberti*. This finding is consistent with the prediction that a low CT_{min} may facilitate the contraction of the southern (downstream, warmer) end of the range of *E. apleurogramma* within the Mpanga River System, while a high CT_{min} in the downstream residents may be constraining them to the south (downstream) of the river system. Whereas most studies of thermal tolerance in fishes have measured CT_{max}, our results suggest that CT_{min} may also be an important thermal metric for understanding and predicting species' range shifts.

Introduction

Range shifts

Across the globe, species are shifting their geographic distributions in order to track their ecological niches (Rubenstein et al. 2023). A species range is a geographically dynamic boundary that comprises the abiotic and biotic factors essential to the survival of a species (Guisan et al. 2017); and thus, ranges may shift in response to variation in climatic conditions as well as changes in biotic interactions (Kawecki 2000; Guisan et al. 2017; Bridle and Hoffmann 2022). Historically, the geographic location of species' has shifted over time as a result of natural changes to the Earth's climate (Wingfield et al. 2015). However, in the light of contemporary climate change, habitat loss, invasive species, pollution, and overexploitation, conditions are changing more rapidly thereby giving rise to novel conditions that can affect species distributions. Understanding processes that may affect distributional shifts has therefore gained importance given the need to predict responses of species to rapid environmental change (Bridle and Hoffmann 2022).

Among the factors underlying the redistribution of species, climate warming has emerged as one of the main drivers of species ranges shifts to higher latitudes and higher altitudes as species track their thermal requirements by seeking cooler habitats (Jones and Schmidt 2019; Punzon et al. 2021; Armstrong et al. 2022; Stuart-Smith et al. 2022; Ramalho et al. 2023). Range-shifts coincident with climate warming have been documented in many taxa including birds, mammals, fishes, and reptiles (Moreno-Rueda et al. 2011; Alofs et al. 2014; Comte et al. 2014; Williams and Blois 2018; Hu et al. 2020; Champion et al. 2021). However, there is variation among taxa and systems in the frequency of range shifts associated with climate change (Rubenstein et al. 2023). In particular, ectotherms show stronger range shift responses to ongoing climate change than endotherms, suggesting greater susceptibility of these species to further changes in climate (Ramalho et al. 2023).

Ectotherms, such as fishes, are particularly sensitive to environmental changes, specifically elevated temperatures, as many of their physiological processes, including their internal body temperature, are regulated by their external environment (Huey and Stephenson 1979; McNab 2002; Deutsch et al. 2008; Li et al. 2013). The ability of a fish to carry out its vital functions over a given range of temperatures is defined as its performance window and can be visually represented by a thermal performance curve (Khelifa et al. 2019). Here, performance (the efficiency of a given physiological function) starts at zero at a fish's lower critical thermal minimum (CT_{min}), increases towards an optimal temperature (T_{opt}), and then rapidly decreases towards its upper critical thermal limit (CT_{max}) (Miller and Stillman 2018). As external temperatures begin to surpass CT_{max} or fall below CT_{min}, species begin to exhibit physiological disorganization and loss of equilibrium that eventually leads to death (Huey and Stephenson 1979; Pörtner 2010; McDonnell and Chapman 2015). The shape of the thermal performance curve can vary both within and between species (Rezende and Bozinovic 2019; Little and Seebacher 2021), and the precise shape is influenced by a multitude of factors, including prior thermal environment in early life, phenotypic plasticity, genetic background, and adaptive background (Schulte et al. 2011; Logan et al. 2019; Little and Seebacher, 2021; Rebolledo et al. 2021; Chapman et al. 2022).

There is a growing body of literature on the thermal tolerance of freshwater fishes and their thermal plasticity (Comte and Olden 2017; Morley et al. 2018; Campos et al. 2020; Chapman et al. 2022). Numerous studies have measured the short-term plasticity, also termed as acclimation capacity, of fishes by exposing fishes to a series of water temperatures (anywhere between a few days to a few weeks) and measuring their thermal tolerance (e.g., McDonnell and Chapman, 2015; Nyboer and Chapman 2017; McKenzie et al. 2020; Johansen et al. 2021; Leonard and Skov 2022; Zillig et al. 2022; Waterbury et al. 2024). For example, both Broad Whitefish (*Coregonus nasus*) and Saffron Cod (*Eleginus gracilis*) acclimated to 15 ˚C for 7 days had a significantly higher mean CT_{max} (27.3 ˚C and 25.9 ˚C, respectively) than fish acclimated to 5 ˚C (23.7 ˚C and 23.2 ˚C, respectively) over the same time frame (Waterbury et al. 2024). Likewise, the mouth-brooding cichlid *Pseudocrenilabrus multicolor* increased its CT_{max} when acclimated to elevated water temperatures with fish acclimated to 32 ˚C having a significantly higher mean CT_{max} (41.1 ˚C) then those acclimated to 26 ˚C (38.5 ˚C) over a 7 day acclimation time (McDonnell and Chapman 2015). A species demonstrating acclimation capacity may be able to adjust its thermal tolerance in response to changing thermal regimes that could facilitate persistence *in situ* (Sandblom et al. 2014; Ruthsatz et al. 2024; Waterbury et al. 2024). Despite evidence of thermal acclimation capacity in many freshwater fishes across latitudes, range contractions and expansions associated with climate change have been reported in many species of fishes in both marine and freshwater systems. For example, systematic species shifts towards higher elevation were reported for 32 species of stream fishes in France between 1980-1992 and 2003-2009 (Comte and Grenouillet 2013). In addition, based on time series databases on climate in conjunction with fish species occurrence data from 10,732 lakes in Ontario, Canada between 1986 and 2017, there is evidence that more fishes are moving into northern lakes than those being lost from their northern limits (Wu et al. 2022).

Given the increasing number of fishes exhibiting range shifts, but also the variability in the rate, direction, and extent of movement (Kirk and Rahel 2022; Conte et al. 2023; Waterbury et al. 2024), there is growing interest in using thermal limits and thermal acclimation capacity to predict persistence *in situ* or range shifts to more favourable thermal regimes (Beitinger and Lutterschmidt 2011; Miller and Stillman 2012; Enders and Durhack 2022). Critical thermal maxima methodology has been used since the 1950s in eco-physiological research to characterize the upper thermal limits of ectothermic species (Cowles and Bogert 1944; Bulger 1984; Baroudy and Elliot 1994; Bennet and Beitinger 1997; Desforges et al 2023). Currently, CT_{max} remains an important metric in evaluating thermal tolerance and among the most fundamental and repeatable ways of assessing the impacts of thermal stress on performance, physiology, and behaviour and in understanding the impacts of climate warming on acclimation capacity, distribution, and development of fishes (Morgan et al. 2020; Leong et al. 2022; Desforges et al. 2023). Because CT_{max} is used to define upper thermal physiological limits, it is often used to assess the potential for range expansion at the cooler end of a species range under future climate change scenarios and to assess the vulnerability of species (Beitinger and Lutterschmidt 2011; Miller and Stillman 2012; Sunday et al. 2012; Desforges 2020; Morgan et al. 2020; Enders and Durhack 2022; Kirk and Rahel 2022; Orsted et al. 2022; Comte et al. 2023; Dressler et al. 2023). Range contractions at the warmer end of a range distribution should be more directly determined by lower thermal limits, often quantified as CT_{min} (lower limit of the thermal window). Despite this, very few studies focus on measuring CT_{min}, especially in freshwater fishes (Yanar et al. 2019; Campos et al. 2020; Song et al. 2023; Blodorn et al. 2024).

Thermal performance curves, bounded by CT_{max} and CT_{min}, are useful in understanding thermal plasticity and adaptation in ectothermic species and can be used in conjunction with species distributions models to gain a better understanding of how species will respond to increased temperatures (Khelifa et al. 2019; Wooliver et al. 2022). For example, comprehensive data sets of species' thermal tolerance limits (CT_{max} and CT_{min}) along with data on distributions and climate-driven range shifts has been used in studies to gain a better understanding of the importance of temperature in shaping geographic ranges at a global scale (Sunday et al. 2012). More specifically, in evaluating tolerance limits (CT_{max} and CT_{min}) for 142 marine and terrestrial ectotherms, studies suggest that marine species' ranges conform more closely to their limits of thermal tolerance than terrestrial species and that range shifts will be more predictable for the former in the context of climate change (Sunday et al. 2012).

Despite the widespread application of CT_{max} as a measure of thermal tolerance, its ecological relevance is debated within the scientific community, due to methodology involving a fast ramping rate and chosen endpoints that have been argued to not accurately represent thermal tolerance (Kingsolver and Umbanhowar 2018; Bartlett et al. 2022; Leong et al. 2022; Bouyoucos et al. 2023). In some cases, CT_{max} lies well above the ambient water temperature experienced by fish in a laboratory or in their natural environment (Enders and Durhack 2022; Radke et al. 2022); though there are cases where fish species are currently living close to their upper thermal limit (Morgan et al. 2019; Morgan 2020). Other thermal metrics can be calculated from thermal maximum trials. The difference between CT_{max} and the maximum temperature of the natural environment of an ectotherm is referred to as the Thermal Safety Margin (TSM) (Sunday et al. 2014). In fishes, there is a general latitudinal trend in the TSM with tropical fishes at low latitude showing a lower TSM (Chapman et al. 2022); and it has been argued that tropical ectotherms are living closer to their upper thermal limit than high latitude ectotherms (Deutsch et al. 2008; Sunday et al. 2010; Nguyen et al. 2011; McDonnel and Chapman 2015; Pincebourde and Suppo 2016; Sunday et al. 2019). However, there are limited data on high altitude tropical fishes that live in cooler waters relative to lowland species. In addition, there is a growing awareness that fish exhibit avoidance behaviour in CT_{max} trials well before the upper critical thermal limit is reached, a point referred to as the Agitation Temperature or T_{ag}. This is defined as the temperature at which a fish first begins to exhibit increased activity and agitation (usually characterized by burst swimming) in response to warming waters (McDonnell and Chapman 2015; McDonnell et al. 2021; Potts et al. 2021; Bouyoucos et al. 2023). This metric is argued to be more ecologically relevant than CT_{max} as it likely reflects the temperature at which fish begin to seek more favourable cooler waters as a behavioural response to thermal stress (Lutterschmidt and Hutchison 1997; McDonnell and Chapman 2015; Bouyoucos et al. 2023). In this study, I explore the relationship between CT_{max}, CT_{min}, T_{ag}, and range shifts in a closely related suite of cyprinid fishes inhabiting a mid-altitude rainforest in western Uganda. Kibale National Park, Uganda, was selected as our study site as long-term studies in the park have documented the recent range-expansion of a native fish species along a thermal gradient (Hunt et al. 2023), making it an ideal site to study the relationship between thermal tolerance and species' range shifts.

Study system

Kibale National Park (0°13′–0°41′ N and 30°19′–30°32′E) situated in western Uganda, is a mid-altitude moist evergreen tropical rainforest ranging between 1,160 m and 2,607 m in altitude and 795 km^2 in area (UWA 2024). The park is a mosaic of mature evergreen forest, portions of which were logged approximately five decades ago, wetlands, open grasslands, and former pine and cypress plantations (Duclos et al. 2013). There are two wet and dry seasons, with May to August and December to February being drier than other months of the year (Hunt et al. 2023). There are two main river systems that drain Kibale National Park, the Dura River and the Mpanga River, both of which feed into the Lake George System (Harniman et al. 2013). The Mpanga River System, the focus of this study, is comprised of large well-oxygenated open streams with minimal vegetation and valley papyrus swamps that are characterized by low dissolved-oxygen (1.82-3.15 mg/L; Chapman et al. 1999; Crispo and Chapman 2008; Joyner-Matos and Chapman 2013). The Rwembaita Swamp System (Fig. 1) is the largest papyrus swamp (4.5 km long) inside Kibale National Park, fed by small high-oxygen streams, that eventually feeds into the Njuguta River, a tributary of the Mpanga River System. This system, which comprises the Rwembaita Swamp and its tributaries, has been the focus of several studies by the Kibale Fish Project since the early 1990's which have focused on comparing environmental and fish faunal characteristics. Monthly sampling of fishes in the Rwembaita Swamp System between June 1990 and May 1991 (Chapman 1995) and between July 1993 and June 1997 (Chapman et al. 1999) captured only two species, the cyprinid *Enteromius neumayeri* (previously *Barbus neumayeri*; Fischer 1884) and the clariid catfish *Clarias liocephalus* (Boulenger 1898). Olowo and Chapman (2006) and Chapman (2005) also only reported *E. neumayeri* and *C. liocephalus* in sites within the Rwembaita Swamp System. In subsequent studies between 1999 and 2011 that focused on *E. neumayeri* (Schaack and Chapman 2003, 2004; Martinez et al. 2004, 2011; Harniman et al. 2013), *E. neumayeri* and *C. liocephalus* were the only species observed during field data collection for these projects (L. Chapman, personal observations). Conversely, downstream southern sites (Kahunge, Bwera (Kiaragura Swamp), Bunoga; Fig. 1) host a number of fish species including populations of cyprinids *E. neumayeri, Enteromius apleurogramma* (previously *Barbus apleurogramma*; Boulenger 1911), *Enteromius alberti* (previously *Enteromius cercops*; *Barbus cercops*; Whitehead 1960) and *Enteromius kerstenii* (previously *Barbus kerstenii*; Peters 1868); *C. liocephalus*; the cichlids *P. multicolor* (Schöller 1903), *Astatoreochromis alluaudi* (Pellegrin 1904), and *Haplochromis* spp.; and the killifishes *Platypanchax modestus* (Pappenheim 1914) and *Nothobranchius* spp. (Raymond et al. 2006; Langerhans et al. 2007; Crispo and Chapman 2008; Binning et al. 2010; Joyner-Matos and Chapman, 2013).

Periodic sampling of fish communities since 2011 has captured the upstream (northwards) range shift of *E. apleurogramma*. This species has expanded its range upstream into the Rwembaita Swamp System where it was first observed in 2015 and has continued to be captured until 2024 (Hunt et al., 2023; L. Chapman, unpubl. data). Within this system it inhabits both the swamp and tributary streams. Sampling expeditions in 2022 and 2023 at downstream (southern) sites did not capture *E. apleurogramma*, suggesting that it is rare or has disappeared from the south of its historical distribution in the Mpanga River. *E. apleurogramma* is one of three native species known to have expanded their range upstream into the Rwembaita Swamp System, the others being the cyprinodontid *P. modestus* (appeared in 2012) and the cichlid *P. multicolor* (first observed in 2022; L. Chapman, unpubl. data). The non-native guppy, *Poecilia reticulata,* has also been captured in the system. It is unlikely that these species moved into the Rwembaita Swamp System by means of human assistance given that it lies deep within Kibale National Park, which has been a protected area since 1993. Further, the species that have shifted their range are not the target of artisanal fisheries, so they are unlikely to have been introduced by fishers. In addition, we should note that co-incident with this range expansion, that there has been an increase in water temperature in the Rwembaita Swamp System of 1.41 °C which aligns with the local air temperature increase of 1.45 °C between 1994 and 2016 (L. Chapman, unpubl. data). Though we do not have long-term data for downstream sites, the historical *E. apleurogramma* populations are between 131 and 146 m lower in elevation (L. Chapman, unpubl data). It is predicted that the average difference in temperature between the upstream and downstream sites is approximately 0.95 °C based on standard atmospheric lapse rate of 6.50 °C/km and site elevation (International Civil Aviation Organization 1993; Hunt et al. 2023). With this in mind, it is possible that the range shift is climate-driven, though we cannot rule out other drivers.

The goal of the study was to quantify and compare the upper and lower thermal limits of *E. apleurogramma* (estimated as CT_{max} and CT_{min}, respectively) to those of its upstream and downstream congeners to gain a better understanding of how thermal tolerance relates to the distribution of species that are closely related. We live-captured fish of different species from different stream and river sites and quantified both their upper and lower thermal limits. We focused on high-oxygen sites only as fish from hypoxic waters tend to show phenotypic differences from conspecifics that inhabit swamps. For example, *E. neumayeri* from swamp sites are characterized by much larger gills than conspecifics from high-oxygen sites (Chapman et al. 1999; Chapman 2007; Langerhans et al. 2007), which could affect their CT_{max} if their upper thermal limit is oxygen dependent (Chapter 2). We tested four predictions: (1) Thermal tolerance is fixed across populations of *E. apleurogramma* and *E. neumayeri*; (2) CT_{max} will be lower than average values for equatorial fishes due to the high elevation of Kibale National Park; (3) If upstream populations have a longer evolutionary history with cooler waters, then upstream populations of *E. neumayeri* should have a lower CT_{max} and a lower CT_{min} than upstream populations of the range-shifting *E. apleurogramma*; and (4) If the north is not yet warm enough for downstream species' to shift their distribution, then downstream congeners *E. alberti* and *E. kerstenii*, should have a higher CT_{min} than upstream populations of *E. apleurogramma*.

Methods

Range shifting species

The East African Red-finned Barb (*E. apleurogramma*) is a small species of ray-finned fish in the family Cyprinidae that is currently listed as a species of least concern by the IUCN (Greenwood 1966; Schmidt 2020). *E. apleurogramma* is widely distributed across East Africa, occurring in

Uganda in lakes Victoria and Edward and associated river systems, as well as in Rwanda and Tanzania (Greenwood 1962; Schmidt et al. 2018). This barb inhabits temporary and permanent streams as well as the marginal water-lily swamps of lakes (Greenwood 1966). There are not many studies which have focused on *E. apleurogramma*, though some studies have found that populations inhabiting low-oxygen conditions have longer gill filaments and larger gills overall than populations inhabiting normoxic streams/rivers (Hunt et al. 2023). Moreover, genetic studies on *E. apleurogramma* have shown that they have high plasticity in gene expression, which could allow for rapid response to new environments (Fox et al. 2024).

Congeneric species

Congeneric species of *E. apleurogramma* that are found within the Mpanga River System include the Neumayer's Barb (*E. neumayeri*), the Luambwa Barb (*E. alberti*), and the Redspot Barb (*E. kerstenii*) all of which are small benthopelagic species of ray-finned fishes of the family Cyprinidae and listed as species of least concern by the IUCN (Whitehead 1960; Greenwood 1966). *E. alberti* is widely distributed throughout Uganda, in Kenya in the affluent rivers of Lake Victoria and in the rivers of the Nyanza Province, and in Rwanda in the Middle Akagera System (Whitehead 1960; Greenwood 1966). *E. kerstenii* is widely distributed across East and Central Africa, occurring in Rwanda, Kenya, Tanzania, Burundi, and in Uganda in lakes Victoria and Edward basins as well as the Kyoga System (Greenwood 1966; Lévêque and Daget 1984). In the Mpanga River drainage both *E. alberti* and *E. kerstenii* are found in high-oxygen open river sites and have not been captured in upstream tributaries of the river system (L. Chapman, pers. obs.). *E. neumayeri* is also distributed widely in East Africa, occurring in Uganda, Kenya, Tanzania, and in Lake Victoria and its tributaries. In the Mpanga River System it inhabits rivers, temporary streams, swamps and occurs across a range of dissolved oxygen conditions (Chapman et al. 1999; Langerhans and Chapman 2007; Harniman et al. 2012).

Fish sampling and collection

Sampling for E. apleurogramma and E. neumayeri comparison

We sampled *E. apleurogramma* and *E. neumayeri* from three different sites – two of which were normoxic stream sites within the Rwembaita Swamp System (Inlet Stream West and Inlet Stream East) and one normoxic site located upstream at the northern boundary of the park in the main section of the Mpanga River (Sebitoli; Fig. 1). For every population we collected 24 fish – giving us a sample size of 12 fish for upper thermal tolerance trials and 12 fish for lower thermal tolerance trials (Table 1). We had initially intended on collecting *E. apleurogramma* from the downstream southern site (Bihehe) to represent the most downstream southern end of the distribution, however, upon sampling we discovered that abundance of *E. apleurogramma* has declined drastically over the past few years, representing only a small proportion (0.4%) of our catch. As such, we used the Rwembaita Swamp System as the new most downstream southern range limit for *E. apleurogramma* and Sebitoli as our most upstream northern site.

Sampling for E. apleurogramma, E. alberti, and E. kerstenii comparison

E. alberti and *E. kerstenii* were collected from Bihehe as this is the closest accessible site to the Rwembaita Swamp System where these two species are found, which likely represents the upstream northern end of their distribution. For each species and each population, we collected 24 fish which gave us a sample size of 12 fish for upper thermal tolerance trials and 12 fish for lower thermal tolerance trials (Table 1**)**. Given our inability to catch *E. apleurogramma* from Bihehe, which has historically represented the upstream northern end of its distribution, we did two comparisons. In the first comparison, we calculated and compared mean thermal tolerance values for *E. apleurogramma* from both Inlet Stream West and Inlet Stream East to *E. alberti* and *E. kerstenii* from Bihehe; and in the second comparison, we compared *E. apleurogramma* from Inlet Stream East only (the site closest to Bihehe) to *E. alberti* and *E. kerstenii*.

Fish capture and water sampling

Water quality parameters, including average water temperature and average dissolved oxygen concentration have been recorded at sites within the Rwembaita Swamp System as part of a long-term (34 year) monitoring program done by the Kibale Fish Project (Lauren Chapman, unpubl. data). Briefly, water temperature and dissolved oxygen data were collected at a set of microsites in the upper 20 cm of the water column using Polaris water temperature and dissolved oxygen probes. In the closed cover of the forest and papyrus swamp habitats, diel variation in dissolved oxygen and water temperature tends to be low (Chapman and Liem 1995). Therefore, measurements were taken once per sampling day in the mid-morning to mid-day period. We calculated two types of limnological data for the purpose of this study – long-term and short-term. For long-term data we calculated average water temperature and dissolved oxygen for sites within the Rwembaita Swamp System from the period between 2020-2023, which likely represents the environment over which fish developed, although the life-span for these species is not known (Table 2). During the field season from the period between May 2023 and August 2023, we sampled water quality parameters including water temperature and dissolved oxygen on the day of fish capture at all sites, including sites where long-term data were not collected (i.e. Sebitoli and Bihehe; Table 3). The limnological data collected during the field season were used to calculate short-term estimates for water quality parameters including average water temperature and average dissolved oxygen. Fish were live-captured using baited minnow traps and brought back to the Makerere University Biological Field Station. At the field station, fish were held in 38 L coolers with untreated rainwater and held under normoxia (6.37 ± 0.07 mg/L; mean ± SEM), ambient temperatures (19.54 ± 0.10 °C), and exposed to a natural photoperiod. Coolers were equipped with air stones, sponge filters, and refuge in the form of leaf cover. Across all coolers, temperature variation ranged by 0.27 °C and dissolved oxygen concentration ranged by 0.98 mg/L (Table 4). Coolers were separated by population and catch day, and held a maximum of 30 fish. Water quality was monitored daily for ammonia and nitrate, and half tank water changes were done as necessary. Fish were fed Tropical Tetraflakes, a food readily consumed by all the species in this study.

Critical Thermal Maximum Trials (CT_{max})

CT_{max} was measured following routine methods (as in Fangue et al. 2006 with *Fundulus heteroclitus*; Mcdonnell and Chapman 2015 with *P. multicolor*; McDonnell et al. 2021 with *Notropis anogenus*). Tests were performed within 4-5 days post-capture to minimize potential acclimation effects that could potentially alter the CT_{max} if a species has plasticity in its thermal limits, so by measuring fish within this time frame we increase the probability that measures more accurately represent the thermal tolerance of fish in their natural setting (Fangue et al. 2006; Mcdonnell and

Chapman 2015; McDonnell et al. 2021). In order to induce a post-absorptive state, fish were starved for 24 hours prior to the experimental trials. Three fish were placed in separate experimental chambers within a larger Plexiglass aquarium (58 L x 25 W x 20 H cm). The compartments contained shelter consisting of rocks and were perforated to allow the mixing of water freely between the chamber and the buffer tank. Initially, water temperature in the experimental tank was at ambient temperature (~17-18 °C), and water was kept well oxygenated (>7.85 mg/L) during acclimation and throughout experimental trials by using battery operated bubblers. Fish were left to acclimate under these conditions for a period of 2 hours, after which water temperature was slowly increased at a standard rate of 0.3 °C/min using a temperature control system (Witrox-4 unit and DAQ-M device; AutoResp; Loligo Systems). This rate has been shown to be low enough to not shock the fish while being high enough to minimize acclimation effects (Cox et al. 1974; Lutterschmidt and Hutchison 1997; Fangue et al. 2006; Chen et al. 2013). Throughout the experiment, the tank was supplied with constant aeration to maintain high dissolved oxygen levels. Water temperature and dissolved oxygen concentration were carefully monitored for the acclimation period and throughout the experiment using handheld Polaris water temperature and dissolved oxygen probes. CT_{max} was defined as the temperature at which the fish lost equilibrium for 30 consecutive seconds (Fangue et al. 2006; McDonnell and Chapman 2015; McDonnell et al. 2021). Loss of equilibrium in fish is characterized by the inability to maintain an upright position in the water column, which impacts the ability of fish to swim and/or move normally (Calfee et al. 2016). We also recorded agitation temperature (T_{ag}), the temperature at which fish begin to swim in an agitated manner, usually characterized by burst swimming (McDonnell et al. 2021; Potts et al. 2021). With these metrics we were able to calculate thermal safety margin (TSM_{max}), which measures the difference between a species' maximum tolerance to heat and the maximum temperature it regularly experiences in its natural environment (CT_{max} - T_{env}). We also calculated the TSM for agitation temperature (TSM_{tag}; $T_{ag} – T_{env}$). For the purpose of this study, we calculated the maximum water temperature as the average of the 10 highest water temperature readings over the past 3 years (Table 2**)**. This metric was not calculated for species from Bihehe or Sebitoli due to lack of long-term environmental data. Following trials, fish were allowed to fully recover in oxygenated water after which they were euthanized using clove oil (0.03%). For every fish, weight (g) and standard length (cm) were recorded post-euthanasia to allow us to test for any body size effects.

Critical Thermal Minimum Trials (CT_{min})

The experimental set-up for CT_{min} was similar to that of CT_{max}. However, for CT_{min}, the water was pumped through a coil that was sitting in an ice bath within a freezer. The ice bath and coil were both placed in the freezer two hours prior to the start of the experiment to allow the water in the ice bath to sufficiently cool without freezing in the coil and blocking the flow of water. The freezer was then left slightly ajar to allow for water circulation through inflow and outflow tubing, while maintaining the temperature within the freezer as constant as possible. Water temperature in the experimental tank was decreased at an approximate rate of 0.3 °C/min using a temperature control system (Witrox-4 unit and DAQ-M device; AutoResp; Loligo Systems). Icepacks were added along the perimeter of the experimental tank when necessary to supplement the cooling from the temperature control system. The behaviour for CT_{max} was species dependent and slightly different than the loss of equilibrium behaviour we saw with CT_{max}. When fish became sedentary in the corner of the chamber and ceased all motion for more than 90 seconds they were lightly probed to check for responsiveness. CT_{min} was then defined as the temperature at which a fish either lost equilibrium or became unresponsive after multiple probes. There was no obvious T_{ag}, so this was not included for CT_{min}. We also calculated thermal safety margin (TSM_{min}), which measures the difference between a species' maximum tolerance to cold water and the temperatures it regularly experiences in its natural environment ($T_{env} – CT_{min}$). For the purpose of this study, we calculated the minimum environmental temperature as the average of the 10 lowest water temperature readings over the past 3 years (Table 2**)**. This metric was again not calculated for Sebitoli or Bihehe due to lack of long-term environmental data. Permission to carry out this research was approved by McGill University, the Uganda Wildlife Authority, and the Uganda National Council for Science and Technology. Procedures used for live fish in this experiment were also approved by McGill University Animal Care Committee (AUP 5029).

Statistical Analyses

We used a one-way ANOVA analysis to test for differences in body mass, standard body length, and total body length among species. All statistical analyses were done using R software version 4.3.2 (The R Foundation for Statistical Computing 2023).

We also used a one-way ANOVA to test for differences in water temperature and dissolved oxygen concentration among study sites. This was done using short-term limnology, which represents the limnological data collected during the field season from May to August 2023. The long-term limnological data for Inlet Stream West and Inlet Stream East were only used to calculate the thermal safety margins for *E. apleurogramma* and *E. neumayeri*.

We included the following thermal metrics in our analysis: CT_{max}, T_{ag}, TSM_{max}, TSM_{tag}, CT_{min}, and TSM_{min}. To detect differences in thermal metrics among populations and species, and any interactions between the two, we used either linear models (ANOVA; package *car* v.3.1-2; Fox and Weisberg 2019) or linear mixed models (LMM; package *lme4* v.1.1.35; Bates et al. 2023). Initially, body size (mass or length) were included in all models but were found to have no significant effect on any of the metrics collected. Thus, body size was removed from all models below. We tested for any random effects of trial number because three fish were tested in the same chamber on the same day and therefore were not independent. If the random effect of trial was significant, we utilized a linear mixed model (LMM) to analyze the data rather than an ANOVA. To calculate p-values in the LMMs, we used an analysis of variance under a chi-square distribution, which is robust to violations of assumption that a mixed model might make of an F-distribution. In the case where trial number was found to have no effect on a collected metric it was excluded from the model in which case linear models (ANOVA) were utilized instead. We used Akaike Information Criterion (AICc) to select the best fit model for effects of populations, species, sites, and any interactions. AIC is an estimator of prediction error that estimates the amount of information lost in a model, and hence, the quality of a given model relative to other models (Stoica and Selen 2004; McElreath 2016; Matt 2019). For linear models, post-hoc analyses were performed using the TukeyHSD() function in order to interpret the statistical significance of the difference between pairs of means (package *multcomp* 1.4-25; Hothorn 2010; Lane 2010). Sum of square values (Type III) are reported for all p-values using the ANOVA() function (package *car* v.3.1-2; Fox and Weisberg 2019). For linear mixed models, post-hoc analyses were performed using the glht() function which is the equivalent of the TukeyHSD() function for mixed models (package *multcomp* 1.4-25; Hothorn 2010). All model outputs are summarized in Tables 5 and 7 in the supplementary materials in Tables 1 and 8.

Results

Body size

Body mass, standard body length, and total body length differed significantly among species (mass: $p = 0.003$, $F = 4.81$, $df = 3,189$; length: $p < 0.0001$, $F = 28.24$, $df = 3,189$; and standard length: $p < 0.0001$, $F = 18.82$, $df = 3$; Tables 1, 5, and 6). Of the four species in this study, *E. alberti* was the largest in terms of both mass and length. As noted in Methods, despite being different among species, body mass and body length had no significant effect on any of the thermal metrics examined.

Environmental Data

Both average water temperature and average dissolved oxygen concentration differed among sites (temperature: $p < 0.0001$, $F = 5.79 \times 10^{2}$, $df = 3,76$; dissolved oxygen: $p < 0.001$, $F = 3.04 \times 10^{30}$, $df = 3,76$; Tables 7 and 8). For data collected during the field season at sites for which thermal metrics were measured, Inlet Stream East was the coolest site and was characterized by the lowest dissolved oxygen concentration of all sites, and Bihehe was the warmest site and characterized by the highest dissolved oxygen concentration (Inlet Stream East temperature: 18.56 ± 0.22 ˚C; mean ± SEM, dissolved oxygen: 3.51 ± 0.22 mg/L; Bihehe temperature: 21.34 ± 0.08 ˚C, dissolved oxygen: 6.94 ± 0.02 mg/L; Table 3). The maximum water temperature for Inlet Stream West and Inlet Stream East (calculated as the average of the highest 10 values over the past 3 years) was 21.34 ± 0.08 ˚C and 21.53 ± 0.62 ˚C, respectively, and the lowest water temperature (calculated as the average of the lowest 10 values) was 16.65 ± 0.24 ˚C and 15.51 ± 0.06 ˚C, respectively (Table 2).

Upstream congener comparison: E. apleurogramma **and** *E. neumayeri*

The best fit model CT_{max} of *E. apleurogramma* and *E. neumayeri* included population and species, and both had significant effects on CT_{max} (population: $p = 0.0082$, $F = 5.16$, $df = 2,84$; species: $p = 0.0071$, $F = 7.71$, $df = 1,84$; Fig 2; Supp Mat. Tables 1 and 2). Fish from Inlet

Stream West had the lowest CT_{max} (Tukey $p = 0.0084$; Fig. 2; all other p-values reported in Supp Mat. Table 2). Across sites *E. neumayeri* had a lower CT_{max} than *E. apleurogramma* ($p = 0.0071$, $F = 7.71$, df = 1,84; Fig. 2; Supp Mat. Table 1; *E. apleurogramma* – mean $CT_{max} = 35.09 \pm 0.16$ °C for all sites; *E. neumayeri* - mean $CT_{max} = 34.26 \pm 0.27$ °C for all sites, Supp Mat. Table 3 and 4).

For T_{ag}, the best fit model included population and species and their interaction. There was a significant interaction between species and population on T_{ag} (population*Species: $p = 0.0151$, $F = 4.47$, df = 2,66; Fig. 3; Supp Mat. Tables 1 and 2). For fish from Inlet Stream West, T_{ag} was lower in *E. neumayeri* (Tukey $p = 0.0386$; Fig. 3; Supp Mat. Table 2). There was no significant difference between species at the other sites (*E. neumayeri* Inlet Stream West - mean $T_{ag} = 29.22 \pm 0.68$ °C; *E. apleurogramma* Inlet Stream West - mean $T_{ag} = 31.51 \pm 0.68$ °C, Supp Mat. Table 3 and 4; all other p-values reported in Supp Mat. Table 2).

The best fit model for CT_{min} included species, population, and the random effect of trial. Both population and species had a significant effect on CT_{min} (population: $p = <0.0001$, $X^2 = 26.72$, df = 2; species: $p = 0.0166$, $X^2 = 5.74$, df= 1; Supp Mat. Table 1). Fish from Inlet Stream East had the lowest CT_{min} and fish from Inlet Stream West had the highest CT_{min} (Tukey $p < 0.0001$; Fig. 4; all other p-values reported in Supp Mat. Table 5). Overall, CT_{min} was lower in *E. apleurogramma* than in *E. neumayeri* ($p = 0.0166$, $X^2 = 5.74$, df= 1; Fig. 4; Supp Mat. Table 1; *E. neumayeri* - mean $CT_{min} = 7.33 \pm 0.18$ °C; *E. apleurogramma* - mean $CT_{min} = 6.71 \pm 0.20$ °C; Supp Mat. Tables 6 and 7).

