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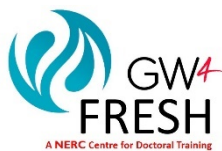
The role of behavioural mechanisms in the invasion success and impact of Nile tilapia (*Oreochromis niloticus*) in Tanzania

Toby Champneys

*A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of
PhD in the Faculty of Life Sciences in the School of Biological Sciences*

Submitted on the 23rd of December 2022

Word Count 39,500



Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: *Toby Champneys* DATE: 23rd December 2022

Abstract

The introduction of species to areas outside of their native range has had wide reaching impacts on global biodiversity. The impacts of introduced species are driven by causal mechanisms which can range across all levels of biological organisation, from the genetic (i.e. hybridisation) to the ecosystem level (i.e. eutrophication). Behavioural interactions underpin many of these causal mechanisms and investigating behavioural interactions between introduced and native species can help us to better understand the success and impact of invasive species. In this thesis I explore the role of behavioural mechanisms in the success and impact of Nile tilapia (*Oreochromis niloticus*), a cichlid species with a pan-tropical non-native distribution. Negative impacts have been reported in many ecosystems across the non-native range of Nile tilapia, however, in many cases there is still a poor understanding of the mechanisms which drive these negative impacts. In Tanzania, introduced populations of *O. niloticus* exist in sympatry with a range of functionally similar and closely related native tilapia species, which is expected to increase the prevalence of interspecific interactions and potentially exacerbate negative outcomes resulting from *O. niloticus* introduction. In chapter two I investigate interference competition over shelter between *O. niloticus* and native tilapia. In chapter three I investigate how naivete might shape interactions between *O. niloticus* and native tilapia during initial encounters. In chapter four, I investigate how grouping with *O. niloticus* affects the social behaviour and group decision making of native tilapia and explore the benefits that *O. niloticus* might derive from grouping with native species. In chapter five I investigate the growth rate of wild caught *O. niloticus* and a native species, to investigate competition induced outcomes on the fitness of these sympatric populations and their hybrids. Overall, my research highlights how behavioural interactions with *O. niloticus* may threaten native tilapia in Tanzania, and how the behavioural responses of naïve native tilapia during early encounters may shape these interactions. I also highlight the role of ecological context and multiple stressors in shaping the prevalence and outcome of such behavioural interactions *in situ*.

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Author Contributions and Publications

Chapter two:

An adapted version of this chapter is published in *Hydrobiologia*.

Champneys, T., Genner, M.J. & Ioannou, C.C. (2021) Invasive Nile tilapia dominates a threatened indigenous tilapia in competition over shelter. *Hydrobiologia* **848**, 3747–3762. <https://doi.org/10.1007/s10750-020-04341-8>

I designed the experiment, conducted experimental trials, analysed the videos, conducted statistical analysis and wrote the paper, with supervisory input from Christos C. Ioannou and Martin J. Genner.

Chapter three:

An adapted version of this chapter is published in *Biological Invasions*:

Champneys, T., Ferry, K., Tomkinson, S., Genner, M. J., & Ioannou, C. C. (2022). Simulated encounters with a novel competitor reveal the potential for maladaptive behavioural responses to invasive species. *Biological Invasions*, **24**(3), 845-860.

I processed the data from behavioural observations, conducted statistical analysis and wrote the paper with supervisory input from Christos C. Ioannou and Martin J. Genner. Sophie Tomkinson and Katie Ferry conducted behavioural trials and analysed the videos as part of their undergraduate research projects with input from Christos C. Ioannou and me.

Chapter four:

I designed the experiment, conducted experimental trials, analysed the videos, conducted statistical analysis, and wrote the paper, with supervisory input from Christos C. Ioannou and Martin J. Genner.

Chapter five:

I planned and conducted fieldwork, processed, and genotyped specimens in the lab, conducted microscopy and measured the images, conducted statistical analysis and wrote the paper, with supervisory input from Martin J. Genner and Christos C. Ioannou. Asilatu Shechonge and Patroba Matiku aided with planning and undertaking fieldwork, and Andy Saxon helped with processing and genotyping the lab specimens from 2016. Specimens from 2016 were collected by Tabitha Blackwell and Benjamin Ngatunga.

Contributions to published work not included in this thesis

I aided with the experimental design, statistical analysis and writing of a research article in *Behavioral Ecology and Sociobiology*:

Wing, J.D.B., Champneys, T.S. & Ioannou, C.C. The impact of turbidity on foraging and risk taking in the invasive Nile tilapia (*Oreochromis niloticus*) and a threatened native cichlid (*Oreochromis amphimelas*). *Behavioral Ecology and Sociobiology* **75**, 49 (2021). <https://doi.org/10.1007/s00265-021-02984-8>

Ethical Note

The research undertaken in this thesis was in accordance with the ethical standards of the University of Bristol and ethical approval was granted by the University's Animal Welfare and Ethical Review Body (UIN number: UB 18 067).

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1 | General Introduction



The southern shore of Lake Rutamba, Tanzania

1.1 Non-native species and their impacts in recipient ecosystems

1.1.1 What is a 'native' species range?

The range limits of a species can be defined as an expression of its ecological niche within space (Sexton et al. 2009). Defining these limits, and thus the native range of a given species, is central to a number of ecological and conservation-focussed questions such as categorising extinction risk status or creating food webs (McGeoch and Latombe 2016). Numerous biogeographic factors constrain species to specific environments (Vermeij 1991). In some cases, species are unable to move outside of their native range due to physiological tolerance limits (i.e. to pH or temperature) or the inability to overcome physical barriers (i.e. water or land), while in some cases it can be due to complex ecological interactions tied to the functioning of whole ecosystems (i.e. availability of key prey).

1.1.2 The origins of species invasions

Species ranges change over time, shifting, expanding and contracting in response to fluctuations in, and adaptations to, the factors which govern them (Davis and Shaw 2001). Typically, these range shifts are small and occur slowly, often lagging behind the environmental changes which drive them (Svenning et al. 2008). However, when the factors which restrict species to a given environment change drastically, species are capable of undergoing rapid expansion into novel environments (Mooney and Cleland 2001). These drastic changes can happen via natural processes, for example, tectonic changes led to the connection of previously isolated land masses and seas, leading to species expansions in terrestrial and marine realms (Vermeij 1991). However, the leading contemporary cause of changes in the distribution of species is the spread and subsequent activities of humans. The rapid colonisation of different continents by humans since their initial migration from Africa has facilitated the introduction of a multitude of species to areas outside of their previously inhabited range (Brown and Sax 2004). Subsequent human population

growth, technological innovations and habitat alteration have resulted in the continual translocation of species between ecosystems which were once entirely separated (Elton 2020).

1.1.3 The four stages of a biological invasion

Human mediated species introductions can occur intentionally (i.e. through intentional stocking; Pringle 2005) or accidentally (i.e. through transport in ships; Keller et al. 2011), and the fate of the resulting “propagules” is highly variable. Predicting the outcome of these propagules is a central goal of invasion biology but is challenging due to the wide array of factors which govern the ability of species to pass through the various stages of a biological invasion (Hayes and Barry 2008). Under the unified framework proposed by Blackburn et al. (2011), the stages of a biological invasion are defined as: transport (which is sometimes divided into uptake and transit as in Chapple et al. (2012)), introduction, establishment and spread (Blackburn et al. 2011; Figure 1.1). At each of these stages, species face novel challenges to pass to the next stage, and it is thought that species with certain characteristics should have an advantage, and these characteristics will differ with invasion stage (Hayes and Barry 2008). Species which are intentionally introduced bypass the transport stage of invasion, meaning that traits which are favourable for uptake and transit are no longer necessary for successful introduction. For example, the cane toad (*Bufo marinus*) was introduced to Caribbean and Pacific islands as a biological control agent for insect pests and is now listed among the world’s 100 worst invasive species (Lowe et al. 2008; Shanmuganathan et al. 2010).

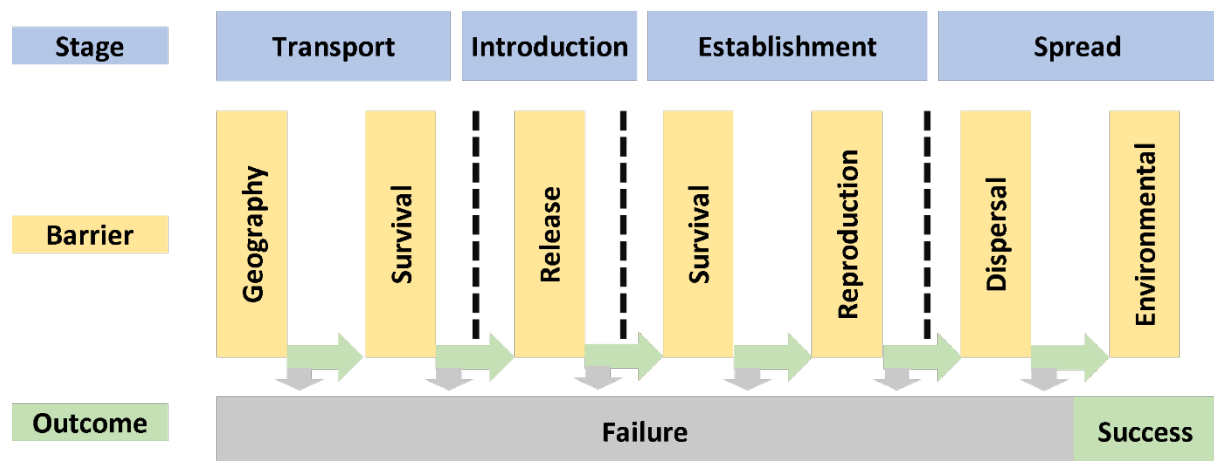


Figure 1.1 An outline of the invasion process of species introductions adapted from Blackburn et al. (2011) and Chapple et al. (2012). The process of invasion follows four sequential stages (blue rectangles), and examples of the barriers necessary to pass to the next stage are indicated below (yellow rectangles). Horizontal (green) and vertical (grey) arrows represent the invasion outcome following success or failure to pass on the next stage.

1.1.4 Do successful invaders possess similar traits?

The large degree to which invaders can differ in their expression of various traits, and the complex feedback loops that can arise from interactions with their novel environment, means that predicting invasion success is a highly complex issue (Hayes and Barry 2008). In light of this complexity a number of studies have attempted to identify traits which are common amongst successful invaders to target research effort towards species which may be ‘predisposed’ to invasion success (Kolar and Lodge 2001). Historically, invasive species have been predicted to be abundant generalists, with wide geographic ranges and high abiotic tolerance limits (Williamson and Fitter 1996; Ricciardi and Rasmussen 1998). To test such predictions, the most common approach is to compare two sets of species within a given region, for example, native vs established introductions (Williamson and Fitter 1996; Vila-Gispert et al. 2005), invasive vs non-invasive

introduced species (Kolar and Lodge 2001) or successful vs unsuccessful introductions (Forsyth et al. 2004; Marchetti et al. 2004).

Marchetti et al. (2004) used abundance data from every fish species inhabiting catchments in California and compared successful vs unsuccessful invaders (109 species in total) across eight traits related to the ecology or biology of the species, and two related to use of the species by humans. The authors found that successful and unsuccessful invaders from their dataset were generally distinguishable by certain species-level characteristics. Firstly, they found that species with broader physiological tolerances were able to establish more successfully. This finding was consistent with those of Kolar and Lodge (2002) who identified that within the Great Lakes of North America, species with wider tolerances to temperature and salinity were more successful in their invasion success. Marchetti et al. (2004) also found that prior invasion success was a strong predictor of success vs failure within their dataset, highlighting that ecological generalists which are favoured by humans are considerably more likely to become successful invaders. Several traits specific to invasion stage were also identified by the authors, with physiological tolerance and parental care both robust biological contributors to success at the establishment phase.

Evidence of the importance of parental care in the establishment of non-native Californian fish species highlights the role that behavioural interactions and the expression of behavioural traits, can play in invasion success. While prior research into traits thought to be common among successful invaders were typically focused on morphology, diet and physiological tolerance (Williamson and Fitter 1996), the role of behavioural traits in invasion success is increasingly recognised (Chapple et al. 2012). Research into this field greatly expanded following the publication of an influential paper by Holway and Suarez (1999), which encouraged researchers to incorporate behavioural metrics into the study of biological invasions (Holway et al. 1999). Subsequently, variation in behavioural traits has been shown to directly influence success across

all stages of a biological invasion (Schöpf Rehage et al. 2005; Short and Petren 2008; Weis 2010). In a meta-analysis of 69 bird species, Sol et al. (2009) found that behavioural flexibility and the ability for species to undertake foraging innovations were major determinants of invasion success (Sol et al. 2002). Thus, identifiable traits across successful invaders appear to be present within groups of organisms such as fish (Marchetti et al. 2004) and birds (Sol et al. 2002).

Personality, which is defined as individual differences in behaviours which show stability over time and context (Sih et al. 2004), has been linked to intraspecific variation in success by a number of studies (Juette et al. 2014; Brand et al. 2021). For example, invasive dispersal occurred over greater distances in mosquitofish (*Gambusia affinis*) which repeatedly expressed higher sociality than the mean from their founding population. Similarly, dispersal tendency in an invasive lizard (*Lampropholis delicata*) was linked to repeatedly expressed aggression behaviour, with more aggressive individuals dispersing further (Michelangeli et al. 2017). Behavioural syndromes, defined as the correlation of multiple personality traits, have also been linked to invasion success (Conrad et al. 2011). Galib et al. (2022) found that signal crayfish (*Pacifastacus leniusculus*) expressing consistently higher levels of boldness, activity, exploration and willingness to climb were more likely to disperse into novel habitats. Population density and local habitat complexity were also found to be important determinants, highlighting that personality traits which may benefit success in one context, cannot be assumed to improve invasion success in others (Galib et al. 2022). Personality dependent dispersal across multiple behavioural traits, in multiple taxa, suggests that personality is likely an important determinant of invasion success in many cases (Daniels and Kemp 2022). However, future research into the role of animal personality on invasion success across a range of fitness-related behaviours has been called for. Our understanding of the ubiquity of these mechanisms across species and ecological contexts is still limited, and to better define their role in predicting invasion outcome research into the effect of personality at multiple invasion stages is needed (Chapple et al. 2012).

Studies which have expanded comparisons of traits and invasion success across a wide range of taxa, have often found inconsistencies between taxonomic groups (Kolar and Lodge 2001; Hayes and Barry 2008). For example, Hayes and Barry (2008) conducted a meta-analysis of invader characteristics across birds, finfish, shellfish, insects, mammals, plants, reptiles and amphibians and found no characteristics that were supported across several of these biological groups. The authors highlighted the confounding effects of phylogeny and residence time, ultimately recommending that site and taxon specific analyses may provide more reliable predictions (Hayes and Barry 2008). Invasive species are consistently non-randomly distributed amongst taxonomic families and, as a result, broad-scale comparative studies can violate assumptions of statistical methods by treating closely related species with numerous overlapping traits as independent data (Alcaraz et al. 2005). Phylogenetic effects on invasion traits can also be observed within less broad taxonomic classifications. In a review of life-history data, Vila-Gispert et al. (2002) showed that taxonomic order was a stronger predictor of life-history traits than habitat or geographical region in 301 fish species. Similarly, Alcaraz et al. (2005) compared invasive and native fish species (a total of 69 species) found in the Iberian Peninsula across 26 quantitative and qualitative variables related to ecology, life-history and human-usage. They found that a number of trait differences vanished once phylogeny was accounted for within their analysis, while other differences were only observable once phylogeny was controlled for (Alcaraz et al. 2005).

Comparing species across a range of taxonomic groups also results in challenges when attempting to compare traits in a quantitative manner. Quantitative analyses are advocated by many invasion ecologists to better understand the underlying mechanisms of invasion success (Kolar and Lodge 2001; Marchetti et al. 2004). For example, while species may be broadly profiled in their reproductive strategy (i.e. sexual or asexual), measuring reproductive output in a unit which can be subject to quantitative comparison between birds, fish, and plants for example, is statistically challenging (Marchetti et al. 2004). Further, most research has focused on already established

populations of non-native species, thus traits which may predict invasion success in the earlier stage of biological invasions are frequently overlooked (Chapple et al. 2012). The selective filter hypothesis proposes that each stage of a biological invasion may select for different traits, and populations may consistently differ in their expression of certain traits because of this filtering (Tingley et al. 2010). Chapple et al. found that populations of an invasive lizard (*Lampropholis delicata*) outside of its native range are consistently different in their expression of boldness and exploratory behaviour and show higher within-individual variation (behavioural plasticity) than individuals from within the native range of the species (Chapple et al. 2022). Most invasive species research has been conducted on long-established populations of invasive species (Kolar and Lodge 2001). This, in conjunction with evidence for selective filtering across invasion stages, the resulting changes in traits expressed by those individuals which are initially introduced, and those which become established and spread, reveals a likely bias in the traits thought to be most important in determining invasion success. To address this, research investigating traits which drive successful establishment during the early stage of biological invasions has been highlighted as a clear future goal of invasive species research (Chapple et al. 2022).

While there are several challenges to identifying traits common across broad taxonomic groupings of successful invaders, “propagule pressure”, defined as the number of introduced individuals or the number of introductions, has emerged as a consistent predictor of invasion success (Lockwood et al. 2005). The likelihood of establishment following introduction is consistently found to increase with the number of individuals that are introduced, and propagule pressure has been termed the null model for biological invasions (Colautti et al. 2006). Criticism of adopting this ‘null model’ has also emerged, following research that shows behavioural traits can determine invasions success regardless of propagule size (Chapple et al. 2012). An argument that is backed up by examples of species which are frequently released, but unable to establish (Mack et al. 2008). Thus, while certain traits appear to be linked to invasion success within taxa, and in some cases across

taxa, we are yet to arrive at a trait-based unified framework for predicting invasion success with sufficient certainty. Incorporating phylogeny, behavioural traits, and propagule pressure into a combined approach may lead to the most accurate predictions. However, incorporating these variables into a single dataset, while avoiding statistical assumption violations and biases relating to invasion stage, is still highly challenging.

1.1.5 The impacts of non-native species on recipient ecosystems

Arguably the most important goal in invasion biology is to understand and predict the impacts that non-native species have on the ecosystems into which they are introduced (Parker et al. 1999). While the factors which dictate impact can overlap with those of successful invaders, and the impact of a non-native species is likely to scale with its ability to spread within a recipient ecosystem, there are many notable examples of widespread non-native species with relatively benign or in some cases even positive impacts (Gurevitch and Padilla 2004; Schlaepfer et al. 2011; Anton et al. 2019). For example, where ecosystem engineers have been eradicated from a given habitat, it has been suggested that introduced species may catalyse ecosystem restoration and benefit populations of native species (Ricardo et al. 2009). The introduction of Aldabra giant tortoises (*Aldabrachelys gigantea*) to several small islands in Mauritius provided a successful substitute for the ecosystem functioning of now extinct tortoise species in their role as herbivores and seed dispersers (Griffiths et al. 2010).