Thermal Safety Margin

For our upstream congener comparison, we found a significant effect of population and species on TSM. Fish from Inlet Stream East showed a larger TSM than fish from Inlet Stream West ($p = 0.0009$, $F = 12.69$, df = 1,45; Fig. 5; Supp Mat. Table 1); and *E. apleurogramma* showed a larger TSM for their CT_{max} (TSM_{max}) than *E. neumayeri* ($p = 0.0119$, $F = 6.87$, df = 1,45; Fig. 5; Supp Mat. Table 1; *E. apleurogramma* - mean $TSM_{max} = 13.62 \pm 0.24$ °C; *E. neumayeri* - mean $TSM_{max} = 12.68 \pm 0.32$ °C; Supp Mat. Tables 3 and 4). TSM for T_{ag} (TSM_{tag})

did not differ between *E. apleurogramma* and *E. neumayeri* ($p = 0.2346$, $F = 1.4517$, df =1,45; Supp Mat. Table 1). We found a significant effect of population and species on TSM for CT_{min} (TSM_{min}). Fish from Inlet Stream East had a larger TSM_{min} than fish from Inlet Stream West ($p < 0.0001$, $X^2 = 63.86$, df = 11; Fig. 6; Supp Mat. Table 1). *E. apleurogramma* showed a larger TSM_{min} than *E. neumayeri* across sites (species: $p = 0.0051$, $X^2 = 7.84$, df = 1; Fig. 6; Supp Mat. Table 1; *E. neumayeri* - mean TSM_{min} = 8.59 ± 0.35 ˚C; *E. apleurogramma* – mean TSM_{min} = 9.56 ± 0.35 ˚C; Supp Mat. Tables 6 and 7).

Downstream congener comparison: E. apleurogramma* and *E. alberti* and *E. kerstenii

There was no significant difference in the CT_{max} across species for *E. apleurogramma*, *E. kerstenii*, and *E. albertii*. This pattern held true when we compared CT_{max} for *E. apleurogramma* across Inlet Stream West and Inlet Stream East to *E. kerstenii* and *E. alberti*, or whether we compared *E. apleurogramma* just from Inlet Stream East (Inlet Stream East and West: $p = 0.27$, $F = 1.35$, df = 2,44; Inlet Stream East only: $p = 0.83$, $F = 1.90$, df = 2,33; Fig. 7; Supp Mat. Tables 3, 4, and 8).

There was a significant effect of species on T_{ag} which again held true for both comparisons between *E. apleurogramma* populations (Inlet Stream East only, Inlet Stream East and Inlet Stream West) to those of *E. kerstenii* and *E. alberti* (Inlet Stream East and West: $p = 0.0066$, $X^2 = 10.05$; df = 2; Inlet Stream East only: $p < 0.0001$; $X^2 = 15.04$, df = 2; Fig. 8; Supp Mat. Table 8). *E. apleurogramma* had the lowest T_{ag} (Inlet Stream East and West: Tukey $p_{EA-EC} = 0.0122$; Inlet Stream East only: Tukey $p_{EC-EA} = <0.001$ and Tukey $p_{EA-EK} = 0.0066$; Fig. 8; all other p-values reported in Supp Mat. Table 9; *E. apleurogramma* - mean T_{ag} = 30.03 ± 0.34 ˚C, *E. kerstenii* - mean T_{ag} = 32.53 ± 0.45 ˚C, and *E. alberti* - mean T_{ag} = 33.13 ± 0.56 ˚C; Supp Mat. Tables 3 and 4).

In terms of CT_{min}, there was a significant difference among the three species, which held true for both the average CT_{min} for *E. apleurogramma* (Inlet Stream West and Inlet Stream East) and the CT_{min} for this species from Inlet Stream East only (Inlet Stream East and West: $p < 0.0001$, $X^2 = 20.17$, df = 2; Inlet Stream East only: $p < 0.0001$, $X^2 = 68.81$, df = 2; Fig. 9; Supp Mat. Table 8). *E. apleurogramma* had the lowest CT_{min} followed by *E. kerstenii* and *E. alberti*

(Inlet Stream East and West: Tukey $p_{EA\text{-}EC} < 0.001$; Inlet Stream East only: $p_{EK\text{-}EC} = 0.0003$, $p_{EA\text{-}EC} < 0.0001$, and $p_{EK\text{-}EA} = <0.0001$; all other p-values reported in Supp. Mat. Table 9; *E. apleurogramma* - mean $CT_{min} = 6.71 \pm 0.20$ ˚C, *E. kerstenii* – mean $CT_{min} = 7.49 \pm 0.25$ ˚C and *E. alberti* - mean $CT_{min} = 9.06 \pm 0.16$ ˚C; Supp Mat. Tables 6 and 7).

Discussion

We detected both inter-populational variation and interspecific variation in the upper thermal limits of *E. apleurogramma* and *E. neumayeri*, which suggests that thermal tolerance is not fixed across populations for either of these species. There is a growing number of studies that show inter-populational variability in upper thermal tolerance in many species of temperate and tropical fishes including Nile Perch (*Lates niloticus*; Chretien and Chapman 2016; Nyboer et al. 2020), Common Killifish (*F. heteroclitus*; Fangue et al. 2006*)*, Rainbow Trout (*Oncorhynchus mykiss*; Dressler et al. 2023*)*, Channel Catfish (*Ictalurus punctatus*; Stewart and Allen 2014), and Chinook Salmon (*Oncorhynchus tshawytscha*; Zillig et al. 2014). These studies show that populations inhabiting warmer environments have higher upper thermal tolerance than their cold water counterparts. For example, for the Common Killifish (*F. heteroclitus)*, the CT_{max} of southern populations was found to be 1.5 ˚C higher than the CT_{max} of northern populations (Fangue et al. 2006). With this in mind, it is possible that the lower CT_{max} at Inlet Stream West than at Inlet Stream East may relate to the lower average water temperature at this site over the past 3 years than at Inlet Stream East, meaning that fish would have developed in a cooler environment at Inlet Stream West than East. However, it should be noted that the water temperature recorded during the sampling of fish at this site (May to August, 2023) was 0.75 ˚C higher at Inlet Stream West than at Inlet Stream East. Long-term data on water temperature are not available for our Sebitoli site, but data on water temperature collected during the fish sampling indicated the highest temperature of the three sites, which may have contributed to the observed inter-site variation in CT_{max} for the two focal species.

Across sites, the CT_{max} of *E. apleurogramma* was greater than that of *E. neumayeri*. This is reflective of other studies that have suggested that interspecific variation in upper thermal tolerance varies according to a species' latitude and evolutionary history (Nati et al. 2021). Based on long-term sampling of fish communities in the Rwembaita Swamp System over the past 34 years, we speculate that *E. neumayeri* has had a longer evolutionary history in the upstream northern sites than *E. apleurogramma*, and that cooler waters may have selected for a lower $\mathrm{CT}_{\mathrm{max}}$ across populations. The fact that *E. apleurogramma* has a higher $\mathrm{CT}_{\mathrm{max}}$ supports the hypothesis that the range-expanding populations of *E. apleurogramma* stem from a more downstream southern portion of the river system, as downstream populations adapted to warmer waters would presumably have a higher $\mathrm{CT}_{\mathrm{max}}$. Variation across populations and between species could reflect multiple factors, including local adaptation, plasticity in thermal limits, and genetic variation (McKenzie et al. 2020; Debes et al. 2021). In terms of plasticity in thermal limits, many studies focusing on measuring short-term plasticity in freshwater fishes have shown that fish have thermal acclimation capacity by which they are able to increase their $\mathrm{CT}_{\mathrm{max}}$ when exposed or acclimated to higher water temperatures (Comte and Olden 2017; Morley et al. 2018; Rahman et al. 2021; Cicchino et al. 2023; Stewart et al. 2023; Waterbury et al. 2024). There is also evidence that a longer acclimation time (days versus weeks) can improve thermal tolerance (Nyboer and Chapman 2017; 2018). Some studies have also suggested that there is heritable genetic variation in tolerance of warming within fish populations (McKenzie et al. 2020; Debes et al. 2021). For example, one study focusing on Atlantic Salmon (*Salmo salar*) in the context of aquaculture showed that tolerance to high temperature is a heritable trait and that selective breeding for tolerance to higher temperatures is possible (Gonan et al. 2024). The source of variation in $\mathrm{CT}_{\mathrm{max}}$ among sites and between species in our study is not known; future studies that address acclimation capacity of these species to elevated water temperature and heritability of thermal tolerance would be a useful next step that builds on our field data.

The $\mathrm{CT}_{\mathrm{max}}$ values that we observed for *E. neumayeri* and *E. apleurogramma* are lower than the average $\mathrm{CT}_{\mathrm{max}}$ of low-latitude fishes inhabiting lower elevations, suggesting that the high elevation of Kibale National Park may be contributing to a lower $\mathrm{CT}_{\mathrm{max}}$ (Illing et al. 2020; Yanar et al. 2023; Debnath 2024). For fish located less than 25 degrees absolute latitude, studies have reported an average $\mathrm{CT}_{\mathrm{max}}$ 39.6 ˚C, with a range of upper thermal tolerance limits between 32.5 ˚C and 45.6 ˚C (Chapman et al. 2022). Despite that *E. neumayeri* and *E. apleurogramma* are both low-latitude tropical fishes, their low upper critical thermal limits are not surprising given that the fishes of the Northern region of Kibale National Park are from an altitude of approximately 4,800 feet, with cool water temperatures. However, these CT_{max} values are still high given the average water temperature of the sites, which is reflected in their high thermal safety margins (13.62 ± 0.24 °C; mean ± SEM for *E. apleurogramma* and 12.68 ± 0.32 °C for *E. neumayeri*). Across a broad latitudinal range, the TSM is smaller for tropical than temperate fishes (Campos et al. 2021; Chapman et al. 2022). One study examining the upper thermal limits of 106 South American fishes across a broad latitudinal gradient reported that for tropical fishes at an absolute latitude of site $\leq$ 23 degrees, acclimated to $\geq$30 degrees in latitude, the TSM ($CT_{max} – T_{acclimation}$) averaged 8.8 °C and ranged between 3.3 °C and 16 °C (Supplementary data in Campos et al. 2021). For fishes acclimated to < 25 °C, the TSM was higher (mean = 10.0°C, range = 4.9 °C to 15.2 °C). For temperate fishes at an absolute latitude of site > 23 degrees acclimated to < 25 °C, the mean TSM calculated from supplementary data in Campos et al. (2021) was 15 °C (range = 9.2 °C to 24 °C). The water temperature experienced by *E. neumayeri* and *E. apleurogramma* is relatively cool for an equatorial region given the high altitude of the site, however, their thermal safety margins fell between the ranges for tropical and high latitude fishes acclimated to < 25 °C reported in Campos et al. (2021).

T_{ag} and the TSM_{tag} were quite variable both among populations and between *E. neumayeri* and *E. apleurogramma*, though *E. apleurogramma* had both a lower mean T_{ag} and lower mean TSM_{tag} than *E. neumayeri.* Studies measuring T_{ag} are still few, but this metric may be an important and ecologically relevant metric in assessing a species’ thermal tolerance as it is thought to reflect the temperature at which fish begin to exhibit behavioural responses to thermal stress (Lutterschmidt and Hutchison 1997; McDonnell and Chapman 2015; Bouyoucos et al. 2023). As such, agitated fish have higher activity levels and consequently higher metabolic demands a well as a decrease in shelter use which could increase predation risk (Ros et al. 2006; McDonnell and Chapman 2015; Barki et al. 2022; Axling et al. 2023). For *E. neumayeri* the T_{ag} was an average of 4.8 °C below the CT_{max} across the three sampled sites; and for *E. apleurogramma* the T_{ag} was an average of 5.2 °C below the CT_{max} across the same sample sites. Thus, fish are becoming agitated several degrees prior to their CT_{max}, a difference referred to as the agitation window (McDonnell et al. 2021). The agitation window for *E. apleurogramma* and *E. neumayeri* fell within the range of other studies. For example, for Pugnose Shiner (*Miniellus* *anogenus*) studies have reported an agitation window of 5.4 ˚C for fish acclimated to 16 ˚C and an agitation window of 2.3 ˚C and 2.2 ˚C for fish acclimated to 19 ˚C and 22 ˚C, respectively; whereas a study of six Brook Trout (*Salvelinus fontinalus*) populations yielded much larger agitation windows varying from approximately 11.1 ˚C to 12.2 ˚C (McDonnell et al. 2021; Wells et al. 2016). Regardless, the T_{ag}, the TSM_{tag}, and the agitation window experienced by both *E. apleurogramma* and *E. neumayeri* provides them with a relatively large buffer in dealing with warming water temperatures in the sense that these species are able to withstand a large range of water temperatures before experiencing agitation behavior and an even larger range of water temperatures before experiencing physiological distress and loss of equilibrium. Freshwater tropical ectotherms are believed to be particularly vulnerable to increases in water temperature as they are thought to have relatively narrow thermal tolerance windows and are therefore already living close to their physiological optima (Sunday et al. 2014; Pincebourde and Suppo 2016). However, this does not seem to be the case for *E. apleurogramma* and *E. neumayeri*.

CT_{min} values experienced by each population of *E. neumayeri* and *E. apleurogramma* were far lower than the environmental water temperature at every given site and across sites CT_{min} was lowest overall at Inlet Stream West, the coolest of the three sites according to our long-term limnological data (Table 2). There are very few studies that report CT_{min} values for freshwater fishes, however for ornamental fishes it is reported that CT_{min} values range between 2.55 ˚C and 11.66 ˚C for fish acclimated to 20 ˚C and between 6.54 ˚C and 13.94 ˚C for fish acclimated to 28 ˚C (Yanar et al. 2019). Thus, the CT_{min} values measured for *E. neumayeri* and *E. apleurogramma* fall well within the range for fishes acclimated to 20 ˚C. Among the few studies which focus on measuring CT_{min}, there are even fewer that have studied inter-populational variation of lower thermal limits in freshwater fishes. However, one study focusing on inter-populational variation of CT_{min} in Atlantic Killifish (*F. heteroclitus*) reported that southern populations haver higher CT_{min} values than northern populations (Fangue et al. 2006). In addition, studies focusing on the genetic mechanisms of cold resistance genes in aquaculture of Nile tilapia (*Oreochromis niloticus)* found that CT_{min} ranged between 6.6 ˚C and 8.2 ˚C and that acclimation to low water temperatures enhanced cold resilience by significantly decreasing CT_{min} (Blodorn et al. 2024; Song et al. 2024). Taken together, the findings from these studies seem to suggest an element of local adaptation and acclimation capacity in terms of lower critical thermal limits. Quite valuably, there are genetic studies in our focal species, albeit for different traits. For *E. apleurogramma*, genetic studies on dissolved oxygen adaptation suggest a high plasticity in gene expression which could allow them to have a rapid response to new environments (Fox et al. 2024). Thus, it may be possible that the lower CT_{min} values exhibited by populations of *E. neumayeri* and *E. apleurogramma* inhabiting cooler waters is the result of local adaptation. Again, we did not test for plasticity in thermal tolerance and/or genetic differences between populations so we cannot address the source of the variation between populations from different sites.

Mean CT_{min} was lower in *E. apleurogramma* than in *E. neumayeri*, which goes against the hypothesis that upstream populations of *E. neumayeri* would have a lower CT_{min} than upstream populations *E. apleurogramma* as a reflection of longer evolutionary history with cooler water. The lower CT_{min} in *E. apleurogramma* is surprising, but consistent with the current distribution of both species. *E. neumayeri* is still common in the warmer waters of Bihehe, whereas *E. apleurogramma* is quite rare (R. Pahulje and L. Chapman, personal observations). On the other hand, long-term sampling of fish communities suggests that *E. apleurogramma* is shifting its range upstream to cooler waters where it has, since its initial appearance in the Rwembaita Swamp System in 2015, increased in abundance and formed established populations. If CT_{min} is associated with contraction of the downstream southern (warmer) limits of the range, the higher CT_{min} in *E. neumayeri* may facilitate a more downstream southerly distribution whereas the lower CT_{min} in *E. apleurogramma* may have facilitated its upstream expansion. *E. neumayeri* was reported at the Kahunge site in the Mpanga River in 2007 (Langerhans et al. 2007; Fig. 1), although periodic sampling in more recent years has not recovered *E. neumayeri* at sites south of Bihehe, suggesting that this species has also exhibited range contraction of its southern distributional limits but not to the degree exhibited by *E. apleurogramma*. Many studies focusing on modelling species' distribution, adaptation, and/or performance in response to climate utilize CT_{max} (Morgan et al. 2020; Desforges et al. 2023). However, our findings seem to suggest that a species' ability to expand its range upstream to cooler waters may be better predicted by its CT_{min} than its CT_{max}. For example, in the case where a species has a higher CT_{min} it may not be able to withstand cooler waters, which would prevent it from shifting its distribution. Not only does this limit a species range, but it also means that this species may be forced to perform under suboptimal conditions if temperatures in the limits of its distribution start to exceed its upper thermal limits. Thus, measuring CT_{min} in combination with CT_{max} may be a more thorough method when trying to understand and predict the ability and extent of species' range shifts.

E. apleurogramma, E. alberti, and E. kerstenii

Overall, CT_{max} did not differ significantly among *E. apleurogramma*, *E. alberti*, and *E. kerstenii*; however, CT_{max} of *E. apleurogramma* was lower than that of *E. neumayeri*. This is consistent with the idea that upstream populations of *E. apleurogramma* likely stemmed from a downstream population of the river system that inhabited warmer water than the water temperature in the Rwembaita Swamp System. It would be interesting to continue to monitor the CT_{max} of *E. apleurogramma* and *E. neumayeri* through time to see if the CT_{max} of *E. apleurogramma* eventually becomes more similar to that of *E. neumayeri*.

For CT_{min} we found a significant level of interspecific variation with *E. apleurogramma* having the lowest CT_{min} followed by *E. kerstenii* and *E. alberti*. This finding is consistent with the hypothesis that a low CT_{min} may be leading to contraction at the downstream southern end of the range of *E. apleurogramma* within the Mpanga River System, while a high CT_{min} in the downstream residents may be constraining them to the south of the river system. If this is true, then we may expect to see *E. kerstenii* shift its distribution in the near future if waters continue to warm under future climate scenarios. Again, it is difficult to say if the lower CT_{min} is the driver of the range shift or simply the result of plastic or heritable change in response to local conditions in the north i.e. the upstream range shift may have resulted from another driver followed by phenotypic change in CT_{min} in the upstream cooler water. Cold tolerance in freshwater fishes is under strong selection and differences in cold tolerance can be heritable, because of this cold tolerance has the ability to rapidly evolve in freshwater fishes (Barrett et al. 2011). Thus, we cannot reject the possibility that upstream populations of *E. apleurogramma* have a low CT_{min} due to their plastic and/or heritable response to cooler waters.

Climate niche modeling assumes that species latitudinal ranges reflect their thermal tolerance (Stevens 1989; Sunday et al. 2012). Geographic range boundaries of marine ectotherms are said to be closely aligned with their potential ranges based on thermal tolerance and extreme temperature (Sunday et al. 2012). In fact, in using global observations of range shifts in marine ectotherms, studies have shown that assemblages of marine ectotherms have contracted their range near the equator and expanded their range at their northern limits with similar frequency (Sunday et al. 2012). In the case of *E. apleurogramma*, the thermal limits as estimated by CT_{max} and CT_{min} are far above and below the actual water temperature inhabited by this species. The TSM suggests a large buffer between the critical upper and lower limits and the maximum and minimum water temperatures for the two sites for which we were able to calculate the TSM. This is the case for many freshwater fishes, where TSM values are relatively high (e.g. Campos et al. 2020; Chapman et al. 2022).

Limitations and Future Directions

It is important to note that range-shifts can be risky given the novel environment and interactions that a range-shifting species must face (Arenas et al. 2011; Fitt and Lancaster 2017). In shifting upstream, it is likely that *E. apleurogramma* competes for resources with *E. neumayeri* given their close phylogenetic relationship and similar ecologies (Greenwood 1966; Hunt et al. 2023; Fox et al. 2024). A study of the relative abundance of species in the Rwembaita Swamp System between 2019 and 2020 found that *E. apleurogramma* and *P. modestus* made up an average of 22.8% and 24.0% of the fish captured across sites, respectively; while native resident species *C. liocephalus* and *E. neumayeri* made an average of 18.5% and 34.8%, respectively (L. Chapman and A. Buchanan, unpubl. data). A survey using the same methods in 1993 found that *E. neumayeri* comprised 73.2% of the fish captured across the same set of sites in the Rwembaita Swamp System (L. Chapman and A. Buchanan, unpubl data). Thus, in terms of relative abundance, *E. neumayeri* has shown a dramatic decline. Following these assemblages in the future will be of extreme importance to monitor the appearance of new species into the Rwembaita Swamp System and changes in the fish community structure.

Future studies should also focus on evaluating changes in fitness-related traits over time in both the range shifting species and those that have not yet shifted their range since it is also possible that CT_{max} and CT_{min} correlate with functional traits that drive range shifts. Functional thermal tolerance limits can be defined as temperatures where key fitness-related traits (e.g., growth, reproduction, predator avoidance) become restricted (Farrell 2009; Schulte 2015; Dressler et al. 2023). For example, inter-populational differences in CT_{max} along with other performance-related traits including aerobic scope, metabolic rate, and exercise recovery, have been recorded for two populations of Rainbow Trout *(O. mykiss)* (Dressler et al. 2023). Thus, it would be interesting to evaluate changes in fitness-related traits over time in the system (e.g., fish condition, fecundity) and/or to explore the relationship between CT_{min}, CT_{max}, and fitness-related traits.

A limitation of this study was that we did not test for plasticity in upper thermal tolerance, which meant that we were unable to address the source of variation between populations and species. Future studies should focus on measuring short term plasticity or acclimation capacity of these fishes by exposing them to a series of warmer water temperatures before measuring their upper thermal limits. Rearing studies across a range of temperatures would also be insightful. Since we do not have any previous data on the thermal limits of *E. apleurogramma* we cannot reject the possibility that upstream populations have acclimated to the cooler waters of the Rwembaita Swamp System. Studies that focus on the role of plasticity in determining thermal limits would be valuable to better understand how these species' are able to adapt and acclimate to changing environments.

Lastly, our study focuses on one drainage system, but the distribution of *E. apleurogramma* and its congeners are much larger; and it is possible that across a very broad latitudinal range, one might find a tighter relationship between range and thermal limits. Future studies in East Africa should consider species distributions on a larger scale and across multiple river systems to gain a better understanding of inter-populational and inter-specific variation in thermal tolerance and its relationship with species distribution.

Conclusion

There are very few studies that have measured CT_{min} in freshwater and tropical fishes, and even fewer which have measured CT_{min} on field populations, and none, to my knowledge that have measured CT_{min} in response to rearing under divergent temperature regimes. As far as I am aware, this study also represents the first time that CT_{max} and CT_{min} were measured in a field setting for tropical fishes. Overall, our results suggest that CT_{min} may be a predictor of range contraction at the downstream southern distribution of *E. apleurogramma* in the Mpanga River System. However, the TSM for CT_{min} is large, suggesting that CT_{min} may correlate with functional traits that have population level consequences. It may not be CT_{min} per se, but other traits correlated with CT_{min}, not measured in our study, which are driving range shifts. Thus, future studies on thermal tolerance should include a combination of both lower and upper critical thermal limits, plasticity of thermal limits, and changes in fitness related-traits in order to get a better understanding of species thermal windows and their ability to shift their distribution in the face of environmental change.

TABLES

Table 1: Average body mass (g), standard and total length (cm), and sample size (n) for each species. Values are reported as mean ± SEM. Total sample size of 24 fish per population, 12 for CT_{max} trials and 12 for CT_{min} trials.

Species	Mass (g)	Standard length (cm)	Total length (cm)	Total sample size (n)	Number of sites	# fish per site
E. apleurogramma	1.32 ± 0.06	3.78 ± 0.06	4.58 ± 0.08	72	3	24
E. neumayeri	1.52 ± 0.15	3.82 ± 0.07	4.76 ± 0.09	72	3	24
E. alberti	2.12 ± 0.12	4.69 ± 0.10	6.02 ± 0.12	24	1	24
E. kerstenii	1.49 ± 0.10	3.99 ± 0.09	5.03 ± 0.11	24	1	24

Table 2: Summary of long-term limnological data for Inlet Stream East and Inlet Stream West. Site temperature (°C) and dissolved oxygen (DO; mg/L) concentration are given as mean ± SEM calculated from the monthly sampling (2020-2023). Average environmental maximum and minimum (Avg. Max. Temp. and Avg. Min. Temp.) were calculated based on the 10 highest and lowest temperatures for each respective site between 2020-2023.

Long-Term Limnological Data (2020-2023)

Site name	Temperature (°C)	DO (mg/L)	Avg. Max. Temp (°C)	Avg. Min. Temp (°C)
Inlet Stream East	18.92 ± 0.08	3.65 ± 0.11	21.34 ± 0.08	16.65 ± 0.24
Inlet Stream West	17.96 ± 0.09	5.85 ± 0.13	21.53 ± 0.62	15.51 ± 0.06

Table 3: Summary of short-term limnological data for every sampling site. Site temperature (°C) and dissolved oxygen (DO; mg/L) are given as mean ± SEM calculated from the sampling done during the field season between May and August 2023.

Short-Term Limnological Data (May- August 2023)

Site name	Habitat type	Temperature (°C)	DO (mg/L)
Inlet Stream East	Stream	18.56 ± 0.22	3.51 ± 0.22
Inlet Stream West	Stream	19.31 ± 0.42	5.96 ± 0.17

Sebitoli	River	20.63 ± 0.20	6.57 ± 0.09
Bihehe	River	21.34 ± 0.08	6.94 ± 0.02

Table 4: Mean temperature (˚C) and dissolved oxygen (DO; mg/L) of each experimental cooler used to hold fish at the Makerere University Biological Field Station for a maximum of five days. Values are reported as mean ± SEM.

Cooler #	Average Temperature (˚C)	Average DO (mg/L)
1	18.68 ± 0.11	6.64 ± 0.20
2	18.76 ± 0.11	6.40 ± 0.20
3	18.70 ± 0.11	6.31 ± 0.23
4	18.80 ± 0.15	5.99 ± 0.22
5	18.53 ± 0.12	6.73 ± 0.18
6	18.80 ± 0.22	6.97 ± 0.18

Table 5: Results of ANOVA testing for differences in mass (g), total length (cm), and standard length (cm) among species (*Enteromius apleurogramma* vs *Enteromius neumayeri* vs *Enteromius kerstenii* vs *Enteromius alberti*). Reported as p-values and F-values. Significant p-values (p<0.05) are shown in bold.

Mass: Mass ~ Species

Effect	Sum of Squares	Mean Square	df	F value	*p*
Species	11.16	3.72	3	4.81	**0.0030**
Residual	146.13	0.77	189		

Length: Length ~ Species

Effect	Sum of Squares	Mean Square	df	F value	*p*
Species	38.07	12.69	3	28.24	**<0.0001**
Residual	84.94	0.45	189		

Standard length: Standard Length ~ Species

Effect	Sum of Squares	Mean Square	df	F value	*p*
Species	15.81	5.27	3	18.82	**<0.0001**
Residual	52.92	0.28	189		

Table 6: Results of post-hoc Tukey test (Tukey HSD) for mean mass (g), standard length (cm), and total length (cm) for all species. Reported as adjusted p-values under the diagonal line and difference values over the diagonal line. Species abbreviated as EA (*E. apleurogramma*); EN (*E. neumayeri)*; EK (*E. kerstenii)*; and EC *(E. alberti*, formerly *E. cercops).* Significant p-values (p<0.05) are shown in bold.

Mass

Species	EC	EA	EK	EN
EC		**0.7971**	-0.6281	**-0.6001**
EA	**0.00112**		0.1690	0.1969
EK	0.0638	0.8339		0.02780
EN	**0.02558**	0.5366	0.9905	

Standard length

Species	EC	EA	EK	EN
EC		**1.1897**	**-1.5736**	0.5728
EA	**0.0006**		0.4053	**0.6169**
EK	**<0.0001**	0.5589		**1.0009**
EN	0.2138	**0.0146**	**0.0018**	

Total length

Species	EC	EA	EK	EN
EC		0.9113	**-0.8627**	-0.1253
EA	**<0.0001**		0.2128	0.0486
EK	**<0.0001**	0.2940		-0.1642
EN	**<0.0001**	0.9462	0.5317	

Table 7: Results of ANOVA analysis testing for differences in temperature and dissolved oxygen concentration between sites. Results are based on short-term limnology only. Reported as p-values and F-values. Significant p-values ($p<0.05$) are shown in bold.

Average Temperature: Average Temperature ~ Site

Effect	Sum of Squares	Mean Square	df	F value	*p*
Site	89.93	29.98	3	5.79x10^{29}	**<0.0001**
Residual	0	0	76		

Dissolved Oxygen: Dissolved oxygen ~ Site

Effect	Sum of Squares	Mean Square	df	F value	*p*
Site	148.4	49.47	3	3.04x10^{30}	**<0.0001**
Residual	0	0	76		

Table 8: Results of post-hoc Tukey test (Tukey HSD) for average environmental water temperature and dissolved oxygen concentration for all study sites. Reported as adjusted p-values under the diagonal line and difference values over the diagonal line. Sites abbreviated as ISE – Inlet Stream East; ISW – Inlet Stream West; SEB – Sebitoli; and BIH – Bihehe. Significant p-values ($p<0.05$) are shown in bold.

Average Temperature

Species	ISE	BIH	SEB	ISW
ISE		**-2.7854**	**2.0724**	**0.7490**
BIH	**<0.0001**		**-0.7130**	**-2.0363**
SEB	**<0.0001**	**<0.0001**		**1.3234**
ISW	**<0.0001**	**<0.0001**	**<0.0001**	

Average Dissolved Oxygen

Species	ISE	BIH	SEB	ISW
ISE		**-3.4271**	**3.0581**	**2.4421**
BIH	**<0.0001**		**-0.3689**	**-0.9850**
SEB	**<0.0001**	**<0.0001**		**0.6160**

ISW	**<0.0001**	**<0.0001**	**<0.0001**	

FIGURES

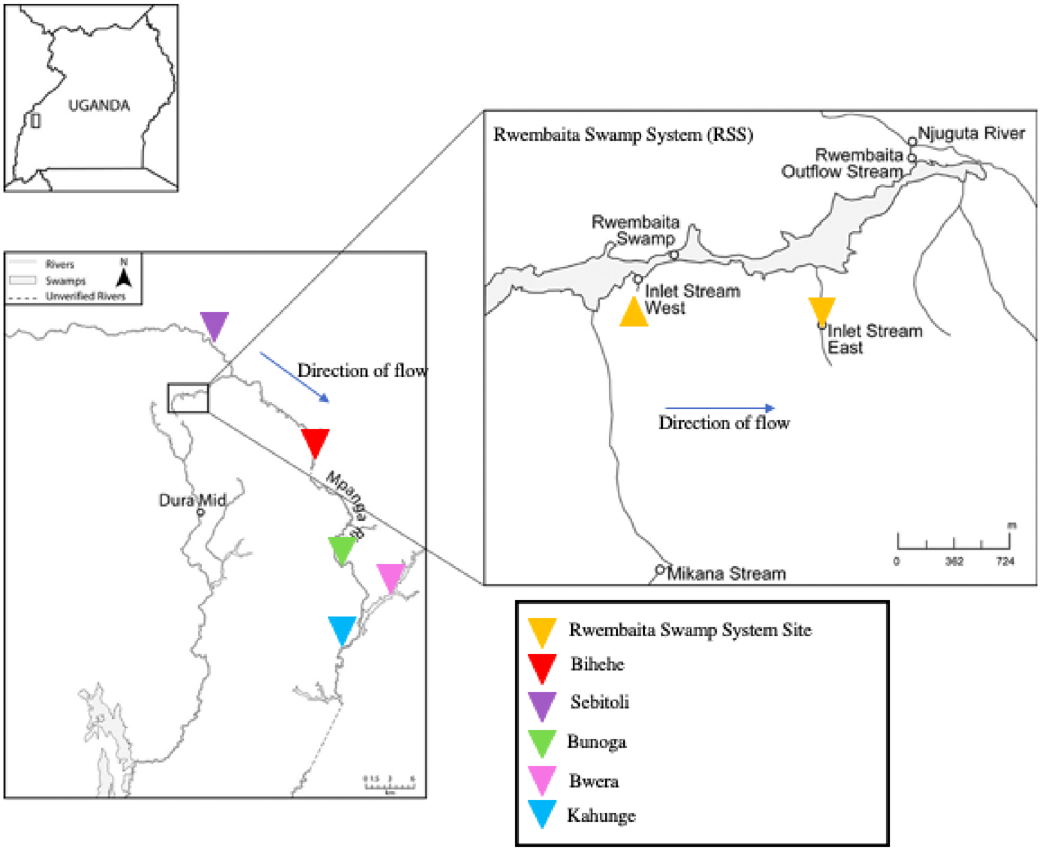

Figure 1: Map of the Mpanga River System and study sites. Sampling sites include: Inlet Stream East, Inlet Stream West, Bihehe, and Sebitoli. Bwera, Kahunge and Bunoga are sites where *E. apleurogramma* was captured prior to colonizing the Rwembaita Swamp System.

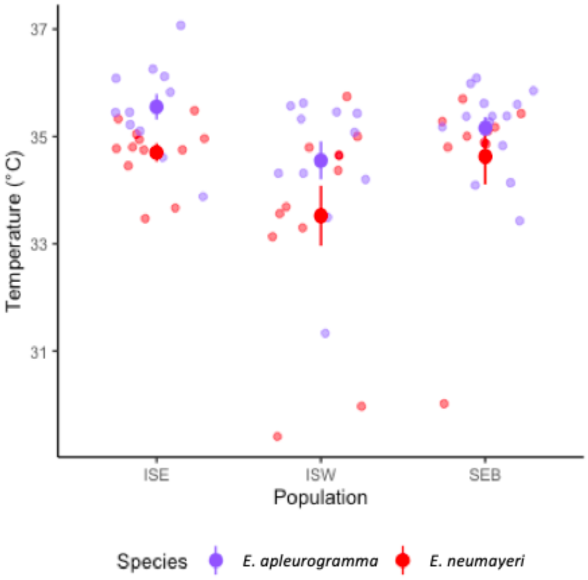

Figure 2: CT$_{max}$ of *E. apleurogramma* and *E. neumayeri* for Rwembaita Swamp System Sites (Inlet Stream West and East) and Sebitoli. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), and SEB (Sebitoli). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

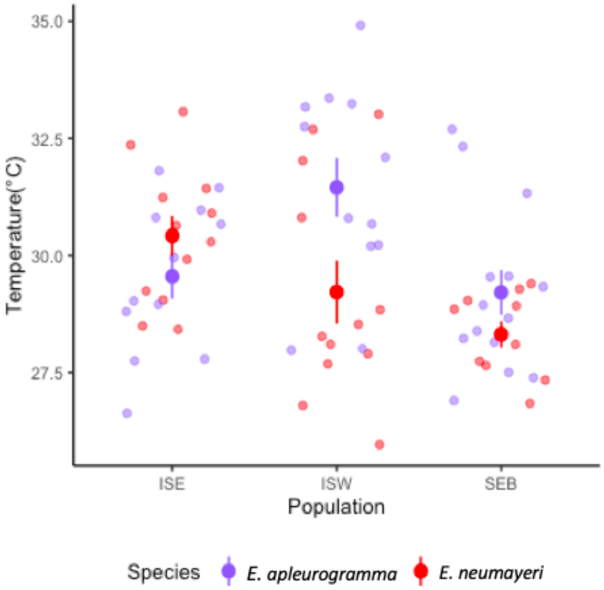

Figure 3: T$_{ag}$ of *E. apleurogramma* and *E. neumayeri* for Rwembaita Swamp System Sites (Inlet Stream West and East) and Sebitoli. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), and SEB (Sebitoli). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

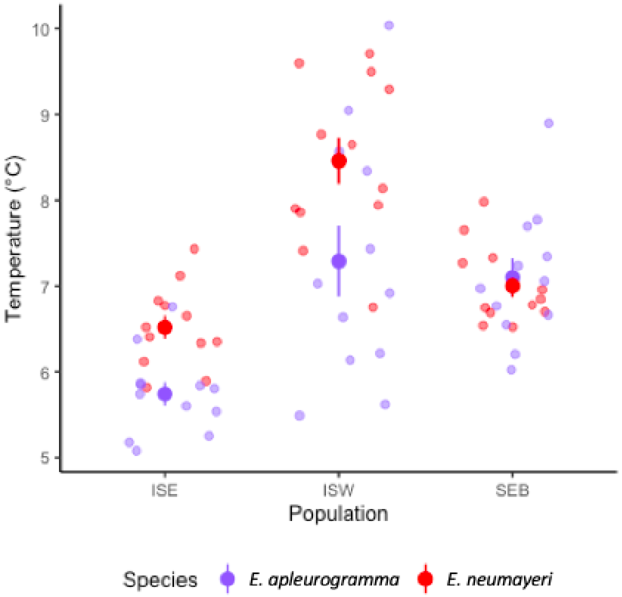

Figure 4: CT_{min} of *E. apleurogramma* and *E. neumayeri* for Rwembaita Swamp System Sites (Inlet Stream West and East) and Sebitoli. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), and SEB (Sebitoli). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

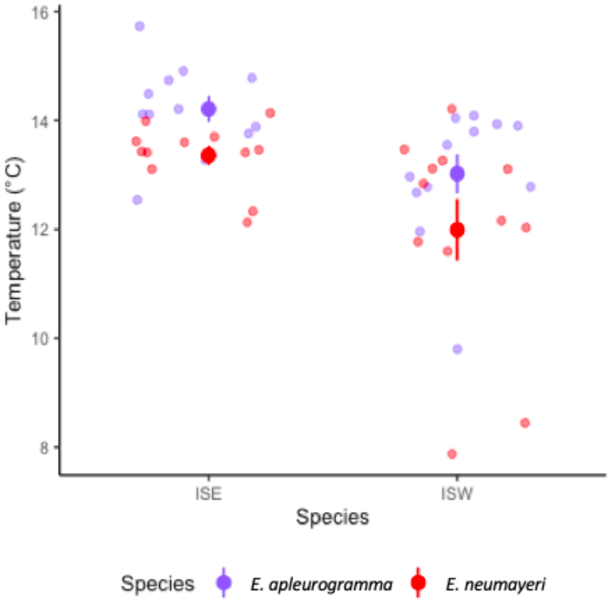

Figure 5: TSM_{max} of *E. apleurogramma* and *E. neumayeri* for Rwembaita Swamp System Sites (Inlet Stream West and East) where long-term limnological data are available. Sites abbreviated as ISE (Inlet Stream East) and ISW (Inlet Stream West). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

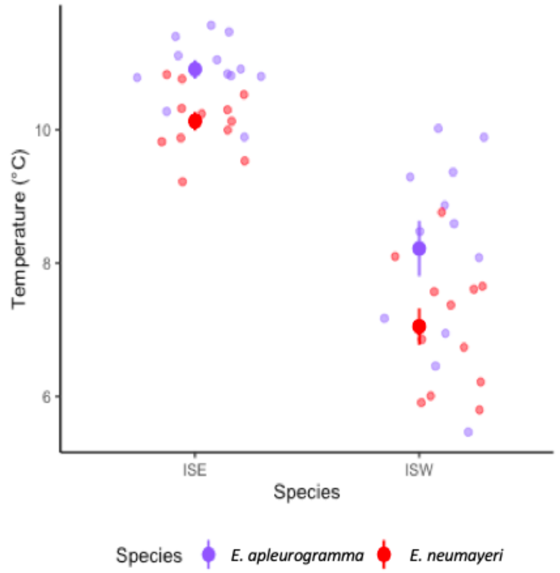

Figure 6: TSM$_{min}$ of *E. apleurogramma* and *E. neumayeri* for Rwembaita Swamp System Sites (Inlet Stream West and East) where long-term limnological data are available. Sites abbreviated as ISE (Inlet Stream East) and ISW (Inlet Stream West). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

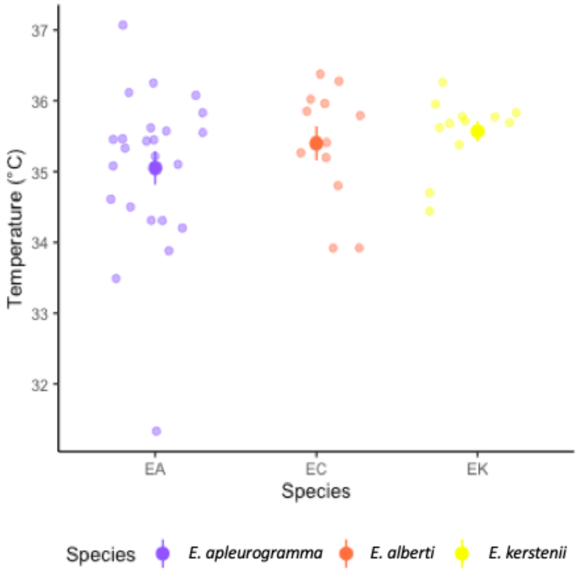

Figure 7: CT$_{max}$ of *E. apleurogramma*, *E. alberti*, and *E. kerstenii*. *E. apleurogramma* includes populations from Inlet Stream West and East. Species abbreviated as EA (*E. apleurogramma*), EC (*E. alberti, synonym - E. cercops*), and EK (*E. kerstenii*). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

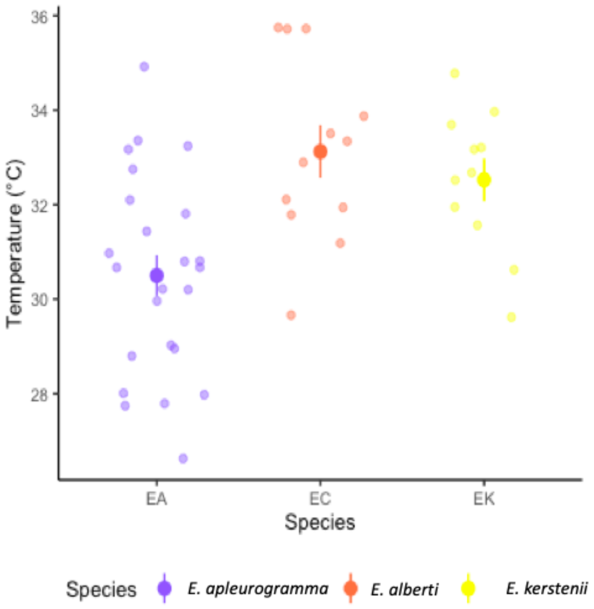

Figure 8: T_{ag} of *E. apleurogramma*, *E. alberti*, and *E. kerstenii*. *E. apleurogramma* includes populations from Inlet Stream West and East. Species abbreviated as EA (*E. apleurogramma*), EC (*E. alberti, synonym - E. cercops*), and EK (*E. kerstenii*). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

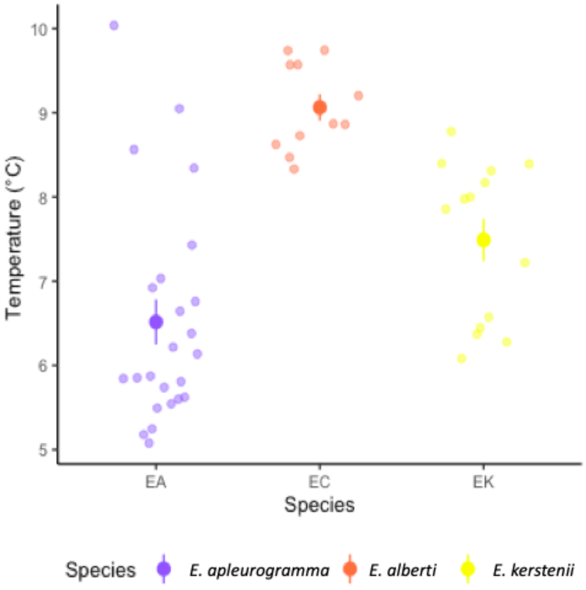

Figure 9: CT_{min} of *E. apleurogramma*, *E. alberti*, and *E. kerstenii*. *E. apleurogramma* includes populations from Inlet Stream West and East. Species abbreviated as EA (*E. apleurogramma*), EC (*E. alberti, synonym - E. cercops*), and EK (*E. kerstenii*). Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

SUPPLEMENTARY MATERIAL

Table 1: Results of linear modelling and linear mixed modelling testing for differences in CT_{max}, T_{ag}, TSM_{tag}, TSM_{max}, CT_{min}, and TSM_{min} among populations of *Enteromius apleurogramma* and *Enteromius neumayeri*. Reported as p-values and F-values for linear models and X^2 values for mixed models. Best-fit models were determined using AIC (see methods). Significant p-values ($p<0.05$) are shown in bold.

CT_{max}: CT_{max} ~ Population + Species

Effect	Sum of Squares	df	F value	*p*
Population	15.45	2	5.16	**0.0082**
Species	11.60	1	7.71	**0.0071**
Residual	113.115	84		

*T_{ag}: T_{ag} ~ Population * Species*

Effect	Sum of Squares	df	F value	*p*
Population	32.00	2	4.92	**0.0101**
Species	10.06	1	3.10	0.0831
Population * Species	29.07	2	4.47	**0.0151**
Residuals	214.484	66		

Thermal safety margin (agitation temperature): TSM_{tag} ~ Population + Species

Effect	Sum of Squares	df	F value	*p*
Population	0.251	1	0.0581	0.8107
Species	6.271	1	1.4517	0.2346
Residuals	194.402	45		

Thermal safety margin (max): TSM_{max} ~ Population + Species

Effect	Sum of Squares	df	F value	*p*
Population	19.64	1	12.69	**0.0009**
Species	10.62	1	6.87	**0.0119**

Residuals	69.61	45	

CT_{min}: CT_{min} ~ Population + Species + (1| trial)

Effect	X^2	df	*p*
Population	26.72	2	**<0.0001**
Species	5.74	1	**0.0166**

Thermal safety margin (min): TSM_{min} ~ Population + Species + (1| trial)

Effect	X^2	df	*p*
Population	63.86	1	**<0.0001**
Species	7.84	1	**0.0051**

Table 2: Results of post-hoc Tukey test (Tukey HSD) for CT_{max} and T_{ag} for *Enteromius apleurogramma* and *Enteromius neumayeri*. Reported as adjusted p-values under the diagonal line and difference values over the diagonal line. Species abbreviated as EA (*E. apleurogramma*) and EN (*E. neumayeri).* Sites abbreviated as ISE – Inlet Stream East; ISW – Inlet Stream West; SEB – Sebitoli; and BIH – Bihehe. Significant p-values (p<0.05) are shown in bold.

CT_{max}

Population	ISW	ISE	SEB
ISW		**-1.0892**	**0.8988**
ISE	**0.0084**		-0.1904
SEB	**0.0355**	0.8531	

T_{ag}

Population	ISW	ISE	SEB
ISW		0.3483	**-1.4975**
ISE	0.7820		-1.1491
SEB	**0.0147**	0.0772	

T_{ag}

Species/Population	EA-ISW	EA-ISE	EA-SEB	EN-ISW	EN-ISE	EN-SEB
EA-ISW		1.9000	**-2.2417**	**-2.2333**	-1.0300	**-3.1357**
EA-ISE	0.1163		-0.3417	-0.3333	0.8700	-1.236

EA-SEB	**0.0275**	0.9967		0.0083	1.2117	-0.8940
EN-ISW	**0.0386**	0.9975	>0.9999		-1.2033	-2.1057
EN-ISE	0.7271	0.8438	0.5312	0.5788		-0.9023
EN-SEB	**0.0018**	0.6009	0.8364	0.8498	0.0832	

Table 3: CT$_{max}$, T$_{ag}$, and thermal safety margins (°C), for all species across all collection sites. Values are reported as mean ± SEM. Sites abbreviated as ISE – Inlet Stream East; ISW – Inlet Stream West; SEB – Sebitoli; and BIH – Bihehe.

Species	Pop.	CT$_{max}$ (°C)	T$_{ag}$ (°C)	TSM$_{max}$ (°C)	TSM$_{tag}$ (°C)
E. apleurogramma	ISE	35.55 ± 0.24	29.55 ± 0.47	14.21 ± 0.24	8.27 ± 0.48
E. apleurogramma	ISW	34.85 ± 0.21	31.51 ± 0.68	13.02 ± 0.35	9.92 ± 0.63
E. apleurogramma	SEB	35.16 ± 0.14	29.21 ± 0.48	NA	NA
E. neumayeri	ISE	34.70 ± 0.17	30.42 ± 0.43	13.36 ± 0.17	9.05 ± 0.43
E. neumayeri	ISW	33.52 ± 0.56	29.22 ± 0.68	11.99 ± 0.56	7.69 ± 0.68
E. neumayeri	SEB	34.63 ± 0.52	28.32 ± 0.28	NA	NA
E. alberti	BIH	35.40 ± 0.24	33.13 ± 0.56	NA	NA
E. kerstenii	BIH	35.57 ± 0.15	32.53 ± 0.45	NA	NA

Table 4: Average CT$_{max}$, T$_{ag}$, and thermal safety margins (°C), for each species. Values are reported as mean ± SEM. For *E. apleurogramma* and *E. neumayeri* average CT$_{max}$ and T$_{ag}$ are calculated for populations from Inlet Stream West, Inlet Stream East and Sebitoli whereas average TSM values are calculated for populations from Inlet Stream West and Inlet Stream East only due to lack of long-term environmental data.

Species	CT$_{max}$ (°C)	T$_{ag}$ (°C)	TSM$_{max}$ (°C)	TSM$_{tag}$ (°C)
E. apleurogramma	35.09 ± 0.16	30.03 ± 0.34	13.62 ± 0.24	9.10 ± 0.17
E. neumayeri	34.26 ± 0.27	29.38 ± 0.32	12.68 ± 0.32	8.37 ± 0.96
E. alberti	35.40 ± 0.24	33.13 ± 0.56	NA	NA
E. kerstenii	35.57 ± 0.15	32.53 ± 0.45	NA	NA

Table 5: Results of post-hoc Tukey test (Tukey glht) for CT_{min} for *Enteromius apleurogramma* and *Enteromius neumayeri*. Reported as adjusted p-values under the diagonal line and differences between averages over the diagonal line. Species abbreviated as EA (*E. apleurogramma*) and EN (*E. neumayeri*). Sites abbreviated as ISE – Inlet Stream East; ISW – Inlet Stream West; and SEB – Sebitoli. Significant p-values ($p<0.05$) are shown in bold.

CT_{min}

Population	ISW	ISE	SEB
ISW		**1.744**	-0.8246
ISE	**<0.0001**		**0.9196**
SEB	0.0528	**0.0240**	

Table 6: CT_{min} and TSM_{min} (°C) for all species across all collection sites. Values are reported as mean ± SEM. Sites abbreviated as ISE – Inlet Stream East; ISW – Inlet Stream West; SEB – Sebitoli; and BIH – Bihehe.

Species	Population	CT_{min} (°C)	TSM_{min} (°C)
E. apleurogramma	ISE	5.74 ± 0.14	10.91 ± 0.14
E. apleurogramma	ISW	7.32 ± 0.45	8.22 ± 0.41
E. apleurogramma	SEB	7.10 ± 0.23	NA
E. neumayeri	ISE	6.52 ± 0.14	10.13 ± 0.14
E. neumayeri	ISW	8.46 ± 0.27	7.05 ± 0.27
E. neumayeri	SEB	7.00 ± 0.13	NA
E. alberti	BIH	9.06 ± 0.16	NA
E. kerstenii	BIH	7.49 ± 0.25	NA

Table 7: Average CT_{min} and TSM_{min} (°C) for each species across all collection sites. Values are reported as mean ± SEM. For *E. apleurogramma* and *E. neumayeri* average CT_{min} is calculated for populations from Inlet Stream West, Inlet Stream East and Sebitoli whereas average TSM values are calculated for populations from Inlet Stream West and Inlet Stream East only due to lack of long-term environmental data.

Species	CT_{min} (°C)	TSM_{min} (°C)
E. apleurogramma	6.71 ± 0.20	9.56 ± 0.35
E. neumayeri	7.33 ± 0.18	8.59 ± 0.35

E. alberti	9.06 ± 0.16	NA
E. kerstenii	7.49 ± 0.25	NA

Table 8: Results of CT_{max}, T_{ag}, and CT_{min} models for *E. apleurogramma* and *E. alberti* and *E. kerstenii* comparison. Reported as p-values and F-values for linear models and X^2 values for linear mixed models. Best-fit models were determined using AIC (see methods). Significant p-values ($p<0.05$) are shown in bold.

CT_{max} (average of ISE and ISW for E. apleurogramma): CT_{max} ~ Species

Effect	Sum of Squares	df	F value	*p*
Species	2.42	2	1.35	0.27
Residuals	25.9076	44		

CT_{max} (ISE only for E. apleurogramma): CT_{max} ~ Species

Effect	Sum of Squares	df	F value	*p*
Species	0.207	2	1.90	0.83
Residuals	17.9714	33		

T_{ag} (average of ISE and ISW for E. apleurogramma): T_{ag} ~ Species + (1| trial)

Effect	X^2	df	*p*
Species	10.05	2	**0.0066**

T_{ag} (ISE only for E. apleurogramma): T_{ag} ~ Species + (1| trial)

Effect	X^2	df	*p*
Species	15.04	2	**<0.0001**

CT_{min} (average of ISE and ISW for E. apleurogramma): CT_{min} ~ Species + (1| trial)

Effect	X^2	df	*p*
Species	20.17	2	**<0.0001**

CT_{min} (ISE only for E. apleurogramma): CT_{min} ~ Species + (1| trial)

Effect	X^2	df	*p*
Species	68.81	2	**<0.0001**

Table 9: Results of post-hoc Tukey test (Tukey glht) for T_{ag} and CT_{min} for *Enteromius apleurogramma*, *Enteromius alberti*, and *Enteromius kerstenii.* Reported as adjusted p-values under the diagonal line and estimate values over the diagonal line. Species abbreviated as EA (*E. apleurogramma*); EC (*E. alberti, synonym cercops*); and EK (*E. kerstenii).* Significant p-values ($p<0.05$) are shown in bold.

T_{ag} (average of ISE and ISW for E. apleurogramma)

Species	EC	EK	EA
EC		-0.5345	**2.6233**
EK	0.8714		2.089
EA	**0.0122**	0.0637	

T_{ag} (ISE only for E. apleurogramma)

Species	EC	EK	EA
EC		-0.5245	**3.5733**
EK	0.8600		**3.0491**
EA	**<0.001**	**0.0066**	

CT_{min} (average of ISE and ISW for E. apleurogramma)

Species	EC	EK	EA
EC		-1.2354	**2.3609**
EK	0.0559		1.1254
EA	**<0.001**	0.0507	

CT_{min} (ISE only for E. apleurogramma)

Species	EC	EK	EA
EC		**-1.346**	**3.2044**
EK	**0.0003**		**1.8585**
EA	**<0.0001**	**<0.0001**	

REFERENCES

Alofs, K.M., Jackson, D.A., and Lester, N.P. 2014. Ontario freshwater fishes demonstrate differing range-boundary shits in a warming climate. Diversity and Distributions 20: 123-136.

Arenas, M., Ray, N., Currat, M., and Excoffier, L. 2011. Consequences of range contractions and range shifts on molecular diversity. Molecular Biology and Evolution 29: 207-218.

Armstrong, J.B., Fullerton, A.H., Jordan, C.E., Ebersole, J.L., Bellmore, J.R., Arismendi, I., Penaluna, B., and Reeves, G.H. 2022. The importance of warm habitat to the growth regime of cold-water fishes. Nature Climate Change 11: 354- 361.

Ashe-Jepsen, E., Cobo, S.A., Basset, Y., Bladon, A.J., Kleckova, I., Laird-Hopkins, B.C., Mcfarlane, A., Sam, K., and Savage, A.F. 2023. Tropical butterflies use thermal buffering and thermal tolerance as alternative strategies to cope with temperature increase. Journal of Animal Ecology 92: 1759-1770.

Axling, J., Vossen, L.E., Peterson, E., and Winberg, S. 2023. Boldness, activity, and aggression: Insights from a large-scale study in Baltic Salmon (*Salmo salar L*). PLosOne 18: no.0287836.

Baroudy, E., and Elliott, J.M. 1994. The critical thermal limits for juvenile Arctic charr *Salvelinus alpinus*. Journal of Fish Biology 45: 1041-1053.

Bates, A.E., and Morley, S.A. 2020. Interpreting empirical estimates of experimentally derived physiological and biological thermal limits in ectotherms. Canadian Journal of Zoology 98: 237-244.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., Fox, J., Bauer, A., Krivitsky, P.N., Tanaka, E., and Jagan, M. 2024. Fit linear and generalized linear mixed-effects models. The models and their components are represented using S4 classes and methods. The core computational algorithms are implemented using the 'Eigen' C++ library for numerical linear algebra and 'RcppEigen' "glue".

Barki, A., Cnaani, A., and Biran, J. 2022. How does temperature affect aggression during and after dominance hierarchy formation in Nile Tilapia? Applied Animal Behaviour Science 247: no.105563.

Bartlett, C.B., Garber, A.F., Gonen, S., and Benfey, T.J. 2022. Acute critical thermal maximum does not predict chronic incremental thermal maximum in Atlantic salmon (*Salmo salar*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 266: no.111143.

Barrett, R.D.H., Paccard, A., Healy, T.M., Bergek, S., Schulte, P.M., Schluter, D. and Rogers, S.M. 2011. Rapid evolution of cold tolerance in stickleback. Proceedings of the Royal Society B: Biological Sciences 278: 233-238.

Beitinger, T.L., and Lutterschmidt, W.I., 2011. Temperature: Measures of thermal tolerance. Encyclopedia of Fish Physiology: 1695-1702.

Bennett, W.A., and Beitinger, T.L. 1997. Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. Copeia 1997: 77-87.

Binning, S.A., Chapman, L.J., and Dumont, J. 2010. Feeding and breathing: Trait correlations in an African cichlid fish. Journal of Zoology 282: 140-149.

Blaustein, A.R., Belden, L.K., Olson, D.H., Green. D.M., Root, T.L., and Kiesecker, J.M. 2001. Amphibian breeding and climate change. Conservation Biology 15: 1804-1809.

Blaustein, A.R., Walls, S.C., Bancroft, B.A., Lawler, J.J., Searle, C.L., and Gervasi, S.S. 2010. Direct and indirect effects of climate change on amphibian populations. Diversity 2: 281-313.

Blodorn, E.B., Martins, A.W.S., Dellagostin, E.N., Nunes, L.S., da Conceicao, R.C.S., Pagano, A.D., Goncalves, N.M., dos Reis, L.F.V., Nascimento, M.C., Quispe, D.K.B., Froes, C.N., Tavares, R.A., Giongo, J.L., Vaucher, R.A., Robaldo, R.B., Domingues, W.B., Collares, G.L., Pinhal, D., and Campos, V.F. 2024. Toward new biomarkers of cold tolerance: microRNAs regulating cold adaptation in fish are differentially expressed in cold-tolerant and cold-sensitive Nile tilapia (*Oreochromis niloticus*). Aquaculture 589: no.740942.

Bouyoucos, I.A., Weinrauch, A.M., Jeffries, K.M., and Anderson, G. 2023. Physiological responses to acute warming at the agitation temperature in a temperate shark. Journal of Experimental Biology 226: jeb246304.

Bridle, J., and Hoffmann, A. 2022. Understanding the biology of species' ranges: when and how does evolution change the rules of ecological engagement? Philosophical Transactions of the Royal Society B: Biological Sciences 377: no1848.

Buckley, L.B., and Huey, R.B. 2016. How extreme temperature impacts organisms and the evolution of their thermal tolerance. Integrative and Comparative Biology 56: 98-109.

Bulger, A.J. 1984. A daily rhythm in heat tolerance in the salt marsh fish *Fundulus heteroclitus*. Journal of Experimental Zoology 230: 11-16.

Calfee, R.D., Puglis, H.J., Little, E.E., Brumbaugh, W.G., and Mebane, C.A. 2016. Quantifying fish swimming behavior in response to acute exposure of aqueous copper using computer assisted video and digital image analysis. Journal of Visualized Experiments 108: no.53477.

Campana, S.E., Stefansdottir, R.B., Jakobsdottir, K., Solmundsson, J. 2020. Shifting fish distributions in warming sub-Arctic oceans. Scientific Reports 10: no.16448.

Campos, D.F., Amanajas, R.D., Almeida-Val, V.M.F., Val, A.L. 2020. Climate vulnerability of South American freshwater fish: Thermal tolerance and acclimation. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 335: 1-12.

Capon, S.J., Koster, B.S., and Bunn, S.E. 2021. Future of freshwater ecosystems in a 1.5 ºC warmer world. Frontiers in Environmental Science 9: no.784642.
Carosi, A., Padula, R., Ghetti, L., and Lorenzoni, M. 2019. Endemic freshwater fish range shifts related to global climate changes: A long-term study provides some observational evidence for the Mediterranean area. Water 11: no.2349.

Champion, C., Brodie, S., and Coleman, M.A. 2021. Climate-driven range shifts are rapid yet variable among recreationally important coastal-pelagic fishes. Frontiers in Marine Science 8: no.622299.

Chapman, L.J. 1995. Seasonal dynamics of habitat use by an air-breathing catfish (*Clarias liocephalus*) in a papyrus swamp. Ecology of Freshwater Fish 4: 113-123.

Chapman, L.J., Chapman, C.A., Brazeau, D., McGlaughlin, B., and Jordan, M. 1999. Papyrus swamps and faunal diversification: Geographical variation among populations of the African cyprinid *Barbus neumayeri*. Journal of Fish Biology 54: 310-327.

Chapman, L.J., Nyboer, E.A., and Fugère, V. 2022. Fish response to environmental stressors in the Lake Victoria Basin Ecoregion. Pages 273-324. In Fangue, N. and Cooke, S., editors. Fish Physiology, Conservation Physiology for the Anthropocene – Issues and Applications. Volume 39.

Chretien, E., and Chapman, L.J. 2016. Tropical fishes in a warming world: Thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. Conservation Physiology 4: cow062.

Chen, Z., Antilla, K., Wu, J., Whitney, C.K., Hinch, S.G., and Farrell, A.P. 2013. Optimum and maximum temperatures of sockeye salmon (*Onchorhynchus nerka)* populations hatched at different temperatures. Canadian Journal of Zoology 91: 263-274.

Cicchino, A.S., Shah, A.A., Forester, B.R., Dunham, J.B., Poff, N.L., Ghalambor, C.K., and Funk, W.C. 2023. Acclimation capacity of critical thermal maximum varies among populations: Consequences for estimates of vulnerability. Ecosphere 14: e4691.

Comte, L., Buisson, L., Daufresne, M., and Grenouillet, G. 2013. Climate-induced changes in the distribution of fish: observed and predicted trends. Freshwater Biology 58: 625-639.

Comte, L., Murienne, J., and Grenouillet, G. 2014. Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes. Nature Communications 5: no.5053.

Comte, L., and Grenouillet, G. 2015. Distribution shifts of freshwater fish under variable climate: comparing climatic, bioclimatic and biotic velocities. Diversity and Distributions 21: 1014-1026.

Comte, L., and Olden, J. 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Global Change Biology 23: 728-736.

Comte, M., Felipe de Campos, D., and Eme, J. 2023. Effective practices for thermal tolerance polygon experiments using mottled catfish *Corydoras paleatus*. Journal of Thermal Biology 115: no.103616.

Cowan, Z.L., Andreassen, A.H., De Bonville, J., Green, L., Binning, S.A., Silva-Garay, L., Jutfelt, F., and Sundin, J. 2023. A novel method for measuring acute thermal tolerance in fish embryos. Conservation Physiology 11: coad061.

Cowles, R.B., and Bogert, C.M. 1944. A preliminary study of the thermal requirements of desert reptiles. Bulletin of the American Museum of Natural History 83: no.5.

Cox, D. K. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. Pages 158-163. In W. Gibbons and R. R.Sharitz, editors. Thermal Ecology. Springfield, IL: National Technical Information Service.

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

Debes, P.V., Solberg, M.F., Matre, I.H., Dyrhovden, L., and Glover K.A. 2021. Genetic variation for upper thermal tolerance diminishes between and within populations with increasing acclimation temperature in Atlantic salmon. Heredity 127: 455- 466.

Debnath, C. 2024. Assessing the thermal limits and metabolic profiles of small indigenous fish species: Informing conservation and aquaculture in a changing climate. Aquaculture Reports 39: no.102396.

Desforges, E.M. 2020. On applying critical thermal maxima method to investigate ecologically-relevant questions in wild fishes. Masters Thesis. Carlton University, Ottawa.

Desforges, J.E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K.M., Eliason, E.J., Dressler, T.L., McKenzie, D.J., Bates, A.E., Lawrence, M.J., Fangue, N., and Cooke, S.J. 2023. The ecological relevance of critical thermal maxima methodology for fishes. Journal of Fish Biology 102: 1000-1016.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Shelson, K.S., Ghalambor, C.K., Haak, D.C., and Martin, P.R. 2008. Impact of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences of the United States of America 105: 6663-6672.

Diamond, S.E. 2018. Contemporary climate-driven range shifts: Putting evolution back on the table. Functional Ecology 32: 1662-1665.

Dressler, T.L., Han Lee, V., Klose, K., and Eliason, E.J. 2023. Thermal tolerance and vulnerability to warming differ between populations of wild *Oncorhynchus mykiss* near the species' southern range limit. Scientific Reports 13: no.14538.

Duan, R-Y., Kong, X-Q., Huang, M-Y., Varela, S., and Ji, X. 2016. The potential effects of climate change on amphibian distribution, range fragmentation and turnover in China. PeerJ 4: e2185.

Duclos, V., Boudreau, S., and Chapman, C.A. 2013. Shrub cover influence on seedling growth and survival following logging of a tropical forest. Biotropica 45: 419-426.

Enders, E.C., and Durhack, T.C. 2022. Metabolic rate and critical thermal maximum of CTmax estimates for westslope cutthroat trout, *Oncorhynchus clarkii lewiki*. Conservation Physiology 10: coac071.

Fangue, N.A., Hofmeister, M., and Schulte, P.M. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. Journal of Experimental Biology 209: 2859-2872.

Fitt, R.N.L., and Lancaster, L.T. 2017. Range shifting species reduce phylogenetic diversity in high latitude communities via competition. Journal of Animal Ecology 86: 543-555.

Fox, J., and Weisberg, S. 2019. An R Companion to Applied Regression, Third Edition, Sage.

Fox, J., Hunt, D.A.G.A., Hendry, A.P, Chapman, L.J., and Barrett, R.D.H. 2024. Counter-gradient variation in gene expression between fish populations facilitates colonization of low-dissolved oxygen environments. Molecular Ecology: no.17419.

Gonen, S., Benfey, T.J., and Garber, A.F. 2024. The genomic architecture of a high temperature tolerance in a year class of Atlantic Salmon. Aquaculture 578: no.740020.

Greenwood, P.H. 1962. A revision of certain *Barbus* species (Pisces, Cyrprinidae) from east, central and south Africa. Bulletin of the British Museum (Natural History) Zoology 8: 151-208.

Greenwood, P.H. 1966. The Fishes of Uganda. The Uganda Society, Kampala, Uganda.

Guisan, A., Thuiller, W., and Zimmermann, N.E. 2017. What drives species distributions? Pages 21-40. In Usher, M., Saunders, D., Peet, R., and Dobson, A., editors. Habitat Suitability and Distribution Models: With Application in R. Cambridge University Press, Cambridge, United Kingdom.