The use of biological control agents has also been highlighted as a potentially positive outcome of non-native species introduction, whereby non-native predators consume other non-native predators reducing the deleterious effects of previous invasions (Ewel and Putz 2004). However, this approach has been criticised due to notable examples of negative effects resulting from the introduction of biological control agents e.g. the impacts of cane toads on native predators in Australia (Jolly et al. 2016) and the impact of invasive cactus moths (*Cactoblastis cactorum*) on native

cacti in southern Florida (Johnson and Stiling 1996). In the case of the cactus moth, this species was purposefully selected due to its host specificity and specific dietary niche, highlighting that risk assessments may fail to consider the possibility for unpredictable effects or impacts on adjacent regions (Louda and Stiling 2004). It is increasingly acknowledged that potentially beneficial interactions between non-native and native species should be considered when discussing biological invasions (Goodenough 2010). However, caution has been advised given the complex nature of the ecological effects that can result from species introductions (Ricardo et al. 2009). Notably, effects initially deemed to be positive may result in negative consequences over a longer time frame and positive interactions with native species may be overridden by negative interactions with others (Ricardo et al. 2009). Black rats (*Rattus rattus*) were found to pollinate some native species in New Zealand following the extinction of native pollinators, however this species greatly contributed to the extinction of native pollinators in the first place. Thus, their positive effects may be greatly overestimated if they are not viewed in the context of the totality of their invasion (Ricardo et al. 2009; Pattemore and Wilcove 2012).

Invasive species impacts are also highly dependent on ecological context; the same species can cause extinctions if introduced into one ecosystem and have relatively little impact in another (Ruffino et al. 2009). Once again this is illustrated by the black rat, which has been introduced to more than 80% of the world's major islands where it preys on eggs, chicks and adults of numerous seabird species (Jones et al. 2008; Ruffino et al. 2009). As a result of this predation it is estimated that rats are a leading cause of extinction risk in 68% of seabirds, and have driven rapid extinctions in Hawaii, New Zealand and Australia (Atkinson 1985). However, in the Mediterranean, the same seabird species have been able to persist through 2,000 years of coexistence with black rats, and limited effects on their populations have been documented on several islands (Zotier et al. 1999). It is hypothesised that the distinct biogeography of many of these Mediterranean islands reduces interactions between rats and the vulnerable breeding sites of seabirds through intra-island refuge

areas (Ruffino et al. 2009). In this case, a change in the physical structure of a habitat appears to modulate the impact of an introduced species and has facilitated long-term coexistence with an invasive predator. In another example, intraspecific variation in the impact of an invasive species has been shown to occur over far smaller geographic ranges. Evangelista et al. (2019) observed intraspecific variation in the impact of an invasive red swamp crayfish (*Procambarus clarkii*) across a 20km gradient within a single lake, linked to changes in predation pressure, competition, and resource availability (Evangelista et al. 2019). These examples highlight the degree to which ecological context may shape the impact of a single introduced species, and how environmental conditions (i.e. landscape or temperature) and ecological effects (i.e. predation pressure or the behavioural responses of prey) can drive this variation.

In conclusion, the ability of non-native species to pass through all four stages of biological invasion does not necessarily ensure that they will have a negative impact. This highlights that a) the mechanisms underlying invasion success (sometimes termed ‘functional response traits’ as in Evangelista et al. 2019) and invasion impact (‘functional effects traits’) are not inherently correlated, and b) the impacts of a given non-native species are likely to depend greatly on the characteristics of the recipient ecosystem.

1.1.6 Targeting the most harmful invaders; a mechanism driven approach

Despite clear variation in invasion impact with species and ecological context, it is also clear that the impacts of certain invasive species, in certain contexts, are highly destructive. Their introduction can result in the extinction of native species (Clavero and García-Berthou 2005), fundamental changes to ecosystem function (Weidenhamer and Callaway 2010), habitat engineering and erosion (Anderson et al. 2014), along with harmful socioeconomic impacts resulting from these changes (Pejchar and Mooney 2009; Paini et al. 2016; Cuthbert et al. 2022). Effective management of such invasions is both challenging and costly (Martins et al. 2006) and

can be socially opposed in areas where non-native species have acquired a cultural value to humans (Mączka 2019). Currently the most widely employed methods are release prevention, containment, targeted eradication, and biological control (Hanley and Roberts 2019). Much like the impacts of invasive species themselves, eradication measures will vary in their effectiveness with species and environmental context and must be carefully designed based on an understanding of these factors (Simberloff 2001). Even effective measures are likely to incur a high economic cost. In Australia over a single financial year (2011-2012) an estimated \$3.8 billion Australian dollars were spent on invasive species management (Hoffmann and Broadhurst 2016). Considering these costs, it is important that limited available resources are targeted towards the most harmful invasive species, in the context where they are likely to have the greatest negative impact. Additionally, predicting harmful effects before or at the early stage of biological invasions may prevent or limit incurring the considerable costs associated with invasive species management. This raises the question: how do we predict, or assess the impact of an invasive species in a given context?

Driving the negative impacts of invasive species are causal mechanisms which can operate across all levels of biological organisation. These mechanisms range from the genetic level (i.e. through hybridization with native species; Deines et al. 2014) to the ecosystem level (i.e. through causing eutrophication; Starling et al. 2002). It is a result of these specific mechanisms that a single species can have entirely different effects in different ecosystems. For example, the impact of invasive mammalian predators is known to be greater on islands, where species such as birds have evolved in the absence of such predation (Ruffino et al. 2009). Understanding the causal mechanisms which drive the impacts of invasive species can help to achieve two key goals in addressing the threat of invasive species: 1) to design strategies to mitigate the negative effects of current invasions and 2) to design predictive risk models which can inform policies to prevent the introduction of potentially harmful species (Vander Zanden et al. 2004; Kulhanek et al. 2011). Invasion ecologists

recognise that our ability to achieve these goals is still inhibited by a poor understanding of the mechanisms which drive the negative impacts of many invasive species (Parker et al. 1999).

As with traits which may benefit invasion success, behavioural traits are also tied to the impact of invasive species and underpin the mechanisms which drive negative impacts of many non-native species (Dick et al. 1995; Salo et al. 2007). It has long been understood that inter and intraspecific variation in behavioural traits play a key role in shaping complex ecological processes such as predator prey interactions, competition between individuals over shared resources, habitat selection and reproduction. These behavioural processes are closely related to survival and thus also drive the negative outcomes of many invasive species. Indeed, the impact of non-native species is thought to be best predicted by the strength and character of interactions with native species (Carthey and Banks 2014). Therefore, investigating behavioural interactions between native and non-native species can provide useful information about the potential for mechanisms which may result in negative impacts of invasive species. It is important to note that variation in the behavioural responses of native species to non-native species is also a crucial determinant of non-native species impact, and should be considered an aspect of the ecological context which may shape the impact of invaders in a given ecosystem (Harrington et al. 2009; Heavener et al. 2014).

Experimental approaches within laboratory settings allow for a high degree of control over environmental conditions, therefore, allowing manipulation of specific variables to understand the contexts in which certain mechanisms are likely to be most impactful. In the long term, such experiments could be used to design mitigation strategies for ongoing invasions. For example, if predation by an invasive species is linked to shelter availability under experimental conditions, then conservation and restoration of shelter habitats provides a potential conservation measure which may mitigate harmful impacts and limit the spread of an ongoing invasion. Conversely, the artificial control of environmental variables can result in an oversimplified interpretation of the ecological

effects resulting from interactions between native and non-native species, therefore it is important to interpret findings with this in mind.

The potential for large variation, in both the characteristics of an invader (within and between species), and the ecosystem into which its introduced, makes it hard to predict and mitigate the impacts of introduced species solely through investigating traits associated with successful invaders (Hayes and Barry 2008). It is a likely result of this complexity that invasion biology is criticised for a lack of principles which provide consistent explanatory power (Lockwood et al. 2005; Colautti et al. 2006). However, if we understand the mechanisms through which a given species may cause harm (i.e hybridisation, predation, or interference competition), and find evidence for such mechanisms through experimental approaches, then these can be combined with invader and ecosystem traits to improve our ability to predict and prevent the release of harmful invaders, and better tackle the threat of ongoing invasions.

1.2 The decline of biodiversity in freshwater ecosystems

1.2.1 Freshwater biodiversity

Freshwater habitats comprise only two percent of the earth's surface yet support almost ten percent of described species, including at least one quarter of vertebrate species (Reid et al. 2019). As a result of this biodiversity, inland waters are highly valuable in both scientific and socioeconomic terms (Dudgeon et al. 2006). Over the last century, there has been a steep rise in the demands that humans place on freshwater habitats, and they are now directly exploited for capture fisheries, aquacultural production, recreation, irrigation, transport, industry, and energy supply (Reid et al. 2019). Additionally, human civilisations are disproportionately situated in close proximity to freshwater (Sala et al. 2000) which results in extensive riparian modification and subsequent input from sediments, contaminants, and nutrients (Amoatey and Baawain 2019).

A multitude of threats to freshwater biodiversity have accompanied this rise in anthropogenic activity, and as a result, freshwater biodiversity is declining at rates far beyond those seen in terrestrial and marine ecosystems (Sala et al. 2000). These declines are exacerbated by global climatic changes, to which freshwaters are particularly vulnerable, because water temperature and availability are highly dependent on climate (Woodward et al. 2010). The resulting loss in biodiversity and widespread extinction of freshwater species is well documented, and freshwater habitats are now among the most endangered on earth (Dudgeon et al. 2006). Dudgeon et al. (2006) grouped the major threats facing freshwater ecosystems into five main categories: over-exploitation, water pollution, habitat degradation, flow modification, and species invasion. Later, twelve threats to freshwater ecosystems were outlined by Reid et al. (2019) which have either emerged or intensified since the initial review by Dudgeon et al. (2006). These were: changing climates, e-commerce and invasions, infectious diseases, harmful algal blooms, expanding hydropower, emerging contaminants, engineered nanomaterials, microplastic pollution, light and noise, freshwater salinisation, declining calcium, and cumulative stressors. Both lists highlight the threat of invasive species to freshwater habitats.

1.2.2 Aquatic invasive species

According to global biodiversity scenarios for the year 2100, invasive species are expected to be the fourth largest driver for biodiversity loss globally (Sala et al. 2000). However, the impact of aquatic invasive species, especially in freshwater ecosystems, are predicted to be particularly severe (Moorhouse and Macdonald 2015). This is thought to be a result of their high biodiversity in conjunction with increased ‘invasibility’, owing to a lack of dispersal barriers (Lodge et al. 1998), and an array of potential transport vectors which can deposit particularly large quantities of non-native species at a given time (Padilla and Williams 2004) resulting in high propagule pressure (Lockwood et al. 2009).

1.3 Nile tilapia as a non-native species

1.3.1 Nile tilapia and its role in modern aquaculture

Nile tilapia (*Oreochromis niloticus*) is a freshwater cichlid endemic to Africa, with a native range that primarily spans West Africa and the Nile basin (Zengeya et al. 2013, 2015). The species is tolerant to a wide range of temperature, salinity, and dissolved oxygen conditions (Zale and Gregory 1989; Avella et al. 1993; Martin et al. 2010). This broad environmental tolerance, combined with their high fecundity, fast growth rate and largely microphagous diet, make *O. niloticus* a well-suited candidate for aquaculture production (Canonico et al. 2005). Presently, Nile tilapia production comprises approximately 9% of inland finfish aquaculture production globally (FAO 2022). Aquaculture has been encouraged as a sustainable solution for protein production that can enhance food-security, while reducing the detrimental effects associated with other forms of farming (i.e. nutrient run-off and habitat fragmentation) and avoiding the deleterious effects that can result from exploiting wild resources through marine or freshwater fish harvesting (Canonico et al. 2005). Consequently, aquaculture is responsible for a rapidly increasing proportion of aquatic production, and now exceeds capture fisheries (Tacon 2020). With this increase has come an increase in poorly managed, unsustainable aquaculture which has had myriad adverse impacts on global ecosystems. Among the most prominent of these impacts is the accidental release of non-native species (Naylor et al. 2001).

1.3.2 The origins of *O. niloticus* introduction

Despite clear benefits within aquaculture, the trophic adaptability, broad environmental tolerance, and rapid population growth rates of *O. niloticus* predispose it as a species with high invasive potential (Ehrlich 1989). *O. niloticus* are also maternal mouthbrooders, and parental care giving is a trait linked with invasion success at multiple stages in comparative analyses of invasive fish (Marchetti et al. 2004). Further, their invasion potential is greatly escalated when combined with a demand for global production, and it is notable that 98% of tilapia production occurs outside of

their native range (Shelton, 2002). It is as a combined result of these factors that tilapia have successfully invaded every environment that they been cultured in (Costa-Pierce, 2003), and *O. niloticus* is now one of the most widespread invasive fish species (Zengeya et al., 2015).

Considering recent growth, aquaculture is likely to have an increasing impact on the proliferation of invasive tilapia. It is not, however, the only source from which non-native populations have been introduced and established. In Indonesia, an aquarium release of Mozambique tilapia (*Oreochromis mossambicus*) in the 1930's is believed to be the first introduction of the species into natural water bodies of the region, before it established as an invasive species (Courtenay and Williams 1992). This spread was exacerbated when the species was transported and released to promote food production for Japanese soldiers during World War II (Canonico et al. 2005). Lake Victoria, Africa, saw the first release of *O. niloticus*, where it was intentionally introduced along with Nile perch (*Lates niloticus*) to boost fisheries (Ogutu-Ohwayo 1990). These introductions had devastating effects on local biodiversity, resulting in the extinction of an estimated 200 endemic haplochromine cichlid species, largely attributed to predation by Nile perch (Goldschmidt et al., 1993). The effects of this introduction on human society were also severe, causing a shift in fisheries income towards larger corporations forcing many local fishers out of business (Kasulo, 2000). Furthermore, this invasion precipitated a broader spread of *O. niloticus* across eastern and southern Africa where it was adopted for both aquaculture and capture fisheries improvement (Skelton, 2001). It is now widespread across natural waterbodies of the region. For example, escapees from fish farms in Zambia in the mid 1990's are now common in the Nata, Buzi, Runde-Save, Limpopo and Middle Zambezi River systems (Schwank 1995; Van der Waal & Bills 2000; Wise et al. 2007).

1.3.3 Mechanisms through which introduced Nile tilapia can impact native ecosystems

Given the widespread distribution of invasive Nile tilapia there is growing interest in the ecological impacts they have on native ecosystems. Many studies have demonstrated declines in native populations following the establishment of *O. niloticus*. Such effects have been reported in Lake Victoria and the Limpopo River System in Africa (Van der Waal & Bills, 2000; Balirwa et al. 2003), in Tanzania (Turner et al. 2018), Colombia (Leal-Flórez, 2003), Brazil (Starling et al., 2002), Nicaragua (McCrary et al., 2001), China (Gu et al. 2015), and Madagascar (Lévêque, 1997). However, in many cases the mechanisms underlying such declines in native species are not well understood. This is despite a growing understanding that knowledge of the mechanisms underlying the negative effects of *O. niloticus* on non-native ecosystems may prove crucial for preventing further impacts. Specifically, there has been little research focussing on the behavioural traits which may allow *O. niloticus* to successfully invade novel ecosystems during the early stage of biological invasion (introduction and establishment).

1.4 Thesis overview

In the research presented in this thesis I investigate the role of behaviour in the establishment and impact of invasive *O. niloticus* on native populations of tilapia. I focus on native *Oreochromis* species from Tanzania, which is home to highly biodiverse freshwaters that support many endemic tilapia species (Turner et al. 2001; Zengeya et al. 2015). These species are often closely related and functionally similar to *O. niloticus* resulting in the potential for strong niche overlap, which increases the potential for behavioural interactions over shared resources (Zengeya et al. 2015). The role of behaviour in biological invasions is increasingly being recognised as a crucial component of both invasion success and impact (Holway and Suarez 1999; Chapple et al. 2012). I sought to understand the degree to which behavioural mechanisms may underpin the successful invasion and the resulting negative impacts of *O. niloticus* in Tanzania. Given the widespread introduction of *O. niloticus* across Tanzanian freshwater and the abundance of native species which it encounters

across this range, evidence of harmful effects from these mechanisms could have wide reaching consequences for freshwater biodiversity, fisheries productivity, and aquaculture potential in Tanzania. This thesis is comprised of four data chapters, which, using a combination of behavioural experiments, genetic analyses, and fieldwork data, aim to investigate a number of behavioural mechanisms which may underpin the negative impacts of a widespread invasive species in a region which is highly biodiverse and likely to be particularly vulnerable.

In chapter two, I investigate interference competition between *O. niloticus* and a native tilapia species Manyara tilapia (*Oreochromis amphimelas*) under laboratory conditions. The aim of this study was to explore how interference competition over shared resources could shape the resource use of invasive *O. niloticus* and sympatric populations of threatened endemic tilapia and to explore the potential outcomes that may result from competitive dominance by either species.

In chapter three, I investigated how naïvete can affect the behavioural response of *O. amphimelas* to *O. niloticus* during early encounters using a laboratory approach. Few studies have investigated how naïvete can affect the outcome of fitness-related behavioural interactions that are not related to predation (i.e. competition). Further, little is known about how naïve native species are likely to respond to *O. niloticus* during early encounters. Therefore, this study provides novel insights into the mechanisms which may shape the successful establishment of a widespread invasive species during a poorly understood stage of biological invasions.

In chapter four, I investigated the relative costs and benefits of heterospecific grouping for invasive *O. niloticus* and native *O. amphimelas* using a laboratory approach. During biological invasions, species are typically introduced in small numbers with little to no access to information about their new environment. It has therefore been suggested that following their introduction, non-native species can derive benefits from joining groups of native species with a similar ecological niche,

giving them access to social information about the environment and avoiding the negative consequences associated with living in small groups. Using a combination of tracking and observational methods I investigated how group-decision making and social behaviour changed before, during and after grouping by *O. niloticus*.

In chapter five, I collected juvenile specimens from Lake Rutamba in Tanzania, where a newly discovered genetically unique lineage of the native Korogwe tilapia (*Oreochromis korogwe*) is threatened by hybridisation with sympatric populations of invasive *O. niloticus* (Blackwell et al. 2021). The potential for competition between these species to negatively affect the *O. korogwe* population has not yet been explored, and more broadly few studies have compared the relative fitness of native species to that of *O. niloticus* in a field setting. In this chapter I use growth rate as a determinant of fitness, using verified scale measurement techniques. I compare the relative performance of *O. niloticus* and *O. korogwe* across a size range encompassing juvenile and subadult fishes. I also provided the first assessment of a fitness related measure of hybrids within this population to provide insight into potential long-term outcome of hybridisation on the two parental species.

2 | Invasive Nile tilapia dominates a threatened indigenous tilapia in competition over shelter



Littoral reed bed, Lake Tandangongoro Tanzania

2.1 Abstract

Invasive species are one of the greatest threats to freshwater ecosystems globally. However, the causal mechanisms that drive negative impacts of many invasive species are poorly understood. In Tanzania, non-native Nile tilapia (*Oreochromis niloticus*) exists in sympatry with a diverse range of native species, many of which are congeners with strong niche overlap. It has been suggested that *O. niloticus* can displace native species from preferred habitat through dominance during interference competition, yet interference competition between *O. niloticus* and a native tilapia species has never been directly tested under experimental conditions. In this study juvenile *O. niloticus* and Manyara tilapia (*Oreochromis amphimelas*), a functionally similar but endangered Tanzanian cichlid, were size matched in conspecific and heterospecific pairs. We presented pairs with limited shelter and recorded competitive interactions. We found that *O. niloticus* were more aggressive and faster to initiate agonistic interactions than *O. amphimelas*. Furthermore, *O. niloticus* showed a strong competitive dominance in their interactions with *O. amphimelas*. One-sided dominance hierarchies can drive fundamental changes in resource use by subordinate individuals, potentially resulting in habitat displacement over the long term. Based on this experimental evidence, we conclude that *O. niloticus* may threaten native tilapia species through dominance in interference competition.

2.2 Introduction

Invasive species pose a major threat to aquatic ecosystems and can cause biodiversity loss, species extinction and adverse socioeconomic changes (Kaufman 1992; Pringle 2005). Underlying these impacts are causal mechanisms that explain why an invasive species has a given effect in an ecosystem. These mechanisms can operate from the genetic level (i.e. through hybridization; Moralee et al. 2000) to the ecosystem level (i.e. through eutrophication; Starling et al. 2002). An understanding of these mechanisms is crucial in order to develop management strategies to mitigate the effects of current invasions (Vander Zanden et al. 2004), design predictive risk models which may inform policies that prevent future invasions (Kulhanek et al. 2011), and allocate limited resources to efficiently target the most harmful invasive species (Parker et al. 1999). Despite the importance of biological invasions, the poor understanding of mechanisms driving the impacts of many invasive species greatly reduces our ability tackle the threat of aquatic invasive species (Parker et al. 1999; Kulhanek et al. 2011).

The Nile tilapia (*Oreochromis niloticus*) is a freshwater fish in the family Cichlidae, native to Western Africa and the Nile basin. It now has a pan-tropical non-native distribution (Canonico et al. 2005), with the spread largely due to its role in aquaculture. It is estimated that 98% of *O. niloticus* production occurs outside its native range, and accidental releases are frequent (Shelton 2002; Naylor et al. 2001). *O. niloticus* gained popularity as an aquaculture species due to its broad environmental tolerance, fast growth rate and high fecundity (Zale and Gregory 1989; Avella et al. 1993). These characteristics are favourable in aquaculture but give the species high invasive potential. Successful establishment of non-native populations has taken place in the majority of countries where culture has been initiated (Ehrlich 1989; Costa-Pierce 2003).

Given the widespread distribution of *O. niloticus*, research into its effect on ecosystems outside of their native range is growing (Canonico et al. 2005). A number of studies have demonstrated declines in populations of native fish following the establishment of *O. niloticus*, with examples from mainland Africa (Van der Waal and Bills 2000; Balirwa et al. 2003), Madagascar (Lévêque 1997), Brazil (Starling et al. 2002), Nicaragua (McCrary et al. 2001) and China (Gu et al. 2015). However, despite the potential threat posed by invasive *O. niloticus*, the mechanisms driving such declines remain poorly understood, and the need for further research into these mechanisms has been highlighted (Canonico et al. 2005; Bradbeer et al. 2019).

It has been proposed that *O. niloticus* can aggressively displace native species from their preferred habitat through dominance in interference competition (Goudswaard et al. 2002). This has been demonstrated under laboratory conditions, where *O. niloticus* decreased the shelter use of the native sunfish species *Lepomis miniatus* and increased the risk of sunfish mortality from predation (Martin et al. 2010). A similar experiment demonstrated that *O. niloticus* dominates competitive interactions with the native Brazilian pearl cichlid *Geophagus brasiliensis*, even when competing with larger pearl cichlid individuals (Sanches et al. 2012). Such studies clearly demonstrate the aggressive phenotype of *O. niloticus*, its tendency to dominate other species in competitive interactions, and the harmful impacts that can result from such interactions. However, given the extensive non-native range of *O. niloticus*, and the diversity of species it encounters, more work is needed to explore the ubiquity of this mechanism. This is especially important in light of the ecological consequences that can result from competitive dominance by invasive species (Case et al. 1994). Hence, examining how more species interact with *O. niloticus* could lend novel insights into the wider impacts of this species.

In Tanzania, non-native *O. niloticus* populations are now well-established (Ogutu-Ohwayo 1990; Njiru et al. 2004), and yet interference competition between *O. niloticus* and native Tanzanian fish

species has never been directly tested. Unlike the studies of interactions between *O. niloticus* and species native to the Americas, Tanzania is home to a number of native congeneric tilapia species which are both closely related and functionally similar to *O. niloticus* (Zengeya et al. 2015), and it is not clear whether these species can be outcompeted by *O. niloticus*. Interference competition is speculated to have driven the declines of native tilapia species in Lake Victoria (Goudswaard et al. 2002), but direct evidence supporting this is lacking. Furthermore, it has been proposed that *O. niloticus* was responsible for the local extinction of the native tilapia species *Oreochromis urolepis* from Lake Hombolo, Tanzania (Turner et al. 2019). These studies that implicate *O. niloticus* as the causal agent in the eradication of a native fish populations highlight the potential vulnerability of functionally similar tilapia species to *O. niloticus* invasion, and consequently interactions between *O. niloticus* and closely related species warrant investigation.

The freshwater habitats of Tanzania are home to an unprecedented biodiversity of fish species and provide a valuable system for the study of evolution and social behaviour (Turner 2007). Tanzanian fish stocks are a crucial source of food and income for a growing human population and are maximally exploited in many cases (FAO 2018). As a result, investigations into the impacts of introduced *O. niloticus* are especially important in Tanzania, where further ecological damage from invasive species could have negative consequences for local biodiversity and socioeconomic systems. This study focuses on interactions between *O. niloticus* and a functionally similar native Tanzanian cichlid, the Manyara tilapia *Oreochromis amphimelas*. *O. amphimelas* is endemic to Tanzania and is found in sympatry with non-native *O. niloticus* in lakes Manyara, Sulungali, Eyasi, Singidani and Kitangiri (Shechonge et al. 2019). Currently *O. amphimelas* is classified as Endangered by the IUCN due to threats from overharvesting, pollution and climate change (Bayona 2006), and therefore this species is vulnerable to further ecological stressors such as invasive species. Like *O. niloticus*, *O. amphimelas* are maternal mouthbrooders and are largely microphagous, suggesting strong resource overlap between these species (Trewavas and Fryer 1965). Littoral vegetation plays an

important role in the life cycle of many tilapia species, particularly as shelter for juveniles (Donnelly 1969), therefore competitive interactions over shared resources appear likely, and displacement from preferred habitat could affect individual and population fitness.

Direct evidence of interference competition between *O. niloticus* and *O. amphimelas* over habitats has not yet been demonstrated *in situ*, and field survey data are necessary to quantify space use and interactions. Nevertheless, it is important to understand the potential for behavioural mechanisms to influence resource use patterns in the species. Therefore, the aim of this study is to conduct the first direct test of interference competition between *O. niloticus* and a closely related native fish species. Under laboratory conditions *O. niloticus* and *O. amphimelas* were size-matched in conspecific and heterospecific pairs. Fish were presented with limited shelter resources and we recorded all competitive interactions in two experiments, one containing a single shared shelter and the other containing two separated shelters. The two experiments enabled us to determine how competitive interactions between *O. niloticus* and *O. amphimelas* vary with resource distribution, a known regulator of invasive species impacts.

2.3 Methods

2.3.1 Subjects and housing

Non-native populations of *O. niloticus* typically originate from intentional stocking events or escapees from commercial aquaculture (Canonico et al. 2005). Thus, we compared behaviour of *O. niloticus* descended from commercial stock, with wild-type (1st generation from wild) *O. amphimelas*. The *O. niloticus* subjects were purchased from Fish Farm UK (London, UK) and the *O. amphimelas* subjects were provided by Bangor University. Subjects from both species were raised in recirculating aquaculture facilities prior to receipt. Precise ages and parentages were not known when received. However, individuals from both species came from numerous spawning pairs and at the time of the experiment, were smaller than the size threshold typical for mature individuals (Al Hafedh et al. 1999; Froese and Pauly 2019). All fish were housed in a recirculating aquarium at the University of Bristol for four weeks prior to testing. Housing tanks were 190 L, with *O. amphimelas* and *O. niloticus* each housed in two separate tanks. By pairing fish from different tanks, even in the same-species tests, we maximised unfamiliarity between individuals. This removed any effect of social hierarchy established during the four weeks before testing began. Housing tanks were separated by perforated dividers, preventing movement of individuals between tanks, while allowing olfactory cue transmission. All housing tanks were held at similar densities, 55-65 individuals per tank, because housing density can affect aggression and boldness in tilapia (Champneys et al. 2018). Lighting was maintained on a 12:12h light:dark cycle to mimic natural conditions in the tropics. Fish were fed daily with a mixture of ZM Large Premium Granular feed (Tecniplast, London, UK), TetraMin flake (Tetra, Melle, Germany), frozen bloodworm (CC Moore & Co, Templecombe, UK) and Gamma™ Krill Pacifica, chopped prawn, Mysis Shrimp, Brineshrimp, and Vegetable Diet (Tropical Marine Centre, Chorleywood, UK).

2.3.2 Experimental setup

Four 36 litre experimental tanks (tank dimensions: 45 x 32 x 25 cm length x width x height) were filled each day to a depth of 14 cm with 20 litres of water from the housing tanks (temperature range: 24-26°). The water temperature of the experimental tanks was measured before each individual trial so that any change throughout the day could be accounted for in the statistical analysis. Shelters consisted of artificial plastic vegetation fixed to a plastic board, which was covered with the white aquatic gravel (Pettex Ltd, Ilford, UK) that also lined the bottom of the experimental tanks. Eight stems, each consisting of 14 14-19 cm long green leaves, were attached to each board, providing a stem density of 160 per m². This stem density is in line with high density areas of *Phragmites* (Uddin and Robinson 2017), an emergent macrophyte found in Tanzanian freshwater systems. Identical shelters were also placed in each of the housing tanks to remove novelty and to encourage individuals to associate the structure with shelter. To prevent disturbance during experiments, experimental tanks were visually isolated from each other with opaque plastic boards, and the surrounding room with opaque plastic sheet hanging from a metal frame.

In experiment one, tanks were divided into three zones of equal size (15x32 cm), one consisting entirely of shelter and the other two of bare substrate, with the area of bare substrate divided into two by a removable door (Figure 2.1a). In experiment two, tanks were also divided into three zones of equal size (15x32 cm): two half-shelter sections consisting of a smaller shelter (15x16 cm) and half substrate (15x16 cm), and a third section of bare substrate between them. The shelters within the half-shelter sections were positioned in opposing corners and a removable door separated one half-shelter section from the other two thirds of the tank (Figure 2.1b). Therefore, overall habitat cover and complexity was the same in both experiments. However, we hypothesised that the spatial separation of the shelter could mitigate aggression between the species and provide insights into potential conservation measures.

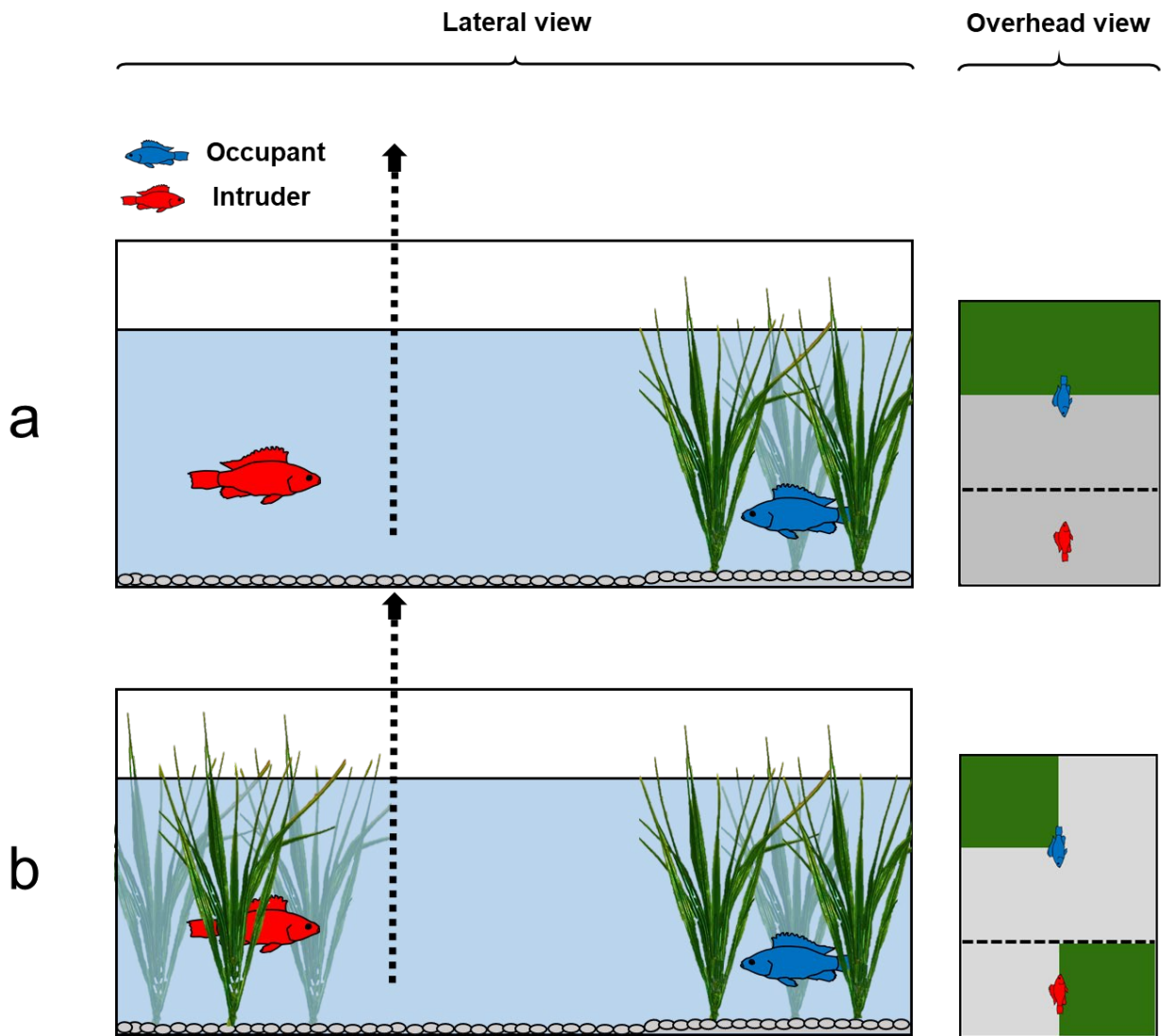


Figure 2.1 Lateral and overhead view of experimental tank set up for **a)** experiment one and **b)** experiment two (not to scale). In both views, dashed lines represent the opaque removable door, green areas represent shelter and grey areas represent bare sediment. Red fish represent intruders and blue fish represent occupants.

2.3.3 Experimental protocol

Experiment one (Figure 2.1a) consisted of an ‘occupant’ which was acclimated within the two thirds of the tank containing bare substrate and the shelter, and an ‘intruder’ which was acclimated behind an opaque removable door in the third of the tank containing bare substrate. To test how *O. niloticus* and *O. amphimelas* interact over a single shelter resource, three treatments were used: (1)

O. amphimelas occupant and *O. amphimelas* intruder (n = 19 trials), (2) *O. niloticus* occupant and *O. niloticus* intruder (n = 18), and (3) *O. amphimelas* occupant and *O. niloticus* intruder (n = 16). Due to a limited sample size of *O. amphimelas* subjects, a fourth treatment consisting of an *O. niloticus* occupant and a *O. amphimelas* intruder was not included in either experiments. Thus, our experiment simulates the introduction of *O. niloticus* individuals where *O. amphimelas* is already a resident species. Experiment one was conducted between the 14th and 19th January 2019.

Experiment two (Fig 2.1b) consisted of an ‘occupant’ which was acclimated within two thirds of the tank containing a bare substrate and a half-shelter section, and an ‘intruder’ which was acclimated behind an opaque removable door in the other third of the tank containing a half-shelter section. To test how *O. niloticus* and *O. amphimelas* interact over two, spatially-divided shelter resources, the same three treatments were used as in experiment one: (1) *O. amphimelas* occupant and *O. amphimelas* intruder (n = 13 trials), (2) *O. niloticus* occupant and *O. niloticus* intruder (n = 18), and (3) *O. amphimelas* occupant and *O. niloticus* intruder (n = 19). Experiment two was conducted between 12th and 19th February 2019 using the same fish as experiment one, meaning that some pairs may have been recombined. No fish was used more than once in each experiment.

Differences in sample size between treatments resulted from discarded trials, where the intruder escaped under the removable door during acclimatisation, or the camera recording failed. Additionally, in experiment two, sample sizes for the *O. amphimelas* - *O. amphimelas* treatment were reduced compared to experiment one because growth rates varied between individuals within the population over the 24 days between experiments one and two, preventing size matching of all available experimental fish. At the start of each trial, an occupant and an intruder were netted haphazardly from their respective housing tanks and size matched by measuring total length (TL) with callipers. If the absolute size difference exceeded 10mm, the intruder was returned, and a different individual was netted.

Table 2.1 Mean total length \pm SD (mm) of occupants and intruders for each treatment in experiments one and two. The mean size difference of occupants compared to intruders \pm SD (%) in each trial is also shown.

Experiment	Trial type	Mean total length of occupants \pm SD (mm)	Mean total length of intruders \pm SD (mm)	Mean per trial size difference (occupant length – intruder length) \pm SD (mm)
One	<i>O. amphimelas</i> – <i>O. amphimelas</i>	58.9 \pm 3.88	58.74 \pm 4.07	0.16 \pm 2.05
One	<i>O. niloticus</i> – <i>O. niloticus</i>	55.69 \pm 3.59	56.34 \pm 3.81	-0.65 \pm 1.94
One	<i>O. amphimelas</i> – <i>O. niloticus</i>	59.43 \pm 5.73	58.3 \pm 5.51	1.13 \pm 2.09
Two	<i>O. amphimelas</i> – <i>O. amphimelas</i>	59.06 \pm 4.62	59.05 \pm 4.01	0.02 \pm 3.02
Two	<i>O. niloticus</i> – <i>O. niloticus</i>	67.41 \pm 5.58	67.39 \pm 5.03	0.01 \pm 1.77
Two	<i>O. amphimelas</i> – <i>O. niloticus</i>	63.11 \pm 3.96	63.15 \pm 4.38	-0.05 \pm 1.21

In both experiments, four experimental setups were run simultaneously, with one of each treatment and an additional trial of a randomly selected treatment. Treatments were randomly assigned to one of the four experimental tanks. Occupant individuals were placed in the larger section of the tank containing a bare sediment and a shelter section in experiment one and a bare sediment and half-shelter section in experiment two. Intruders were placed behind the opaque

removable door in the remaining third of the tank, which contained a bare sediment section in experiment one and a mixed section in experiment two (Figure 2.1). The trial began with a 30-minute acclimation period. Both species initiate feeding in this time in a similar experimental setup (unpublished data). Following acclimation, the door was raised by hand, while the rest of the experimenter was obscured from view, and the fish were able to explore the tank and interact for 15 minutes.