Harniman, R., Merritt, T.J.S., Chapman, L.J., and Martinez-Garcia, M. 2013. Population differentiation of African cyprinid *Barbus neumayeri* across dissolved oxygen regimes. Ecology and Evolution 3: 1495-1506.

Hothorn, B. 2010. Simultaneous tests and confidence intervals for general linear hypotheses in parametric models, including linear, generalized linear, linear mixed effects, and survival models. CRC Press.

Hu, R., Gu, Y., Luo, M., Wei, M., and Zhong, J. 2020. Shifts in bird ranges and conservation priorities in China under climate change. PLoS ONE 15: e0240225.

Huey, R.B., and Stevenson, R.D. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. American Zoologist 19: 357-366.

Hunt, D.A.G.A., Hendry, A.P., and Chapman, L.J. 2023. Are we there yet? Inter- and intraspecific approaches to evaluating phenotypic optima in a range expanding East African fish, *Enteromius apleurogramma* (Cyprinidae). Biological Journal of the Linnean Society 140: 296-312.

Illing, B., Downie, A.T., Beghin, M., and Rummer, J.L. 2020 Critical thermal maxima of early stages of three tropical fishes: Effects of rearing temperature and experimental heating rate. Journal of Thermal Biology 2020: no.102582.

International Civil Aviation Organization. 1993. Manual of the ICAO Standard Atmosphere: extended to 80 kilometres (262500 feet). Montreal, QC: International Civil Aviation Organization.

Johansen, J.L., Nadler, L., Habary, A., Bowden, A.J., and Rummer, J. 2021. Thermal acclimation of tropical coral reef fishes to global heat waves. eLife 10: e59162.

Jones, N., and Schmidt, B. 2019. Thermal habitat: Understanding stream temperature and thermal classifications. Ontario Ministry of Natural Resources and Forestry, Science and Research Branch, Peterborough, ON. Science and Research Information Report IR-18. 13p.

Joyner-Matos, J. and Chapman, L.J. 2013. Persisting in papyrus: size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia. Comparative Biochemistry and Physiology Part A 165: 405–416.

Kawecki, T.J. 2000. Adaptation to marginal habitats: contrasting influence of the dispersal rate on the fate of alleles with small and large effects. Proceedings: Biological Sciences 267: 1315-1320.

Khelifa, R., Blanckenhorn, W.U., Roy, J., Rohner, P.T., and Mahdjoub, H. 2019. Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability. Journal of Animal Ecology 88: 1901-1912.

Kingsolver, J.G., and Umbanhowar, J. 2018. The analysis an interpretation of critical temperatures. Journal of Experimental Biology 221: jeb167858.

Kirk, M.A., and Rahel, F.J. 2022. Assessing climate-induced range shifts of stream fishes using a consensus framework. Ecology of Freshwater Fish 32: 270-281.

Korkmaz, M., Mangit, F., Dumlupinar, I., Colak, M.A., Akpinar, M.B., Koru, M., Pacheco, J.P., Garcia-Ramirez, A., Yilmaz, G., Amorim, C.A., Ozgencil, I.K., Innal, D., Yerli, S.V., Ozkan, K., Akyurek, Z., Baklioglu, M, and Jeppesen, E. 2023. Effects of climate change on the habitat suitability and distribution of freshwater fish species in semi-arid central Anatolian Ecoregion in Turkiye. Water 15: no.1619.

Kraskura, K., Hardison, E.A., and Eliason, E.J. 2023. Body size and temperature affect metabolic and cardiac thermal tolerance in fish. Scientific Reports 13: no.17900.

Lane, D. 2010. Tukey's honestly significant difference (hsd). Pages 1566-1570. Encyclopedia of Research Design. Sage Publications, Volume 0.

Langerhans, R.B., Chapman, L.J., and DeWitt, T.J. 2007. Complex phenotype-environment associations revealed in an East African cyprinid. Journal of Evolutionary Biology 20: 1171-1181.

Langerhans, R.B., Gifford, M.E., and Joseph, E.O. 2007. Ecological speciation in *Gambusia* fishes. Evolution 61: 2056-2074.

Lévêque, C., and Daget, J. 1984. Cyrpinidae. Pages 217-242. In J. Daget., D.E.F., Gosse, and T, Van den Auendaerde, editors. Check-list of the freshwater fishes of Africa (CLOFFA). ORSTOM, Paris and MRAC, Tervuren, Volume 1.

Leonard, J.N., and Skov, P.V. 2022. Capacity for thermal adaptation in Nile tilapia (*Oreochromis niloticus)*: Effects on oxygen uptake and ventilation. Journal of Thermal Biology 105: no.103206.

Leong, C.M., Tsang, T.P.N., and Guenard, B. 2022. Testing the reliability and ecological implications of ramping rates in the measurement of critical thermal maximum. PLoS ONE 17: e0265361.

Little, A.G., and Seebacher, F. 2021. Physiological performance curves: When are they useful? 2021. Frontiers in Physiology 12: no.805102.

Logan, M., Ane-Minnaar, I., Keegan, K., and Clusella-Trullas, S. 2019. The evolutionary potential of an insect invader under climate change. International Journal of Organic Evolution 74: 132-144.

Lutterschmidt, W. I., & Hutchinson, V. H. 1997. The critical thermal maximum: History and critique. Canadian Journal of Zoology 75: 1567–1574.

Madeira, C., Mendonca, V., Leal, M.C., Flores, A.A.V., Cabral, H.N., Diniz, M.S., and Vinagre, C. 2017. Thermal stress, thermal safety margins, and acclimation capacity in tropical shallow waters – An experimental approach testing multiple end-points in two common fish. Ecological Indicators 81: 146-158.

Martinez, M.L., L.J. Chapman, J.M. Grady, and Rees, B.B. 2004. Interdemic variation in hematocrit and lactate dehydrogenase in the African cyprinid Barbus neumayeri. Journal of Fish Biology 65: 1056-1069.

Martinez, M.L., Raynard, E.L., Rees, B.B., and Chapman, L.J. 2011. Oxygen limitation and tissue metabolic potential of the African fish *Barbus neumayeri*: roles of native habitat and acclimatization. BMC Ecology 11: 2.

Matt, T. 2019. The AIC is an estimate for OOS deviance. P. 90. Business Data Science: Combining Machine Learning and Economic to Optimize, Automate, and Accelerate Business Decisions. McGraw Hill, New York.

McDonnell, L.H., and Chapman, L.J. 2015. At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. Conservation Physiology 3: cov050.

McDonnell, L.H., Mandrak, N.E., Kaur, S., and Chapman., L.J. 2021. Effects of acclimation to elevated water temperature and hypoxia on thermal tolerance of the threatened Pugnose Shiner (*Notropis anogenus*). Canadian Journal of Fisheries and Aquatic Sciences 78: 1257-1267.

McElreath, R. 2016. AIC provides a surprisingly simple estimate of the average out-of-sample deviance. Pages 189. Stastistical Rethinking: A Bayesian Course with Examples in R and Stan. CRC Press.

McKenzie, D.J., Zhang, Y., Eliason, E.J., Schulte, P.M., Claireaux, G., Blasco, F.R., Nati, J.J.H., and Farrell, A.P. 2020. Intraspecific variation in tolerance of warming in fishes. Journal of Fish Biology 98: 1536-1555

McKenzie, D.J., Geffroy, B., and Farrell, A.P. 2021. Effects of global warming on fishes and fisheries. Journal of Fish Biology 98: 1489-1492.

McNab, B.K. 2002. The physiological ecology of vertebrates: A view from energetics. Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

Moreno-Rueda, G., Pleguezuelos, J.M., Pizarro, M., and Montori, A. 2011. Northward shifts of the distribution of Spanish reptiles in association with climate change. Conservation Biology 26: 278-283.

Miller, N.A., and Stillman, J.H. 2018. Physiological optima and critical limits. Nature Education Knowledge 10: 1.

Morgan, R., Finnoen, M.H., and Jutfelt, F. 2018. CT_{max} is repeatable and doesn't reduce growth in zebrafish. Scientific Reports 8: no.7099.

Morgan, R., Sundin, J., Finnoen, M.H., Dresler, G., Ventdrell, M.M., Dey, A., Sarkar, K., and Jutfelt, F. 2019. Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zerbrafish populations. Conservation Physiology 7: coz036.

Morgan, R. 2020. Physiological plasticity and evolution of thermal performance in zebrafish. Ph.D. Thesis. Norwegian University of Science and Technology, Trondheim, Norway.

Morgan, R., Finnoen, M.H., Jensen, H., Pelabon, C., and Jutfelt, F. 2020. Low potential for evolutionary rescue from climate change in a tropical fish. Proceedings of the National Academy of Science 117: 33365-33372.

Morley, S.A., Peck , L.S., Sunday, J.M., Heiser, S., and Bates, A.E. 2018. Physiological acclimation and persistence of ectothermic species under extreme heat events. Global Ecology and Biogeography 28: 1018-1037.

Nati, J.J.H., Svendsen, M.B.S., Marras, S., Killen, S.S., Steffensen, J.F., McKenzie, D.J., and Domenici, P. 2021. Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. Scientific Reports 11: no.21272.

Nguyen, K.D.T., Morley, S.A., Lai, C.H., Clark, M.S., Tan, K.S., Bates, A.E., Peck, L.S. 2011. Upper temperature limits of tropical marine ectotherms: global warming implications. PLoS ONE 6: e29340.

Nuon, V., Chea, R., Lek, S., So, N., Hgueny, B., and Grenouillet, G. 2024. Climate change drives contrasting shifts in fish species distribution in the Mekong Basin. Ecological Indicators 160: no.111857

Nyober, E.A. and Chapman, L.J. 2017. Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. Journal of Experimental Biology 220: 3782-3793.

Nyober, E.A. and Chapman, L.J. 2018. Cardiac plasticity influences aerobic performance and thermal tolerance in a tropical, freshwater fish at elevated temperatures. Journal of Experimental Biology 221: no.178087.

Nyober, E.A., Chretien, E., and Chapman, L.J. 2020. Divergence in aerobic scope and thermal tolerance is related to local thermal regime in two introduced populations of Nile perch (*Lates niloticus*). Journal of Fish Biology 97: 231-245.

Olowo, J.P. and Chapman, L.J. 1996. Papyrus swamps and variation in the respiratory behaviour of the African fish *Barbus neumayeri*. African Journal of Ecology 34: 211-222.

Orsted, M., Jorgensen, L.B., and Overgaard, J. 2022. Finding the right thermal limit: a framework to reconcile ecological, physiological and methodological aspects of CTmax in ectotherms. Journal of Experimental Biology 225: jeb244514.

Pincebourde, S., and Suppo, C. 2016. The vulnerability of tropical ectotherms to warming is modulated by the microclimatic heterogeneity. Integrative and Comparative Biology 56: 85-97.

Piczak, M.L., Perry, D., Cooke, S.J., Harrison, I., Benitez, S., Koning, A., Peng., L., Limbu, P., Smokorowski, S.S-R., Koehn, J.D., and Creed, I.F. 2023. Protecting and restoring habitats to benefit freshwater biodiversity. Environmental Reviews 00: 1-19.

Potts, L., Mandrak, N.M., and Chapman, L.J. 2021. Coping with climate change: Phenotypic plasticity in the imperiled freshwater fish in response to elevated water temperature. Aquatic Conservation 31: 2726-2736.

Pörtner, H.O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213: 881-893.

Punzon, A., Lopez-Lopez, L., Gonzalez-Irusta, J.M., Preciado, I., Hidalgo, M., Serrano, A., Tel, E., Somavilla, R., Polo, J., Blanco, M., Ruiz-Pico, S., Ferndandez-Zapico, O., Velasco, F., and Massuti, E. 2021. Tracking the effect of temperature in marine demersal fish communities. Ecological Indicators 121: no.107142.

Quinn, N.L., McGowan, C.R., Cooper, G.A., Koop, B.F., and Davidson, W.S. 2011. Identification of genes associated with heat tolerance in Arctic charr exposed to acute thermal stress. Physiological Genomics 43: 685- 696.

Radtke, G., Wolnicki, J., Kapusta, A., Przybylski, M., and Kaczkowski, Z. 2022. Critical thermal maxima of three small-bodied dish species (Cypriniformes) of different origin and protection status. The European Zoological Journal 89: 1351-1361.

Rahman, M.M., Lee, Y.D., and Baek, H.J. 2021. Acclimation temperature influences the critical thermal maxima (CT_{max}) of red-spotted grouper. Fisheries and Aquatic Sciences 24: 235-242.

Ramalho, Q., Vale, M.M., Manes, S., Diniz, P., Malecha, A., and Prevedello, J.A. 2023. Evidence of stronger range shift response to ongoing climate change by ectotherms and high-latitude species. Biological Conservation 279: no.109991.

Raymond, K., Chapman, L.J., and Lanciani, C. 2006. Host, macrohabitat, and microhabitat specificity in the gill parasite *Neodiplozoon polycotyleus* [Monogenea]. Journal of Parasitology 92: 1211-1217.

Rebolledo, A.P., Srgo, C.M., and Monro, K. 2021. Thermal performance curves are shaped by prior thermal environment in early life. Frontiers in Physiology 12: no.738338.

Rezende, E.L., and Bozinovic, F. 2019. Thermal performance across levels of biological organization. Philosophical Transactions of the Royal Society B: Biological Sciences 374: no.1778.

Ros, A.F.H., Becker, K., and Oliveira, R.F. 2006. Aggressive behaviour and energy metabolism in a cichlid fish, *Oreochromis mossambicus*. Physiology and Behavior 89: 164-170.

Rubenstein, M.A., Weiskopf, S.R., Bertrand, R., Carter, S.L., Comte, L., Eaton, M.J., Johnson, C.G., Lenoir, J., Lynch, A.J., Miller, B.W., Morelli, T.L., Rodriguez, M.A., Terando, A., and Thompson, L.M. 2023. Climate change and the global redistribution of biodiversity: substantial variation in empirical support for expected range shifts. Environmental Evidence 12: no.7.

Ruthsatz, K., Dahlke, F., Alter, K., Wohlrab, S., Eterovick, P.C., Lyra, M.L., Gippner, S., Cooke, S.J., and Peck, M.A. 2024. Acclimation capacity to global warming of amphibians and freshwater fishes: Drivers, patterns, and data limitations. Global Change Biology 30: e17318.

Sandblom, E., Grans, A., Axelsson, M., and Seth, H. 2014. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. Proceedings of the Royal Society B Biological Sciences 281: no.1794.

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

Schaack, S.R. and Chapman, L.J. 2004. Interdemic variation in the foraging ecology of the African cyprinid, *Barbus neumayeri*. Environmental Biology of Fishes 70: 95-105.

Schmidt, R. 2020. *Enteromius apleurogramma*. The IUCN Red List of Threatened Species 2020: e.T162471510A162471538. Jan 15th 2022. https://dx.doi.org/10.2305/IUCN.UK.2020-1.RLTS.T162471510A162471538.en.

Schmidt, R.C., Bart, H., and Nyingi, W.D. 2018. Integrative taxonomy of the red-finned barb, *Enteromius apleurogramma* (Cyprininae: Smiliogastrini) from Kenya, supports recognition of *E. amboseli* as a valid species. Zootaxa 4482: no.566.

Schulte, P.M., Healy, T.M., and Fangue, N.A. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integrative and Comparative Biology 51: 691-702.

Song, G., Liu, R., Chen, S., Li, Q., Zongbin, C., and Long, Y. 2023. Comparative transcriptomic and epigenetic analyses to identify the cold resistance-associated genes and disclose the regulator mechanism in tilapias. Aquaculture 587: no.740858.

Stevens, G. C. 1989. The latitudinal gradient in geographical range how so many species coexist in the tropics. The American Naturalist 133: no.240256.

Stewart, H.A., and Allen, P.J. 2014. Critical thermal maxima of two geographic strains of channel and hybrid catfish. North American Journal of Aquaculture 76: 104-111.

Stewart, E.M.C., Frasca, V.R., Wilson, C.C., and Raby, G.D. 2023. Short-term acclimation dynamics in cold water fish. Journal of Thermal Biology 11: no.103482.

Stoica, P, and Selen, Y. 2004. Model-order selection: a review of information criterion rules. IEEE Signal Processing Magazine 21: 36-47.

Stuart-Smith, R.D. 2021. Climate change: Large scale abundance shifts in fishes. Current Biology 31: 1445-1447.

Stuart-Smith, R.D., Edgar, G.J., Clausius, E., Oh, E.S., Barrett, N.S., Emslie, M.J., Bates, A.E., Bax, N., Brock, D., Cooper, A., Davis, T.R., Day, P.B., Dunic, J.C., Green, A., Hasweera, N., Hicks, J., Holmes, T.H., Jones, B., Jordan, A., Knott, N., Larkin, M.F., Ling, S.D., Mooney, P., Pocklington, J.B., Seroussi, Y., Shaw, I., Shields, D., Smith, M., Soler, G.A., Stuart-Smith, J., Turak, E., Turnbull, J.W., and Mellin, C. 2022. Tracking widespread climate-driven change on temperature and tropical reefs. Current Biology 32: 4128- 4138.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. 2010. Global analysis of thermal tolerance and latitude in ectotherms. Proceedings of the Royal Society B: Biological Sciences 278: 1823–1830.

Sunday, J.M., Bates, A.E., and Dulvy, N.K. 2012. Thermal tolerance and the global redistribution of animals. Nature Climate Change 2: 686-690.

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., and Huey, R.B. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences 111: 5610-5615.

Sunday, J., Bennett, J. M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A. L., Leiva, F. P., Verberk, W. C., Olalla-Tárraga, M. A., & Morales-Castilla, I. 2019. Thermal tolerance patterns across latitude and elevation. Philosophical Transactions of the Royal Society B: Biological Sciences 374: no.20190036.

Tewksbury, J.J. Huey, R.B. Deutsch, C.A. 2008. Putting the heat on tropical animals. Science 320: 1296-1297.

UWA. Kibale: The Primate Capital of the World. July 27th, 2024. https://ugandawildlife.org/national-parks/kibale-national-park/

Waterbury, C.R., Sutton, T., Kelley, A.L., Lopez, J.A. 2024. Effects of temperature acclimation on the upper thermal tolerance of two Arctic fishes. Conservation Physiology 12: coae001.

Wells, Z.R.R., McDonnell, L., Chapman, L.J., and Fraser, D.J. 2016. Limited variability in upper thermal tolerance among pure and hybrid populations of a cold-water fish. Conservation Physiology 4: cow63.

Williams, J.E., Blois, J.L. 2018. Range shifts in response to past and future climate change: Can climate velocities and species' dispersal capabilities explain variation in mammalian range shifts? Journal of Biogeography 45: 2175-2189.

Wingfield, J.C., Krause, J.S., Perez, J.H., Chmura, H.E., Nemeth, Z., Word, K.R., Calisi, R.M., and Meddle, S.L. 2015. A mechanistic approach to understanding range shifts in a changing world: What makes a pioneer? General and Comparative Endocrinology 222: 44-53.

Wooliver, R., Vtipilthorpe, E.E., Wiegmann, A.M., and Sheth, S.N. 2022. A viewpoint on ecological and evolutionary study of plant and thermal performance curves in a warming world. Journal of the Annals of Botany PLANTS 14: plac016.

Wu, T., Imrit, M.A., Movahedinia, Z., Kong, J., Woolway, R.I., and Sharma, S. 2022. Climate tracking by freshwater fishes suggests that fish diversity in temperate lakes may be increasingly threatened by climate warming. Diversity and Distributions 29: 300-315.

Yanar, M., Erdogan, E., and Kumlu, M. 2019. Thermal tolerance of thirteen popular ornamental fish species. Aquaculture 501: 382-386.

Yanar, M., Evliyaoglu, E., and Tekelioglu, B.K. 2023. Sex differences in thermal tolerance of nine ornamental fish species from the Poeciliidae, Cichlidae and Cyprinidae family. Turkish Journal of Fisheries and Aquatic Sciences 23: no.22738.

Zillig, K.W., Lusardi, R.A., Cocherelle, D.E., and Fangue, N.A. 2022. Interpopulation variation in thermal physiology among seasonal runs of Chinook salmon. Canadian Journal of Fisheries and Aquatic Sciences 80: 1-13.

LINKING STATEMENT

In the first chapter of my thesis, I focused on how thermal tolerance relates to the distribution of species that are closely related. More specifically, I compared the upper and lower thermal tolerance of a native range shifting species, *E. apleurogramma*, to those of its upstream congener (*E. neumayeri*) and its downstream congeners (*E. alberti* and *E. kerstenii*) who have not undertaken the same distributional shifts. For upper thermal limits, *E neumayeri* had a significantly lower CT_{max} then *E. apleurogramma* across all populations, which supports the hypothesis that upstream populations have a longer evolutionary history in cooler waters which may allow them to be better adapted to cooler environments. We also found that downstream congeners *E. alberti* and *E. kerstenii*, have a higher CT_{min} than upstream populations of *E. apleurogramma*, which could be constraining them to the downstream warmer waters of the Mpanga River. Taken together, our results highlight the importance of CT_{min} as a predictor for range contractions at the southern end of a species' distribution.

There was a large focus on the effect of increasing water temperatures on fish physiology for the first chapter of my thesis. However, outside of the laboratory, environmental stressors are likely to co-occur and interact in freshwater systems to have a combined effect on fishes. In evaluating species' range shifts, it is important to consider multiple ecological gradients as species' shifting their range according to their thermal limits might run into a second environmental stressor, which might require a rapid response or might serve as a barrier, thereby limiting changes in distribution. Therefore, in my second chapter I explore how range expansion along a thermal gradient is affected by response to a second strong environmental gradient, here dissolved oxygen. To answer this question, I compare the thermal tolerance of the range shifting *Platypanchax modestus* across its distribution (both upstream and downstream populations) as well as across divergent oxygen habitats (normoxic streams and hypoxic swamps). Based on the potential for oxygen dependency of thermal tolerance, I ask whether there is a relationship between CT_{max} and dissolved oxygen concentration of habitats where fish were captured. I also ask whether there is evidence for phenotypic divergence between low- and high-oxygen habitats by comparing the gill size of *P. modestus* between a low-oxygen and two high-oxygen habitats.

The results of this chapter highlight the importance of considering multiple environmental stressors in understanding and predicting species' range shifts.

CHAPTER 2

Title: Thermal tolerance of a range-shifting killifish in the face of hypoxia

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Abstract

Distributional shifts through range contractions and expansions have been reported in many species of fishes coincident with climate warming. However, environmental stressors may interact to have a combined effect on fish distributions, and range shifts. Here, we explore how range shifts along a thermal gradient in the killifish *Platypanchax modestus* may be affected by response to a second strong environmental gradient – dissolved oxygen. Sampling of fish communities in the Rwembaita Swamp System of Kibale National Park, Uganda between 1990 and 2024, has captured the upstream range shift of this species into both high-oxygen streams and low-oxygen swamps. In this study, we measured gill size in populations of *P. modestus* from its historical downstream distribution and the upstream hypoxic and normoxic sites to determine if *P. modestus* colonizing low- and high-oxygen sites exhibits divergence in oxygen uptake capacity (gill size). We also quantified the relationship between CT_{max} across seven populations of *P. modestus* and both average environmental water temperature and average dissolved oxygen concentration to test whether upper thermal tolerance is temperature and/or oxygen dependent. The population of *P. modestus* from the downstream normoxic river site (putative source population) had the smallest total gill filament length, whereas the upstream hypoxic population had the largest total gill filament length and the upstream normoxic population had an intermediate gill filament length. These results suggest that gill size variation between high and low dissolved oxygen populations can be driven by environmental effects. For thermal tolerance, we detected a weak positive relationship between CT_{max} and environmental dissolved oxygen but not for water temperature. This is consistent with theories on the oxygen dependency of thermal tolerance, though other factors may differ among populations that are correlated with dissolved oxygen. Overall these results suggest that range shifting *P. modestus* may increase gill size in response to environmental dissolved oxygen concentration, but still show evidence of oxygen dependency across populations.

Introduction

Range shifts

Among the multitude of factors that contribute to species' range shifts, climate warming has emerged as one of the main drivers as species attempt to track their thermal requirements by moving to cooler habitats at higher latitudes and/or altitudes (Jones and Schmidt 2019; Punzon et al. 2021; Armstrong et al. 2022; Stuart-Smith et al. 2022; Ramalho et al. 2023). Climate-driven range shifts have already been documented in many taxa including a number of species' of plants, birds, mammals, fishes, and reptiles with the intensity and frequency of shifts being both species and taxa dependent (Alofs et al. 2014; Boyle et al. 2016; Williams and Blois 2018; Beyer and Manica 2020; Moran et al. 2022; Rubenstein et al. 2023; Hallfors et al. 2024). For example, in fishes changes in abundance and species composition have been documented for a number of pelagic fishes in the Pacific Arctic which have been tightly coupled to recent changes in temperature and sea ice (Levine et al. 2023).

Another factor that is important in predicting and understanding range shifts is the consideration of multiple ecological gradients. Species shifting their range according to one factor such as their thermal requirements might run into a second environmental stressor, which might require a rapid response or might serve as a barrier, thereby limiting changes in distribution. For example, aquatic hypoxia (low dissolved oxygen, <2 mg O_2/L; ~30% of air saturation; Levin et al. 2009), is a stressor of increasing importance to aquatic organisms worldwide. Hypoxia occurs naturally in many systems characterized by low light and low mixing such as dense tropical swamps, the bottom waters of deep lakes, and floodplain pools (Helly and Levin 2004; Diaz and Brietburg 2009; Rabalais et al. 2010; Chapman 2015; Chapman et al. 2021; Tellier et al. 2022). In addition, hypoxia has become more prevalent due to anthropogenic perturbations, including human-mediated eutrophication (Diaz and Brietburg 2009; Chapman 2015). Hypoxia and elevated water temperature are likely to have interactive effects on aquatic ectotherms like fishes, because both affect aerobic metabolism (McBryan et al. 2013; Earhart et al. 2022; Firth et al. 2023, see *Temperature and hypoxia*, below). Considering the importance of understanding species range shifts and the potential interactions between different ecological variables, we explored the effects of hypoxia on the upper thermal limits and respiratory morphology of a range shifting tropical killifish in Uganda.

Freshwater fishes and water temperature

Ectothermic organisms, including fishes, are those that rely on external heat sources to regulate their body temperature; and thus, their internal body temperature closely tracks that of their environment (Huey and Stephenson 1979; Miller and Stillman 2012; Ohlberger 2013; Haesemeyer 2020; Volkoff and Ronnestad 2020). Given that body temperature affects a number of physiological processes including metabolism (Li et al. 2023), development, and reproduction (Akin 2011; Clarke and Zani 2012), ectothermic organisms are particularly vulnerable to changes in temperature. As a result, ectotherms must remain within the range of temperatures that allows them to carry out their vital functions, which has been defined as a performance window (Khelifa et al. 2019). The shape of the performance window differs across species and taxa, though is always bounded by a lower critical thermal limit (CT_{min}) and an upper critical thermal limit (CT_{max}) (MacLean et al. 2019; Little and Seebacher 2021; Morley et al. 2022; Claunch et al. 2023). When environmental temperatures exceed a species' critical thermal boundaries, species begin to exhibit physiological disorganization and loss of equilibrium, which may eventually lead to death (Huey and Stephenson 1979; Pörtner 2010; McDonnell and Chapman 2015).

The need for ectotherms such as fishes to shift their distribution in response to increasing temperatures will depend, at least in part, on how fast the species can respond *in situ* to elevated water temperature through plastic and/or heritable change. Some ectothermic organisms are able to acclimate to warmer temperatures, which can allow them to mitigate some of the physiological impairments associated with increased temperatures (Gilbert and Farrell 2021; Gilbert et al. 2022). In fishes, there are a number of studies that have exposed fish to a series of increasingly warmer water temperatures over a period of a few days to a few weeks in order to measure their short term-plasticity, or acclimation capacity (Comte and Olden 2017; Morley et al. 2018; Campos et al. 2020; Chapman et al. 2022). In general, these studies show that many fishes are able to increase their upper thermal limits with increased acclimation temperature, thereby demonstrating acclimation capacity (Comte and Olden 2017; Morley et al. 2018; Campos et al. 2020; Chapman et al. 2022), which may contribute to their persistence *in situ*. Despite this acclimation capacity, range contractions and expansions coincident with climate change have already been reported in several fish species (Alofs et al. 2014; Carosi et al. 2019; Kirk and Rahel 2022; Cowan et al. 2023; Nuon et al. 2024; Waterbury et al. 2024). For example, the northern range boundaries of freshwater sport fishes across 1527 lakes in Ontario, Canada have experienced a northward shift of approximately 12.9-17.5 km per decade within the past 30 years as expected based on observed climate warming (Alofs et al. 2014). Additionally, in the Mekong River in South East Asia, studies based on occurrence data and species distribution models have predicted that climate change will be the primary driver of future change in fish species distributions and of the 195 fish species examined 84% are expected to undergo a northward distributional shift (Nuon et al. 2024). Given the increasing number of fishes exhibiting range shifts or predicted to experience range shifts coincident with climate warming, there is a need to explore the response of range-shifting species to abiotic and biotic factors that may co-occur with thermal gradients.

In order to test how species respond to thermal stress, critical thermal maxima is among the most widely used techniques across taxa (Cereja 2020). CT_{max} was first used by Cowles and Bogert in 1944 to characterize the thermal limits of desert reptiles and, to this day, remains one of the key methods for evaluating upper thermal tolerance of ectotherms (Cowles and Bogert 1944; Lutterschmidt and Hutchison 1997; Morgan et al. 2020; Leong et al. 2022; Cowan et al. 2023; Desforges et al 2023). The ecological relevance of CT_{max} has been debated within the scientific community (Kingsolver and Umbanhowar 2018; O'Donnell et al. 2020) though despite this, it remains an integrative metric with repeatable outcomes and has been used in characterizing upper thermal limits for fish species across the globe (Beitinger et al. 2000; Hillyard 2017; Bartlett et al. 2022; Bouyoucos et al. 2023).

Freshwater fishes and dissolved oxygen

Dissolved oxygen can be a strong driver of intraspecific and interspecific variation in many physiological, morphological, molecular, and life history traits related to oxygen uptake and delivery (Chapman 2015). For example, for several species of East African fishes, fish from hypoxic swamps are characterized by larger gill surface area and/or gill filament length than conspecifics living under normoxic conditions (Chapman 2007; 2015; 2021; Chapman et al. 2022). Hypoxia tolerance is shaped by both evolutionary adaptation as well as phenotypic plasticity (Gilmore et al. 2019; Borowiec et al. 2020; Zhang et al. 2023). In general, prolonged exposure to hypoxia (hypoxia acclimation) improves hypoxia tolerance in freshwater fishes and hypoxia tolerant fish show acclimation response to normoxic conditions (Gilmore et al. 2019; Borowiec et al. 2020; Mucha et al. 2023; Zhang et al. 2023). As such, fish from normoxic sites that shift their range into cooler, but hypoxic, sites in response to warming waters may show evidence of increased oxygen uptake (e.g., increased gill size) and/or delivery (increased cardiac output) to facilitate persistence (Borowiec et al. 2020).

Temperature and hypoxia

Increases in water temperature and hypoxia are two stressors that are likely to have interactive effects on ectothermic aquatic organisms as both elevated water temperature and hypoxia affect aerobic metabolism. Increases in water temperature can increase the rate of oxygen consumption in aquatic ectotherms, while hypoxia can limit oxygen supply (McBryan et al. 2013; Islam et al. 2019; Volkoff and Ronnestad 2020; Wilco et al. 2022). Many studies have found evidence for interactions between temperature and hypoxia on freshwater fishes (Wei et al. 2015; Jung 2018; Earhart et al. 2020; Jung et al. 2020; Wilco et al. 2022). The functional association between hypoxia and thermal tolerance has often been explored in the context of oxygen- and capacity-limited thermal tolerance (Pörtner 2010), whereby, under warming water temperatures, the maximum rate of oxygen consumption exceeds the capacity of the cardio-respiratory system to supply oxygen to tissues, manifested as a reduction in aerobic scope (difference between standard and maximum metabolic rate, measured as rate of oxygen consumption). Consequently, the upper thermal limits of fishes are hypothesized to be highly sensitive to hypoxia, with the corollary that changes that increase oxygen uptake (e.g., increased gill size) and delivery (e.g., improved cardiac output) may improve thermal tolerance. However, support for the oxygen dependency of thermal limits in fishes is mixed (Ern et al. 2018, McDonnell et al. 2019). There is evidence that acute hypoxia exposure reduces CT_{max} (Islam et al. 2020; Potts et al. 2021; Earhart et al. 2023; Reemeyer and Chapman 2024), and some studies have reported a positive correlation between thermal and hypoxia tolerance (Li et al. 2019). In contrast, there is also evidence that thermal and hypoxia tolerance are not linked (Brijs et al. 2015; Motyka et al. 2017) or that there is no effect until extreme hypoxia occurs (Ern et al. 2016, 2017). If thermal tolerance is oxygen-dependent, we might predict a positive association between the dissolved oxygen concentration of a habitat and thermal tolerance. However, if fish from hypoxic populations have been able to adapt to hypoxia with increased oxygen uptake and delivery relative to normoxic populations, or if CT_{max} is not oxygen-dependent, there might be a weak relationship or no relationship.