2.3.4 Recording and video analysis

In both experiments the 15-minute interaction period was recorded on two overhead GoPro Hero 6 cameras (linear field of view, 30 frames per second, 1280 x 720, GoPro Inc., San Mateo, CA, USA), each recording two of the four tanks. Video recordings were analysed using BORIS version 7.4.6, by one reviewer (Friard and Gamba 2016). It was not possible for this reviewer to be blind to the treatment due to the nature of the experiment. Thus, given that a subset of additional videos was not scored by a second observer who was blind to the hypotheses of the experiment, we cannot completely rule out the possibility of unconscious bias. The time spent outside of shelter by the occupant was recorded, along with all agonistic interactions between the occupant and the intruder. Being out of shelter was defined as when more than half of the individual's body length was both past the edge of the plastic board at the base of the shelter and less than half covered by a leaf from one of the stems. The agonistic interactions recorded were biting, chasing, and mouth fighting, and were defined according to a published ethogram on *O. niloticus* (Alvarenga and Volpato 1995). The behaviour definitions are as follows: biting (termed nipping by Alvarenga and Volpato 1995, but renamed to avoid confusion with non-aggressive interactions) "the aggressor swims towards the opponent and bites", chasing "the aggressor swims towards the opponent, while the opponent swims away from the aggressor, without any physical contact", mouth fighting "both fish approach frontally with the mouth open and bite the opponent's mouth" (Alvarenga and Volpato, 1995). A number of behaviours associated with aggression in cichlids such as gill

spreads, tail beats and lateral displays were not recorded in this experiment as they were hard to definitively identify with the overhead camera set up. These behaviours carry a low injury risk while providing an assessment of fighting ability (Enquist et al. 1990). Physical displays of aggression are thought to be a highly escalated form of competitive interaction in cichlids (Enquist et al. 1990), and these are the focus of our analysis.

The initiator (occupant or intruder) was recorded for each interaction. This information was used to calculate the time taken for the two fish to first interact and the number of agonistic interactions. The number of agonistic interactions was also used to analyse the level of competitive dominance shown by occupants. Based on our definition of chasing, the aggressor always forced the other fish to swim away (Alvarenga and Volpato, 1995). Biting typically resulted in a similar avoidance response from the recipient, but when retaliation occurred, this was recorded as a separate event. Typically, the losers of competitive interactions in cichlid fish are defined by an avoidance response and/or a lack of retaliation (Oliveira et al. 2009; Reddon and Hurd 2009) following an agonistic interaction. We were therefore confident that the number of agonistic interactions initiated by the occupant relative to the intruder could act as a measure of dominance in our analysis. This is similar to methods outlined in Bailey et al. (2000) and Sanches et al. (2012) who defined dominance by the proportion of agonistic interactions initiated by an individual. The definition of mouth fighting outlined by Alvarenga and Volpato (1995) states that “both fish approach frontally” meaning that an initiator cannot be clearly identified. As a result, mouth fighting was recorded as an agonistic interaction directed by both individuals. This meant that it did not affect the number of interactions directed by the occupant relative to the intruder (dominance), while still providing information on the total number of agonistic interactions occurring in each treatment.

2.3.5 Statistical analysis

All analyses were conducted using R version 3.6.0 (R Core Team 2019). The time taken for the first interaction between occupant and intruder was analysed in both experiments with a Cox Proportional-Hazards Model using the *coxph* function in the package *survival* (Therneau 2015). This analyses how the probability of an event occurring is affected by a given set of risk factors at any given time. Here, the event was the first interaction between occupant and intruder, the risk factors are the model covariates: treatment, temperature and intruder length (TL), and the time is the experiments' duration (900 s; Table 2.2). Survival analysis allows both the time taken for the first interaction to occur and whether or not an interaction did occur within the 900 s to be included. The effect of each covariate on the probability of a first interaction is calculated while taking all other covariates into account. A hazard is the likelihood of the event occurring at a specific time, and in a Cox Proportional-Hazards Model, hazards are assumed to be consistent over time. In the data from experiment one, an initial test using the *cox.zph* function revealed a violation of this proportional hazard assumption through significant test results for the intruder length (TL) covariate ($P < 0.001$) and the global test ($P = 0.004$). In experiment two, a significant test result was also found for the intruder length (TL) covariate ($P = 0.04$), while the global test was non-significant ($P = 0.1$). Plotting residuals over time revealed intruder length (TL) to have a time varying effect for both experiments, so time was stratified into three separate 5-minute time windows (0-300s, 300-600s and 600-900s). Thus, in both experiments, the effect of intruder length (TL) was analysed separately for each time window using a *strata* argument in the model formula (method outlined by Zhang et al. 2018). Commands *ggcoxdiagnostics* and *ggcoxfunctional* in the package *survminer* were used to test for influential observations and non-linearity (Kassambara et al. 2019), and the assumptions were satisfied for both experiments. Packages *survival* and *survminer* were used to visualise the results.

The number of agonistic interactions initiated by each fish was analysed using Generalised Linear Mixed Models (GLMMs in the *glmmadmb* package) with a negative binomial family (Skaug et al.

2016) for each experiment separately. In each of these models, trial number was included as a random effect to account for the non-independence of data from the two fish tested in the same trial. Models with and without correction for zero-inflation were compared using AICc (Akaike Information Criterion corrected (AICc) for small sample sizes) and the results showed that models not corrected for zero-inflation were more likely. The time spent outside of shelter by the occupant was analysed for both experiments using negative binomial Generalised Linear Models (*glm.nb*) in the package 'MASS' (Venables and Ripley 2002).

For the number of agonistic interactions, and the time spent in the open by the occupant, five to seven models were constructed based on *a priori* hypotheses, each containing different combinations of seven explanatory variables and interaction terms (Tables 2.3 and 2.4). These models were compared using the AICc to indicate the strength of support for each model. Water temperature in the experimental tanks and size difference between intruder and occupant were included as fixed effects because relatively small variations in temperature (Cerqueira et al. 2016) and size difference (Sanches et al. 2012) have been shown to affect the behaviour of *O. niloticus*. The treatment \times size difference and treatment \times temperature interaction terms were included because we hypothesised that size differences and temperature may affect the two species differently. For the analysis of the number of agonistic interactions, role was included as a fixed effect as we predicted that occupants and intruders may differ in their aggression levels. This difference in aggression level between intruders and occupants provided a measure of dominance by measuring the number of agonistic interactions initiated by occupants relative to that of intruders. The inclusion of a treatment \times role interaction term tested whether the degree of dominance shown by the occupant differed between the three treatments. Temperature and absolute size difference were standardised in this analysis using the *scale* function to improve model convergence.

Table 2.2 Definitions and range/levels of the five explanatory variables used across analyses

Explanatory variable	Definition	Range/Levels
Treatment	Species	“ <i>O. amphimelas</i> - <i>O. amphimelas</i> ” “ <i>O. niloticus</i> - <i>O. niloticus</i> ”, “ <i>O. amphimelas</i> - <i>O. niloticus</i> ”
Role	Role of the individual	“Occupant”, “Intruder”
Size difference	Percentage total length difference between occupant and intruder	Experiment one: -7.88 to +7.33 (%), Experiment two: -9.66 to +8.61 (%)
Absolute size difference	Absolute percentage total length difference between occupant and intruder	Experiment one: 0 to 7.88 (%), Experiment two: 0 to 9.66 (%)
Intruder length	Total length of the intruder	Experiment one: 49 to 66.33 (mm), Experiment two: 51 to 74.9 (mm)
Temp	Temperature of the experimental tank water	Experiment one: 24 to 26 (°C), Experiment two: 24.6 to 26 (°C)
Trial number	Experimental trial number	Experiment one: 1 to 59 Experiment two: 1 to 51

2.4 Results

2.4.1 Latency for intruder to interact with occupant - experiment one

The probability of an agonistic interaction was lower in the *O. amphimelas* - *O. amphimelas* treatment than in the *O. amphimelas* - *O. niloticus* and *O. niloticus* - *O. niloticus* treatments, with these latter two treatments not differing significantly from each other (Figure 2.2a). Therefore, *O. amphimelas* were more likely to be involved in aggression when the intruder was an *O. niloticus* rather than a conspecific (comparison: coef = -2.3, exp(coef) = 0.099, lower 95% CI = 0.037, upper 95% CI = 0.267, $P < 0.001$). *O. niloticus* intruders engaged in agonistic interactions in 91% of trials, and the probability of an interaction occurring did not depend on the occupant species (comparison: coef = -0.47, exp(coef) = 0.61, lower 95% CI = 0.29, upper 95% CI = 1.33, $P = 0.22$). Warmer temperature was associated with an increased probability of first interaction (coef = 0.81, exp(coef) = 2.27, lower 95% CI = 1.35, upper 95% CI = 3.82, $P = 0.002$). The effect of intruder length (TL) was not consistent over time. In the first time period (0-300s), larger intruders were more likely to interact (coef = 0.15, exp(coef) = 1.16, lower 95% CI = 1.01, upper 95% CI = 1.34, $P = 0.03$), in the second (300-600s) there was no significant relationship between intruder length (TL) and the probability of an interaction (coef = -0.06, exp(coef) = 0.94, lower 95% CI = 0.83, upper 95% CI = 1.06, $P = 0.33$), and in the third (600-900s), larger intruders had a reduced chance of first interaction (coef = -0.3, exp(coef) = 0.74, lower 95% CI = 0.57, upper 95% CI = 0.96, $P = 0.02$). The overall model fit was highly significant (Likelihood ratio test = 47.83, d.f = 6, $P < 0.001$, $n = 114$, events = 38).

2.4.2 Latency for intruder to interact with occupant - experiment two

When shelters were separated in experiment two, the probability of an agonistic interaction differed significantly between all treatments (Figure 2.2b). *O. amphimelas* - *O. amphimelas* still had the lowest probability of an interaction and again, *O. amphimelas* occupants were significantly more

likely to be involved in aggression when the intruder was an *O. niloticus* rather than a conspecific (comparison: $\text{coef} = -1.64$, $\exp(\text{coef}) = 0.19$, lower 95% CI = 0.05, upper 95% CI = 0.76, $P = 0.018$). *O. niloticus* intruders engaged in agonistic interactions in 70.3% of trials, a lower proportion than experiment one, and the latency depended significantly on the occupant species with aggression being more likely to occur when paired with a conspecific rather than an *O. amphimelas* (comparison: $\text{coef} = -0.92$, $\exp(\text{coef}) = 0.40$, lower 95% CI = 0.16, upper 95% CI = 0.97, $P = 0.043$). The effect of intruder length (IL) had no significant effect on the likelihood of interaction at any of the three time periods (0-300s, 0-600s, and 600-900s). Warmer temperature had no significant effect on the likelihood of first interaction. The overall model fit was significant (Likelihood ratio test = 21.18, d.f = 6, $P = 0.02$, $n = 123$, events = 29).

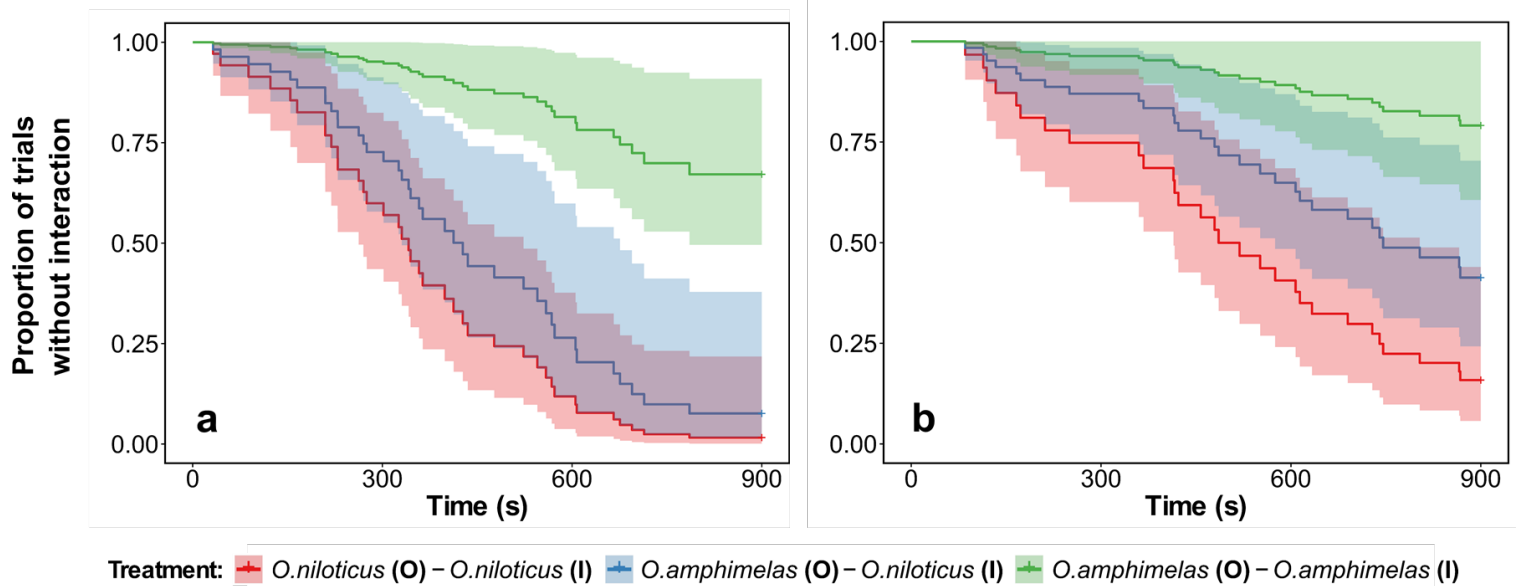


Figure 2.2 Latency to first interact (s \pm 95 CI) in **a)** experiment one and **b)** experiment two. Each line represents one of the three experimental treatments. Colours represent treatments as indicated by the figure legend, (O) represents the occupant and (I) the intruder in each treatment.

2.4.3 Number of agonistic interactions - experiment one

Biting was the most common agonistic interaction, followed by chasing (Figure 2.3). Mouth fighting was more prevalent than chasing in the *O. niloticus* - *O. niloticus* treatment but was scarce in the other treatments (Figure 2.3). The AICc values and Akaike weights indicated 83% support for the model containing the treatment and role (intruder or occupant) interaction term, in addition to the main effects contained in all models (Table 2.3). The strong preference for this model suggests that the difference in aggression by occupants and intruders, a measure of dominance, varied between the treatments. Pairwise comparisons revealed that occupants initiated significantly lower numbers of agonistic interactions relative to intruders in the *O. amphimelas* - *O. niloticus* treatment than in the *O. niloticus* - *O. niloticus* (estimate = -1.32, SE = 0.58, z value = -2.27, P = 0.023) and *O. amphimelas* - *O. amphimelas* (estimate = -2.48, SE = 0.8, z value = -3.12, P = 0.002) treatments. Thus, dominance was skewed towards *O. niloticus* in the *O. amphimelas* - *O. niloticus* treatment, but was significantly more balanced in the conspecific treatments (Figure 2.4a). The *O. amphimelas* - *O. amphimelas* treatment had the lowest levels of aggression, with cases of aggression only occurring in 37% of trials (Figure 2.4a).

2.4.4 Number of agonistic interactions - experiment two

The AICc values and Akaike weights indicate 76% support for the model containing the treatment and role interaction term, in addition to the main effects contained in all models (Table 2.3). This strong preference for this model suggests that the difference in dominance between intruders and occupants differed significantly between treatments. Similarly to experiment one, pairwise comparisons reveal that the number of agonistic interactions initiated by occupants relative to intruders was significantly lower in the *O. amphimelas* - *O. niloticus* treatment than in the *O. niloticus* - *O. niloticus* (estimate = -3.61, SE = 0.53, z value = -6.79, P < 0.001) and *O. amphimelas* - *O. amphimelas* (estimate = -6.58, SE = 1.32, z value = -5, P < 0.001) treatments. Therefore, balanced

dominance levels were observed between conspecifics, but in the *O. amphimelas* - *O. niloticus* treatment, *O. niloticus* showed significant competitive dominance (Figure 2.4b).

Table 2.3 Model comparison for the negative binomial Generalised Linear Mixed Models used to analyse the number of agonistic interactions in experiment one and two. The random factor was trial number. Temp refers to water temperature.

Experiment	Model	Explanatory variables	AICc	Δ AICc	df	Akaike weight
One	m1.2	Treatment * Role + Absolute size difference + Temp	616.2	0	10	0.83
	m1.1	Treatment * Role + Treatment * Absolute size difference + Temp	620.8	4.7	12	0.08
	m1.4	Treatment + Role + Absolute size difference + Temp	621.2	5	8	0.07
	m1.5	Treatment + Absolute size difference + Temp	624.5	8.3	7	0.01
	m1.3	Role + Treatment * Absolute size difference + Temp	625.6	9.4	10	0.01
	m1.6	Role+ Absolute size difference + Temp	648.6	32.4	6	0
	m1.7	Absolute size difference + Temp	653.9	37.7	5	0
Two	m2.2	Treatment * Role + Absolute size difference + Temp	464.4	0	10	0.76
	m2.1	Treatment * Role + Treatment * Absolute size difference + Temp	466.7	2.3	12	0.24
	m2.3	Role + Treatment * Absolute size difference + Temp	492.8	28.4	10	0
	m2.5	Treatment + Absolute size difference + Temp	499.6	35.2	7	0
	m2.4	Treatment + Role + Absolute size difference + Temp	499.8	35.4	8	0
	m2.6	Role+ Absolute size difference + Temp	520.8	56.4	6	0
	m2.7	Absolute size difference + Temp	522.5	58.2	5	0

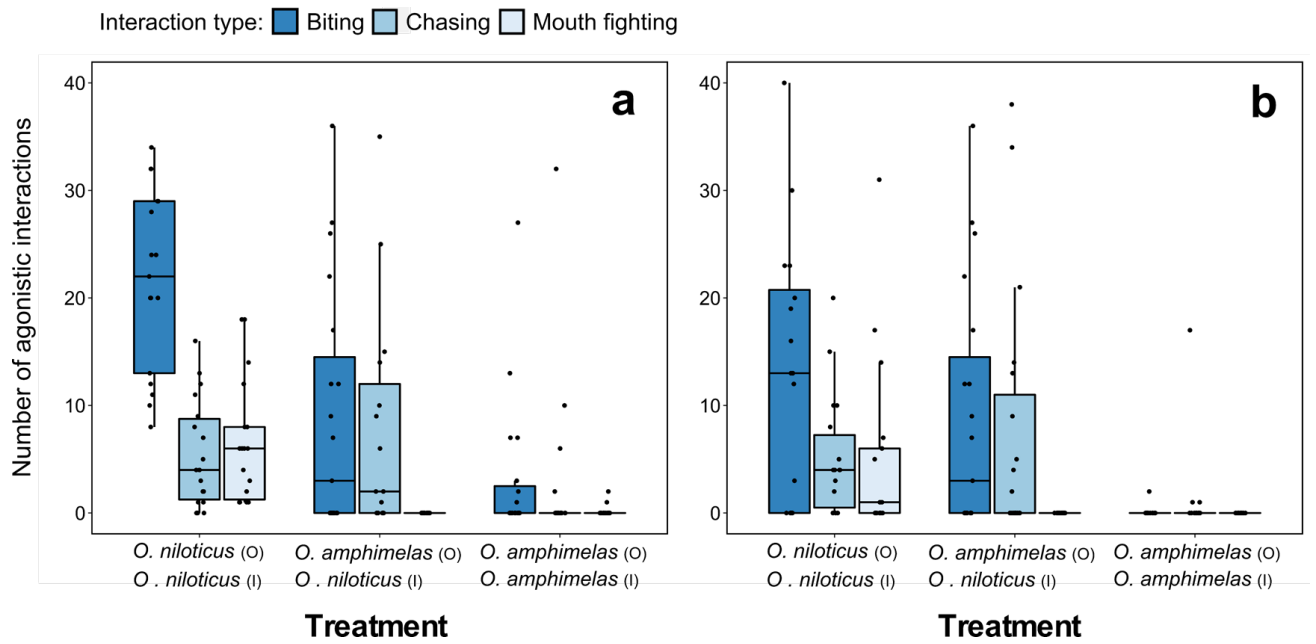


Figure 2.3 Number of the three types of agonistic interaction in each treatment in **a)** experiment one and **b)** experiment two. The roles of occupant and intruder are represented in each treatment by (O) and (I) respectively. The distance between the top and bottom of each box represents interquartile range, whiskers extend to data points within 1.5 times the interquartile range, and the line through the centre of each box represents the median. Individual data points are scattered over their corresponding treatment with added jitter for clarity, and those above or below the whiskers represent outliers.