Study system

To address potential effects of hypoxia on the thermal tolerance of range-shifting fishes, we focus on the small killifish (*Platypanchax modestus*) from Kibale National Park in western Uganda. Kibale National Park was selected as our study site as we have captured the range expansion of *P. modestus* along a thermal and oxygen gradient, making it an ideal system to study the link between range-shifts in response to multiple environmental stressors (L. Chapman, unpubl. data). Kibale National Park (0°13′–0°41′ N and 30°19′–30°32′E), is a mid-altitude moist evergreen tropical rainforest that lies between 1,160 m and 2,607 m in altitude (UWA 2024). The park, which is 795 km^2 in area is comprised of mature forest, portions of which were logged approximately five decades ago, wetlands, grasslands, and former pine and cypress plantations (Duclos et al. 2013). There are two wet and dry seasons in this equatorial region, with May to August and December to February characterized by drier conditions (Hunt et al. 2023). There are two river systems that drain Kibale National Park, the Dura River and the Mpanga River, both of which eventually feed into the Lake George System (Harniman et al. 2013). The Mpanga River System, the focus of this study, is comprised of large well-oxygenated open stretches and valley papyrus swamps that are characterized by naturally hypoxic water (Chapman et al. 1999). The Rwembaita Swamp System (Fig. 1) is the largest hypoxic swamp (4.5 km in length) inside Kibale National Park and is fed by small normoxic streams. The Rwembaita Swamp System eventually feeds into the Njuguta River, a tributary of the Mpanga River. This system, which comprises the Rwembaita Swamp and its tributaries, has been the focus of several studies by the Kibale Fish Project since the early 1990’s that have compared environmental features and fish faunal characteristics. Monthly sampling of fishes in the Rwembaita Swamp System between June 1990 and May 1991 (Chapman 1995) and between July 1993 and June

1997 (Chapman et al. 1999) captured only two species of fishes, the cyprinid *Enteromius neumayeri* (previously *Barbus neumayeri*; Fischer 1884) and the clariid catfish *Clarias liocephalus* (Boulenger 1898). Olowo and Chapman (2006) and Chapman (2005) also only reported *E. neumayeri* and *C. liocephalus* in sites within the Rwembaita Swamp System. In subsequent studies between 1999 and 2011 that focused on *E. neumayeri* (Schaack and Chapman 2003, 2004; Martinez et al. 2004, 2011; Harniman et al. 2013), *E. neumayeri* and *C. liocephalus* were the only species observed during the field research that was carried out for these studies (L. Chapman, personal observations). Conversely, downstream southern sites (Kahunge, Bwera (Kiaragura Swamp), Bunoga; Fig. 1) host a number of fish species including populations of *E. neumayeri, Enteromius apleurogramma* (previously *Barbus apleurogramma*; Boulenger 1911), *Enteromius alberti* (previously *Enteromius cercops*; *Barbus cercops*; Whitehead 1960) and *Enteromius kerstenii* (previously *Barbus kerstenii*; Peters 1868); *C. liocephalus*; the cichlids *Pseudocrenilabrus multicolor* (Schöller 1903), *Astatoreochromis alluaudi* (Pellegrin 1904), and *Haplochromis* spp.; and the killifishes *P. modestus* (previously *Hypsopanchax deprimozi*; Pappenheim 1914) and *Nothobranchius* spp. (Raymond et al. 2006; Langerhans et al. 2007; Crispo and Chapman 2008; Binning et al. 2010; Joyner-Matos and Chapman, 2013).

Periodic sampling of fish communities by the Kibale Fish Project has captured the range shift of a native fish species, the cyprinodontid *P. modestus*. This species was formerly restricted to the downstream (southern) part of the Mpanga drainage and remains abundant at these sites based on field observations, but has recently expanded its range upstream (northwards) into the Rwembaita Swamp System, where it was first observed in 2012 and has continued to be captured throughout both dry and wet seasons until 2024 (L. Chapman, unpubl. data). It is likely that the putative source population of these *P. modestus* is a downstream normoxic river population based on the geography of the Mpanga River and the position of the Rwembaita Swamp. Within the Rwembaita Swamp System, this species has shifted its range into both the swamp (low oxygen) and tributary streams (high oxygen) (L. Chapman, unpubl. data). *P. modestus* is one of three native species known to have expanded its range upstream into this system, the others being the cyprinid *E. apleurogramma* (appeared in 2015; Hunt et al. 2023) and the cichlid *P. multicolor* (first observed in 2022; L. Chapman, unpubl. data). The non-native *Poecilia reticulata* has also been captured in the system (L. Chapman, unpubl. data). It is unlikely that these species moved into the Rwembaita Swamp System by means of human intervention as the swamp lies deep within Kibale National Park, which has been a protected area since 1993. In addition, these species are not the target of artisanal fisheries in this area of Uganda, so it is unlikely that they have been translocated by fishers. Co-incident with this range expansion, there has been an increase in water temperature in the Rwembaita Swamp System of 1.41 °C between 1994 and 2016, which aligns with the local air temperature increase of 1.45 °C over that period (L. Chapman, unpubl. data). Though we do not have long-term data for downstream sites, the historical *P. modestus* populations are approximately 200 m lower in elevation (L. Chapman, unpubl. data). It is predicted that the average difference in temperature between the upstream and downstream sites is approximately 1.3 °C based on standard atmospheric lapse rate of 6.50 °C/km and site elevation (International Civil Aviation Organization 1993; Hunt et al. 2023). With this in mind, it is possible that the range shift is climate-driven, though we cannot rule out other ecological drivers affecting freshwater systems which may not have been measured in this study such as water turbidity and forest cover.

Here, we explore how range expansion of *P. modestus*, along its thermal gradient is affected by response to a second strong environmental gradient – dissolved oxygen. We test whether fishes that have shifted their range upstream into hypoxic habitats show evidence of increased oxygen uptake capacity (larger gills) than conspecifics that have shifted their range upstream into normoxic habitats, and whether there is a relationship between CT_{max}, oxygen concentration, and water temperature of the habitat. We tested three hypotheses: (1) there is a positive relationship between CT_{max} and water temperature in the expanded range; (2) there is a negative relationship between thermal tolerance (CT_{max}) and dissolved oxygen concentration among populations in the expanded range (if CT_{max} is oxygen dependent); and (3) there is divergence in oxygen uptake capacity (gill size) between populations of *P. modestus* from the historical downstream distribution and the upstream hypoxic and normoxic sites.

Methods

Study Species

The Ruwenzori Lampeye (*P. modestus)* is a small cypriniforme killifish of the family Procatopodidae that is currently listed as a species of least concern by IUCN (Greenwood 1966; Huber 1996; Natugonza et al. 2022). *P. modestus* is widely distributed across Uganda and the Democratic Republic of Congo, occurring in the Ruwenzori and western Rift Valley slopes in the affluent rivers of Lake Edward and Lake George, and also in the Semliki River and the upper Ituri River Systems (Huber 1996). This killifish inhabits the shore regions of lakes and inflowing rivers and streams (Greenwood 1966; Huber 1996).

Fish sampling and holding conditions

Sampling for P. modestus for CT_{max} Trials

We sampled *P. modestus* from seven different upstream sites: three stream sites within the Rwembaita Swamp System (Mikana-Up Stream, Inlet Stream West and Inlet Stream East), three swamp sites located within the system (Rwembaita Swamp Up, Rwembaita Swamp Mid, and Rwembaita Swamp Low), and one river site (Njuguta) located at the outflow of the Rwembaita Swamp (Fig. 1). For every population, we collected 12 fish for upper thermal tolerance trials (n= 12 CT_{max}; Table 1). Fish were live-captured using baited minnow traps and brought back to the Makerere University Biological Field Station. At the field station, fish were held in 37 L coolers with untreated rainwater and held under normoxia (6.00 ± 0.07 mg/L; mean ± SEM), ambient temperatures (17.96 ± 0.06 °C), and exposed to a natural photoperiod. Coolers were equipped with air stones, sponge filters, and refuge in the form of leaf cover. The average water temperature in the coolers closely matched the mean long-term temperature across all sites with coolers only approximately 0.65 °C below the mean water temperature. Across all coolers, temperature variation ranged by 0.94 °C and dissolved oxygen concentration ranged by 1.2 mg/L (Table 2). Coolers were separated by population and catch day, and held a maximum of 30 fish. Water quality was monitored daily for ammonia and nitrate, and half tank water changes were done as necessary. Fish were fed Tropical Tetraflakes. In all populations, there was a subset of fish that showed evidence of cyst-like growths that were visible through the skin of the fish likely to be a tumour caused by a defect in a ribosomal protein gene (Amsterdam et al. 2004). These fish were not included in the study. In addition, *P. modestus* exhibits strong sexual dimorphism in colour and body shape. For this study, we focused on male fish that could be easily distinguished from female and juvenile fish.

Water temperature and dissolved oxygen concentration have been collected monthly for 34 years by the Kibale Fish Project in Kibale National Park, Uganda at a series of representative habitats for all sites in the Rwembaita/Njuguta System (Lauren Chapman, unpubl. data). Briefly, water temperature and dissolved oxygen data were collected at a set of microsites in the upper 20 cm of the water column using water temperature and dissolved oxygen probes. Under the closed cover of the forest and papyrus swamp habitats, diel variation in dissolved oxygen and water temperature tends to be low (Chapman and Liem 1995). Therefore, measurements were taken once per sampling day in the mid-morning to mid-day period. We calculated average water temperature and dissolved oxygen concentration for sites within the Rwembaita Swamp System for the period between 2020-2022 (Table 3). The lifespan of *P. modestus* is currently unknown, but is likely at least 2 years based on killifishes in the aquarium trade, and therefore the two year average represents the potential environment over which the fish developed (Cellerino et al. 2015; Furness 2015; Polačik et al. 2023; Ruparelia 2023).

Critical Thermal Maximum Trials (CT_{max})

CT_{max} was measured following routine methods on healthy male fish 4-5 days post-capture to minimize potential acclimation effects from being held under normoxic conditions (e.g., Fangue et al. 2006; Mcdonnell and Chapman 2015; McDonnell et al. 2021). Acclimation could potentially alter the CT_{max} if *P. modestus* has plasticity in its thermal limits, so by measuring fish within this time frame we increase the probability that measures more accurately represent the thermal tolerance of fish in their natural setting (as in Fangue et al. 2006 with *Fundulus heteroclitus*; Mcdonnell and Chapman 2015 with *P. multicolor*; McDonnell et al. 2021 with *Notropis anogenus*). To induce a post-absorptive state, fish were starved for 24 hours prior to the experimental trials. Three fish were placed in individual experimental chambers that were separated by opaque dividers within a larger Plexiglass aquarium (58 L x 25 W x 20 H cm). The compartments contained shelter consisting of rocks and were perforated to allow the mixing of water freely between the chamber and the buffer tank. Initially, water temperature in the experimental tank was at ambient temperature (~17-18 °C), and water was kept fully oxygenated (>7.85 mg/L) during acclimation and throughout experimental trials by using battery operated bubblers. Fish were left to acclimate under these conditions for a period of 2 hours, after which water temperature was slowly increased at a standard rate of 0.3 °C/min using a temperature control system (Witrox-4 unit and DAQ-M device; AutoResp; Loligo Systems). This rate has been shown to be low enough to not shock the fish while being high enough to minimize acclimation effects (Cox et al. 1974; Lutterschmidt and Hutchison 1997; Fangue et al. 2006; Chen et al. 2013). Water temperature and dissolved oxygen were carefully monitored for both the acclimation period and throughout the experiment using handheld Polaris water temperature and dissolved oxygen probes. CT_{max} was defined as the temperature at which the fish first lost equilibrium for 30 consecutive seconds (Fangue et al. 2006; McDonnell and Chapman 2015; McDonnell et al. 2021). In fish, loss of equilibrium is characterized by the inability to maintain an upright position in the water column, which impacts the ability of fish to swim and/or move normally (Calfee et al. 2016). Following trials, fish were allowed to fully recover in oxygenated water after which they were euthanized using clove oil (0.03%). For every fish, weight and standard length were recorded post-euthanasia. Permission to carry out this research was approved by McGill University, the Uganda Wildlife Authority, and the Uganda National Council for Science and Technology. Procedures used for live fish in this experiment were also approved by McGill University Animal Care Committee (AUP 5029).

Gill Size

Gill analysis was done following Hunt et al. (2023) for three populations: Bunoga (historical downstream river site, mean dissolved oxygen = 7.5 mg/L; Crocker et al. 2012); Inlet Stream West (upstream normoxic site); and Rwembaita Swamp Mid (upstream hypoxic site). Sample size for gill analysis are reported in Table 8. Specimens were from a collection in 2018 that were preserved in 10% formalin and transferred to McGill University. Total gill filament length was selected as a metric, because there are a number of studies that have shown that some East African fishes from hypoxic swamps have a larger gill filament length than conspecifics from normoxic waters (reviewed L. Chapman 2007; 2015; 2021). Further, previous studies have found that for various fish species, populations with increased total gill filament length are characterized by a larger gill surface area (*P. multicolor*, Chapman et al. 2000; 2008; *Poecilia* *latipinna*, Timmerman and Chapman 2004; *A. alluaudi*, Chapman et al. 2007). We also measured the total number of gill filaments. To quantify total gill filament length and number, each of the four gill arches from the left side of the branchial basket was dissected out, laid flat on a microscope slide, and photographed with a camera (Lumenera Scientifica Infinity) connected to a stereoscope. The photos were examined using ImageJ v.1.50i. For each hemibranch, the length of every fifth gill filament was measured across the gill arch, with additional measurements at each end to increase resolution. Two successive measurements were averaged to estimate the length of intermediate filaments. Gill filament lengths were then summed for the eight hemibranchs and multiplied by two to produce an estimate of the total gill filament length. The total filament number was counted for each hemibranch on one side of the fish and multiplied by two. According to standard procedure, both total gill filament length and number were log 10 transformed and standardized to the mean body mass of the samples in the analysis using to the formula below (Reist 1986; Hendry and Taylor 2004). β is the common within-group slope that was generated from a linear model with varying intercepts.

$$Gill\ traitvalue_{adj} = Gill\ traitvalue_{raw} \times \left(\frac{mass_{mean}}{mass_{individual}}\right)^{\beta}$$

Statistical Analyses

We used a one-way ANOVA analysis to test for differences in body mass and standard body length among populations. We also used a one-way ANOVA to test for differences in mean water temperature and dissolved oxygen concentration among study sites. All statistical analyses were done using R software version 4.3.2 (The R Foundation for Statistical Computing, 2023).

To detect differences in CT_{max} we used a linear mixed model (LMM; package *lme4* v.1.1.35; Bates et al. 2023). Initially, body size (mass and/or length) was included in the model but was found to have no significant effect on CT_{max}, and thus was removed from the model. We tested for the random effect of trial number because three fish were tested in the same chamber on the same day and therefore were not independent. Because trial number was included as a random effect, we needed to use a linear mixed model rather than an ANOVA. To calculate p-values in the linear mixed model we used an analysis of variance under a chi-square distribution, which is robust to violations of assumption that a mixed model might make of an F-distribution. To interpret the statistical significance of the difference between pairs of means, post-hoc analyses were performed using the glht() function, which is the equivalent of the TukeyHSD() function for mixed models (package *multcomp* 1.4-25; Hothorn 2010). Wald chi-square values (Type III) are reported for all p-values using the Anova() function (package *car* v.3.1-2; Fox and Weisberg 2019).

We used linear models (ANOVA) and partial regression plots to detect relationships between CT_{max}, average water temperature, and average dissolved oxygen. Partial regression analyses were used to test the significance of predictor variables. Normal probability plots and collinearity diagnostics were employed to explore model assumptions using the effect package (package *eemeans* v.4.2-2; Fox and Weisberg 2019).

To analyze mass-adjusted total gill filament length and number, we used the lm() function to produce linear models (ANOVA) including population as a fixed factor. Post-hoc analyses were performed using the TukeyHSD() function to interpret the statistical significance of the difference between pairs of means (package *multcomp* 1.4-25; Hothorn 2010; Lane 2010). Sum of square values (Type III) are reported for all p-values using the ANOVA() function (package *car* v.3.1-2; Fox and Weisberg 2019). All model outputs are summarized in Tables 4, 6, 9, 11, and 13.

Results

Body size

Body mass and standard body length differed significantly among populations (mass: $p < 0.0001$, $F = 6.29 \times 10^{29}$, df = 7,80; standard length: $p < 0.0001$, $F = 5.48 \times 10^{28}$, df = 7,80; Tables 1, 4, and 5). Fish sampled from Rwembaita Swamp Low were smallest (0.41 ± 0.04 g; mean ± SEM) and fish from Rwembaita Swamp Up were the largest (0.68 ± 0.06 g). As noted above, despite being different among populations, body mass and body length had no significant effect on upper thermal limits.

Environmental Data

Both average water temperature and average dissolved oxygen concentration differed among sites (temperature: $p < 0.0001$, $F = 5.14\text{x}10^{26}$, df = 6,2057; dissolved oxygen: $p < 0.0001$, $F = 3.88\text{x}10^{29}$, df = 6,2057; Tables 3, 6, and 7). Mikana-Up Stream was the coolest site and was characterized by the highest dissolved oxygen concentration of all sites, and Inlet Stream East was the warmest site characterized by the lowest dissolved oxygen concentration among stream sites (Mikana-Up Stream temperature: 17.75 ± 0.04 °C; dissolved oxygen: 6.29 ± 0.04 mg/L; Inlet Stream East temperature: 18.97 ± 0.08 °C; dissolved oxygen: 3.66 ± 0.13 mg/L; Table 3). The three swamp sites were characterized by low oxygen conditions ranging from 1.83 ± 0.01 mg/L at Rwembaita Swamp Mid to 3.15 ± 0.26 mg/L at Rwembaita Swamp Up, an area of the swamp that receives inflow from a small stream. Temperatures were very similar among the swamp sites ranging from 18.30 ± 0.05 °C at Rwembaita Swamp Mid to 18.42 ± 0.06 °C at Rwembaita Swamp Low (Table 3).

Critical Thermal Maximum

CT_{max} differed significantly across sites ($p = 0.0273$, $X^2 = 14.22$; df = 6; Tables 8 and 9). We used multiple regression and partial regression plots to detect relationships between CT_{max}, average water temperature, and average dissolved oxygen concentration across all sites. There was no relationship between CT_{max} and average water temperature (Table 10; Fig. 2). However, we detected a weak positive relationship between CT_{max} and dissolved oxygen concentration after controlling for the linear effects of water temperature using partial regression analysis ($p = 0.0686$, $F = 3.18$, df = 1,4; Table 10; Fig 3).

Gill size

Total gill filament length differed significantly across the three populations ($p < 0.0001$, F= 28.62, df =2,23; Tables 11, 12, and 13; Fig. 4). Total gill filament length was smallest in the normoxic Bunoga population; and fish from the normoxic Inlet Stream West population, showed a marginally smaller total gill filament length (Tukey p = 0.0627; Table 11, 12, and 13; Fig. 4) than fish from the hypoxic Rwembaita Swamp Mid population. Total filament number also differed across sites (p = 0.03467, F = 3.91, df =2,23), with normoxic Bunoga fish having the highest total gill filament number, though not significantly greater than hypoxic Rwembaita Swamp Mid fish (Tables 11, 12, and 13; Fig. 5).

Discussion

CT_{max}

We found evidence for inter-populational variation in upper thermal limits for *P. modestus*. Previous studies have shown evidence for inter-populational variability in upper thermal tolerance in many species of temperate and tropical fishes including Nile Perch (*Lates niloticus*; Chretien and Chapman 2016; Nyober et al. 2020), Common Killifish (*F. heteroclitus*; Fangue et al. 2006), Rainbow Trout (*Oncorhynchus mykiss*; Dressler et al. 2023), Channel Catfish (*Ictalurus punctatus*; Stewart and Allen 2014), and Chinook Salmon (*Oncorhynchus tshawytscha*; Zillig et al. 2014). Variation between populations of the same species may be due to plasticity in thermal limits and/or heritable genetic variation (McKenzie et al. 2020; Debes et al. 2021). Since we did not test for plasticity in upper thermal tolerance we are unable to address the source of inter-populational variation. Future studies should focus on measuring short term plasticity or acclimation capacity of *P. modestus* by exposing each population to warmer water temperatures before measuring their upper thermal limits. Rearing studies across a range of temperatures would also be insightful.

Though we detected a significant difference in upper thermal tolerance among populations, we did not detect a significant relationship between CT_{max} and water temperature in the Rwembaita Swamp System. Our results therefore suggest that environmental water temperature is not a significant predictor of CT_{max}, in this system. This may be due to the geographical proximity of our study sites. The average temperature of the sites chosen in this study ranged between 17.75 °C and 20.15 °C and variation in CT_{max} across sites, which was significant, was driven primarily by the difference between Njuguta (warm site) and Rwembaita Swamp Mid (cool site). It is possible that with this range of water temperatures that the thermal gradient was not strong enough to detect a relationship between CT_{max} and site temperature. *P. modestus* is widely distributed across East Africa, and it is possible that across a very large latitudinal range and across a larger temperature range that one may find different results. In our study, we focused on a single drainage system. However, we are also beginning to see similar upstream range shifts for *P. modestus* in the Dura River System, the other major river that drains Kibale (L. Chapman, personal observations). In addition, many studies have reported seasonal variation in thermal tolerance, which highlights the importance of environmental temperature when measuring CT_{max} (Laye and Claussen 1982; Leclair et al. 2020; Turko et al. 2020). Thus, future studies should consider species distributions on a larger scale and across multiple river systems to gain a better understanding of inter-populational variation in thermal tolerance and its relationship with environmental water temperature.

We detected a weak effect of average dissolved oxygen concentration on upper thermal tolerance with CT_{max} increasing with oxygen concentration after controlling for the linear effects of temperature using partial regression. This supports the hypothesis for the oxygen dependency of upper thermal tolerance in *P. modestus*. The functional association between dissolved oxygen concentration and thermal tolerance has often been explored in the context of oxygen- and capacity-limited thermal tolerance (Pörtner 2010), whereby, under warming water temperatures, the maximum rate of oxygen consumption exceeds the capacity of the cardio-respiratory system to supply oxygen to tissues. Consequently, the upper thermal limits of fishes are thought to be highly sensitive to hypoxia. However, support for oxygen dependency of thermal tolerance is mixed. Some studies have found no relationship between oxygen availability and CT_{max} (Brijs et al. 2015; Motyka et al. 2017; Christen et al. 2020; Ern et al. 2023; Montgomery et al. 2024) or no effect until severe hypoxia is reached (Ern et al. 2016; 2017; Jutfelt et al. 2024), while other studies have found that acute hypoxia exposure reduces CT_{max} (Rutledge and Beitinger 1989;

Ellis et al. 2013; Healy and Schulte 2012; Leeuwis et al. 2021; Potts et al. 2021; Andreassen et al. 2022; Jutfelt et al. 2024; Reemeyer and Chapman 2024). For example, for Pugnose Shiner (*Miniellus anogenus*) acute hypoxia exposure reduces CT_{max} in comparison to fish measured under normoxic conditions (Potts et al. 2021). Similarly, for Redside Dace (*Clinostomus elongatus*) acclimated to normoxia (>90 % air saturation), acute hypoxia exposure decreased CT_{max} (Reemeyer and Chapman 2024). However, this effect can be reduced if fish are acclimated to hypoxia rather than being acutely exposed (Reemeyer and Chapman 2024). The same relationship holds true for Sablefish (*Anoplopoma fimbria*) whereby fish reared under hypoxic conditions for 4-6 months exhibited a CT_{max} which was 3 °C lower than the CT_{max} of normoxia-reared fish over the same time frame (Leeuwis et al. 2021). In our study, *P. modestus* seems to be following the same trend with hypoxic populations having slightly lower CT_{max} values. There are a few factors that may contribute to the weak relationship between CT_{max} and dissolved oxygen. First, we found evidence that *P. modestus* populations are diverging in gill size. Larger gills may be an adaptive response to hypoxia that also affects CT_{max} if CT_{max} is oxygen dependent. Studies have reported that gill size (e.g., total gill filament length) increases with rearing temperature in Pugnose Shiner which may serve as an adaptation to increase oxygen uptake capacity and support increased metabolic demands in warmer waters (Potts et al. 2021). So, the fact that gills are larger in hypoxic sites may contribute to the fish's ability to maintain a high CT_{max}. A limitation of this study is that CT_{max} was measured under normoxic conditions for fish from all sites. As noted above, hypoxia exposure has been shown to reduce CT_{max} in other studies; therefore, it is possible that the CT_{max} for fish from hypoxic sites would have been even lower if measured under hypoxia.

Gill analysis

Gill analysis suggests that there is indeed evidence for divergence in oxygen uptake capacity between populations of *P. modestus* from the putative normoxic downstream source population and the upstream hypoxic and normoxic populations. Results revealed that the high-oxygen downstream population from Bunoga had the smallest total gill filament length whereas the upstream hypoxic swamp populations had the largest total gill filament length. Populations from the upstream normoxic stream site had an intermediate gill filament length that was closer to that of the upstream hypoxic population. Gill size variation between high- and low- dissolved oxygen populations of *P. modestus* may be driven by environmental effects. There are many studies that show divergence in gill size between populations of the same species living under hypoxic and normoxic conditions. For East African species this includes, among others: the haplochromine cichlid *P. multicolor* (Chapman et al. 2000; 2008; 2021); the haplochromine cichlid *A. alluaudi* (Chapman et al. 2007); the cyprinid *E. neumayeri* (Chapman et al. 1999; Langerhans et al. 2007; Hunt et al. 2023); the cyprinid *E. apleurogramma* (Hunt et al. 2023); the mormyrid *Petrocephalus catostoma* (Chapman and Hulen 2001); and the cyprinid *Rastrineobola argentea* (Sharpe et al. 2018). Divergence in gill size across dissolved oxygen concentrations in the field is likely an adaptation for increased oxygen uptake, and is one of many mechanisms exhibited by fish under hypoxia (Chapman 2015).

Acclimation and rearing studies suggest a strong element of plasticity in gill size. Some studies have reported that for the weakly electric fish *Petrocephalus degeni* metrics of gill size decreased, with gill filament length exhibiting a 14% reduction and hemibranch area exhibiting an 18% reduction, when fish from hypoxic swamps were acclimated under normoxia for 75 days (Mucha et al. 2023). Similarly, studies on hypoxic populations of *P. multicolor* from the Mpanga River drainage reported that fish reared under hypoxia exhibit gills that are on average 35% larger in total gill filament length and 56% larger in hemibranch area (Crispo and Chapman 2010). High levels of developmental plasticity in gill size and shape have also been reported for other haplochromine cichlids of the Lake Victoria basin of East Africa including the endemic *Haplochromis (Yssichromis) pyrrhocephalus*, which showed an increase of 80% in gill surface area in full sibs raised at 10% saturation versus full sibs reared at 80-90% saturation (Rutjes 2006). Likewise, similar findings have been reported for the East African cichlid *A. alluaudi* whereby full sibs reared under hypoxia increased their total gill filament length by 27% and their total hemibranch area by 35% in comparison to full sibs reared under normoxic conditions (Chapman et al. 2007). The source of variation in gill size of *P. modestus* in this study is unknown, but may reflect an element of plasticity.

Interestingly, we found that the total gill filament length of *P. modestus* from the normoxic Inlet Stream West fish was larger than those from the normoxic Bunoga population;

and more similar to the hypoxic Rwembaita Swamp Mid population. This may be due to the swamp serving as a natural barrier to the stream meaning that fish that have currently expanded their range into the normoxic stream site had to have passed through the hypoxic swamp. In our study, we found that average dissolved oxygen concentration at Inlet Stream West was 5.84 ± 0.14 mg/L (mean ± SEM) whereas Crispo and Chapman (2008) reported that the average dissolved oxygen at Bunoga was 7.7 mg/L so it is possible that the difference between *P. modestus* from Bunoga versus Inlet Stream West reflects the dissolved oxygen concentration of the two sites. Finally, it is possible that upstream populations of *P. modestus* are at an early phase of divergence in their oxygen uptake capacity. A similar study quantified total gill filament length of *E. apleurogramma*, a small species of cyprinid inhabiting the Mpanga river drainage, which has experienced a similar range shift into the Rwembaita Swamp System where it now occupies both swamp and stream sites (Hunt et al. 2023). More specifically, this study compared total gill filament length of *E. apleurogramma* from its home range to its upstream northern congener *E. neumayeri* and to expanding upstream populations of *E. apleurogramma*. For every population of both species, total gill filament length was measured across high and low dissolved oxygen conditions. The study found evidence for larger total gill filament length in hypoxic populations relative to normoxic populations. However, in range-expanding populations, which are presumably not yet fully adapted to their environment, total gill filament length was intermediate to historical populations of *E. apleurogramma* and *E. neumayeri* (Hunt et al. 2023). With this in mind, it is possible that the intermediate total gill filament length observed for *P. modestus* is reflective of an early divergence in oxygen uptake capacity. One limitation of this study is that the gill samples used in this analysis were collected in 2018. It is possible that there has been continued divergence between swamp and stream sites. It would be informative in future studies to continue to monitor the magnitude and the direction of the divergence between the low- and high-oxygen sites in the Rwembaita Swamp System. In addition, we do not know the source of the inter-populational variation in gill size, but this would be an interesting avenue for future investigation.

Conclusion

Understanding the ability of species to adapt to changing environmental conditions and their ability to shift their distribution is become increasingly important. For species of fishes that inhabit landlocked water and/or have limited dispersal abilities, their ability to respond adaptively *in situ* may be critical in assuring their persistence in their current habitat. For species that cannot respond *in situ*, their ability to shift their distribution to match their requirements will be of great importance. In order to better understand species range shifts we must consider multiple ecological gradients and environmental stressors that species may encounter while shifting their distribution. Our results suggest that species which are potentially shifting their distribution according to their thermal environment can potentially diverge in their oxygen uptake capacity to help them deal with low oxygen conditions, which would then allow them to diverge across a second environmental gradient in their expanded range. Many other stressors interact with warming water temperatures including deforestation and associated increases in turbidity (Ellison et al. 2017; Illa et al. 2018; Shi et al. 2022). This study then represents an important first step to exploring potential interactions between increasing water temperatures and co-occurring environmental variation in other abiotic factors. Future studies examining thermal tolerance and range shifts should consider the interaction between multiple environmental stressors in order to gain a better insight into how species may shift their distribution in the face of environmental change.

TABLES

Table 1: *Platypanchax modestus* body mass (g), standard length (cm), and sample size (n) for every collection site for which CT_{max} was measured. Values are reported as mean ± SEM.

Population	Mass (g)	Standard length (cm)	Sample size (n)
Inlet Stream East	0.63 ± 0.08	3.02 ± 0.13	12
Inlet Stream West	0.51 ± 0.07	2.89 ± 0.12	12
Mikana-Up Stream	0.50 ± 0.03	3.06 ± 0.10	12
Rwembaita Swamp Mid	0.53 ± 0.10	2.78 ± 0.11	12
Rwembaita Swamp Up	0.68 ± 0.06	3.16 ± 0.09	12
Rwembaita Swamp Low	0.41 ± 0.04	2.69 ± 0.13	12
Njuguta	0.61 ± 0.06	3.15 ± 0.07	12

Table 2: Mean temperature (˚C) and dissolved oxygen (DO; mg/L) of each experimental cooler used to hold *Platypanchax modestus* at the Makerere University Biological Field Station for a maximum of five days. Values are reported as mean ± SEM.

Cooler #	Average Temperature (˚C)	Average DO (mg/L)
1	18.27 ± 0.19	5.95 ± 0.12
2	18.19 ± 0.13	5.67 ± 0.22
3	18.07 ± 0.16	5.81 ± 0.20
4	17.87 ± 0.14	6.11 ± 0.17
5	17.89 ± 0.14	6.54 ± 0.12
6	17.87 ± 0.15	6.19 ± 0.15
7	17.59 ± 0.21	5.33 ± 0.31
8	17.33 ± 0.15	6.14 ± 0.31

Table 3: Summary of long-term limnological data for all sites and measures of CT_{max} (˚C) for *Platypanchax modestus* for each site. Site temperature (˚C) and dissolved oxygen (DO; mg/L) are given as mean ± SEM calculated from the monthly sampling (2020-2022).

Site name	Habitat type	Temperature (˚C)	DO (mg/L)	CT_{max} (˚C)
Inlet Stream East	Stream	18.97 ± 0.08	3.66 ± 0.13	33.72 ± 0.44
Inlet Stream West	Stream	17.80 ± 0.08	5.84 ± 0.14	33.52 ± 0.35
Mikana-Up Stream	Stream	17.75 ± 0.04	6.29 ± 0.04	33.70 ± 0.34
Rwembaita Swamp Mid	Swamp	18.30 ± 0.05	1.83 ± 0.01	32.18 ± 0.51
Rwembaita Swamp Up	Swamp	18.34 ± 0.12	3.15 ± 0.26	33.67 ± 0.48
Rwembaita Swamp Low	Swamp	18.42 ± 0.06	2.01 ± 0.10	33.70 ± 0.45
Njuguta	River	18.55 ± 0.05	5.60 ± 0.10	34.76 ± 0.25

Table 4: Results of ANOVA testing for differences in mass and standard length among populations of *Platypanchax modestus*. Reported as p-values and F-values. Significant p-values ($p<0.05$) are shown in bold.

Mass: Mass ~ Population

Effect	Sum of Squares	Mean Square	df	F value	*p*
Population	0.6396	0.1066	6	1.20×10^{30}	**<0.0001**
Residual	0	0	80		

Standard Length: Standard Length ~ Population

Effect	Sum of Squares	Mean Square	df	F value	*p*
Population	2.435	0.4058	6	4.18x10^{28}	**<0.0001**
Residual	0	0	80		

Table 5: Results of post-hoc Tukey test (Tukey HSD) for average mass and standard length for all populations of *Platypanchax modestus*. Reported as adjusted p-values under the diagonal line and difference values over the diagonal line. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), MIK (Mikana-Up Stream), RSWM (Rwembaita Swamp Mid), RSWU (Rwembaita Swamp Up), RSWL (Rwembaita Swamp Low), and NJ (Njuguta). Significant p-values (p<0.05) are shown in bold.