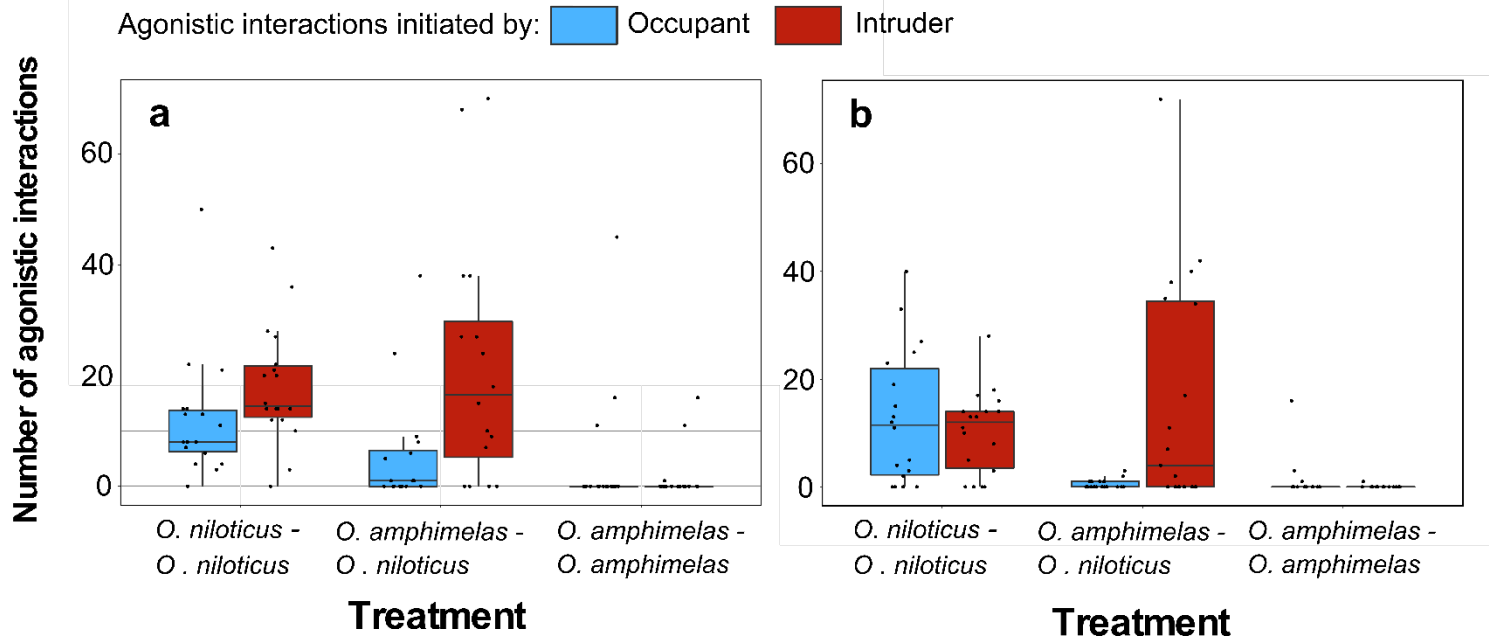


Figure 2.4 Agonistic interactions initiated by the intruder and the occupant across the three treatments in **a**) experiment one and **b**) experiment two. The distance between the top and bottom of each box represents interquartile range, whiskers extend to data points within 1.5 times the interquartile range, and the line through the centre of each box represents the median. Individual data points are scattered over their corresponding treatment with added jitter for clarity, and those above or below the whiskers represent outliers.

2.4.5 Time spent out of shelter by the occupant - experiment one and two

The AICc values and Akaike weights indicate 90% support in experiment one and 85% support in experiment two for the simplest model that lacked the treatment variable (Table 2.4). This suggests that in both experiments the time spent out of shelter by the occupant did not vary considerably between treatments (Figure 2.5), and the effects of temperature and size difference were not treatment specific as the models with interaction terms were not well supported by the data.

Table 2.4 Model comparison for the five negative binomial Generalised Linear Models used to analyse the time spent in the open by the occupant in experiment one and two.

Experiment	Model	Explanatory variables	AICc	Δ AICc	df	Akaike weight
One	m3.5	Size difference + Temp	55.6	0	4	0.9
	m3.4	Treatment + Size difference + Temp	60.4	4.8	6	0.08
	m3.3	Treatment * Temp + Size difference	65.3	9.7	8	0.01
	m3.2	Treatment * Size difference + Temp	65.5	9.9	8	0.01
	m3.1	Treatment * Size difference + Treatment * Temp	70.9	15.3	10	0
Two	m4.5	Size difference + Temp	63.9	0	4	0.85
	m4.4	Treatment + Size difference + Temp	67.7	3.8	6	0.13
	m4.3	Treatment * Temp + Size difference	72.8	8.8	8	0.01
	m4.2	Treatment * Size difference + Temp	73.1	9.2	8	0.01
	m4.1	Treatment * Size difference + Treatment * Temp	78.7	14.8	10	0

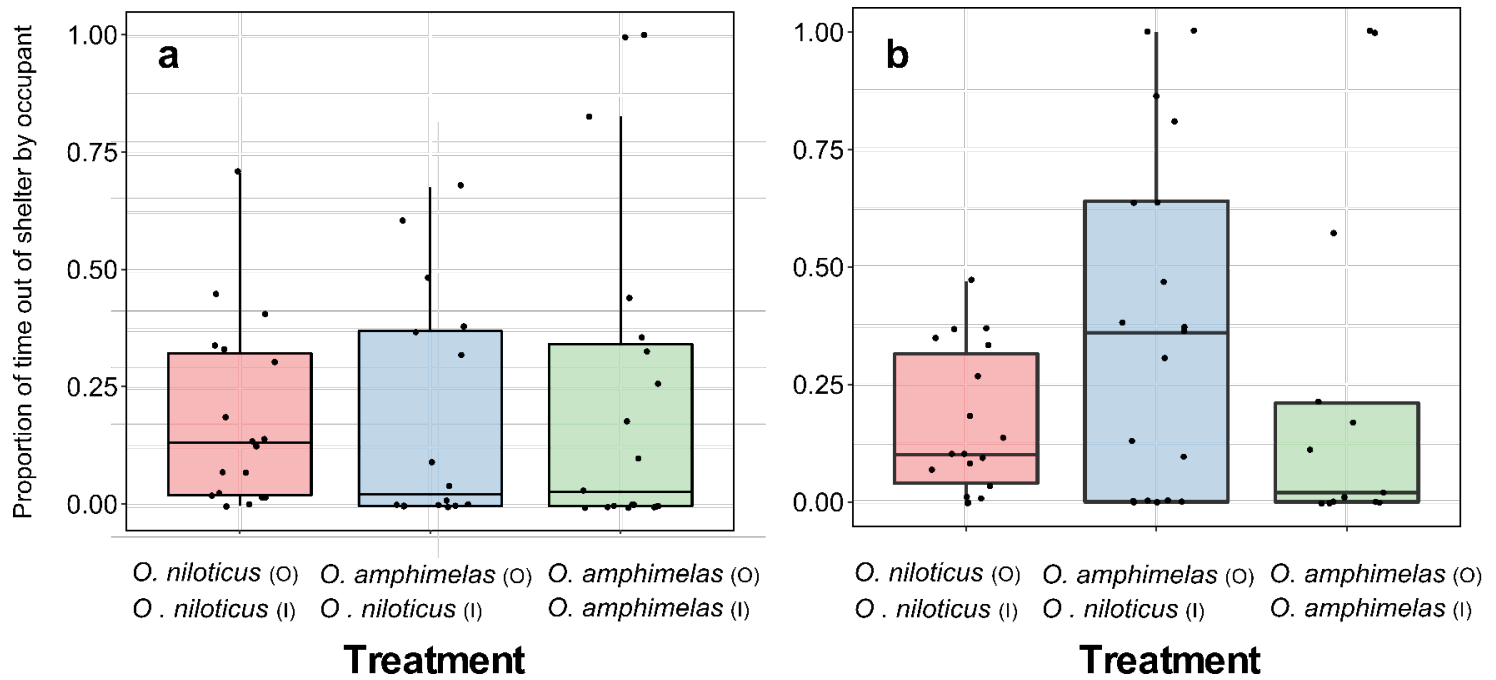


Figure 2.5 The time spent out of the shelter by the occupant across the three treatments in **a)** experiment one and **b)** experiment two. The roles of occupant and intruder are represented in each treatment by (O) and (I) respectively. The distance between the top and bottom of each box represents interquartile range, whiskers extend to data points within 1.5 times the interquartile range, and the line through the centre of each box represents the median. Individual data points are scattered over their corresponding treatment with added jitter for clarity, and those above or below the whiskers represent outliers.

2.5 Discussion

In both experiments, when *O. amphimelas* individuals were paired with an *O. niloticus*, agonistic interactions were initiated more quickly and were more frequent across the trial than when *O. amphimelas* were paired with a conspecific. Thus, *O. niloticus* dominated competitive interactions with *O. amphimelas*, while in the conspecific pairings, dominance between occupants and intruders was significantly more balanced. Differences in competitive ability between the two species appears to drive dominance of *O. niloticus* over *O. amphimelas*, rather than the roles of intruder or occupant. Providing two spatially separated shelters appeared to reduce the likelihood of an interaction occurring across all treatments, although *O. niloticus* still showed competitive dominance over *O. amphimelas*. Thus, it appears that the probability of competition occurring may be reduced through resource partitioning, however when competition does occur, *O. niloticus* will dominate competitive encounters with *O. amphimelas*.

The competitive dominance of *O. niloticus* demonstrated here presents a mechanism through which invasive *O. niloticus* could negatively affect native species in the biodiverse freshwater systems of East Africa. The outcome of interspecific competition can fundamentally change the resource use of individuals, which can drive habitat displacement at the population level (Morse, 1974; Nakano, 1995). Displacement from preferred habitat by invasive species has been linked to superiority in competitive interactions over food (Petren and Case 1996), space (Carlton et al. 1999) and shelter (Case et al. 1994; Mooney and Cleland 2001), and has caused declines in the diversity and abundance of native species (Porter and Savignano 1990). When combined with strong niche overlap, competitive dominance by *O. niloticus* could result in similar effects under field conditions and discourage native species from accessing resources. If interspecific competition is found to occur over shelter, the ecological consequences for displacement are likely to be strong due to an increase in predation (Martin et al. 2010), a key driver for population reduction and in some cases,

extinction (Blackburn et al. 2004; Sax and Gaines, 2008). We therefore recommend that field surveys investigating the habitat use of *O. niloticus* and *O. amphimelas* are undertaken, as accurate predictions on the likelihood of ecological consequences require habitat use data.

In neither experiment did we find any difference in the time spent out of shelter by the occupant between treatments. Laboratory studies on a variety of fish species have reported habitat displacement as a result of interference competition (Mills et al. 2004; Martin et al. 2010; Grabowska et al. 2004). Therefore, it is particularly surprising that *O. amphimelas* occupants did not spend more time out of shelter when paired with *O. niloticus* compared to when they were paired with another *O. amphimelas*, since they received much more aggression when paired with *O. niloticus*. It is likely that the 15-minute recording period, and lack of alternative habitats, may not have been sufficient to capture longer-term behavioural outcomes of the competitive interactions such as changes in habitat use (Morse 1974). With a longer trial length where alternative habitats were available and competition could be evaded by moving to a different habitat, we predict that *O. amphimelas* would be displaced from its preferred habitat under laboratory conditions in order to avoid competition with dominant *O. niloticus*. In addition, shelter use is known to consistently vary between individuals within populations (Ioannou and Dall 2016), and individuals can also vary in how they change their shelter use when other individuals are present (Bevan et al. 2018). This inter-individual variation would have contributed to within-treatment variation in our experiments, possibly concealing any effect between treatments.

Mean size differences between occupants and intruders were below 3% in both experiments, and never exceeded 10% in any individual trial (Table 2.1). *In situ* however, *O. niloticus* have especially high growth rates, and typically reach considerably larger sizes than *O. amphimelas* and other sympatric tilapia species (Froese and Pauly 2019). The maximum standard lengths reported for *O. niloticus* and *O. amphimelas* are 60 cm and 28 cm respectively (Froese and Pauly 2019). The

advantage of increased body-size in competitive interactions is well established in fish (Francis 1983; Abbott et al. 1985) and thus the dominance shown by *O. niloticus* in this experiment could be heightened *in situ*. Consequently, the results from this experiment may represent a near best-case scenario, where competitive interactions between *O. niloticus* and native Tanzanian species occur at equal body-sizes. While small body-size differences can be overridden by differences in other competitive traits such as aggression (Hasegawa et al. 2004), this is unlikely to benefit *O. amphimelas*, which we found to be considerably less aggressive than *O. niloticus*.

If prolonged, one-sided dominance hierarchies such as the one observed in this experiment can result in the monopolisation of resources in favour of the dominant individual or species (Harwood et al. 2003). For example, under laboratory conditions, *O. niloticus* can prevent subordinate conspecifics from accessing food patches (Barreto et al. 2006). Mesocosm experiments revealed that *O. niloticus* can reduce the growth rate of native species with a similar diet when they are cultured alongside one another (Gu et al. 2015), highlighting the potential for a positive feedback loop between growth rate and competitive dominance. In such a mechanism, the effects of dominance could reduce access to resources, slowing growth rate, heightening size differences between species and increasing dominance by *O. niloticus* in future interactions. The tendency for tilapia to move from shallow shelter areas to open water as their body-size increases may present one mitigating factor by reducing competition over shelter when body-size discrepancies become more apparent (Lowe-McConnell 2000). However, further evidence describing the extent of resource overlap between *O. niloticus* and native species at different body-sizes and in different habitat types is imperative in order to make more detailed predictions regarding the outcomes of competitive dominance by *O. niloticus*.

Here we show that *O. niloticus* dominate competitive encounters with *O. amphimelas*, providing the first experimental evidence that competition with *O. niloticus* may result in harmful consequences

for native tilapia species, as has been theorised (Goudswaard et al. 2002; Canonico et al. 2005). Behavioural studies such as this one can provide direct evidence for mechanisms which may underpin negative effects of *O. niloticus* on native species. Such evidence is highly important in order to design effective management strategies, allocate resources, and implement policy decisions surrounding *O. niloticus* invasions. Ideally, these investigations would be undertaken *in situ*, however this is often not feasible due to limitations in visibility, especially in turbid or vegetated areas. As a result, laboratory experiments must be designed to most closely and accurately replicate natural conditions, and to preserve the relevant behaviours of experimental subjects. With this in mind, the interpretation of results from this study could benefit from two key areas of research. Firstly, field survey data on the resource use of *O. niloticus* and native tilapia would improve the accuracy of predictions about the implications of the behavioural mechanisms demonstrated here. Secondly, studying the behaviour of *O. niloticus* and *O. amphielmas* *in situ* or with wild caught individuals could help to compare the differences between captive and wild phenotypes, and serve to verify the use of laboratory-reared individuals in studies of invasive species. Given the results of our experiment, the potential severity of competitive dominance by invasive species, and the threatened status of many indigenous tilapia, future research in these two areas, in conjunction with laboratory experiments, could form an important component in limiting the harmful effects of *O. niloticus* across its non-native range.

3 | Simulated encounters with a novel competitor reveal the potential for maladaptive behavioural responses to invasive species



Juvenile Nile tilapia caught during fieldwork sampling

3.1 Abstract

During the early stage of biological invasions, interactions occur between native and non-native species that do not share an evolutionary history. This can result in ecological naïveté, causing native species to exhibit maladaptive behavioural responses to novel enemies, leading to negative consequences for individual fitness and ecosystem function. The behavioural response of native to non-native species during novel encounters can determine the impact of non-native species, and restrict or facilitate their establishment. In this study we simulated novel encounters between a widespread invasive fish species, the Nile tilapia (*Oreochromis niloticus*), and a threatened native Manyara tilapia (*Oreochromis amphimelas*). In the first experiment single adult *O. niloticus* were presented with a stimulus chamber (a transparent plastic cylinder) which was empty during control trials and contained a pair of juvenile *O. amphimelas* in stimulus trials. In the second experiment, the reciprocal set up was used, with pairs of juvenile *O. amphimelas* as the focal species and adult *O. niloticus* as the stimulus. Both species approached the stimulus chamber more readily during stimulus trials, a behavioural response which would increase the prevalence of interspecific interactions *in situ*. This included physical aggression, observed from the competitively dominant *O. niloticus* towards *O. amphimelas*. Despite an initial lack of fear shown by *O. amphimelas*, close inspection of the stimulus chamber often resulted in an energetically costly dart response. Under field conditions we predict that naïve native individuals may readily approach *O. niloticus*, increasing the likelihood of interactions and exacerbating widely reported negative outcomes.

3.2 Introduction

The establishment of species in areas outside of their native range can have wide ranging effects on ecosystems (Clavero and García-Berthou 2005). In some cases, non-native species have driven population declines and extinctions of native species following their establishment (Sax and Gaines 2008), while in others they have had limited impact or even provided desirable ecosystem functions (Richardson et al. 2000; Schlaepfer et al. 2011; Anton et al. 2019). This variation in impact is thought to be determined largely by the character and strength of interactions with native species (Carthey and Banks 2014). Studying interactions between native and non-native species can therefore be used to target mitigation strategies towards the most harmful invaders (Parker et al. 1999) and to inform policy decisions which may limit the spread of species with high potential for negative impact (Kulhanek et al. 2011).

During the early stage of invasion, novel interactions occur between native and non-native species (Schlaepfer et al. 2005). This can result in ecological naïveté, where native species are unable to respond appropriately to the level of threat posed by non-native species and exhibit maladaptive behavioural responses as a result (Carthey and Banks 2014). Naïveté can affect the outcome of any antagonistic interaction, but the majority of research to date has focused on predator-prey interactions (Carthey and Banks 2014). This work has highlighted how naïveté to both non-native predators and prey can result in increased mortality for native species, impacting ecosystem function (Llewelyn et al. 2009; Robbins et al. 2013; Papacostas and Freestone 2019; Anton et al. 2020). Alternatively, misidentification of non-predatory invasive species can result in unnecessary energy expenditure or missed opportunities to forage (Carthey and Blumstein 2018). While the selective forces relating to predator-prey interactions are particularly strong, naïveté can affect the outcome of any antagonistic interaction, resulting in consequences for individual and population fitness. For example, exploitative and interference competition shape community structure within

ecosystems (Rowles and O'Dowd 2007), and naïveté to a novel competitor can influence the outcome of competitive interactions (Harrington et al. 2009; Heavener et al. 2014). Across all antagonistic interactions, maladaptive behavioural responses resulting from naïveté can lead to a number of harmful outcomes including predation (Salo et al. 2007), ingestion of toxic prey (Letnic et al. 2008), restricted access to resources (Heavener et al. 2014), increased disease and parasite transmission (Chalkowski et al. 2018), and hybridisation (Bleeker et al. 2007). These outcomes are closely linked to fitness and survival, and the need to explore how naïveté influences a wide range of antagonistic interactions has been highlighted in several publications (Carthey and Banks 2014; Heavener et al. 2014).

The initial stages of invasion are thought to play a particularly important role in determining the outcome of biological invasions (Chapple et al. 2012). This heightens the importance of early encounters, where an appropriate response to novel species may limit the establishment and impact of these non-native species, while maladaptive behavioural responses may lead to harmful outcomes for native species and facilitate the spread of the non-native species. Furthermore, naïveté is highest during early-encounters, limiting the available time for adaptive changes in response to non-native species (Phillips and Shine 2004). The majority of research on biological invasions has focused on well-established invasive populations, yet it is estimated that less than 10% of non-native species that are released will become established (Kolar and Lodge 2001). Research into interactions between native and non-native species during early encounters may therefore improve understanding of the mechanisms that govern success and failure at the establishment step of invasions. Investigating such interactions in the field is challenging because the precise timing of non-native species introductions is not typically known, and so interactions between native and non-native species have already occurred frequently before they can be investigated. Experimental approaches can therefore be used to investigate interspecific interactions which allows for control of prior experience.

In this study, we simulated novel interactions between a widespread invasive species (Nile tilapia *Oreochromis niloticus*) and a threatened tilapia native to Tanzania (Manyara tilapia *Oreochromis amphimelas*). *Oreochromis niloticus* has been introduced to several catchments containing *O. amphimelas*, and the two can be found in sympatry (Shechonge et al. 2019). Currently, little is known about the timing and history of this specific invasion, and limited research has been conducted on the natural history of *O. amphimelas*. However, both *O. niloticus* and *O. amphimelas* are mouthbrooders and are largely microphagous (Trewavas and Fryer 1965), suggesting strong resource overlap and the potential for competition over the limited resources of food and spawning areas. There is limited evidence for predation of native congeners by *O. niloticus*, yet the potential for harmful effects resulting from interactions with *O. niloticus* has been documented for many species (Martin et al. 2010; Sanches et al. 2012), including *O. amphimelas* (Champneys et al. 2020; Wing et al. 2020). For example, *O. niloticus* and *O. amphimelas* have been shown to compete aggressively for shelter under experimental conditions, with *O. niloticus* dominating these interactions (Champneys et al. 2020). Thus, naivete during early encounters with this novel competitor could lead to harmful outcomes for naïve *O. amphimelas in situ*.

In the first experiment, we presented an adult *O. niloticus* with a pair of juvenile *O. amphimelas* in a clear chamber, enabling us to determine how *O. niloticus* may first respond to native species when entering a novel habitat. Based on previous research we predicted *O. niloticus* to boldly approach the stimulus area and show aggression towards the chamber when it contained *O. amphimelas*. In the second experiment we presented pairs of juvenile *O. amphimelas* with an adult *O. niloticus* in a clear chamber, providing insight into the response of *O. amphimelas* as a naïve native species. Little is known about how native tilapia species respond to novel competitors and collectively the results of the two experiments allow discussion of the potential impact that different behavioural responses could have on the fitness and survival of the native species.

3.3 Methods

3.3.1 Experimental subjects and housing

Oreochromis niloticus subjects were purchased from a commercial aquaculture supplier (Fish Farm UK, London) and *O. amphimelas* subjects were reared at the University of Bristol and were second generation from wild caught stock. Wild individuals were originally caught from Lake Manyara (3°36'29.5"S 35°49'01.2"E, precise coordinates unknown). *Oreochromis niloticus* are typically introduced via intentional stocking events, or through unintentional release from aquaculture (Canonico et al. 2005). Therefore, we used adult *O. niloticus* from aquaculture stock in this experiment which are likely to be the first individuals to interact with native species during the early stage of *O. niloticus* colonisation. Juvenile *O. amphimelas* from wild stock were used as this is when the size difference between the two species is largest allowing us to investigate a scenario where *O. amphimelas* may perceive *O. niloticus* as a predator. While little is known about the natural history of *O. amphimelas*, juvenile tilapias are often found in groups and therefore a pair of *O. amphimelas* was used to measure their social response to *O. niloticus* which is likely to be an ecologically relevant response to threat *in situ*. Prior to this experiment, the *O. niloticus* subjects were used in one experiment where they interacted with sized matched *O. amphimelas* for a single 15-minute trial (Champneys et al 2020), while the *O. amphimelas* had no prior experience interacting with *O. niloticus*. *Oreochromis niloticus* subjects had an average total length of 83.13 ± 7.21 mm and *O. amphimelas* had an average total length of 34.77 ± 7.26 mm.

Fish were housed in a recirculating aquarium at the University of Bristol for at least three months prior to testing. *O. niloticus* were housed in 190L tanks with 30-40 individuals per tank and *O. amphimelas* were housed in 90L housing tanks with 100-110 individuals per tank. Housing tanks contained a variety of plastic plants and pipes to provide enrichment, reduce stereotypic behaviours and improve welfare (Favero Neto and Giaquinto 2020). Both species were fed daily

with a mixed diet consisting of chopped prawn, brine shrimp, mysis shrimp and vegetable diet (Tropical Marine Centre, Chorley Wood, UK), ZM Large Premium Granular feed (Tecniplast, London, UK), TetraMin flake (Tetra, Melle, Germany), frozen bloodworm (CC Moore & Co, Templecome, UK) and Gamma TM Krill Pacifica. On trial days, fish were only fed after experiments finished to standardise hunger during trials. Housing tanks were maintained at a temperature of 26-28°C. Lighting conditions were 12:12h light : dark cycle to replicate conditions in the tropics. Trials took place during October and November 2019.

3.3.2 Experimental set up and protocol

A single experimental tank (136 cm long x 72 cm wide x 40 cm high) was divided lengthways with a 136 cm long x 30 cm high opaque central divider into two identical experimental sections (Figure 3.1). The divider was a fixed structure sealed to the tank to prevent water movement between the sections. Each section was filled daily with 49L of housing tank water to a depth of 10cm. Each section of the tank had two distinct zones and three virtual zones which were added digitally for the purposes of analysis (Figure 3.11). The shelter zone (21cm x 36cm) was situated at one of the long ends of the tank, covered by a 5mm black mesh plastic sheet 20cm above the water level. At the opposite end was the stimulus zone (32cm x 36cm), which had a clear plastic cylinder in its centre (16cm diameter x 25 high) which was filled with water to the same height as the rest of the tank. The section in between contained the three additional virtual zones (zone 1: 19cm x 36cm, zone 2: 26cm x 36cm and zone 3: 27cm x 36cm). A water heater and filter (Eheim 2213) were used to maintain water quality and temperature and were switched off during trials to avoid disturbance, with water temperature remaining at a constant 26°C.

The experimental setup was used to run two separate experiments concurrently. In experiment one, a single *O. niloticus* was the focal subject, and the clear cylinder in the stimulus zone contained two size-matched *O. amphimelas* in stimulus trials and was empty in control trials. In experiment

two, a size-matched pair of *O. amphimelas* were the focal species, with one randomly designated to be the focal subject and have its behaviour monitored. The clear cylinder contained a single *O. niloticus* in the stimulus trials and was empty in control trials. Experiment one took place in section A and experiment two in section B (Figure 3.1). Stimulus individuals were always focal individuals from the previous trial so were partially acclimatised to the arena. Each trial day thus began with a control treatment in both experiments. From the first experiment of the day onwards, control or stimulus treatments were randomly selected before each trial at a ratio of two stimulus trials to one control trial. This ratio was used to increase the sample size of stimulus trials, allowing separate analyses on this subset of the data to be run with sufficient statistical power, which included the size of the stimulus fish as a covariate.

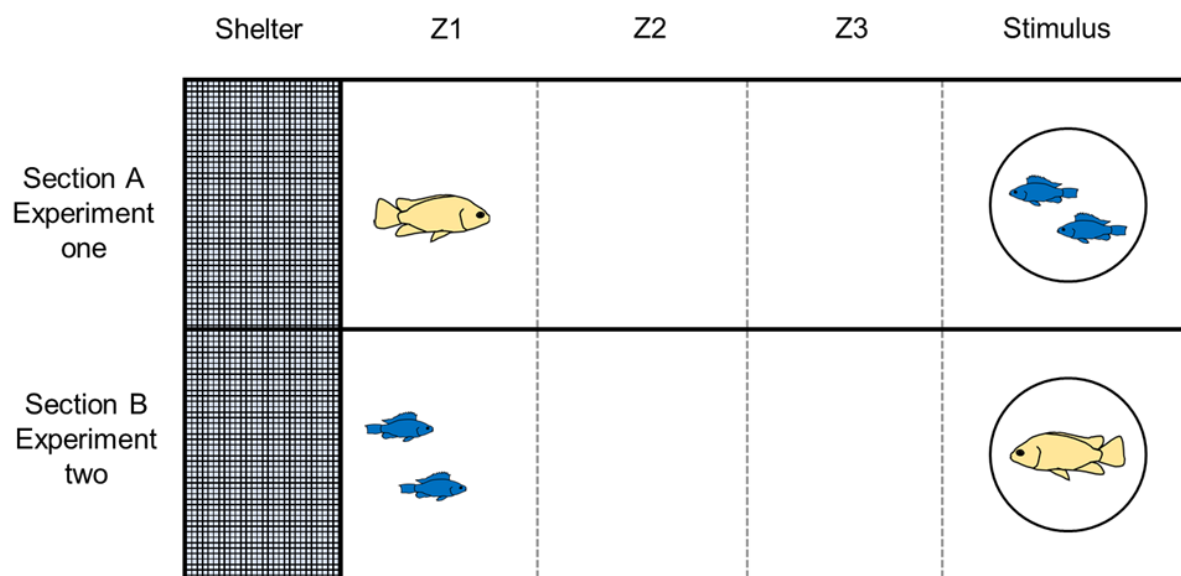


Figure 3.1 Overhead view of the experimental set up used in experiments one and two (not to scale). The tank was split lengthways using an opaque divider. Z1, Z2 and Z3 represent the three virtual zones in the centre of the tank which were used for video analysis dashed grey lines represent digital divisions black circles represent the transparent cylinders which contained the stimulus subjects in stimulus trials and were empty in control trials

At the start of each trial, focal individuals from the previous trial were either placed in the clear cylinder of the other section (for stimulus trials) or placed into dedicated housing tanks to avoid reusing individuals (for control trials). Focal individuals were then netted haphazardly from their housing tank using a hand net and transferred to the shelter zone of the experimental tank. Once all fish for both experiments had been transferred, the 12-minute recording period began. After use as either focal or stimulus, all individuals had their total length measured using callipers and were transferred to the housing tanks for used fish.

3.3.3 Video recording and analysis

The 12-minute trial was recorded by an overhead GoPro Hero 6 camera (linear field of view, 30 frames per second, 1280 x 720 resolution, GoPro Inc., San Mateo, CA, USA) situated 76cm above the water surface. This filmed both experiments in a single video. Each video was analysed separately for each experiment using BORIS software v 9.7.4 by two observers (Friard and Gamba 2016). Each observer scored a separate set of videos which were allocated randomly. The mean values of the two datasets were compared to identify clear inconsistencies which we did not find. However, we cannot rule out the possibility of unconscious bias given that the two reviewers were not blind to treatment. This could be improved by having a subset of videos analysed by an additional reviewer who was unaware of the hypotheses of the experiment. The results from this reviewer and those of the two non-blind reviewers could then be tested for repeatability. This software is used manually by the observer to record a previously defined ethogram of behaviours as either single time point or state events. In experiment two, one of the two *O. amphimelas* was randomly assigned as the test fish to be monitored and only the behaviour of this individual was recorded. The ethogram of behaviours was designed based on similar studies of fish behaviour and the full definition of each behaviour can be found in Table 3.1. In experiment one, the number of times the focal *O. niloticus* approached the stimulus, circled the stimulus, or showed aggression towards the stimulus was recorded. In experiment two, the number of times the focal *O. amphimelas*

approached the stimulus and showed a flight response (i.e. darted away) from the stimulus was recorded. The behaviours recorded differed between the species due to unique behaviours being exhibited by each species. Flight responses were not exhibited by *O. niloticus* and circle and aggression behaviours were not exhibited by *O. amphimelas*. The time taken for each of these behaviours to first occur in each trial was also recorded for both experiments. In both experiments, the location of the focal subject (shelter, zone 1, zone 2, zone 3 or stimulus zone) was recorded throughout the trial, and this provided the time taken to first leave the shelter and the time taken to first enter the stimulus zone. In experiment two, the duration of time that the focal *O. amphimelas* spent in the same zone as the other *O. amphimelas* individual was recorded to provide a measure of sociability. The duration of time spent stationary by the focal individual was also recorded in both experiments to provide a measure of activity. The behaviours recorded are relevant in the context of ecological naivete and interactions between competitors because a willingness to approach a novel competitor would increase the frequency of interactions while avoidance behaviour would decrease the frequency of interactions. Thus, approach and dart events along with the time taken to leave the shelter and enter the stimulus zone provided a measure of this willingness to approach the novel species. Additionally, circling and aggressive behaviours provided a measure of the likely outcome of interactions between the species, if the plastic chamber had not prevented physical contact. The final data set included 79 trials for experiment one (53 stimulus, 23 control), and 74 for experiment two (49 stimulus, 25 control), after one from experiment one and six from experiment two were discarded due to video recording failures.

Table 3.1 Definitions of behaviours recorded from the videos

Behaviour	Definition	Used in
Approach	The focal subject swims towards the stimulus until it is within less than one of its own body lengths (Frommen et al. 2009; Bevan et al. 2018)	Experiment one and experiment two
Circle	The focal subject circles around the entire circumference of the stimulus	Experiment one
Aggression	The focal subject swims rapidly towards the stimulus until it makes contact with the stimulus chamber (Ros et al. 2006; Champneys et al. 2018; Szopa-Comley et al. 2020)	Experiment one
Dart	The focal subject swims rapidly away from the stimulus (Kimbell and Morrell 2015)	Experiment two

3.3.4 Statistical analysis

All analyses were conducted using R version 3.6.3 (R Core Team 2020). In both experiments, the time to response variables were analysed using Cox Proportional-Hazards Models using the ‘*coxph*’ function in the package ‘*survival*’ (Therneau 2020). This tests how the likelihood of an event is influenced by a set of risk factors at any given time over the measured duration. In experiment one, the events were the time taken to first enter the stimulus zone, approach the stimulus, and circle the stimulus. In experiment two, the events were the time taken for the focal subject to first leave the shelter, enter the stimulus zone, and approach the stimulus. In both experiments, the measured duration was the length of the experiment (720 s). The risk factors were the fixed effects which were treatment (control or stimulus) and test subject body size. In experiment one, subject body size was the total length of the focal *O. niloticus*, in experiment two it was the mean total length of the *O. amphimelas* pair. The data were then subset to only include stimulus trials, and the same dependent variables were analysed using the same method, removing the treatment term but

including stimulus body size as a model covariate to assess whether behavioural responses were influenced by the size of the stimulus fish.

The proportional hazards assumption is a central assumption of these models, and we tested it using the *cox.zph* function which revealed no violation for any of the models. The '*ggcoxdiagnostics*' command in the package '*survminer*' was used to test for influential observations (Kassambara et al. 2019) and this assumption was satisfied in all cases. To test for non-linearity, models were run with a smoothing parameter fitted to the linear covariate focal subject body size using a penalised spline method with the function *pspline* function in the package '*survival*'. This method smooths covariates which have non-linear effects on the dependent variable, improving the evaluation of the relationship between the predictor variable and the dependent variable. Models with and without spline terms were then compared using AICc (Akaike Information Criterion corrected (AICc) for small sample sizes) with the function *AICctab* in package '*bbmle*' (Bolker, 2020; following methods in Roshani and Ghaderi, 2016). In all cases, models without the spline term were more likely, confirming that fitting linear relationships was more appropriate. Packages '*survival*' and '*survminer*' were used to visualise the results.

In experiment one, the time spent stationary (a measure of activity) by the *O. niloticus* and the number of approach, circle and aggression events were analysed using Generalised Linear Models (GLMs). A Gaussian family was used for activity using the *glm* function with the default family, while a negative binomial family was used for approach, circle and aggression events using the *glm.nb* function in the package '*MASS*' (Venables and Ripley 2002). In experiment two, the time spent in the same zone by the two *O. amphimelas* (sociability), the time spent stationary by the focal *O. amphimelas* (activity), the number of approach events, and whether the trial contained at least one dart event, were also analysed using GLMs. A Gaussian family was used for sociability, a binomial family was used for dart events and a negative binomial family was used for activity and

approaches. In both experiments, treatment (stimulus or control), focal body size (total length of the focal *O. niloticus* or mean total length of the *O. amphimelas* pair) and an interaction term between these two variables were included as covariates. In order to assess the effect of stimulus body size within stimulus trials, the data were then subset to only include stimulus trials. With this dataset, the same four dependent variables per experiment were analysed using GLMs, and in both experiments, the body size of the stimulus fish, focal body size, and an interaction term including these two variables were included as covariates. For this analysis, negative binomial GLMs were used for all four dependent variables in experiment one. In experiment two, negative binomial GLMs were used for sociability, activity and number of approaches while GLMs with a binomial family were used for dart events.

The default link functions were used in each GLM. Plots of fitted values versus residuals and the distribution of residuals versus a normal distribution using a QQ plot were used to test the model assumptions in the Gaussian models. The overdispersion statistic was calculated for the negative binomial models to ensure it fell between 0.5 and 2, indicating that there was no overdispersion. Once these assumptions were satisfied, a set of models containing all combinations of the covariates and their interaction terms were run. These models were compared based on the Akaike information criterion corrected for small sample sizes (AICc) using the function *AICcTab* to determine the models with most support. A difference in AICc between two models greater than two (i.e. $\Delta AICc > 2$) indicates strong support for the model with lower AICc being the more likely model given the data (Burnham and Anderson 2004). Akaike weights were also calculated, these provide a conditional probability for each model further aiding interpretation of the level of support for each model (Wagenmakers and Farrell 2004). Therefore, by running a set of models with and without explanatory variables of interest, the variables which provide the best explanation of variation in the dependent variable can be inferred.

The chosen response variables in this study allowed us to consider a suite of potential behavioural responses to the stimulus chamber. We deemed that our null hypotheses, namely that there was no difference in a given behavioural response between control and stimulus trials, was sufficiently unique across our response variables that we did not require correction for multiple testing. However, we acknowledge that correction in the cases where response variables were most indistinct, i.e. the number of approach and circle events, may have reduced the rate of type one error in this analysis.