Mass

Pop.	ISE	ISW	MIK	RSWM	RSWU	RSWL	NJ
ISE		**-0.1283**	**-0.1317**	**-0.1008**	**0.0500**	**-0.2250**	**-0.0267**
ISW	**<0.0001**		**-0.0033**	**0.0275**	**0.1783**	**-0.0967**	**0.1017**
MIK	**<0.0001**	**<0.0001**		**0.0308**	**0.1817**	**-0.0933**	**0.1050**
RSWM	**<0.0001**	**<0.0001**	**<0.0001**		**0.1508**	**0.1242**	**-0.0742**
RSWU	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**0.2750**	**0.0767**
RSWL	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**-0.1983**
NJ	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

Standard Length

Pop.	ISE	ISW	MIK	RSWM	RSWU	RSWL	NJ
ISE		**-0.1250**	**0.0417**	**-0.2333**	**0.1417**	**-0.3250**	**0.1300**
ISW	**<0.0001**		**0.1667**	**-0.1083**	**0.2667**	**-0.2000**	**0.2550**
MIK	**<0.0001**	**<0.0001**		**-0.2750**	**0.1000**	**-0.3667**	**0.0883**
RSWM	**<0.0001**	**<0.0001**	**<0.0001**		**0.3750**	**0.0917**	**-0.3633**
RSWU	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**0.4667**	**0.0117**
RSWL	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**-0.4550**
NJ	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

Table 6: Results of ANOVA analysis testing for differences in average temperature and dissolved oxygen between sites. Reported as p-values and F-values. Significant p-values ($p<0.05$) are shown in bold.

Average Temperature: Average Temperature ~ Site

Effect	Sum of Squares	Mean Square	df	F value	*p*
Site	338.6	56.44	6	5.14x10^{26}	**<0.0001**
Residual	0	0	2057		

Average Dissolved Oxygen: Average Dissolved Oxygen ~ Site

Effect	Sum of Squares	Mean Square	df	F value	*p*
Site	7154	1192	6	3.88x10^{29}	**<0.0001**
Residual	0	0	2057		

Table 7: Results of post-hoc Tukey test (Tukey HSD) for average environmental water temperature and dissolved oxygen for all study sites. Reported as adjusted p-values under the diagonal line and difference values over the diagonal line. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), MIK (Mikana-Up Stream), RSWM (Rwembaita Swamp Mid), RSWU (Rwembaita Swamp Up), RSWL (Rwembaita Swamp Low), and NJ (Njuguta). Significant p-values ($p<0.05$) are shown in bold.

Average Temperature

Site	ISE	ISW	MIK	RSWM	RSWU	RSWL	NJ
ISE		**-1.1758**	**-1.2191**	**-0.6755**	**-0.6363**	**-0.5551**	**-0.4245**
ISW	**<0.0001**		**-0.04332**	**0.5003**	**0.5395**	**0.6207**	**0.7513**
MIK	**<0.0001**	**<0.0001**		**0.5436**	**0.5827**	**0.6640**	**0.7947**
RSWM	**<0.0001**	**<0.0001**	**<0.0001**		**0.0392**	**-0.1204**	**-0.2511**
RSWU	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**-0.0811**	**-0.2118**
RSWL	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**-0.1307**
NJ	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

Average Dissolved Oxygen

Site	ISE	ISW	MIK	RSWM	RSWU	RSWL	NJ
ISE		**2.1776**	**2.6264**	**-1.8358**	**-0.5108**	**-1.6523**	**1.9307**
ISW	**<0.0001**		**0.4489**	**-4.0134**	**-2.6884**	**-3.8300**	**-0.2469**
MIK	**<0.0001**	**<0.0001**		**-4.4623**	**-3.1372**	**-4.2787**	**-0.6958**
RSWM	**<0.0001**	**<0.0001**	**<0.0001**		**1.3250**	**-0.1835**	**-3.7665**
RSWU	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**1.1415**	**-2.4414**
RSWL	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**-3.5829**
NJ	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

Table 8: Results of CT_{max} model for *Platypanchax modestus* across populations. Reported as X^2 values and p-values. Significant p-values ($p<0.05$) are shown in bold.

CT_{max}: CT_{max} ~ Population + (1| trial)

Effect	X^2	df	*p*
Population	14.22	6	**0.0273**

Table 9: Results of post-hoc Tukey test (Tukey glht) for *Platypanchax modestus* CT_{max} comparison between populations. Reported as adjusted p-values under the diagonal line and estimate values over the diagonal line. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), MIK (Mikana-Up Stream), RSWM (Rwembaita Swamp Mid), RSWU (Rwembaita Swamp Up), RSWL (Rwembaita Swamp Low), and NJ (Njuguta). Significant p-values ($p<0.05$) are shown in bold.

CTmax

Pop.	ISE	ISW	MIK	RSWM	RSWU	RSWL	NJ
ISE		-0.2042	-0.0258	-1.538	-0.0742	-0.1308	0.9951
ISW	1.000		0.1783	-1.3341	0.1300	0.0733	1.1993
MIK	1.000	1.000		-1.5125	-0.0484	-0.1050	1.0209
RSWM	0.3043	0.4843	0.3248		1.4641	-1.4075	**-2.5334**

RSWU	1.000	1.000	1.000	0.3524		0.0566	-1.0693
RSWL	1.000	1.000	1.000	0.4020	1.000		-1.1259
NJ	0.7602	0.5637	0.7372	**0.0033**	0.6807	0.6245	

Table 10: Results of multiple regression and partial regression used detect relationships between CT_{max}, and both average site water temperature, and average site dissolved oxygen concentration. Reported as p-values and F-values. Significant p-values ($p<0.05$) are shown in bold.

CT_{max}: Average CT_{max} ~ Average Temperature + Average Oxygen

Effect	Sum of Squares	df	F value	*p*
Average Temperature	1.03	1	3.18	0.1491
Average Oxygen	1.98	1	6.13	0.0686
Residuals	1.2901	4		

Table 11: *Platypanchax modestus* body mass (g), standard length (cm), sample size (n), mean total gill filament length (mm), and mean total gill filament number for every site for which gill data were collected. Values are reported as mean ± SEM.

Population	Mass (g)	Standard length (cm)	Sample size (n)	Mean total gill filament length (mm)	Mean total gill filament number (#)
Inlet Stream West	0.63 ± 0.08	3.22 ± 1.05	10	146.74 ± 2.97	209.71 ± 3.31
Rwembaita Swamp Mid	0.65 ± 0.06	3.57 ± 0.68	6	162.85 ± 6.90	213.59 ± 3.04
Bunoga	0.52 ± 0.05	3.25 ± 0.92	10	115.10 ± 4.32	223.55 ± 4.33

Table 12: Linear model testing for differences in total gill filament length and total gill filament number among three populations of *Platypanchax modestus*: a normoxic downstream river population (Bunoga), an upstream normoxic population (Inlet Stream West), and an upstream hypoxic swamp population (Rwembaita Swamp Mid). Values were adjusted to the mean body size of 3.35 cm for analyses. Significant p-values ($p<0.05$) are shown in bold.

Total gill filament length: Total gill filament length ~ Population

Effect	Sum of Squares	df	F value	*p*
Population	9711.1	2	28.62	**< 0.0001**
Residuals	3902.2	23		

Total gill filament number: Total gill filament number ~ Population

Effect	Sum of Squares	df	F value	*p*
Population	1001.2	2	3.91	**0.03466**
Residuals	2948.1	23		

Table 13: Results of post-hoc Tukey test (Tukey HSD) for *Platypanchax modestus* gill analysis comparison of total gill filament length and total gill filament number between the downstream river population and upstream normoxic and hypoxic populations. Reported as adjusted p-values under the diagonal line and difference values over the diagonal line. Sites abbreviated as ISE (Inlet Stream East), ISW (Inlet Stream West), and Bunoga (Bunoga). Significant p-values (p<0.05) are shown in bold.

Total gill filament length

Population	ISW	RSWM	Bunoga
ISW		16.1056	**31.6432**
RSWM	0.0627		**47.7488**
Bunoga	**<0.0001**	**<0.0001**	

Total gill filament number

Population	ISW	RSWM	Bunoga
ISW		3.8882	**-13.8466**
RSWM	0.7858		-9.9583
Bunoga	**0.0305**	0.22543	

FIGURES

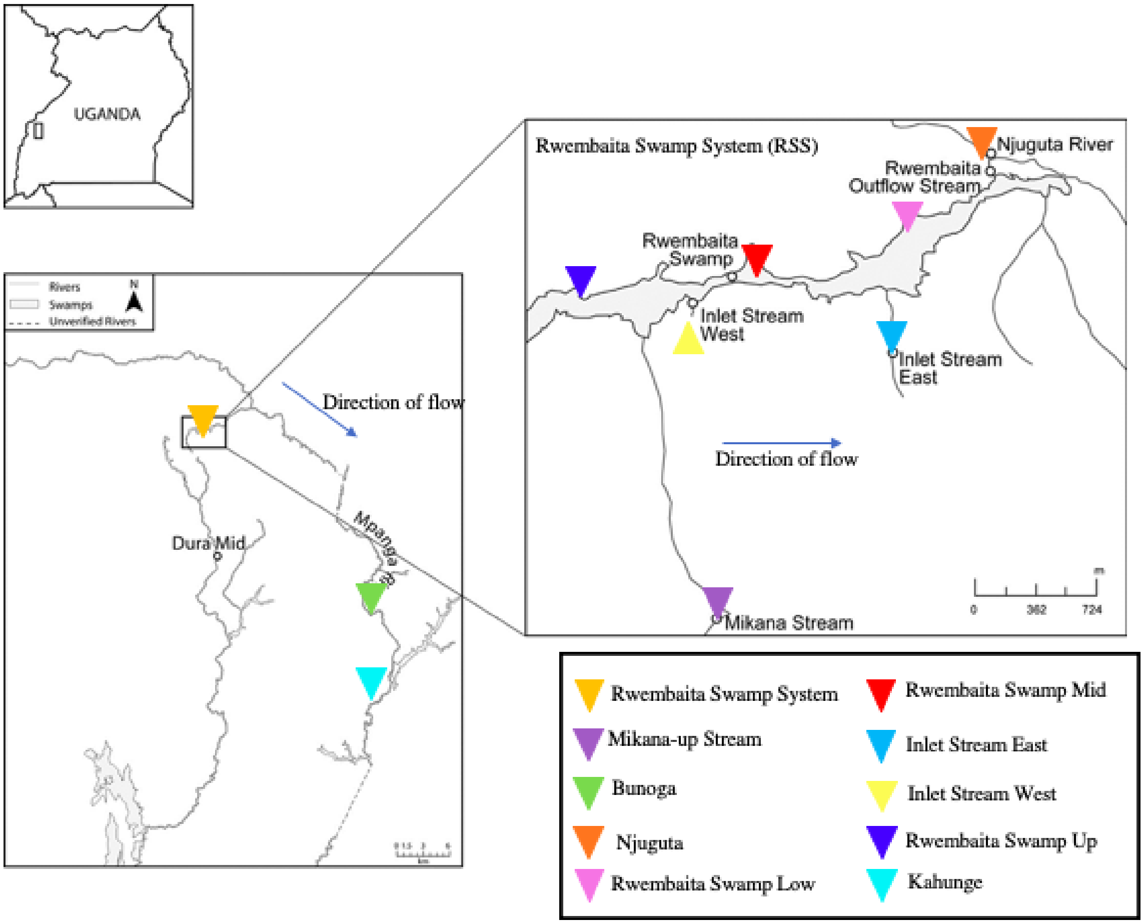

Figure 1: Map of the Mpanga River System and study sites. Coloured flags indicate different sampling sites within and outside of the Rwembaita Swamp System.

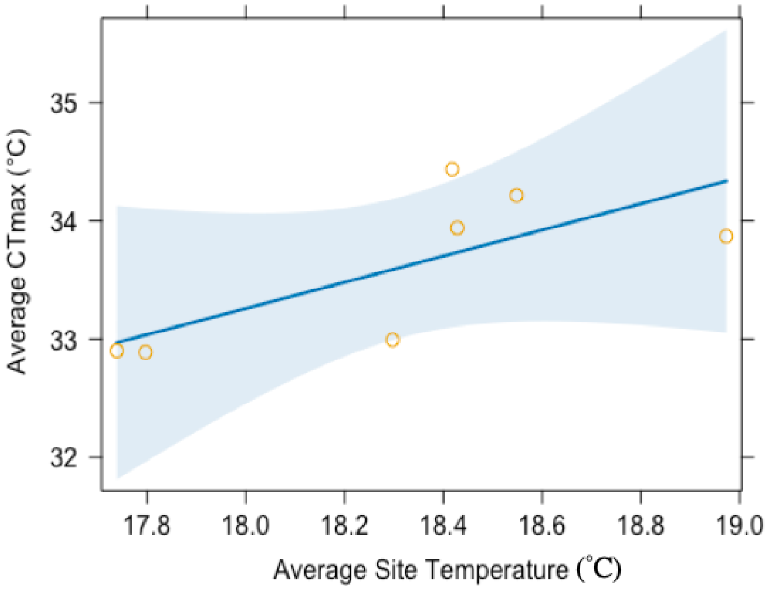

Figure 2: Partial regression plot showing the relationship between average site CT_{max} in °C and average site water temperature in °C across populations of *Platypanchax modestus*, after controlling for the effects of dissolved oxygen concentration.

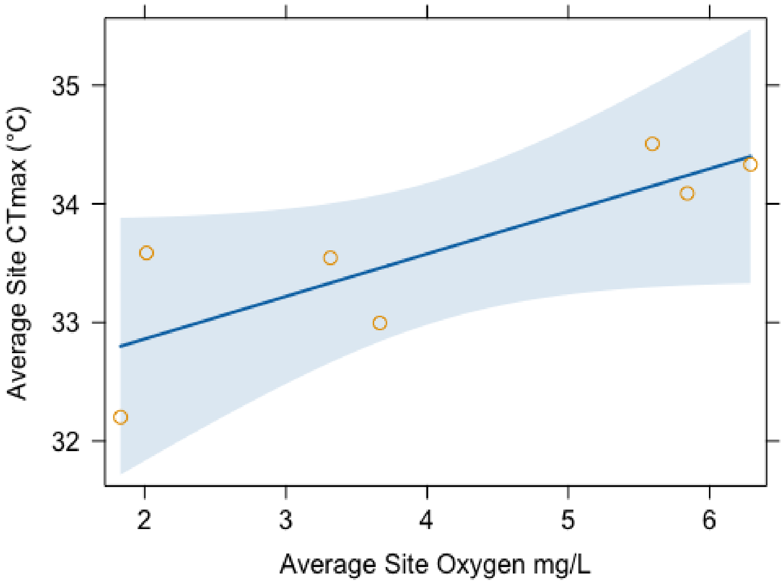

Figure 3: Partial regression plot showing the relationship between average site CT_{max} in °C and average site oxygen concentration in mg/L across populations of *Platypanchax modestus*, after controlling for the effects of water temperature.

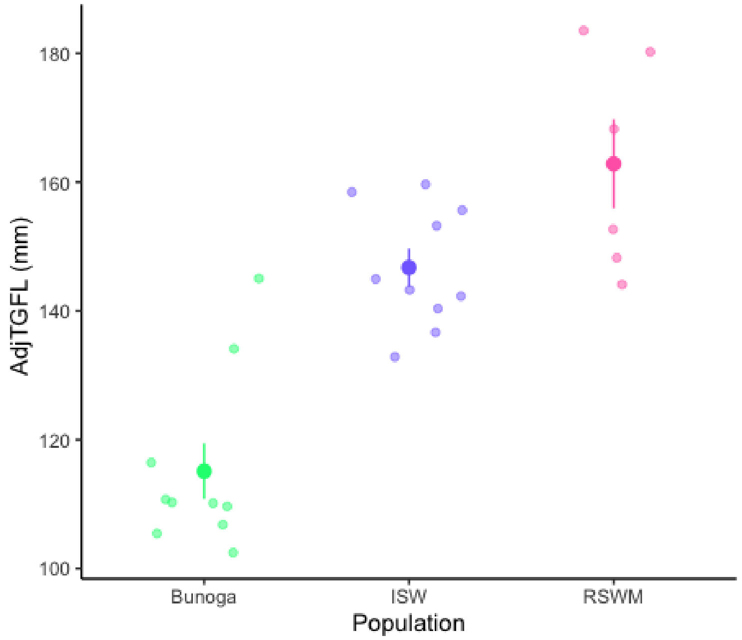

Figure 4: Adjusted total gill filament length (AdjTGFL) in mm for the normoxic river site (Bunoga, downstream historical range) and the normoxic and hypoxic Rwembaita Swamp System sites (Inlet Stream West and Rwembaita Swamp Mid, upstream expanded range) of *Platypanchax modestus*. Sites abbreviated as Bunoga, ISW, and RSWM respectively. Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

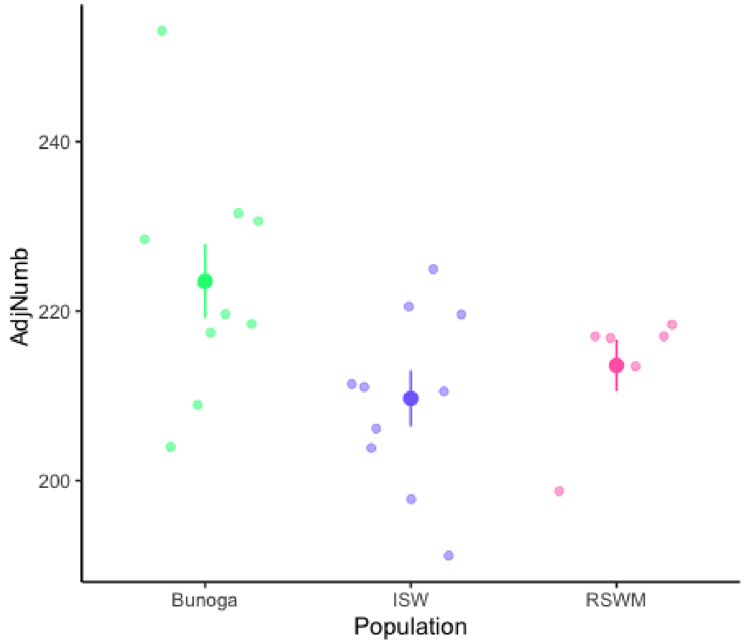

Figure 5: Adjusted total gill filament number (AdjNumb) for the normoxic river site (Bunoga, downstream historical range) and the normoxic and hypoxic Rwembaita Swamp System sites (Inlet Stream West and Rwembaita Swamp Mid, upstream expanded range) of *Platypanchax modestus*. Sites abbreviated as Bunoga, ISW, and RSWM respectively. Darker points and lines on the graph represent mean ± SEM, while lighter points represent the raw data.

REFERENCES

Akin, J.A. 2011. Homeostatic processes for thermoregulation. Nature Education Knowledge 3: 7.

Almela, V.D., South, J., and Britton, J.R. 2021. Predicting the competitive interactions and trophic niche consequences of a globally invasive fish with threatened native species. Journal of Animal Ecology 90: 2651-2662.

Alofs, K.M., Jackson, D.A., and Lester, N.P. 2014. Ontario freshwater fishes demonstrate differing range-boundary shits in a warming climate. Diversity and Distributions 20: 123-136.

Amsterdam, A., Sadler, K.C., Lai, K., Farrington, S., Bronson, R.T., Lees, J.A., and Hopkins, N. 2004. Many ribosomal protein genes are cancer genes in Zebrafish. PLoS ONE Biology 2: 0690-0698.

Andreassen A.H., Hall, P., Khatibzadeh, P., Jutfelt, F., and Kermen, F. 2022. Brain dysfunction during warming is linked to oxygen limitation in larval zebrafish. Proceedings of the National Academy of Science 119: e2207052119.

Armstrong, J.B., Fullerton, A.H., Jordan, C.E., Ebersole, J.L., Bellmore, J.R., Arismendi, I., Penaluna, B., and Reeves, G.H. 2022. The importance of warm habitat to the growth regime of cold-water fishes. Nature Climate Change 11: 354- 361.

Baisero, D., Visconti, P., Pacifici, M., Cimatti, M., and Rondinini C. 2020. Projected global loss of mammal habitat due to land-use and climate change. One Earth 2: 578-585.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., Fox, J., Bauer, A., Krivitsky, P.N., Tanaka, E., and Jagan, M. 2024. Fit linear and generalized linear mixed-effects models. The models and their components are represented using S4 classes and methods. The core computational algorithms are implemented using the 'Eigen' C++ library for numerical linear algebra and 'RcppEigen' "glue".

Bartlett, C.B., Garber, A.F., Gonen, S., and Benfey, T.J. 2022. Acute critical thermal maximum does not predict chronic incremental thermal maximum in Atlantic salmon (*Salmo salar*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 266: no.111143.

Beitinger, T.L., Bennett, W.A., and McCauley, R.W. 2000. Temperature tolerances of north American Freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes 58: 237-275.

Beyer, R.M., and Manica, A. 2020. Historical and projected future range sizes of the world’s mammals, birds, and amphibians. Nature Communications 11: no.5633.

Binning, S.A., Chapman, L.J., and Dumont, J. 2010. Feeding and breathing: Trait correlations in an African cichlid fish. Journal of Zoology 282: 140-149.

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. Journal of Experimental Biology 223: jeb209692.

Bouyoucos, I.A., Weinrauch, A.M., Jeffries, K.M., and Anderson, G. 2023. Physiological responses to acute warming at the agitation temperature in a temperate shark. Journal of Experimental Biology 226: jeb246304.

Boyle, M., Schwanz, L., Hone, J., and Georges A. 2016. Dispersal and climate warming determine range shift in model reptile populations. Ecological Modelling 328: 34-43.

Brijs, J., Jutfelt, F., Clark, T.D., Gräns, A., Ekström, A., and Sandblom, E. 2015. Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. Journal of Experimental Biology 218: 2448–2454.

Campana, S.E., Stefansdottir, R.B., Jakobsdottir, K., Solmundsson, J. 2020. Shifting fish distributions in warming sub-Arctic oceans. Scientific Reports 10: no.16448.

Carosi, A., Padula, R., Ghetti, L., and Lorenzoni, M. 2019. Endemic freshwater fish range shifts related to global climate changes: A long-term study provides some observational evidence for the Mediterranean area. Water 11: no.2349.

Cellerino, A., Valenzano, D.R., and Reichard, M. 2015. From the bush to the bench: the annual *Notobranchius* fishes as a new model system in biology. Biological Reviews 1: 000-000.

Cereja, R. 2020. Critical thermal maxima in aquatic ectotherms. Ecological Indicators 119: no.106856.

Champion, C., Brodie, S., and Coleman, M.A. 2021. Climate-driven range shifts are rapid yet variable among recreationally important coastal-pelagic fishes. Frontiers in Marine Science 8: no.622299.

Chapman, L.J. 1995. Seasonal dynamics of habitat use by an air-breathing catfish (*Clarias liocephalus*) in a papyrus swamp. Ecology of Freshwater Fish 4: 113-123.

Chapman, L.J. and Liem, K.F.. 1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. Environmental Biology of Fishes 44: 183-197.

Chapman, L.J., Chapman, C.A., Brazeau, D., McGlaughlin, B., and Jordan, M. 1999. Papyrus swamps and faunal diversification: Geographical variation among populations of the African cyprinid *Barbus neumayeri*. Journal of Fish Biology 54: 310-327.

Chapman, L.J., Galis, F., and Shinn, J. 2000. Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. Ecology Letters 3: 388-393.

Chapman, L.J., and Hulen, K.G. 2001. Implication of hypoxia for the brain size and gill morphology of mormyrid fishes. Journal of Zoology 254: 461-472.

Chapman, L.J. Morpho-physiological divergence across oxygen gradients in fishes. 2007. Pages 14-39. In: M.N. Fernandes, F.T. Rantin, M.L. Glass, and B.G. Kapoor, editors. Fish Respiration and the Environment. Science Publishers, Inc., Enfield, NH.

Chapman, L.J., DeWitt, T.J., Tzenava, V., and Paterson, J. 2007. Interdemic variation in the gill morphology of a eurytopic African cichlid. Proceedings of the 9th International Symposium on Fish Physiology, Toxicology, and Water Quality. EPA/600/R-07/010. 209-225.

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

Chapman, L.J. 2015. Low-oxygen lifestyles. Pages 683-714. In Riesch, R., Tobler, M., and Plath, M., editors. Extremophile Fishes. Springer, New York, New York, United States.

Chapman, L.J. Respiratory ecology of cichlids. 2021. Pages 683-714. In M.E. Abate and D.L.G Noakes, editors. The Behavior, Ecology and Evolution of Cichlid Fishes: A Contemporary Modern Synthesis. Springer Academic.

Chen, Z., Antilla, K., Wu, J., Whitney, C.K., Hinch, S.G., and Farrell, A.P. 2013. Optimum and maximum temperatures of sockeye salmon (*Onchorhynchus nerka)* populations hatched at different temperatures. Canadian Journal of Zoology 91: 263-274.

Chretien, E., and Chapman, L.J. 2016. Tropical fishes in a warming world: Thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. Conservation Physiology 4: cow062.

Christen, F., Dufresne, F., Leduc, G., Dupont-Cyr, B.A., Vandenburg, G.W., Le Francois, N.R., Tardif, J.C., Lamarre, S.G., and Blier, P.U. 2020. Thermal tolerance and fish heart integrity: fatty acids profiles as predictors of species resilience. Conservation Biology 8: coaa108.

Clarke, D.N., and Zani, P.A. 2012. Effect of night-time warming on temperate ectotherm reproduction: potential fitness benefits of climate change for side-blotched lizards. Journal of Experimental Biology 215: 1117-1127.

Claunch, N.M., Goodman, C.M., Kluever, B.M., Barve, N., Guralnick, R.P., and Romagosa, C.M. 2023. Commonly collected thermal performance data can inform species distributions in data-limited invader. Scientific Reports 13: no.15880.

Comte, L., Buisson, L., Daufresne, M., and Grenouillet, G. 2013. Climate-induced changes in the distribution of fish: observed and predicted trends. Freshwater Biology 58: 625-639.

Comte, L., Murienne, J., and Grenouillet, G. 2014. Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes. Nature Communications 5: no.5053.

Comte, M., Felipe de Campos, D., and Eme, J. 2023. Effective practices for thermal tolerance polygon experiments using mottled catfish *Corydoras paleatus*. Journal of Thermal Biology 115: no.103616.

Cowan, Z.L., Andreassen, A.H., De Bonville, J., Green, L., Binning, S.A., Silva-Garay, L., Jutfelt, F., and Sundin, J. 2023. A novel method for measuring acute thermal tolerance in fish embryos. Conservation Physiology 11: coad061.

Cowles, R.B., and Bogert, C.M. 1944. A preliminary study of the thermal requirements of desert reptiles. Bulletin of the American Museum of Natural History 83: 265-296.

Cox, D. K. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. Pages 158-163. In W. Gibbons and R. R.Sharitz, editors. Thermal Ecology. Springfield, IL: National Technical Information Service.

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

Crispo, E. and Chapman, L.J. 2010. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. Journal of Evolutionary Biology 23: 2091-2103.

Crocker, C., Chapman, L.J., and Martinez M. 2013. Natural variation in enzyme activity of the African cichlid *Pseudocrenilabrus multicolor victoriae*) Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 164: 53-60.

Debes, P.V., Solberg, M.F., Matre, I.H., Dyrhovden, L., and Glover K.A. 2021. Genetic variation for upper thermal tolerance diminishes between and within populations with increasing acclimation temperature in Atlantic salmon. Heredity 127: 455- 466.

Desforges, E.M. 2020. On applying critical thermal maxima method to investigate ecologically-relevant questions in wild fishes. Master's Thesis. Carlton University, Ottawa.

Desforges, J.E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K.M., Eliason, E.J., Dressler, T.L., McKenzie, D.J., Bates, A.E., Lawrence, M.J., Fangue, N., and Cooke, S.J. 2023. The ecological relevance of critical thermal maxima methodology for fishes. Journal of Fish Biology 102: 1000-1016.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Shelson, K.S., Ghalambor, C.K., Haak, D.C., and Martin, P.R. 2008. Impact of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences of the United States of Amercia 105: 6663-6672.

Diamond, S.E. 2018. Contemporary climate-driven range shifts: Putting evolution back on the table. Functional Ecology 32: 1662-1665.

Donelson, J.M., Sunday, J.M., Figueira, W.F., Gaitan-Espitia, J.D., Hobday, A.J., Johnson, C.R., Leis, J.M., Ling, S.D., Marshall, D., Pandolfi, J.M., Pecl, G., Rodgers, G.G., Booth, D.J., and Munday, P.L. 2019. Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. The Philosophical Transactions of the Royal Society B: Biological Sciences 374: no.1768.

Dressler, T.L., Han Lee, V., Klose, K., and Eliason, E.J. 2023. Thermal tolerance and vulnerability to warming differ between populations of wild *Oncorhynchus mykiss* near the species' southern range limit. Scientific Reports 13: no.14538.

Duclos, V., Boudreau, S., and Chapman, C.A. 2013. Shrub cover influence on seedling growth and survival following logging of a tropical forest. Biotropica 45: 419-426.

Earheart, M.L., Blanchard, T.S., Harman, A.A., and Schulte, P.M. 2020. Hypoxia and high temperature as interacting stressors: Will plasticity promote resilience of fishes in a changing world? The Biological Bulletin 243: 149-170.

Earhart, M.L., Blanchard, T.S., Strowbirdge, N., Sheena, R., McMaster, C., Staples, B., Brauner, C.J., Baker, D.W., and Schulte, P.M. 2023. Heatwave resilience of juvenile white sturgeon is associated with epigenetic and transcriptional alterations. Scientific Reports 13: no.15451.

Ellison, D., Morris, C.E., Locatelli, B., Sheil, D., Cohen, J., Murdiyarso, D., Gutierrez, V., Noordwijk, M.V., Creed, I.F., Pokorny, J., Gaveau, D., Spracklen, D.V., Tobella, A.B., Ilstedt, U., Teuling, A.J., Gebrehiwot, S.G., Sands, D.C., Muys, B., Verbist, B., Springgay, E., Sugandi, Y., and Sullivan, C.A. 2017. Trees, forests and water: Cool insights for a hot world. Global Environmental Change 43: 51-61.

Ellis L.E., Sacobie, C.F.D., Kieffer, J.D., and Benfey, T.J. 2013. The effects of dissolved oxygen and triploidy on critical thermal maximum in brook charr (*Salvelinus fontinalis*). Comparative Biochemistry and Physiology A Molecular and Integrative Physiology 166: 426–433.

Ern, R., Norin, T., Gamperi, A.K., and Esbaugh, A.J. 2016. Oxygen dependence of upper thermal limits in fishes. Journal of Experimental Biology 219: 3376-3383.

Ern, R., Andreassen, A.H., and Jutfelt, F. 2023. Physiological mechanisms of acute upper thermal tolerance in fish. Physiology 38: 141-158.

Evans, T.G., Diamond, S.E., and Kelly, M.W. 2015. Mechanistic species distribution modelling as a link between physiology and conservation. Conservation Biology 3: cov056.

Fangue, N.A., Hofmeister, M., and Schulte, P.M. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. Journal of Experimental Biology 209: 2859-2872.

Firth, B.L., Craig, P.M., Drake, A.R., and Power, M. 2023. Seasonal, environmental and individual effects on hypoxia tolerance of eastern sand darter (*Ammocrypte pellucida*). Conservation Physiology 11: coad008.

Fox, J., and Weisberg, S. 2019. An R Companion to Applied Regression, Third Edition, Sage.
Furness, A. 2015. The evolution of an annual life cycle in killifish: Adaption to ephemeral aquatic environments through embryonic diapause. Biological Reviews of the Cambridge Philosophical Society 1: 1-18.

Gilbert, M.J.H., and Farrell, A.P. 2021. The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic charr (*Salvelinus alpinus*), a northern cold-water specialist. Journal of Thermal Biology 95: no.102816.

Gilbert, M.J.H., Adams, O.A., and Farrell, A.P. 2022. A sudden change of heart: Warm acclimation can produce a rapid adjustment of maximum heart rate and cardiac thermal sensitivity in rainbow trout. Current Research in Physiology 5: 179-183.

Gilmore, K.L., Doubleday, Z.A., and Gillanders, B.M. 2019. Prolonged exposure to low oxygen improves hypoxia tolerance in a freshwater fish. Conservation Physiology 7: coz058.

Goldsmit, J., McKindsey, C.W., Shlegel, R.W., Deslauriers, D., and Howland, K.L. 2024. Predicted shifts in suitable habitat of interacting benthic species in a warmer and invaded Canadian Arctic. Elementa Science of the Anthropocene 12: no.00018.

Gomez-Ruiz, E.P., and Lacher, T.E. 2019. Climate change, range shifts, and the disruption of a plant-pollinator complex. Scientific Reports 9: no.14048.

Greenwood, P.H. 1966. The Fishes of Uganda. The Uganda Society, Kampala, Uganda.

Haesemeyer, M. 2020. Thermoregulation in fish. Molecular and Cellular Endocrinology 518: no.110986.

Hallfors, M.H., Heikkinen, R.K., Kuussaari, M., Lehikoinen, A., Luoto, M., Poyry, J., Virkkala, R., Sasstamoinen, M., and Kujala, H. 2024. Recent range shifts of moths, butterflies, and birds are driven by the breadth of their climatic niche. Evolution Letters 8: 89-100.

Healy, T.M., and Schulte P.M. 2012. Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in common killifish (*Fundulus heteroclitus*). Physiological and Biochemical Zoology 85: 107-119.

Helly, J., and Levin, L.A. 2004. Global distribution of naturally occurring marine hypoxia on continental margins. Deep Sea Research Part 1: Oceanographic Research Paper 51: 1159-1168.

Hendry A.P. and Taylor, E.B. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58: 2319–2331.

Hillyard, S.D. 2017. Life in hot water: The desert pupfish. Encyclopedia of Fish Physiology: 1831-1842.

Hothorn, B. 2010. Simultaneous tests and confidence intervals for general linear hypotheses in parametric models, including linear, generalized linear, linear mixed effects, and survival models. CRC Press.

Hu, R., Gu, Y., Luo, M., Wei, M., and Zhong, J. 2020. Shifts in bird ranges and conservation priorities in China under climate change. PLoS ONE 15: e0240225.

Huber, J.H. 1996. Updated checklist of taxonomic names, collecting localities and bibliographic references of oviparous Cyprinodont fishes (Atherinomorpha, Pisces). Société Française d'Ichtyologie, Muséum National d'Histoire Naturelle, Paris, France.

Huey, R.B., and Stevenson, R.D. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. American Zoologist 19: 357-366.