3.4 Results

3.4.1 Experiment one

Time taken to enter the stimulus zone, approach the stimulus and circle the stimulus

The likelihood of the focal *O. niloticus* entering the stimulus zone did not differ significantly between trials with *O. amphimelas* as the stimulus and control trials without *O. amphimelas* (Cox Proportional-Hazards model, $\text{coef} = 0.51$, $\exp(\text{coef}) = 1.66$, lower 95% CI = 0.96, upper 95% CI = 2.86, $P = 0.069$; Figure 3.2a). However, *O. niloticus* were more likely to approach ($\text{coef} = 0.7$, $\exp(\text{coef}) = 2.01$, lower 95% CI = 1.11, upper 95% CI = 3.65, $P = 0.021$; Figure 3.2b) and circle ($\text{coef} = 2.1$, $\exp(\text{coef}) = 8.14$, lower 95% CI = 1.93, upper 95% CI = 34.4, $P = 0.004$; Figure 3.2c) the stimulus in trials with *O. amphimelas*. The size of the *O. niloticus* did not affect the likelihood of entering the stimulus zone ($\text{coef} = 0.01$, $\exp(\text{coef}) = 1.01$, lower 95% CI = 0.98, upper 95% CI = 1.05, $P = 0.53$), approaching ($\text{coef} = 0.006$, $\exp(\text{coef}) = 1.01$, lower 95% CI = 0.97, upper 95% CI = 1.05, $P = 0.76$) or circling the stimulus ($\text{coef} = -0.03$, $\exp(\text{coef}) = 0.97$, lower 95% CI = 0.92, upper 95% CI = 1.03, $P = 0.31$). In the stimulus trials only, the likelihood of entering the stimulus zone ($\text{coef} = 0.01$, $\exp(\text{coef}) = 1.01$, lower 95% CI = 0.97, upper 95% CI = 1.06, $P = 0.48$), approaching the stimulus ($\text{coef} = 0.01$, $\exp(\text{coef}) = 1.01$, lower 95% CI = 0.97, upper 95% CI = 1.06, $P = 0.63$), and circling the stimulus ($\text{coef} = -0.008$, $\exp(\text{coef}) = 0.99$, lower 95% CI = 0.94, upper 95% CI = 1.05, $P = 0.78$) was not significantly affected by the mean body size of the *O. amphimelas* pair used as the stimulus.

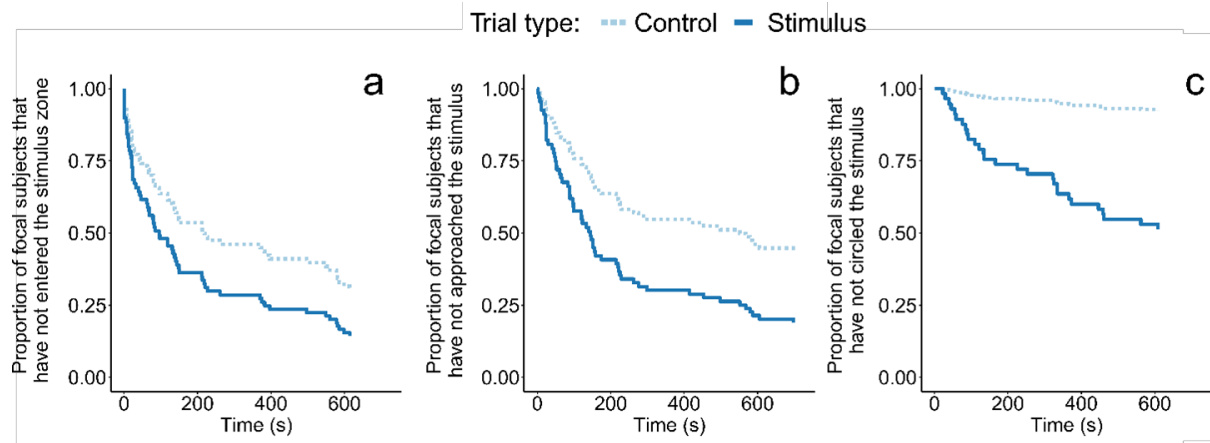


Figure 3.2 The likelihood of *O. niloticus* a) entering the stimulus zone, b) approaching the stimulus, and c) circling the stimulus in control and stimulus trials in experiment one.

3.4.1.1 Activity

For the time spent stationary, AICc values and Akaike weights indicated strongest support for the model containing only focal body size as a covariate (Table 3.2), suggesting that activity levels were not significantly different between treatments (Figure 3.3a). In the stimulus trials only, AICc values and Akaike weights also indicated strong support for the model containing only focal body size, suggesting no effect of stimulus body size on the time spent stationary by *O. niloticus* (Table 3.3). In both analyses, larger fish were shown to have lower activity than smaller fish.

3.4.1.2 Approach events

AICc values and Akaike weights indicated strongest support for the model containing only treatment as a fixed effect (Table 3.2), where approaches were more frequent toward the *O. amphimelas* than the empty cylinder in the control trials (Figure 3.3b). In the stimulus trials only, AICc values and Akaike weights indicate support for the model containing the interaction term between stimulus body size and focal body size. Support for this model suggests that the effect of stimulus body size on the number of approaches may vary depending on focal body size. However, the model including only stimulus body size, also has strong support ($\Delta\text{AICc} < 2$), where the number of approaches increased as stimulus body size increased (Table 3.3).

3.4.1.3 Circle events

AICc values and Akaike weights indicated strongest support for the model containing only treatment as a fixed effect (Table 3.2), where the circle events were more frequent in stimulus than control trials (Figure 3.3c). In the stimulus trials only, AICc values and Akaike weights indicated strong support for the null model (Table 3.3).

3.4.1.4 Aggression events

AICc values and Akaike weights indicated strongest support for the model containing treatment and focal body size as fixed effects (Table 3.2). Strong support is also given to the model containing only treatment ($\Delta\text{AICc} < 2$), with the aggression events being far more frequent in the stimulus than the control trials (Figure 3.3d). In the stimulus trials only, AICc values and Akaike weights indicated strong support for the model containing focal body length as a covariate (Table 3.3), although there was also strong support for the null model ($\Delta\text{AICc} < 2$), suggesting that focal and stimulus body sizes had weak effects on the number of aggression events in the stimulus trials (Table 3.3).

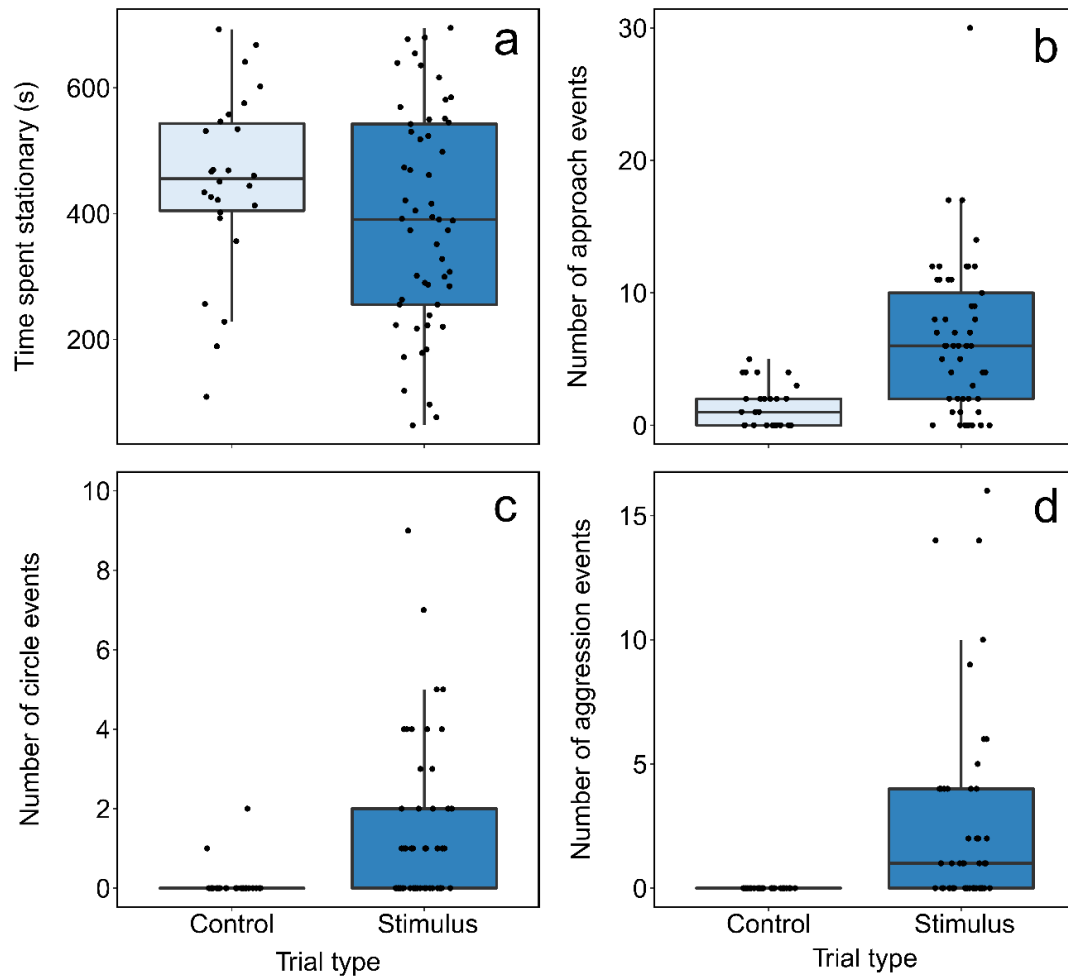


Figure 3.3 Box plots showing a) time spent stationary, b) number of approach events, c) number of circle events, and d) number of aggression events by the focal *O. niloticus* subject in experiment one. The distance between the top and bottom of each box represents interquartile range, whiskers extend to data points within 1.5 times the interquartile range, and the line through the centre of each box represents the median. Individual data points are scattered over their corresponding treatment with added jitter for clarity and those above or below the whiskers represent outliers.

Table 3.2 Model comparisons for GLMs used in experiment one to analyse how the dependent variables of *O. niloticus* activity, approach, circle, and aggression were affected by five different combinations of the fixed effects treatment and focal fish body size.

Dependent variable	Model	Explanatory variables	AICc	Δ AICc	df	Akaike weight
Activity (Gaussian)	m4	Focal body size	1031.4	0	3	0.32
	m2	Treatment + Focal body size	1032	0.5	4	0.25
	m1	Treatment * Focal body size	1032.4	1	5	0.20
	m3	Treatment	1033.5	2	3	0.12
	m5	Null	1033.6	2.2	2	0.11
Approach events (Negative binomial)	m3	Treatment	403.1	0	3	0.48
	m2	Treatment + Focal body size	403.6	0.5	4	0.37
	m1	Treatment * Focal body size	405.4	2.3	5	0.15
	m4	Focal body size	420.3	17.2	3	<0.001
	m5	Null	421.4	18.3	2	<0.001
Circle events (Negative binomial)	m3	Treatment	193.2	0	3	0.53
	m2	Treatment + Focal body size	194.1	0.9	4	0.34
	m1	Treatment * Focal body size	195.9	2.7	5	0.13
	m4	Focal body size	206.4	13.3	3	<0.001
	m5	Null	206.7	13.6	2	<0.001
Aggression events (Negative binomial)	m2	Treatment + Focal body size	205.9	0	4	0.51
	m3	Treatment	206.8	0.9	3	0.33
	m1	Treatment * Focal body size	208.1	2.3	5	0.16
	m4	Focal body size	231	25.2	3	<0.001
	m5	Null	232.7	26.8	2	<0.001

Table 3.3 Model comparisons for GLMs used in experiment one to analyse how the dependent variables of *O. niloticus* activity, approach, circle, and aggression events were affected by five different combinations of the covariates stimulus body size and focal body size in the stimulus trials only.

Dependent variable	Model	Explanatory variables	AICc	Δ AICc	df	Akaike weight
Activity (Negative binomial)	m4	Focal body size	701.2	0	3	0.5
	m2	Stimulus body size + Focal body size	703.2	2	4	0.18
	m5	Null	703.3	2.1	2	0.17
	m3	Stimulus body size	704.7	3.6	3	0.08
	m1	Stimulus body size * Focal body size	705.3	4.1	5	0.06
Approach events (Negative binomial)	m1	Stimulus body size * Focal body size	311.5	0	5	0.37
	m3	Stimulus body size	312.9	1.4	3	0.19
	m2	Stimulus body size + Focal body size	312.9	1.4	4	0.19
	m5	Null	313.6	2	2	0.13
	m4	Focal body size	313.7	2.2	3	0.12
Circle events (Negative binomial)	m5	Null	172.2	0	2	0.39
	m4	Focal body size	172.7	0.5	3	0.31
	m3	Stimulus body size	174.3	2.1	3	0.14
	m2	Stimulus body size + Focal body size	174.6	2.4	4	0.11
	m1	Stimulus body size * Focal body size	176.6	4.4	5	0.04
Aggression events (Negative binomial)	m4	Focal body size	203.8	0	3	0.36
	m5	Null	204.7	0.9	2	0.23
	m2	Stimulus body size + Focal body size	205.1	1.3	4	0.18
	m3	Stimulus body size	206	2.2	3	0.12
	m1	Stimulus body size * Focal body size	206.2	2.3	5	0.11

3.4.2 Experiment two

3.4.2.1 Time taken to leave the shelter, enter the stimulus zone, and approach the stimulus

The likelihood of the focal *O. amphimelas* individual leaving the shelter did not differ significantly between stimulus and control trials (Cox Proportional-Hazards model, $\text{coef} = 0.48$, $\exp(\text{coef}) = 1.62$, lower 95% CI = 0.98, upper 95% CI = 2.69, $P = 0.058$; Figure 3.4a). However, *O. amphimelas* were significantly more likely to enter the stimulus zone ($\text{coef} = 0.55$, $\exp(\text{coef}) = 1.73$, lower 95% CI = 1.01, upper 95% CI = 2.99, $P = 0.045$; Figure 3.4b) and approach the stimulus ($\text{coef} = 1.41$, $\exp(\text{coef}) = 4.11$, lower 95% CI = 2.08, upper 95% CI = 8.11, $P < 0.001$; Figure 3.4c) in trials with *O. niloticus* as the stimulus. The mean body size of *O. amphimelas* pairs had no significant effect on the likelihood of leaving shelter ($\text{coef} = 0.03$, $\exp(\text{coef}) = 1.03$, lower 95% CI = 1, upper 95% CI = 1.07, $P = 0.064$), entering the stimulus zone ($\text{coef} = 0.02$, $\exp(\text{coef}) = 1.02$, lower 95% CI = 0.98, upper 95% CI = 1.05, $P = 0.33$), or approaching the stimulus ($\text{coef} = 0.02$, $\exp(\text{coef}) = 1.02$, lower 95% CI = 0.99, upper 95% CI = 1.06, $P = 0.21$). In the stimulus trials only, the body size of stimulus *O. niloticus* did not affect the likelihood of leaving shelter ($\text{coef} = -0.0007$, $\exp(\text{coef}) = 0.99$, lower 95% CI = 0.97, upper 95% CI = 1.03, $P = 0.96$), entering the stimulus zone ($\text{coef} = -0.0008$, $\exp(\text{coef}) = 0.99$, lower 95% CI = 0.96, upper 95% CI = 1.04, $P = 0.97$), or approaching the stimulus ($\text{coef} = -0.006$, $\exp(\text{coef}) = 0.99$, lower 95% CI = 0.96, upper 95% CI = 1.03, $P = 0.77$).

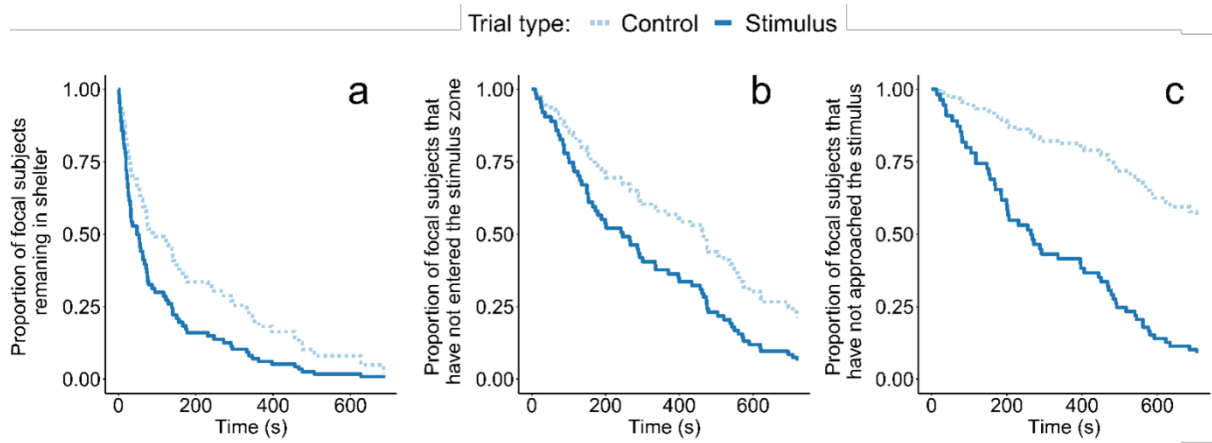


Figure 3.4 The likelihood of *O. amphimelas* a) leaving the shelter, b) entering the stimulus zone, and c) approaching the stimulus in control and stimulus trials in experiment two.

3.4.2.2 Sociability

AICc values and Akaike weights indicated strong support for the null model (Table 3.4), suggesting no effect of treatment or focal body size on the time spent in the same zone by *O. amphimelas* subjects (Figure 3.5a). In the stimulus trials only, AICc values and Akaike weights also indicated strong support for the null model, suggesting no effect of stimulus body size on the sociability of *O. amphimelas* pairs (Table 3.5).

3.4.2.3 Activity

For the time spent stationary, AICc values and Akaike weights indicated strongest support for the model containing only treatment as a fixed effect (Table 4), however, the AICc of the null model was within 1.2 units suggesting only weak evidence for the main effect of treatment (Figure 3.5b). In the stimulus trials only, AICc values and Akaike weights indicated strongest support for the null model, suggesting no effect of stimulus body size on the time spent stationary by focal *O. amphimelas* pairs (Table 3.5).

3.4.2.4 Approach events

The model with treatment and focal body size as fixed effects had the strongest support according to the AICc (Table 3.4), where approach events were significantly more likely in stimulus trials (Figure 3.5c), and the size of focal *O. amphimelas* significantly affected the number of approaches, with larger *O. amphimelas* making more approaches. In the stimulus trials only, AICc values and Akaike weights indicated strong support for the model containing only focal body size as a covariate. This further highlights the significant effect of focal subject body size on the number of approach events, while suggesting no effect of stimulus body size (Table 3.5).

3.4.2.5 Dart events

In the models for the likelihood of a dart event occurring during a trial, AICc values indicated strong support for the model containing only treatment as a fixed-effect (Table 3.4), where dart events were more likely to occur in stimulus trials than control trials (Figure 3.5d). In stimulus trials, AICc values and Akaike weights indicated strongest support for the null model, suggesting no effect of stimulus body size on the likelihood of dart events occurring (Table 3.5).