Hunt, D.A.G.A., Hendry, A., and Chapman, L.J. 2023 Are we there yet? Inter- and intraspecific approaches to evaluating phenotypic optima in a range expanding East African fish, *Enteromius apleurogramma* (Cyprinidae)? Biological Journal of the Linnean Society.140: 296-312.

Ilha, P., Schiesari, L., Yanagawa, F.I., Jankowski, K., and Navas, C.A. 2018. Deforestation and stream warming affect body size of Amazonian fishes. PLoS One 13: no.e0196560.

IPCC. Sixth Assessment Report. March 20th, 2023. https://www.ipcc.ch/assessment-report/ar6/.

International Civil Aviation Organization. 1993. Manual of the ICAO Standard Atmosphere: extended to 80 kilometres (262500 feet). Montreal, QC: International Civil Aviation Organization.

Islam, M.A., Uddin, M.H., Uddin, M.J., and Shahjahan, M. 2019. Temperature changes influenced the growth performance and physiological functions of Thai pangas *Pansasianodon hypophthalmus*. Aquaculture Reports 13: no.100179.

Islam, S.M., Zahangir, M.M., Jannat, R., Hasan, M.N., Suchana, S.A., Rohani, F.R., and Shahjahan, M. 2020. Hypoxia reduced upper thermal limits causing cellular and nuclear abnormalities of erythrocytes in Nile tilapia, *Oreochromic niloticus*. Journal of Thermal Biology 90: no.102604.

Jones, N., and Schmidt, B. 2019. Thermal habitat: Understanding stream temperature and thermal classifications. Ontario Ministry of Natural Resources and Forestry, Science and Research Branch, Peterborough, ON. Science and Research Information Report IR-18. 13p.

Joyner-Matos, J. and Chapman, L.J. 2013. Persisting in papyrus: size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia. Comparative Biochemistry and Physiology Part A 165: 405–416.

Jung, E.H. 2018. The relationship between thermal tolerance and hypoxia tolerance in Amazonian fishes. Ph.D. Dissertation, University of British Columbia: 10.14288/1.0364578.

Jung, E.H., Brix, K.V., Richards, J.G., Val, A.L., and Brauner, C.J. 2020. Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world. The Science of the Total Environment 15: no.748.

Jutfelt, F., Ern, R., Leeuwis, H.J., and Clark, T.D. 2024. Effects of climate warming. Pages 14-31. In: S.L. Alderman and T.E. Gillis, editors. Encyclopedia of Fish Physiology (Second Edition). Oxford Academic Press.

Khelifa, R., Blanckenhorn, W.U., Roy, J., Rohner, P.T., and Mahdjoub, H. 2019. Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability. Journal of Animal Ecology 88: 1901-1912.

Kingsolver, J.G., and Umbanhowar, J. 2018. The analysis an interpretation of critical temperatures. Journal of Experimental Biology 221: jeb167858.

Kirk, M.A., and Rahel, F.J. 2022. Assessing climate-induced range shifts of stream fishes using a consensus framework. Ecology of Freshwater Fish 32: 270-281.

Kulhanek, S.A., Leung, B., and Ricciardi, A. 2011. Using ecological niche models to predict the abundance and impact of invasive species: application to the common carp. Ecological Applications 21: 203-213.

Lane, D. 2010. Tukey’s honestly significant difference (hsd). Pages 1566-1570. Encyclopedia of Research Design. Sage Publications, Volume 0.

Langerhans, R.B., Chapman, L.J., and DeWitt, T.J. 2007. Complex phenotype-environment associations revealed in an East African cyprinid. Journal of Evolutionary Biology 20: 1171-1181.

Langerhans, R.B., Gifford, M.E., and Joseph, E.O. 2007. Ecological speciation in *Gambusia* fishes. Evolution 61: 2056-2074.

Layne, J.R., and Claussen, D.L. 1982. Seasonal variation in the thermal acclimation of critical thermal maxima (CTmax) and minima (CTmin) in the salamander *Eurycea bislineata*. Journal of Thermal Biology 7: 29-33.

Leeuwis, R.H.J., Zanuzzo, F.S., Peroni, E.F.C., and Gamperl, A.K. 2021. Research on sablefish (*Anaplopoma fimbria*) suggests that limited capacity to increase heart function leaves hypoxic fish susceptible to heat waves. Proceedings of the Royal Society B 288: no.1946.

Leclair, A.T.A., Drake, A.R., Pratt, T.C., and Mandrak, N.E. 2020. Seasonal variation in thermal tolerance of redside dace *Clinostomus elongatus*. Conservation Physiology 8: coaa081.
Leong, C.M., Tsang, T.P.N., and Guenard, B. 2022. Testing the reliability and ecological implications of ramping rates in the measurement of critical thermal maximum. PLoS ONE 17: e0265361.

Levin, L.A., Ekau, E., Gooday, A.J. Jorissen, F., Middelburg, J.J., Naqvi, S.W.A., Neira, C., Rabalais, N.N., and Zhang, J. 2009. Effects of natural and human-induced hypoxia on coastal benthos. Biogeosciences 6: 2063-2098.

Levine, R.M., De Robertis, A., Grunbaum, D., Wildes, S., Farley, E.V., Stabeno, P.J., and Wilson, C.D. 2023. Climate-driven shifts in pelagic fish distributions in a rapidly changing Pacific Arctic. Deep Sea Research Part II: Tropical Studies in Oceanography 208: no.105244.

Li, S., Guo, H., Du, C.Y., Tao, Y.X., Feng, J.Y., Xu, H., Pang, X., Li, Y. 2023. Effect of temperature on exercise metabolism, hypoxia tolerance, and RNA-seq analysis in *Sinilabeo rendahli* from the Yangtze River, China. Frontiers in Ecology and Evolution 11: no.1159161.

Little, A.G., and Seebacher, F. 2021. Physiological performance curves: When are they useful? 2021. Frontiers in Physiology 12: no.805102.

Lutterschmidt, W. I., & Hutchinson, V. H. 1997. The critical thermal maximum: History and critique. Canadian Journal of Zoology 75: 1567-1574.

MacLean, H.J., Sorensen, J.G., Kristensen, T.N., Loeschcke, V., Beedholm, K., Kellerman, V., and Overgaard, J. 2019. Evolution and plasticity of thermal performance: an analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. The Philosophical Transactions of the Royal Society B: Biological Sciences 374: no.1778.

Martinez, M.L., L.J. Chapman, J.M. Grady, and Rees, B.B. 2004. Interdemic variation in hematocrit and lactate dehydrogenase in the African cyprinid *Barbus neumayeri*. Journal of Fish Biology 65: 1056-1069.

Martinez, M.L., Raynard, E.L., Rees, B.B., and Chapman, L.J. 2011. Oxygen limitation and tissue metabolic potential of the African fish *Barbus neumayeri*: roles of native habitat and acclimatization. BMC Ecology 11: 2.

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. Integrative and Comparative Biology 53: 648-659.

McDonnell, L.H., and Chapman, L.J. 2015. At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. Conservation Physiology 3: cov050.

McDonnell, L.H., Mandrak, N.E., Kaur, S., and Chapman., L.J. 2021. Effects of acclimation to elevated water temperature and hypoxia on thermal tolerance of the threatened pugnose shiner (*Notropis anogenus*). Canadian Journal of Fisheries and Aquatic Sciences 78: 1257-1267.

McKenzie, D.J.,Zhang, Y., Eliason, E.J., Schulte, P.M., Claireaux, G., Blasco, F.R., Nati, J.J.H., and Farrell, A.P. 2020. Intraspecific variation in tolerance of warming in fishes. Journal of Fish Biology 98: 1536-1555.

McNab, B.K. 2002. The physiological ecology of vertebrates: A view from energetics. Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

McPhee, D., Watson, J.R., Harding, D.J., Prior, A., Fawcett, J.H., Franklin, C.E., and Cramp, R.L. 2023. Body size dictates physiological and behavioural responses to hypoxia and elevated water temperatures in Murray cod (*Maccullochella peelii*). Conservation Physiology 11: coac087.

Miller, N.A., and Stillman, J.H. 2018. Physiological optima and critical limits. Nature Education Knowledge 10: 1.

Montgomery, D.W., Finlay, J., Simpson, S.D., Engelhard, G.H., Birchenough, S.N.R., and Wilson, R.W. 2024. Respiratory acidosis and O2 supply capacity do not affect the acute temperature tolerance of rainbow trout (*Oncorhynchus mykiss*). Conservation Biology 12: coae026.

Moran, E., Thuiller, W., Angert, A.L., and Garzon, M.B. 2022. Predicting and managing climate-driven range shifts in plants. Frontiers in Ecology and Evolution 10: no.856213

Moreno-Rueda, G., Pleguezuelos, J.M., Pizarro, M., and Montori, A. 2011. Northward shifts of the distribution of Spanish reptiles in association with climate change. Conservation Biology 26: 278-283.

Morgan, R., Finnoen, M.H., Jensen, H., Pelabon, C., and Jutfelt, F. 2020. Low potential for evolutionary rescue from climate change in a tropical fish. Proceedings of the National Academy of Science 117: 33365-33372.

Morley, S.A., Chu, J.W.F., Peck, L.S., and Bates, A.E. 2022. Temperatures leading to heat escape responses in Antarctic marine ectotherms match acute thermal limits. Frontiers in Physiology 13: no.1077376.

Motyka R., Norin, T., Petersen, L.H., Huggett, D.B., and Gamperl, A.K. 2017. Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss*), but does not affect their upper thermal tolerance. Journal of Thermal Biology 68: 149–161.

Mucha, S., Chapman, L.J., and Krahe, R. 2023. Normoxia exposure reduces hemoglobin concentration and gill size in a hypoxia-tolerant tropical freshwater fish. Environmental Biology of Fishes 106: 1405-1423.

Natugonza, V., Musinguzi, L., Moelants, T., and Twongo, T.K. 2022. *Platypanchax modestus*. The IUCN Red List of Threatened Species 2022. December 10th, 2024. https://dx.doi.org/10.2305/IUCN.UK.2022-1.RLTS.T60386A47186245.en

Nuon, V., Chea, R., Lek, S., So, N., Hgueny, B., and Grenouillet, G. 2024. Climate change drives contrasting shifts in fish species distribution in the Mekong Basin. Ecological Indicators 160: no.111857.

Nyboer, E.A., Chretien, E., and Chapman, L.J. 2020. Divergence in aerobic scope and thermal tolerance is related to local thermal regime in two introduced populations of Nile perch (*Lates niloticus*). Journal of Fish Biology 97: 231-245.

O'Donnell, M.J., Regish, A.M., McCormick, S.D., and Letcher, B.H. 2020. How repeatable is CTmax within individual brook trout over short- and long-time intervals? Journal of Thermal Biology 89: no.102559.

Ohlberger, J. 2013. Climate warming and ectotherm body size- from individual physiology to community ecology. Functional Ecology 27: 991-1001.

Olowo, J.P. and Chapman, L.J. 1996. Papyrus swamps and variation in the respiratory behaviour of the African fish *Barbus neumayeri*. African Journal of Ecology 34: 211-222.

Ormerod, S.J., Dobson, M., Hildrew, A.G., and Townsend, C.R. 2010. Multiple stressors in freshwater ecosystems. Freshwater Biology 55: 1-4.

Payne, N.L., Smith, J.A., van der Meulen, D.E., Taylor, M.D., Watanabe, Y.Y., Takahashi, A., Marzullo, T.A., Gray, C.A., Cadiou, G., and Suthers, I.M. 2015. Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. Functional Ecology 30: 903-912.

Polačik, M., Garcia, D., Arezo, M.J., Papa, N., Shclueb, H., Blanco, D., Podrabsky, J.E., and Vrtilek, M. 2023. Embryonic development of natural annual killifish populations of the genus *Austrolebias*: Evolutionary parallelism and the role of environment. Freshwater Biology 68: 1726-1738.

Potts, L., Mandrak, N.M., and Chapman, L.J. 2021. Coping with climate change: Phenotypic plasticity in the imperiled freshwater fish in response to elevated water temperature. Aquatic Conservation 31: 2726-2736.

Pörtner, H.O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213: 881-893.

Punzon, A., Lopez-Lopez, L., Gonzalez-Irusta, J.M., Preciado, I., Hidalgo, M., Serrano, A., Tel, E., Somavilla, R., Polo, J., Blanco, M., Ruiz-Pico, S., Ferndandez-Zapico, O., Velasco, F., and Massuti, E. 2021. Tracking the effect of temperature in marine demersal fish communities. Ecological Indicators 121: no.107142.

Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., and Zhang, J. 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7: 585-619.

Radtke, G., Wolnicki, J., Kapusta, A., Przybylski, M., and Kaczkowski, Z. 2022. Critical thermal maxima of three small-bodied dish species (Cypriniformes) of different origin and protection status. The European Zoological Journal 89: 1351-1361.

Ramalho, Q., Vale, M.M., Manes, S., Diniz, P., Malecha, A., and Prevedello, J.A. 2023. Evidence of stronger range shift response to ongoing climate change by ectotherms and high-latitude species. Biological Conservation 279: no.109991.

Raymond, K., Chapman, L.J., and Lanciani, C. 2006. Host, macrohabitat, and microhabitat specificity in the gill parasite *Neodiplozoon polycotyleus* [Monogenea]. Journal of Parasitology 92: 1211-1217.

Reemeyer, J. and Chapman, L.J. 2024. Effects of acute hypoxia exposure and acclimation on the thermal tolerance of an imperiled Canadian minnow. Journal of Experimental Zoology A: Ecological and Integrative Physiology 341: 937-949.

Reist, J.D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Canadian Journal of Zoology 64: 1363–1368.

Ricciardi, A., Iacarella, J.C., Aldridge, D.C., Blackburn, T.M., Calrton, J.T., Catford, J.A., Dick, J.T.A., Hulme, P.E., Jeschke, J.M., Liebhold, A.M., Lockwood, J.L., MacIsaac, H.J., Meyerson, L.A., Pyšek, P., Richardson, D.M., Ruiz, G.M., Simberloff, D., Vila, M., and Wardle, D.A. 2020. Four priority areas to advance invasion science in the face of rapid change environmental change. Environmental Reviews 29: 119-141.

Rolls, R.J., Heino, J., Ryder, D.S., Chessman, B.C., Growns, I.O., Thompson, R.M., and Gido, K.B. 2018. Scaling biodiversity responses to hydrological regimes. Biological Reviews 93: 971-995.

Rubenstein, M.A., Weiskopf, S.R., Bertrand, R., Carter, S.L., Comte, L., Eaton, M.J., Johnson, C.G., Lenoir, J., Lynch, A.J., Miller, B.W., Morelli, T.L., Rodriguez, M.A., Terando, A., and Thompson, L.M. 2023. Climate change and the global redistribution of biodiversity: substantial variation in empirical support for expected range shifts. Environmental Evidence 12: no.7.
Ruparelia, A.A., Salavaty, A., Barlow, C.K., Lu, Y., Sonntag, C., Hersey, L., Eramo, M.J., Krug, J., Reutler, H., Schittenhelm, R.B., Ramialison, M., Cox, A., Ryan, M.T., Creek, D.J., Englert, C., and Currie, P.D. 2023. The African killifish: a short-lived vertebrate model to study the biology of sarcopenia and longevity. Aging Cell 23: e13862.

Rutjes, H.A. 2006. Phenotypic responses to lifelong hypoxia in cichlids. Ph.D. dissertation, Leiden University, The Netherlands.

Rutledge C.J. and Beitinger, T.L. 1989. The effects of dissolved oxygen and aquatic surface respiration on the critical thermal maxima of three intermittent-stream fishes. Environmental Biology of Fishes 24: 137–143.

Samal, P., Srivastava, J., Charles, B., and Singarasubramanian S.R. 2023. Species distribution models to predict the potential niche shift and priority conservation areas for mangroves (*Rhizophora apiculata, R. mucronata*). Ecological Indicators 154: no.110631.

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

Schaack, S.R. and Chapman, L.J. 2004. Interdemic variation in the foraging ecology of the African cyprinid, *Barbus neumayeri*. Environmental Biology of Fishes 70: 95-105.

Sharpe, D.M.T. and L.J. Chapman. 2018. Contemporary phenotypic change in correlated characters in the African cyprinid, *Rastrineobola argentea*. Biological Journal of the Linnean Society 124: 85-98.

Shi, M., Ma, J., and Zhang, K. 2022. The impact of water temperature on in-line turbidity detection. Water 14: no.3720.

Stewart, H.A. and Allen, P.J. 2014. Critical thermal maxima of two geographic strains of channel and hybrid catfish. North American Journal of Aquaculture 76: 104-111.

Stuart-Smith, R.D., Edgar, G.J., Clausius, E., Oh, E.S., Barrett, N.S., Emslie, M.J., Bates, A.E., Bax, N., Brock, D., Cooper, A., Davis, T.R., Day, P.B., Dunic, J.C., Green, A., Hasweera, N., Hicks, J., Holmes, T.H., Jones, B., Jordan, A., Knott, N., Larkin, M.F., Ling, S.D., Mooney, P., Pocklington, J.B., Seroussi, Y., Shaw, I., Shields, D., Smith, M., Soler, G.A., Stuart-Smith, J., Turak, E., Turnbull, J.W., and Mellin, C. 2022. Tracking widespread climate-driven change on temperature and tropical reefs. Current Biology 32: 4128- 4138.

Tellier, J.M., Kalejs, N.I., Leonhardt, B.S., Cannon, D., Höök, T.O., and Collingsworth, P.D. 2022. Widespread prevalence of hypoxia and the classification of hypoxic conditions in the Laurentian Great Lakes. Journal of Great Lakes Research 48: 13-23.

Timmerman, C.M. and Chapman, L.J. 2004. Hypoxia and interdemic variation in the sailfin molly (*Poecilia latipinna*). Journal of Fish Biology 65: 635-650.

Tingley, R., Vallinoto, M., Sequeria, F., and Kearney, M.R. 2014. Realized niche shift during a global biological invasion. Proceedings of the National Academy of Sciences 111: no.28.

Turko, A.J., Nolan, C.B., Balshine, S., Scott, G.R., and Pitcher, T.E. Thermal tolerance depends on season, age and body condition in imperilled redside dace *Clinostomus elongatus*. Conservation Biology 8: coaa062.

United Nations. Climate Action: Causes and Effects of Climate Change. July 27th, 2024. https://www.un.org/en/climatechange/science/causes-effects-climate-change.

Usui, T., Lerner, D., Eckert, I., Angert, A.L., Garroway, C.J., Hargreaves, A., Lancaster, L.T., Lessard, J.P., Riva, F., Schmidt, C., van der Burg, K., and Marshall, K.E. 2023. The evolution of plasticity at geographic range edges. Trends in Ecology and Evolution 38: 831-842.

UWA. Kibale: The Primate Capital of the World. July 27th, 2024. https://ugandawildlife.org/national-parks/kibale-national-park/

Volkoff, H., and Ronnestad, I. 2020. Effects of temperature on feeding and digestive processes in fish. Temperature (Austin) 7: 304-320.

Wallingford, P.D., and Sorte, C.J.B. 2022. Dynamic species interactions associated with the range-shifting marine gastropod *Mexacanthina lugubris*. Oecologia 198: 749-761.

Waterbury, C.R., Sutton, T., Kelley, A.L., Lopez, J.A. 2024. Effects of temperature acclimation on the upper thermal tolerance of two Arctic fishes. Conservation Physiology 12: coae001

Wei, H., Cao, Z.D., and Fu, S.J., 2015. Effect of temperature on hypoxia tolerance and its underlying biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 187: 232-241.

Wilco, C.E., Verkberk, P., Sandker, J.F., van de pol, I.L.E., Urbina, M.A., Wilson, R.W., McKenzie, D.J., and Leiva, F.P. 2022. Body mass and cell size shape the tolerance of fishes to low oxygen in a temperature-dependent manner. Global Change Biology 28: 5695-5707.

Williams, J.E., Blois, J.L. 2018. Range shifts in response to past and future climate change: Can climate velocities and species' dispersal capabilities explain variation in mammalian range shifts?. Journal of Biogeography 45: 2175- 2189.

Wingfield, J.C., Krause, J.S., Perez, J.H., Chmura, H.E., Nemeth, Z., Word, K.R., Calisi, R.M., and Meddle, S.L. 2015. A mechanistic approach to understanding range shifts in a changing world: What makes a pioneer? General and Comparative Endocrinology 222: 44-53.

Wu, T., Imrit, M.A., Movahedinia, Z., Kong, J., Woolway, R.I., and Sharma, S. 2022. Climate tracking by freshwater fishes suggests that fish diversity in temperate lakes may be increasingly threatened by climate warming. Diversity and Distributions 29: 300-315.

Zhang, Y., Mauduit, F., Pettinau, L., Ollivier, H., Lancien, F., Antilla, K., Farrell, A.P., and Claireaux, G. 2023. Respiratory plasticity during acclimation to hypoxia and following a recovery in normoxia. Canadian Journal of Zoology 101: 794-806.

Zillig, K.W., Lusardi, R.A., Cocherelle, D.E., and Fangue, N.A. 2022. Interpopulation variation in thermal physiology among seasonal runs of Chinook salmon. Canadian Journal of Fisheries and Aquatic Sciences 80: 1-13.

GENERAL DISCUSSION

In this thesis, I explored the relationship between thermal tolerance and range expansion of two tropical freshwater fishes (*E. apleurogramma* and *P. modestus*), which have recently shifted their distribution upstream to higher altitude regions of the Mpanga River, situated in Kibale National Park, Uganda. I also explored the relationship between range expansion along a thermal gradient and response to a second environmental gradient – dissolved oxygen. Hypoxia was selected as a second environmental gradient since 1) it is a prevalent stressor in many freshwater ecosystems, 2) it interacts with water temperature, and 3) it naturally occurs in the swamps of the Mpanga river, thereby serving as a potential barrier to the range expansion of these fish species. To accomplish this, I compared multiple field populations of *Enteromius* species as well as *P. modestus* across their distribution in the Mpanga River System.

In the first chapter of my thesis, I explored the relationship between thermal tolerance and range expansion in congeneric species of *Enteromius*. More specifically, I quantified and compared the upper and lower thermal limits of *E. apleurogramma* to those of its upstream and downstream congeners to gain a better understanding of the potential drivers of the range shift in this species. If thermal tolerance is fixed across a species' distributional range, and assuming that temperatures increase at the same rate globally then, in response to climate warming, we would expect species to expand their range at the colder limit and contract their range at the warmer limit (Sunday et al. 2012). Thus, range shifts at the warmer end of a range distribution should be more directly determined by lower thermal limits, often quantified as CT_{min}. A key finding of this study was variation in CT_{min} among species. In comparing the CT_{min} between *E. apleurogramma* and the downstream congeners, *E. alberti* and *E. kerstenii*, we found a significant level of interspecific variation with *E. apleurogramma* having the lowest CT_{min} followed by *E. kerstenii* and *E. alberti*. This aligns with the prediction that a high CT_{min} in the downstream congeners may be constraining them to the warmer downstream waters of the south. Overall, our results suggest that CT_{min} may be a correlate of range contraction at the downstream southern distribution of *E. apleurogramma* in the Mpanga River System. To my knowledge, this study represents the first time that CT_{min} has been measured in a field setting for tropical fishes. Future studies on thermal tolerance should include lower critical thermal limits in order to get a better understanding of species thermal windows and their ability to shift their distribution in the face of ecological change.

In the second chapter of my thesis, I explored how range expansion along a thermal gradient can be affected by responses to other environmental gradients such as hypoxia. In order to accomplish this, I quantified the inter-populational variation in thermal tolerance and gill size from *P. modestus*, a species which is expanding its range into both hypoxic and normoxic upstream cooler waters. I expected to see differences in upper thermal limits between populations given that many other species of freshwater tropical fishes show evidence for inter-populational variability in thermal tolerance (Fangue et al. 2006; Stewart and Allen 2014; Zillig et al. 2014; Chretien and Chapman, 2016; Nyober et al. 2020; Dressler et al. 2023). I also expected to see divergence in gill size among hypoxic and normoxic populations as many other studies have shown evidence for divergence in oxygen uptake capacity (gill size) in response to low oxygen conditions (Chapman et al. 1999; 2000; 2007; 2008; 2021; Chapman and Hulen 2001; Langerhans et al. 2007; Sharpe et al. 2015; Hunt et al. 2023). It is very likely that many fish species exhibiting distributional shifts are experiencing exposure to other environmental gradients. This study represents an important first step to exploring potential interactions between elevated water temperatures and co-occurring environmental variation in other abiotic factors.

I detected a significant difference in total gill filament length between the normoxic downstream population and the upstream expanding normoxic and hypoxic populations of *P. modestus*. Analysis of total gill filament length revealed that the population of *P. modestus* from the downstream normoxic river site had the smallest total gill filament length, whereas upstream populations which have expanded their range in to the hypoxic swamp site had the largest total gill filament length. Upstream populations which have expanded their range in to the normoxic stream site had an intermediate gill filament length, which was closer to that of the range-expanding hypoxic population. There are many studies on freshwater tropical fishes that show a similar divergence pattern in gill size between populations living under hypoxic and normoxic conditions (reviewed in Chapman 2007; 2015; 2021; Chapman et al. 2022). In addition, many studies suggest a strong element of plasticity in gill size (Rutjes 2006; Chapman et al. 2008;

Crispo and Chapman 2010; Chapman et al. 2022). The general trend is that populations reared under hypoxia have a longer total gill filament length and/or greater gill surface area then populations reared under normoxia (Crispo and Chapman 2000; Rutjes 2006; Chapman et al. 2007; Mucha et al. 2023). Our results suggest that gill size variation between high and low dissolved oxygen populations of *P. modestus* can be driven by environmental effects, which aligns well with the findings highlighted in the current literature. We do not know the source of the inter-populational variation in gill size, but this would be an interesting avenue for future investigation.

It is important to note though that the total gill filament length of the range-expanding normoxic population of *P. modestus* was larger than those of the normoxic Bunoga population; and more similar to those captured from the range-expanding hypoxic site. Total gill filament length has also been quantified for *E. apleurogramma,* a species of fish in the Mpanga river System that is also exhibiting the same upstream range expansion into hypoxic and normoxic waters. This study compared total gill filament length of *E. apleurogramma* from its home range to expanding populations of the same species and as well to its upstream congener *E. neumayeri*, across a both high and low dissolved oxygen conditions and found similar trends in gill size divergence (Hunt et al. 2023). It is possible that populations of *P. modestus* that have established in the stream and swamp environment of the Rwembaita Swamp System are at an early phase of divergence and not yet fully adapted to their environment, which is reflected by an intermediate gill filament length.

I also found evidence of inter-populational variation in upper thermal tolerance. This aligns with previous studies which have also shown evidence for inter-populational variation in upper thermal tolerance in a number of freshwater tropical and temperate fish species (Fangue et al. 2006; Stewart and Allen 2014; Zillig et al. 2014; Chretien and Chapman, 2016; Nyober et al. 2020; Dressler et al. 2023). Despite this, my results suggest that environmental water temperature is not a significant predictor of CT_{max}, which may be due to the geographical proximity of our study sites. *P. modestus* is widely distributed across East Africa and it is possible that with a larger thermal gradient one may find different results. However, we did detect a weak effect of average site dissolved oxygen on upper thermal tolerance with CT_{max}

increasing with oxygen concentration after controlling for the linear effects of temperature. This aligns with the framework of the oxygen- and capacity-limited thermal tolerance theory and though other studies show mixed support for the oxygen dependency of thermal tolerance, there are many studies which have shown that hypoxia exposure reduces CT_{max} (Leeuwis et al. 2021; Potts et al. 2021; Andreassen et al. 2022; Reemeyer and Chapman 2024). *P. modestus* seems to be following the same trend with hypoxic populations having slightly lower CT_{max} values (CT_{max} = 33.19 ± 0.29 °C; mean ± SEM) then normoxic populations (CT_{max} = 33.92 ± 0.18 °C), though it is possible that further divergence in CT_{max} may be observed as populations continue to adapt to their environment. Some studies have also found that total gill filament length also increases with rearing temperatures, which can aid in oxygen uptake capacity and support increased metabolic demands exhibited by fish experiencing thermal stress (Potts et al. 2021). It is possible that hypoxic populations with larger gill sizes are able to increase their oxygen uptake capacity to compensate for their lower upper thermal limits, thereby resulting in less divergence among hypoxic and normoxic populations.

Limitations and future directions

In my experiments with *E. apleurogramma*, one limitation was that I did not test for plasticity in upper thermal tolerance, which meant that I was unable to address the source of variation between congeneric *Enteromius* species. Future studies should focus on measuring short term plasticity or acclimation capacity of these fishes by exposing them to warmer water temperatures before measuring their upper thermal limits. Rearing studies across a range of temperatures would also be insightful. It is difficult to say if the low CT_{min} of *E. apleurogramma* is the driver of the range shift or simply the result of plastic or heritable change in response to local upstream conditions. This would be impossible to answer without any previous data on lower thermal limits for this species so we cannot reject the possibility that northern upstream populations of *E. apleurogramma* have a low CT_{min} due to their plastic and/or heritable response to cooler waters. Thus, studies that focus on the role of plasticity in determining lower thermal limits, as proposed above for CT_{max} would be valuable. This limitation also applies to chapter 2, where we measured CT_{max} for multiple populations of *P. modestus*.

There are very few studies that have measured CT_{min} in freshwater and tropical fishes, and even fewer which have measured CT_{min} on field populations, and none, to my knowledge that have measured CT_{min} in response to rearing under divergent temperature regimes. It is important to note that the CT_{min} values recorded in this study were well below the environmental temperature, averaging 6.71 ± 0.20 ˚C (mean ± SEM) as the thermal safety margin for *E. neumayeri*, 7.33 ± 0.18 ˚C for *E. apleurogramma*, 9.06 ± 0.16 ˚C for *E. alberti,* and 7.49 ± 0.25 ˚C for *E. kerstenni*. In some instances, the lower and upper critical thermal limits fall well above and below the environmental temperature (Enders and Durhack 2022; Radke et al. 2022). However, some species of fishes are known to encounter temperatures and heating rates similar to those used in CT_{max} trials under certain natural conditions, such as in intertidal zones, near thermal vents, or when moving through a thermocline (Madeira et al. 2012; Bates and Morley 2020; Desforges 2020; Morgan et al. 2020). The differences among the species in this study, as stated above are consistent with the idea that CT_{min} may be important in predicting range contractions at the warmer end of the range. However, it may not be CT_{min} per se but other traits correlated with CT_{min}, not measured in our study, which are driving range shifts. Future studies should focus on incorporating CT_{min} and CT_{max} to get a better understanding of species range shifts and distributions.

In this study I focused on a single drainage system, however both *E. apleurogramma* and *P. modestus* are widely distributed across East Africa, and it is possible that across a very large latitudinal range and across a larger temperature range one may find different results. In addition, it is important to consider that range shifts as we are seeing in Kibale National Park are likely to reflect climate change, but one needs to rule out other possibilities. Since the Rwembaita Swamp System lies well within the park, there have been no major anthropogenic perturbations that might explain the observed distributional changes. In addition, we are beginning to see similar range shifts in the Dura River, the other major river that drains Kibale, where *P. modestus* has shifted its range to now occupy the Dura River up to its most northern upstream distribution (L. Chapman, personal observations). Future studies in East Africa should consider species distributions on a larger scale and across multiple river systems to gain a better understanding of inter-populational and inter-specific variation in thermal tolerance and its relationship with species distribution.

Other biotic factors must also be taken into consideration, such as interspecific interactions between range-expanding species and those already inhabiting the range-expanded habitat. For example, it is possible that expanding *E. apleurogramma* competes for resources with *E. neumayeri* given their close phylogenetic relationship and the observations that both species include insect larvae in their diet (Greenwood 1966; Schaack and Chapman 2004; Hunt et al. 2023; Fox et al. 2024). A study comparing the relative abundance of species in the Rwembaita Swamp System between 1992-1993 and 2019-2020 found that *E. apleurogramma* and *P. modestus* made up an average of 22.8% and 24.0% of the fish captured across sites, respectively; while native resident species *C. liocephalus* and *E. neumayeri* made up an average of 18.5% and 34.8%, respectively (L. Chapman and A. Buchanan, unpubl. data). A survey using the same methods in 1993 found that *E. neumayeri* comprised 73.2% of the fish captured across the same set of sites (L. Chapman and A. Buchanan, unpubl data). Thus, in terms of relative abundance, *E. neumayeri* has shown a dramatic decline, and certainly it will be important to follow these assemblages in the future to see if (a) more species shift their range into the Rwembaita Swamp System, and (b) how this changes the fish community structure. Future studies should also focus on evaluating changes in fitness-related traits over time in both the original fish species and the range shifting species.

CONCLUSION

Overall, my findings highlight the importance of species thermal tolerance and interactions between environmental stressors in predicting and understanding species range shifts. Even under the most optimistic scenarios projected by the IPCC (AR6 2021) temperatures are predicted to continue to rise well above pre-industrial temperatures. For species that are able to adapt rapidly to these changing environmental conditions, persistence in their current habitat may be possible. However, in the case where change exceeds the rate of adaptive evolution, species will need to shift their distribution to ensure their persistence. Understanding and being able to predict both 1) how species can respond and adapt to environmental changes *in situ* and 2) their ability and the extent to which they can shift their distribution will be crucial in conservation management. With freshwater fishes being among the most threatened taxa as well as their economic and social importance, ensuring their conservation will be extremely important in the face of ecological change.

REFERENCES

Abraham, P., Boivin, G., Moiroux, J., and Brodeur, J. 2017. Behavioural effects of temperature on ectothermic animals: Unifying thermal physiology and behavioural plasticity. Biological Reviews of the Cambridge Philosophical Society 92: 1859-1876.

Agarwal, D., Shanmugam, S.A., Kathiryelpandian, A., Eswaran, S., Rahter, M.A., and Rakkanan, G. 2024. Unravelling the impact of climate change on fish physiology: A focus on temperature and salinity dynamics. Journal of Applied Ichtyology 2024: no.5782274.

Alofs, K.M., Jackson, D.A., and Lester, N.P. 2014. Ontario freshwater fishes demonstrate differing range-boundary shits in a warming climate. Diversity and Distributions 20: 123-136.

Andreassen A.H., Hall, P., Khatibzadeh, P., Jutfelt, F., and Kermen, F. 2022. Brain dysfunction during warming is linked to oxygen limitation in larval zebrafish. Proceedings of the National Academy of Science 119: e2207052119.

Atkinson, D., Johnston, I.A., and Bennett, A.F. 1996. Ectotherm life-history responses to developmental temperature. Pages 183-204. In A.F. Bennett and I.A Johnston, editors. Animals and temperature: phenotypic and evolutionary adaptation. Society for Experimental Biology Seminar Series, Cambridge, Cambridge University Press.

Barbarossa, V., Schmitt, R.J.P., Huijbregts, M.A.J., Zarfl, C., King, H., and Schipper, A.M. 2020. Impacts of current and future large dams on the geographic range connectivity of freshwater fish worldwide. Proceedings of the National Academy of Sciences of the United States of America 117: 3648-3655.

Bates, A.E., and Morley, S.A. 2020. Interpreting empirical estimates of experimentally derived physiological and biological thermal limits in ectotherms. Canadian Journal of Zoology 98: 237-244.

Belleau, C.C., Drolet, D., McKindsey, C.W., and Archambault, P. 2021. Environmental stressors, complex interactions and marine benthic communities’ responses. Scientific Reports 11: no.4191.

Binning, S.A., Chapman, L.J., and Dumont, J. 2010. Feeding and breathing: Trait correlations in an African cichlid fish. Journal of Zoology 282: 140-149.

Biro, P.A., Beckmann, C., and Stamps, J.A. 2010. Small within day increases in temperature affects boldness and alters personality in coral reef fish. Proceedings of the Royal Society B: Biological Sciences 277: 71-77.

Bonacina, L., Fasano, F., Mezzanotte, V., and Fornaroli, R. 2023. Effects of water temperature on freshwater macroinvertebrates: a systematic review. Biological Reviews of the Cambridge Philosophical Society 98: 191-221.

Brodie, E.D., and Russell, N.H. 1999. The consistency of individual differences in behaviour: temperature effects on antipredator behaviour in garter snakes. Animal Behaviour 57: 445-451.

Brown, M.E., Antle, J.M., Backlund, E.R., Carr, W.E., Easterling, M.K., Walsh, C., Ammann, W., Attavanich, C.B., Barrett, M.F., Bellemare, V., Dancheck, C., Funk, K., Grace, J.S.I., Ingram, H., Jiang, H., Maletta, T., Mata, A., Murray, M., Ngugi, D., Ojima, B., O'Neil, B., and Tebaldi, C. 2015. Climate Change, Global Food Security, and the U.S Food System. USDA, 146pp.

Brule, T., Renan, X., and Marrufo, T.C. 2022. Potential impact of climate change on fish reproductive phenology: Case study in gonochoric and hermaphrodite commercially important species from the southern Gulf of Mexico. Fishes 7: no.156.

Campos, D.F., Amanajas, R.D., Almeida-Val, V.M.F., Val, A.L. 2020. Climate vulnerability of South American freshwater fish: Thermal tolerance and acclimation. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 335: 1-12.

Carosi, A., Padula, R., Ghetti, L., and Lorenzoni, M. 2019. Endemic freshwater fish range shifts related to global climate changes: A long-term study provides some observational evidence for the Mediterranean area. Water 11: no.2349.

Champion, C., Brodie, S., and Coleman, M.A. 2021. Climate-driven range shifts are rapid yet variable among recreationally important coastal-pelagic fishes. Frontiers in Marine Science 8: no.622299.

Capon, S.J., Koster, B.S., and Bunn, S.E. 2021. Future of freshwater ecosystems in a 1.5 ^{o}C warmer world. Frontiers in Environmental Science 9: no.784642.

Chapman, L.J. 1995. Seasonal dynamics of habitat use by an air-breathing catfish (*Clarias liocephalus*) in a papyrus swamp. Ecology of Freshwater Fish 4: 113-123.

Chapman, L.J. and Liem, K.F.1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. Environmental Biology of Fishes 44: 183-197.

Chapman, L.J., Chapman, C.A., Brazeau, D., McGlaughlin, B., and Jordan, M. 1999. Papyrus swamps and faunal diversification: Geographical variation among populations of the African cyprinid *Barbus neumayeri*. Journal of Fish Biology 54: 310-327.

Chapman, L.J., Galis, F., and Shinn, J. 2000. Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. Ecology Letters 3: 388-393.

Chapman, L.J., and Hulen, K.G. 2001. Implication of hypoxia for the brain size and gill morphology of mormyrid fishes. Journal of Zoology 254: 461-472.

Chapman, L.J., Schneider, K., Apodaca, C.K., and Chapman, C.A. 2004. Respiratory ecology of macroinvertebrates in a swamp-river system of East Africa. Biotropica 36: 572-585.

Chapman, L.J. Morpho-physiological divergence across oxygen gradients in fishes. 2007. Pages 14-39. In M.N. Fernandes, F.T. Rantin, M.L. Glass, and B.G. Kapoor, editors. Fish Respiration and the Environment. Science Publishers, Inc., Enfield, NH.

Chapman, L.J., DeWitt, T.J., Tzenava, V., and Paterson, J. 2007. Interdemic variation in the gill morphology of a eurytopic African cichlid. Proceedings of the 9th International Symposium on Fish Physiology, Toxicology, and Water Quality. EPA/600/R-07/010. 209-225.

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

Chapman, L.J., and Mckenzie, D.J. 2009. Behavioral responses and ecological consequences. Pages 26-77. In Richards, J.G., Farrell A.P., and Brauner, C.J., editors. Hypoxia. Elsevier Science Publishing, San Diego, United States.

Chapman, L.J. 2015. Low-oxygen lifestyles. Pages 9-33. In Riesch, R., Tobler, M., and Plath, M., editors. Extremophile Fishes. Springer, New York, New York, United States.

Chapman, L.J. Respiratory ecology of cichlids. 2021. Pages 683-714. In M.E. Abate and D.L.G Noakes, editors. The Behavior, Ecology and Evolution of Cichlid Fishes: A Contemporary Modern Synthesis. Springer Academic

Chapman, L.J., Nyboer, E.A., and Fugère, V. 2022. Fish response to environmental stressors in the Lake Victoria Basin Ecoregion. Pages 273-324. In Fangue, N. and Cooke, S., editors. Fish Physiology, Conservation Physiology for the Anthropocene – Issues and Applications. Volume 39.

Chislock, M.F., Doster, E. Zitomer, R.A., and Wilson, A.E. 2013. Eutrophication: causes, consequences, and controls in aquatic ecosystems. Nature Education Knowledge 4: no.10.

Chretien, E., and Chapman, L.J. 2016. Tropical fishes in a warming world: Thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. Conservation Physiology 4: cow062.

Christensen, E.A.F., Norin, T., Tabak, I., van Deurs, M., Behrens, J.W. 2021. Effects of temperature on physiological performance and behavioral thermoregulation in an invasive fish, the round goby. Journal of Experimental Biology 224: no.237669.

Clarke, A., and Fraser, K.P.P. 2004. Why does metabolism scale with temperature? Functional Ecology 18: 243-251.

Clavijo-Baquet, S., Orellana, M.J., Sabat, P., and Bozinovic, F. 2022. How do ectotherms perform in cold environments? Physiological and life-history traits in and Andean viviparous lizard. Frontiers in Ecology and Evolution 10: no.974968.

Colchen, T., Teletchea, F., Fontaine, P., and Pasquet, A. 2017. Temperature modifies activity, inter-individual relationships and group structure in a fish. Current Zoology 63: 175-183.

Comte, L., and Grenouillet, G. 2013. Do stream fish track climate change? Assessing distribution shifts in recent decades. Ecography: 1236-1246.

Comte, L., Buisson, L., Daufresne, M., and Grenouillet, G. 2013. Climate-induced changes in the distribution of fish: observed and predicted trends. Freshwater Biology 58: 625-639.

Comte, L., Murienne, J., and Grenouillet, G. 2014. Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes. Nature Communications 5: no.5053.

Comte, L., and Oldern, J. 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Global Change Biology 23: 728-736.

Coristine, L.E., and Kerr, J.T. 2011. Habitat loss, climate change, and emerging conservation challenges in Canada. Canadian Journal of Zoology 89: 435-451.

Couet, J., Marjakangas, E.L., Santangeli, A., Kalas, J.A., Lindstrom, A., and Lehikoinen, A. 2022. Short-lived species move uphill faster under climate change. Oecologia 198: 877-888.

Crispo, E. and Chapman, L.J.. 2010. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. Journal of Evolutionary Biology 23: 2091-2103.

Da Silva, C.R.B., and Diamond, S.E. 2024. Local climate change velocities and evolutionary history explain multidirectional range shifts in a North American butterfly assemblage. Journal of Animal Ecology 93: 1160-1171.

Debes, P.V., Solberg, M.F., Matre, I.H., Dyrhovden, L., and Glover K.A. 2021. Genetic variation for upper thermal tolerance diminishes between and within populations with increasing acclimation temperature in Atlantic salmon. Heredity 127: 455- 466.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Shelson, K.S., Ghalambor, C.K., Haak, D.C., and Martin, P.R. 2008. Impact of climate warming on terrestrial ectotherms across latitudes. Proceedings of the National Academy of Sciences of the United States of America 105: 6663-6672.

Diamond, S.E. 2018. Contemporary climate-driven range shifts: Putting evolution back on the table. Functional Ecology 32: 1662-1665.

Desforges, E.M. 2020. On applying critical thermal maxima method to investigate ecologically-relevant questions in wild fishes. Masters Thesis. Carlton University, Ottawa.

Diaz, R.J., and Breitburg, D.L. 2009. The hypoxia environment. In: Richards, J.G., Farrell, A.P., and Brauner, C.J.(eds) Hypoxia, fish physiology, vol 27. Elsevier, San Diego, pp 2–23.

Dressler, T.L., Han Lee, V., Klose, K., and Eliason, E.J. 2023. Thermal tolerance and vulnerability to warming differ between populations of wild *Oncorhynchus mykiss* near the species' southern range limit. Scientific Reports 13: no.14538.

Duan, R-Y., Kong, X-Q., Huang, M-Y., Varela, S., and Ji, X. 2016. The potential effects of climate change on amphibian distribution, range fragmentation and turnover in China. PeerJ 4: e2185.

Dupuis, A.P., and Hann, B.J. 2009. Warming spring and summer water temperatures in small eutrophic lakes of the Canadian Prairies: potential implications for phytoplankton and zooplankton. Journal of Plankton Research 31: 489-502.

Enders, E.C., and Durhack, T.C. 2022. Metabolic rate and critical thermal maximum of CTmax estimates for westslope cutthroat trout, *Oncorhynchus clarkii lewiki*. Conservation Physiology 10: coac071.

Ern, R., Norin, T., Gamperi, A.K., and Esbaugh, A.J. 2016. Oxygen dependence of upper thermal limits in fishes. Journal of Experimental Biology 219: 3376-3383.

Fangue, N.A., Hofmeister, M., and Schulte, P.M. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. The Journal of Experimental Biology 209: 2859-2872.

Fernandes, F., Calado, R., Jeronimo, D., and Madeira, D. 2023. Thermal tolerance limits and physiological traits as indicators of *Hediste diversicolor*'s acclimation capacity to global and local change drivers. Journal of Thermal Biology 114: no.103577.

Fox, J., Hunt, D.A.G.A., Hendry, A.P, Chapman, L.J., and Barrett, R.D.H. 2023. Counter-gradient variation in gene expression between fish populations facilitates colonization of low-dissolved oxygen environments. Molecular Ecology: e17419.

Francispillai, E.K. and L.J. Chapman. 2024. Effects of temperature on fish aggression and the combined impact of temperature and turbidity on thermal tolerance. Journal of Thermal Biology 125: no.103987

Greenwood, P.H. 1966. The Fishes of Uganda. The Uganda Society, Kampala, Uganda.

Guo, F., Lenoir, J., and Bonebrake, T.C. 2018. Land use change interacts with climate to determine elevational species redistribution. Nature Communications 9: no.1315.

Hale, S.S., Cicchetti, G., and Deacutis, C.F. 2016. Eutrophication and hypoxia diminish ecosystem functions of benthic communities in a New England estuary. Frontiers in Marine Sciences 3: no.00249.

Hu, R., Gu, Y., Luo, M., Wei, M., and Zhong, J. 2020. Shifts in bird ranges and conservation priorities in China under climate change. PLoS One 15: e0240225.

Huey, R.B., and Stevenson, R.D. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. American Zoologist 19: 357-366.

Hunt, D.A.G.A., Hendry, A.P., and Chapman, L.J. 2023. Are we there yet? Inter- and intraspecific approaches to evaluating phenotypic optima in a range expanding East African fish, *Enteromius apleurogramma* (Cyprinidae). Biological Journal of the Linnean Society 140: 296-312.

Islam, S.M., Zahangir, M.M., Jannat, R., Hasan, M.N., Suchana, S.A., Rohani, F.R., and Shahjahan, M. 2020. Hypoxia reduced upper thermal limits causing cellular and nuclear abnormalities of erythrocytes in Nile tilapia, *Oreochromis niloticus*. Journal of Thermal Biology 90: no.102604.

Jackson, M.C., Wasserman R.J., Grey, J., Ricciardi, A., Dick, J.T.A., and Alexander, M.E. 2017. Chapter two – Novel and disrupted trophic links following invasion in freshwater ecosystems. Advances in Ecological Research 57: 55-97.

Jones, N.A.R., Newton-Youens, J., and Frommen, J.G. 2023. Rise and fall: increasing temperatures have nonlinear effects on aggression in a tropical fish. Animal Behaviour 207: 1-11.

Joyner-Matos, J. and Chapman, L.J. 2013. Persisting in papyrus: size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia. Comparative Biochemistry and Physiology Part A 165: 405–416.

Jung, E.H., Brix, K.V., Richards, J.G., Val, A.L., and Brauner, C.J. 2020. Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world. The Science of the Total Environment 15: no.748.

Jutfeldt, F. 2020. Metabolic adaptation to warm water in fish. Functional Ecology 34: 1138-1141.

Kirk, M.A., and Rahel, F.J. 2022. Assessing climate-induced range shifts of stream fishes using a consensus framework. Ecology of Freshwater Fish 32: 270-281.

Kong, X., Ghaffar, S., Determan, M., Friese, K., Jomaa, S., Mi, C., Shatwell, T., Rinke, K., and Rode, M. 2022. Reservoir water quality deterioration due to deforestation emphasizes the indirect effects of global change. Water Research 221: no.118721.

Korkmaz, M., Mangit, F., Dumlupinar, I., Colak, M.A., Akpinar, M.B., Koru, M., Pacheco, J.P., Garcia-Ramirez, A., Yilmaz, G., Amorim, C.A., Ozgencil, I.K., Innal, D., Yerli, S.V., Ozkan, K., Akyurek, Z., Baklioglu, M., and Jeppesen, E. 2023. Effects of climate change on the habitat suitability and distribution of freshwater fish species in semi-arid central Anatolian Ecoregion in Turkiye. Water 15: no.1619.

Kraskura, K., Hardison, E.A., and Eliason, E.J. 2023. Body size and temperature affect metabolic and cardiac thermal tolerance in fish. Scientific Reports 13: no.17900.

Kremsner, U., and Schnug, E. 2002. Impacts of fertilizers on aquatic ecosystems and protection of water bodies from mineral nutrients. Landbauforshung Völkenrode 52: 81-90.

Langerhans, R.B., Gifford, M.E., and Joseph, E.O. 2007. Ecological speciation in *Gambusia* fishes. Evolution 61: 2056-2074.

Lawlor, J.A., Comte, L., Grenouillet, G., Lenoir, J., Baecher, J.A., Bandara, R.M.W.J., Bertrand, R., Chen, I.C., Diamond, S.E., Lancaster, L.T., Moore, N., Murienne, J., Oliveira, B.F., Pecl, G.T., Pinsky, M.L., Rolland, J., Rubenstein, M., Scheffers, B.R., Thompson, L.M., Amerom, B.V., Villalobos, F., Weiskopt, S.R., and Sunday, J. 2024. Mechanisms, detection and impacts of species redistributions under climate change. Nature Reviews Earth and Environment 5: 351-368.

Leeuwis, R.H.J., Zanuzzo, F.S., Peroni, E.F.C., and Gamperl, A.K. 2021. Research on sablefish (*Anaplopoma fimbria*) suggests that limited capacity to increase heart function leaves hypoxic fish susceptible to heat waves. Proceedings of the Royal Society B 288: no.20202340.

Lema, S.C., Bock, S.L., Malley, M.M., and Elkins, E.A., 2019. Warming water beget smaller fish: evidence for reduced size and altered morphology in a desert fish following anthropogenic temperature change. Biology Letters: no.20190518.

Li, Y., Cohen, J.M., and Rhor, J.R. 2013. Review and synthesis of the effects of climate change on amphibians. Integrative Zoology 8: 145-161.

Lindmark, M., Audzijonyta, A., Blanchard, J.L. and Gardmark, A. 2022. Temperature impacts on fish physiology and resource abundance lead to faster growth but smaller fish sizes and yields under warming. Global Change Biology 28: 6239-6253.

Lonhart, S.I., Jeppesen, R., Beas-Luna, R., Crooks, J.A., and Lorda, J. 2019. Shifts in the distribution and abundance of coastal marine species along the eastern Pacific Ocean during marine heatwaves from 2013 to 2018. Marine Biodiversity Records 12: no.13.

Luthi, D., Etheridge, D.M. et al. 2010. Vostok Ice Core Data/ J.R. Petit et al; NOAA Mauna Loa CO2 Record.

MacLean, H.J., Higgins, J.K., Buckley, L.B., and Kingsolver, J.G. 2016. Morphological and physiological determinants of local adaptation to climate in Rocky Mountain butterflies. Conservation Physiology 4: cow035.

Macusi, E.D., Abreo, N.A.S., Abreo, S., Cuenca, G., Ranara, C.T.B., Ranara, B., Cardona, L.T., Andam, M.B., Guanzon, G.C., Katikiro, R., and Deepananda, K.M.H.A. 2015. The potential impacts of climate change on freshwater fish, fish culture, and fishing communities. Journal of Natural Studies 14: 14-31.

Madeira, C., Mendonca, V., Leal, M.C., Flores, A.A.V., Cabral, H.N., Diniz, M.S., and Vinagre, C. 2017. Thermal stress, thermal safety margins, and acclimation capacity in tropical shallow waters – An experimental approach testing multiple end-points in two common fish. Ecological Indicators 81: 146- 158.

Martinez, M.L., L.J. Chapman, J.M. Grady, and Rees, B.B. 2004. Interdemic variation in hematocrit and lactate dehydrogenase in the African cyprinid *Barbus neumayeri*. Journal of Fish Biology 65: 1056-1069.

Martinez, M.L., Raynard, E.L., Rees, B.B., and Chapman, L.J. 2011. Oxygen limitation and tissue metabolic potential of the African fish *Barbus neumayeri*: roles of native habitat and acclimatization. BMC Ecology 11: 2.

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. Integrative and Comparative Biology 53: 648-659.

McKenzie, D.J., Axelsson, M., Chabot, D., Claireux, G., Cooke, S.J., Corner, R.A., De Boeck, G., Domenici, P., Guerreiro, P.M., Hamer, B., Jorgensen, C., Killen, S.S., Lefevbre, S., Marras, S., Michaelidis, B., Nilsson, G.E., Peck, M.A., Perez-Ruzafa, A., Rijnsdrop, A.D., Shiels, H.A., Steffensen, J.F., Svendsen, J.C., Svendsen, M.B.S., Teal, L.R., van der Meer, J., Wang, T., Wilson, J.M., Wilson, R.W., and Metcalfe, J.D. 2016. Conservation physiology of marine fishes: state of the art and prospects for policy. Conservation Physiology 4: cow046.

McKenzie, D.J., Zhang, Y., Eliason, E.J., Schulte, P.M., Claireaux, G., Blasco, F.R., Nati, J.J.H., and Farrell, A.P. 2020. Intraspecific variation in tolerance of warming in fishes. Journal of Fish Biology 98: 1536-1555.

McKenzie, D.J., Geffroy, B., and Farrell, A.P. 2021. Effects of global warming on fishes and fisheries. Journal of Fish Biology 98: 1489-1492.

McDonnell, L.H., and Chapman, L.J. 2015. At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. Conservation Physiology 3: cov050.

McNab, B.K. 2002. The physiological ecology of vertebrates: A view from energetics. Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

Mitchell, A., Hayes, C., Booth, D.J., and Nagelkerken, I. 2023. Future shock: Ocean acidification and seasonal water temperatures alter the physiology of competing temperate and coral reef fishes. Science of the Total Environment 883: no.163684.

Moreno-Rueda, G., Pleguezuelos, J.M., Pizarro, M., and Montori, A. 2011. Northward shifts of the distribution of Spanish reptiles in association with climate change. Conservation Biology 26: 278-283.

Morgan, R., Finnoen, M.H., Jensen, H., Pelabon, C., and Jutfelt, F. 2020. Low potential for evolutionary rescue from climate change in a tropical fish. Proceedings of the National Academy of Science 117: 33365-33372.

Morley, S.A., Peck , L.S., Sunday, J.M., Heiser, S., and Bates, A.E. 2018. Physiological acclimation and persistence of ectothermic species under extreme heat events. Global Ecology and Biogeography 28: 1018-1037.

Neubauer, P., and Andersen, K.H. 2019. Thermal performance of fish is explained by an interplay between physiology, behaviour and ecology. Conservation Physiology 7: coz025.

Nuon, V., Chea, R., Lek, S., So, N., Huguney, B., and Grenouillet, G. 2024. Climate change drives contrasting shifts in fish species distribution in the Mekong Basin. Ecological Indicators 160: no.111857.

Nyober, E.A. and Chapman, L.J. 2017. Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. Journal of Experimental Biology 220: 3782-3793.

Nyober, E.A. and Chapman, L.J. 2018. Cardiac plasticity influences aerobic performance and thermal tolerance in a tropical, freshwater fish at elevated temperatures. Journal of Experimental Biology 221: no.178087.

Nyober, E.A., Chretien, E., and Chapman, L.J. 2020. Divergence in aerobic scope and thermal tolerance is related to local thermal regime in two introduced populations of Nile perch (*Lates niloticus*). Journal of Fish Biology 97: 231-245.

Olowo, J.P. and Chapman, L.J. 1996. Papyrus swamps and variation in the respiratory behaviour of the African fish *Barbus neumayeri*. African Journal of Ecology 34: 211-222.

Pecl, G.A., Araujo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Cheng, I.C., Clark, T.D., Colwell, R.K., Danielsen, F, Evengard, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R.A., Griffis, R.B., Hobday, A.J., Janion-Scheepers, C., Jarzyna, M.A., Jennings, S., Lenoir, J., Lin-Nevted, H.I., Martin, V.Y., McCormack, P.C., McDonald, J., Mitchell, N.J., Mustonen, T., Pandolfi, J.M., Pettorelli, N., Popova, E., Robinson, S.A., Scheffers, B.R., Shaw, J.D., Sorte, C.J.B., Strugnell, J.M., Sunday, J.M., Tuanmu, M.N., Verges, A., Villanueva, C., Wernberg, T., Wapstra, E., and Williams, S.E. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. Science 355: no.6332.

Perry, A., Low, P.J., Ellis, J., and Reynolds, J.D. 2005. Climate change and distribution shifts in marine fishes. Science 308: 1912-1915.

Piczak, M.L., Perry, D., Cooke, S.J., Harrison, I., Benitez, S., Koning, A., Peng, L., Limbu, P., Smokorowski, S.S.R., Koehn, J.D., and Creed, I.F. 2023. Protecting and restoring habitats to benefit freshwater biodiversity. Environmental Reviews 00: 1-19.

Pilakouta, N., O'Donnell, P.J., Crespel, A., Levet, M., Claireaux, M., Humble, J.L., Kristajansson, K.B., Skulason, S., Lindstrom, J., Metcalfe, N.B., Killen, S.S., and Parsons, K.J. 2022. A warmer environment can reduce sociability in ectotherms. Global Change Biology 29: 206-214.

Pinsky, M.L., Selden, R.L., and Kitchel, Z.J. 2020. Climate-driven shifts in marine species ranges: Scaling from organisms to communities. Annual Review of Marine Science 12: 153-179.

Poesch, M.S., Chavarie, L., Chu, C., Pandit, S.N., and Tonn, W. 2016. Climate change impacts on freshwater fishes: A Canadian perspective. Fisheries 41: 385-391.

Politis, S.N., Mazurais, D., Servili, A., Zambonino-Infante, J.L., Miest, J.J., Sorensen, S.R., Tomkiewicz, J., and Butts, I.A.E. 2017. Temperature effects on gene expression and morphological development of European eel, *Anguilla Anguilla* larvae. PLoS ONE 2017: e0182726.

Pörtner, H.O., Farrell, A.P. 2008. Physiology and climate change. Science 322: 690–692.

Pörtner, H.O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213: 881-893.

Pörtner, H.O., Bock, C., and Mark, F.C. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. Journal of Experimental Biology 220: 2685-2696.

Potts, L., Mandrak, N.M., and Chapman, L.J. 2021. Coping with climate change: Phenotypic plasticity in the imperiled freshwater fish in response to elevated water temperature. Aquatic Conservation 31: 2726-2736.

Radtke, G., Wolnicki, J., Kapusta, A., Przybylski, M., and Kaczkowski, Z. 2022. Critical thermal maxima of three small-bodied dish species (Cypriniformes) of different origin and protection status. The European Zoological Journal 89: 1351-1361.

Ramalho, Q., Vale, M.M., Manes, S., Diniz, P., Malecha, A., and Prevedello, J.A. 2023. Evidence of stronger range shift response to ongoing climate change by ectotherms and high-latitude species. Biological Conservation 279: no.109991.

Raymond, K., Chapman, L.J., and Lanciani, C. 2006. Host, macrohabitat, and microhabitat specificity in the gill parasite *Neodiplozoon polycotyleus* [Monogenea]. Journal of Parasitology 92: 1211-1217.

Reemeyer, J. and Chapman, L.J. 2024. Effects of acute hypoxia exposure and acclimation on the thermal tolerance of an imperiled Canadian minnow. Journal of Experimental Zoology A: Ecological and Integrative Physiology 341: 937-949.

Ricciardi, A., and MacIsaac, H.J. 2011. Impacts of biological invasions on freshwater ecosystems. Pages 210-224. In Richardson, D.M., editor. Fifty Years of Invasion Ecology: The Legacy of Charles Elton. 1st Edition. Blackwell Publishing Ltd.

Roman, M.R., Brandt, S.B., Houde, E.D., and Pierson, J.J. 2019. Interactive effects of hypoxia and temperature on coastal pelagic zooplankton and fish. Frontiers in Marine Science 6: no.00139.

Rubenstein, M.A., Weiskopf, S.R., Bertrand, R., Carter, S.L., Comte, L., Eaton, M.J., Johnson, C.G., Lenoir, J., Lynch, A.J., Miller, B.W., Morelli, T.L., Rodriguez, M.A., Terando, A., and Thompson, L.M. 2023. Climate change and the global redistribution of biodiversity: substantial variation in empirical support for expected range shifts. Environmental Evidence 12: no.7.

Rutjes, H.A. 2006. Phenotypic responses to lifelong hypoxia in cichlids. Ph.D. dissertation, Leiden University, The Netherlands.

Ruthsatz, K., Dahke, F., Alter, K., Wohlradb, S., Eterovik, P.C., Lyra, M.L., Gippner, S., Cooke, S.J., and Peck, M.A. 2024. Acclimation capacity to global warming of amphibians and freshwater fishes: Drivers, patterns and data limitations. Global Change Biology 30: e17318.

Schaack, S.R., and Chapman, L.J. 2004. Interdemic variation in the foraging ecology of the African cyprinid, *Barbus neumayeri*. Environmental Biology of Fishes 70: 95-105.

Schulte, P.M. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. Journal of Experimental Biology 218: 1856-1866.

Segurado, P., Branco, P., Jauch, E., Neves, R., and Ferreira, M.T. 2016. Sensitivity of river fishes to climate change: The role of hydrological stressors on habitat range shifts. Science of the Total Environment 562: 435-445.

Servili, A., Canario, A.V.M., Mouchel, O., and Munez-Cueto, J.A. 2020. Climate change impacts on fish reproduction are mediated at multiple levels of the brain-pituatary-gonad axis. General and Comparative Endocrinology 291: no.113439.

Smith, S.M., Fox, R.J., Donelson, J.M., Head, M.L., and Booth, D.J. 2016. Predicting range-shift success potential for tropical marine fishes using external morphology. Biology Letters 12: no.20160505.

Spies, I., Gruenthal, K.M., Drinan, D.P., Hollowed, A.B., Stevenson, D.E., Tarpey, C.M., and Hauser, L.2020. Genetic evidence of a northward range expansion in the eastern Bering Sea stock of Pacific cod. Evolutionary Applications 13: 362-375.

Stafford, K.M., Farley, E.V., Ferguson, M., Kuletz, K.J., and Levine, R. 2022. Northward range expansion of Subarctic upper trophic level animals into the Pacific Arctic Region. Oceanography 35: 3-4.

Stewart, H.A., and Allen, P.J. 2014. Critical thermal maxima of two geographic strains of channel and hybrid catfish. North American Journal of Aquaculture 76: 104-111.

Sudo, K., Maehara, S., Nakaoka, M., and Fujii, M. 2022. Predicting future shifts in the distribution of topicalization indicator fish that can affect coastal ecosystem services of Japan. Frontiers in Built Environment 7: no.78870.

Sunday, J.M., Bates, A.E., and Dulvy, N.K. 2012. Thermal tolerance and the global redistribution of animals. Nature Climate Change 2: 686-690.

Tewksbury, J.J. Huey, R.B. Deutsch, C.A. 2008. Putting the heat on tropical animals. Science 320: 1296-1297.

Thyrring, J., Tremblay, R., and Sejr, M.K. 2019. Local cold adaptation increases the thermal window of temperate mussels in the Arctic. Conservation Physiology 7: coz098.

UWA. Kibale: The Primate Capital of the World. July 27th, 2024. https://ugandawildlife.org/national-parks/kibale-national-park/

Vaquer-Sunyer, R., and Duarte, C.M. 2008. Thresholds of hypoxia for marine biodiversity. Proceedings of the National Academy of Sciences of the United States of America 105: 15452-15457.

Verbek, W.C.E.P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., and Terblanche, J.S. 2016. Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 192: 64-78.

Verbek, W.C.E.P. Sandker, J.F., van de Pol, I.L.E. Urbina, M.A., Wilson, R.W. McKenzie, D.J., and Leiva, F.P. 2022. Body mass and cell size shape the tolerance of fishes to low oxygen in a temperature-dependent manner. Global Change Biology 28: 5695- 5707.

Volkoff, H., and Ronnestad, I. 2020. Effects of temperature on feeding and digestive processes in fish. Temperature (Austin) 7: 304- 320.

Walczynska, A., and Sobczyk, L. 2017. The underestimated role of temperature-oxygen relationship in large-scale studies on size-to-temperature response. Ecology and Evolution 7: 7434- 7441.

Wesselmann, M., Apostolaki, E.T., and Anton, A. 2024. Species range shifts, biological invasions and ocean warming. Marine Ecological Progress Series 728: 81-83.

Williams, J.E., Blois, J.L. 2018. Range shifts in response to past and future climate change: Can climate velocities and species' dispersal capabilities explain variation in mammalian range shifts?. Journal of Biogeography 45: 2175- 2189.

Womersley, F.C., Sousa, L.L., Humphries, N.E., Abrantes, K., Araujo, G., Bach, S.S., Barnett, A., Berumen, M.L., Lion, S.B., Braun, C.D., Clingham, E., Cochran, J.E.M., Parra, R., Diamant, S., Dove, A.D.M., Duarte, C.M., Dudgeon, C.L., Erdmann, M.V., Espinoza, E., Ferreira, L.C., Fitzpatrick, R., Cano, J.G., Green, J.R., Guzman, H.M., Hardenstine, R., Hasan, A., Hazin, F.H.V., Hearn, A.R., Hueter, R.E., Jaidah, M.Y., Labaja, J., Ladino, F., Macena, B.C.L., Meekan, M.G., Norman, B.M., Palma, P.C.R., Pierce, S.J., Quintero, L.M., Macias, D.R., Reynolds, S.D., Robinson, D.P., Rohner, C.A., Rowat, D.R.L., Sequeira, A.M.M., Sheaves, M., Shivji, M.S., Sianipar, A.B., Skomal, G.B., Soler, G., Syakurachman, I., Thorrold, S.R., Thums, M., Tyminski, J.P., Web, D.H., Wetherbee, B.M., Queiroz, N., and Sims, D.W. 2024. Climate-driven global redistribution of an ocean giant predicts increased threat from shipping. Nature Climate Change 14: 1282-1291.

Woodword, G., Perkins, D.M., and Brown, L.E. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B 365: 2093-2106.

Woolway, R.L., Sharma, S., and Smol, J.P. 2022. Lakes in hot water: The impacts of changing climate on freshwater ecosystems. BioScience 72: 1050-1061.

Wu, T., Imrit, M.A., Movahedinia, Z., Kong, J., Woolway, R.I., and Sharma, S. 2022. Climate tracking by freshwater fishes suggests that fish diversity in temperate lakes may be increasingly threatened by climate warming. Diversity and Distributions 29: 300-315.

Yesuf, G.U., Brown, K.A., Walford, N.S., Rakotoarisoa, S.E., and Rufino, M.C. 2021. Predicting range shifts for critically endangered plants: Is habitat connectivity irrelevant or necessary? Biological Conservation 256: no.109033.

Zillig, K.W., Lusardi, R.A., Cocherelle, D.E., and Fangue, N.A. 2022. Interpopulation variation in thermal physiology among seasonal runs of Chinook salmon. Canadian Journal of Fisheries and Aquatic Sciences 80: 1-13.