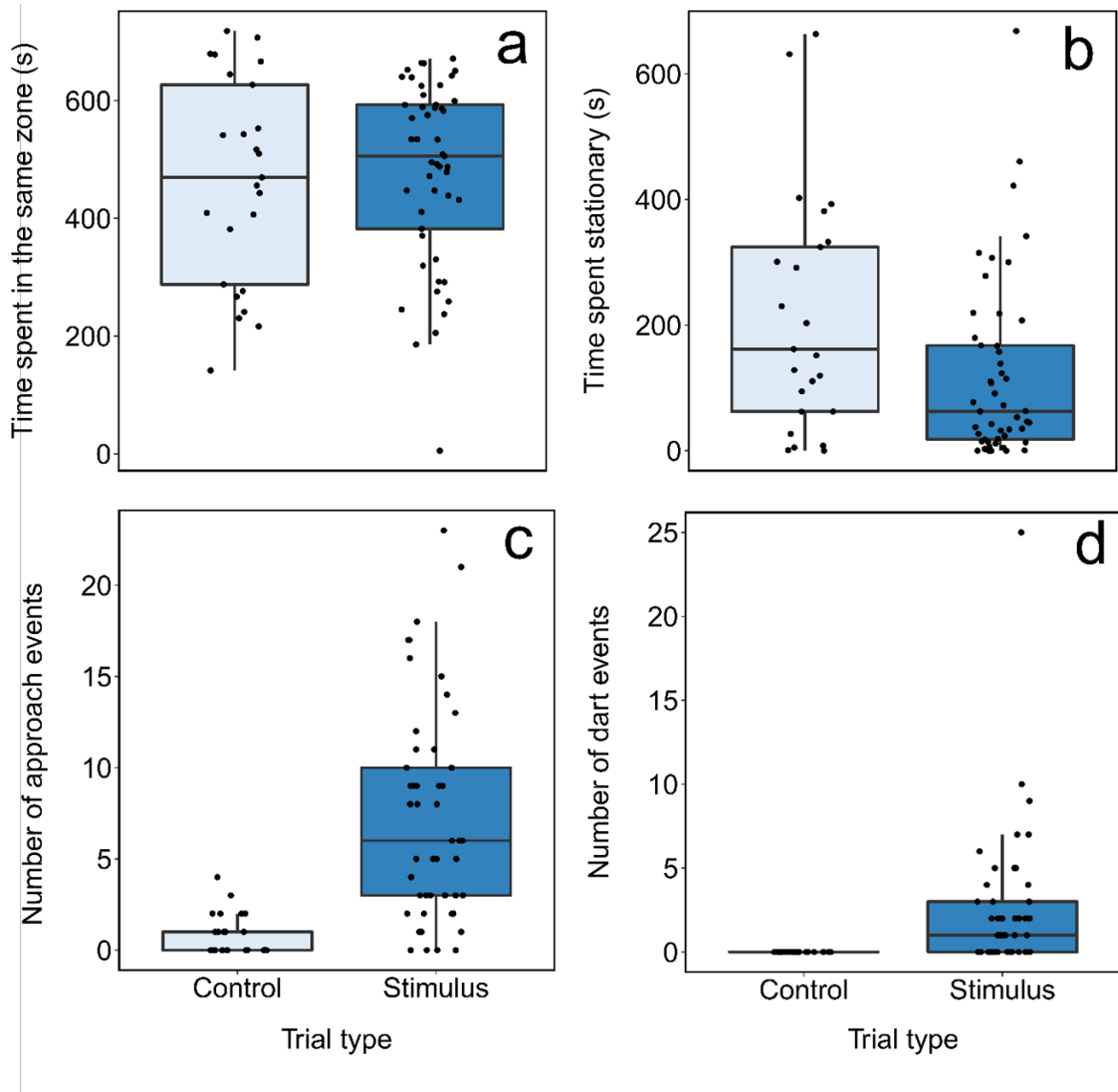


Figure 3.5 Box plots showing a) time spent by *O. amphimelas* in the same zone with the conspecific, b) time *O. amphimelas* spent stationary, c) number of approach events by *O. amphimelas*, and d) number of dart events by the focal *O. amphimelas* in experiment two. The distance between the top and bottom of each box represents interquartile range, whiskers extend to data points within 1.5 times the interquartile range, and the line through the centre of each box represents the median. Individual data points are scattered over their corresponding treatment with added jitter for clarity and those above or below the whiskers represent outliers.

Table 3.4 Model comparisons for GLMs used to analyse how the dependent variables of *O. amphimelas* sociability, activity, dart events and approach events were affected by five different combinations of the fixed effects treatment and focal body size.

Dependent variable	Model	Explanatory variables	AICc	Δ AICc	df	Akaike weight
Sociability (Gaussian)	m5	Null	965.1	0	2	0.52
	m4	Focal body size	967	1.9	3	0.2
	m3	Treatment	967.1	2	3	0.19
	m2	Treatment + Focal body size	669.1	4	4	0.07
	m1	Treatment * Focal body size	671.4	6.3	5	0.02
Activity (Negative binomial)	m3	Treatment	878.2	0	3	0.44
	m5	Null	879.3	1.2	2	0.24
	m2	Treatment + Focal body size	880.3	2.1	4	0.15
	m4	Focal body size	880.9	2.8	3	0.11
	m1	Treatment * Focal body size	882.1	3.9	5	0.06
Number of approaches (Negative binomial)	m2	Treatment + Focal body size	352.4	0	4	0.61
	m1	Treatment * Focal body size	353.3	0.9	5	0.39
	m3	Treatment	363.8	11.4	3	0.002
	m4	Focal body size	396.9	44.5	3	<0.001
	m5	Null	405	52.6	2	<0.001
Dart events (Binomial)	m3	Treatment	68.6	0	2	0.54
	m2	Treatment + Focal body size	69.5	0.9	3	0.35
	m1	Treatment * Focal body size	71.7	3.1	4	0.11
	m4	Focal body size	102.2	33.6	2	<0.001
	m5	Null	102.7	34.1	1	<0.001

Table 3.5 Model comparisons for GLMs used to analyse how the dependent variables of *O. amphimelas* sociability, activity, dart events and approach events were affected by five different combinations of the covariates stimulus body size and focal body size in the stimulus trials only.

Dependent variable	Model	Explanatory variables	AICc	Δ AICc	df	Akaike weight
Sociability (Negative binomial)	m5	Null	667	0	2	0.54
	m3	Stimulus body size	669.1	2.1	3	0.19
	m4	Focal body size	669.1	2.2	3	0.19
	m2	Stimulus body size + Focal body size	671.3	4.3	4	0.06
	m1	Stimulus body size * Focal body size	673.7	6.8	5	0.02
Activity (Negative binomial)	m5	Null	559.1	0	2	0.5
	m3	Stimulus body size	560.5	1.4	3	0.24
	m4	Focal body size	561.3	2.3	3	0.16
	m2	Stimulus body size + Focal body size	562.8	3.8	4	0.08
	m1	Stimulus body size * Focal body size	564.8	5.8	5	0.03
Number of approaches (Negative binomial)	m4	Focal body size	291.4	0	3	0.7
	m2	Stimulus body size + Focal body size	293.8	2.3	4	0.22
	m1	Stimulus body size * Focal body size	296.1	4.7	5	0.07
	m5	Null	299.9	8.4	2	0.01
	m3	Stimulus body size	302.1	10.7	3	0.003
Dart events (Binomial)	m5	Null	66.5	0	1	0.42
	m4	Focal body size	67.4	0.9	2	0.27
	m3	Stimulus body size	68.3	1.8	2	0.18
	m2	Stimulus body size + Focal body size	69.4	2.8	3	0.1
	m1	Stimulus body size * Focal body size	71.7	5.2	4	0.03

3.5 Discussion

In experiment one, *O. niloticus* were more likely to approach and circle the stimulus when the stimulus chamber contained the *O. amphimelas* pair rather than when it was empty. A larger number of approach, circle and aggression events also occurred in these stimulus trials. Thus, despite limited experience interacting with *O. amphimelas*, *O. niloticus* were readily antagonistic towards them, suggesting that aggression towards juvenile *O. amphimelas* by adult *O. niloticus* is likely during novel encounters. In experiment two, the sociability of the *O. amphimelas* pair was unaffected by the presence of *O. niloticus*, and there was little evidence for a difference in activity between stimulus and control trials. Under the risk of potential predation, reduced activity (Sanches et al. 2015; Miyai et al. 2016; Ioannou et al. 2017) and increased sociability (Romenskyy et al. 2020) are known behavioural responses, however neither were observed in this experiment when the stimulus chamber contained the *O. niloticus*. This apparent lack of a response to the potential threat from *O. niloticus* is further highlighted by the fact that *O. amphimelas* entered the stimulus zone and approached the stimulus more readily when it contained an *O. niloticus*, suggesting that naïve individuals of native species may readily approach *O. niloticus*, a behavioural response which would increase the frequency of interactions. *O. amphimelas* also showed more frequent darting during stimulus trials but these were never observed in control trials. Darting is a rapid locomotory response which often occurs when fish are startled by a threat (McCormick et al. 2019). Thus, while *O. amphimelas* showed a lack of fear in approaching the stimulus chamber when it contained an *O. niloticus*, this often resulted in an energetically costly dart response.

The response of *O. amphimelas* in this experiment demonstrates how naïveté to a novel competitor may result in maladaptive behavioural responses by non-native species. The aggression shown by *O. niloticus* in experiment one is in accordance with previous research demonstrating the tendency of *O. niloticus* to be aggressive to other species (Martin et al. 2010; Sanches et al. 2012), including

O. amphimelas (Champneys et al. 2020). Such studies have demonstrated how interactions between *O. niloticus* and native species may lead to harmful outcomes including competition-induced restricted access to resources, increased predation, and parasite transfer (Jiménez-García et al. 2001; Martin et al. 2010). These are a consequence of direct antagonistic interactions, thus native species may reduce the impact of *O. niloticus* through avoidance behaviours. *Oreochromis amphimelas* showed a lack of threat perception in the presence of *O. niloticus* until they reached very close proximity with the stimulus chamber where they often showed darting responses (Kimbell and Morrell 2015). The stimulus chamber prevented physical aggression between the two species; however under natural conditions, such approaches would likely result in direct agonistic interactions, which could lead to injury or unnecessary energy expenditure from flight responses such as darting. Thus, the failure to detect the potential threat of *O. niloticus*, until within very close proximity, may drive harmful impacts for naïve native species.

In experiment one, larger *O. niloticus* were more active and were less aggressive towards the *O. amphimelas* pair. *Oreochromis niloticus* also approached more frequently when the *O. amphimelas* were larger. Similarly, in experiment two, larger *O. amphimelas* made more approaches in stimulus trials. Previous research has shown that *O. niloticus* are highly aggressive towards *O. amphimelas* when they are similar sizes (Champneys et al. 2020). Given that the *O. niloticus* were considerably larger than the *O. amphimelas* in this experiment, increased aggression and approaches when the two species were more similarly sized (smaller *O. niloticus* and larger *O. amphimelas*) is in accordance with prior research. *O. niloticus* are much larger than the majority of native tilapia species in Tanzania (Shechonge et al. 2019), and the results of this experiment suggest that aggression may be reduced as body size differences increase. Conservation measures may therefore be targeted towards limiting the impact of *O. niloticus* on the largest-bodied native species.

Research suggests that naïveté is thought to be lower in response to congeneric or functionally similar species (Anton et al. 2020) because native species may recognise traits which are similar to those of species to which they have had eco-evolutionary exposure (Cox and Lima 2006). The experimental subjects used in this study are congeneric tilapia species and are both maternal mouthbrooders and microphagous, suggesting functional similarity (Trewavas and Fryer 1965). This similarity may reduce naïveté and prevent maladaptive responses to invasive species. However, ecological similarity may also benefit the invasive species, by causing it to be well adapted to the novel ecosystem enabling it to exploit available resources and thrive as a result. The majority of research on this topic has focused on predator-prey naïveté, and future work could benefit understanding of how these principles may apply to naïveté of a native species towards a non-native competitor. Research has shown that *O. niloticus* can be conditioned to show an anti-predatory response to a novel predator stimulus under experimental conditions (Mesquita and Young 2007), suggesting that species within this genus may learn to recognise threats following sufficient exposure. Thus, studies assessing the cognitive abilities of native tilapia species would benefit our understanding of the impact of naïveté during invasions, given that rapid behavioural adaptation to novel enemies could greatly limit their negative impacts.

The results of this study highlight how naïveté during interactions with *O. niloticus* could drive harmful impacts for *O. amphimelas*, and other native fish species. Currently, very little is known about the timing or impacts of this specific invasion *in situ*, and this is the case for many *O. niloticus* invasions across its non-native range. Given that declines of native species following the introduction of *O. niloticus* have been reported in several areas including Tanzania (Canonico et al. 2005), future research focusing on how naïveté may impact early interactions with *O. niloticus* could help target conservation efforts towards vulnerable populations and aid policy decisions which may limit the further spread of this species.

4 | Heterospecific grouping with native species by invasive Nile tilapia: implications for invasion success, social behaviour and group decision making



Lake Rutamba, Tanzania

4.1 Abstract

During biological invasions, non-native species are typically introduced in small numbers with little to no access to information about their environment. It has therefore been suggested that following their introduction, non-native species can derive benefits from joining groups of native species with a similar ecological niche, giving them access to social information about the environment and avoiding the negative consequences associated with living in small groups. The response of native species is likely to shape the outcome of such heterospecific grouping by non-native species, and few studies have considered the costs and benefits for native species when non-native species enter these heterospecific groups. Therefore, the ability for native and non-native species to gain the benefits from group living (e.g. decreased predation risk and access to social information), while avoiding the costs (e.g. increased competition) could be a crucial determinant of establishment success and impact on native species. The Manyara tilapia (*Oreochromis amphimelas*) is a threatened tilapia species endemic to Tanzania, where it lives in sympatry with invasive Nile tilapia (*Oreochromis niloticus*), a globally widespread invasive species. In this experiment we investigated social behaviour and group decision making in groups of three *O. amphimelas* individuals across trials consisting of three parts: before, during and after the introduction of a single *O. niloticus* individual. Each trial part was comprised of a five-minute acclimatisation and a ten-minute recording period. Using a combination of tracking and observational methods, we found evidence that a large size difference between *O. amphimelas* and *O. niloticus* resulted in more frequent conspecific aggression between *O. amphimelas*. We found that both species made a similar number of successful leadership attempts in leaving the refuges, and *O. amphimelas* were just as likely to follow an unfamiliar *O. niloticus* as a familiar conspecific. Our results suggest that *O. niloticus* may readily group with unfamiliar native tilapia species following introduction which may benefit their establishment.

4.2 Introduction

The role of behaviour in biological invasions is receiving increasing attention (Chapple et al. 2012). The behavioural characteristics of invasive species, and those of the native species they encounter, affect the behavioural interactions which occur between them and ultimately the outcome of a biological invasion (Sol et al. 2002; Blackburn et al. 2009; Anton et al. 2020). Unlike morphological adaptations, behavioural changes in response to novel species can be altered by individuals over a short time frame and change the outcome of behavioural interactions (Phillips and Shine 2004). Behavioural responses to unfamiliar species often occur when species are naïve to the potential threats posed by a novel species, due to a lack of evolutionary history and learned experience (Carthey and Banks 2014). Responses by either native or introduced species can be adaptive (i.e. habitat shifts to avoid novel predators; Pangle and Peacor, 2006) or maladaptive (i.e. readily approaching a predator or dominant competitor; Champneys et al., 2022). Therefore, behavioural responses to unfamiliar species can directly affect the outcome of biological invasions (Chapple et al. 2022).

Social interactions are ubiquitous across the animal kingdom and can be mediated by complex processes or simple inter-individual interactions (Krause and Ruxton 2002). Social interactions are intrinsically linked to fitness related processes including foraging, predator avoidance and reproduction (Bush et al. 2022). As a result, changes in behaviour following anthropogenic disturbance can lead to altered social interactions, with a variety of outcomes for affected species (Fisher et al. 2021). One important aspect of social behaviour is group decision making, the use of social information to make decisions. Group decision making is closely linked to the fitness of individuals living in groups. The ability to make good decisions that result in fitness benefits depends on information (Dall et al. 2005), which can be private, collected through individual experience, or social, derived from the behaviour of group members (King and Cowlshaw 2007).

Decision making in groups can range across a spectrum from egalitarian, whereby decisions are equally distributed among group members, to leadership, whereby single or few individuals are responsible for making decisions for the group (Ioannou et al. 2015). The degree to which egalitarianism or leadership are adaptive in a given context depends on access to information, along with the motivation of individuals. In some cases, the disproportionate influence of some individuals within a group can be beneficial to all group members (McComb et al. 2001), while in others it can lead to disproportionate benefits for leading individuals (Fischhoff et al. 2007).

Non-native species introductions typically involve small numbers of individuals, with little to no experience of the habitat they are introduced to (Camacho-Cervantes et al. 2014a). As a result, non-native species can derive benefits from entering groups of native species with a similar ecological niche, giving them access to social information about the environment and avoiding the negative consequences associated with living in small groups (Camacho-Cervantes et al. 2014). A number of studies have found evidence that invasive guppies (*Poecilia reticulata*) preferentially join heterospecific groups of native species, suggesting that the benefits of grouping (i.e. decreased predation risk and access to social information) can outweigh the costs (i.e. increased competition; Camacho-Cervantes et al. 2014a, b; Santiago-Arellano et al. 2021). However, whether this is true of other widespread invasive fish species remains largely unknown. Furthermore, few studies have considered the potential costs and benefits for native species when non-native species associate with them during these early stages. Many of the mechanisms which drive the negative effects of invasive species rely on direct interactions, such as predation, competition, parasitism and hybridisation. It is therefore likely that grouping by non-native species which interact with the native species in these ways would incur heavy costs on the native species group. Grouping by non-native species would also increase the pressure on any shared resources, increasing exploitative competition possibly affecting survival. On the other hand, larger group sizes may benefit individuals by lowering predation risk (Camacho-Cervantes et al. 2014).

Given that *O. niloticus* introductions typically involve small numbers of individuals, invasion outcome could be driven in part by the personality of the introduced individuals. Variation in any of the five major axes of personality variation namely exploration, sociability, activity, aggressiveness and boldness could modify an individual's impact on a recipient ecosystem. The role of personality in invasion outcome has received some attention (Chapple et al., 2012), however few studies have tested for repeatable impacts by individual invaders on the behavioural response of native species. Studies testing for personality variation within *O. niloticus* show contrasting results, with one study finding evidence of highly repeatable inter-individual behaviours (Cerqueira et al. 2016) and another finding little evidence of personality expression in this species (Wing et al 2021). In this study we test for consistent inter-individual variation in fifteen *O. niloticus* individuals over a number of behavioural traits. By testing for consistent inter-individual variation within *O. niloticus* individuals, and testing for repeatable impacts of these individuals on the behaviour of a native species, we hope to provide unique insights into the role of personality on the invasion success and impact of *O. niloticus*.

In this experiment, we investigated the social behaviour of *O. amphimelas* across trials consisting of three parts: before, during, and after the introduction of a single *O. niloticus* individual. Each trial part was comprised of a five-minute acclimatisation and a ten-minute recording period. Using a combination of tracking and observational methods, we compared the behaviour of the three *O. amphimelas* individuals across these three trial parts to investigate how the introduction of an unfamiliar heterospecific competitor affected the social behaviour of a native species, before, during and after, its introduction into the group. We also investigated how behavioural and physical characteristics of the *O. niloticus* affected the response of *O. amphimelas* across the three trial parts. To investigate group decision making in the presence of an unfamiliar *O. niloticus*, we recorded the frequency of leadership attempts (the initiation of movement from shelter by one individual), and follower events during the second part of the trial. Finally, we tested whether *O. niloticus* showed

consistent inter-individual variation across three trials in which they were tested, and whether this resulted in consistent behavioural responses from *O. amphimelas*.

4.3 Methods

4.3.1 Housing

The *O. niloticus* subjects were the second generation from commercially bought stock and were raised in the laboratory at the University of Bristol. *O. amphimelas* subjects were reared at the University of Bristol and were second generation from wild caught stock. Wild individuals were originally caught from Lake Manyara (3°36'29.5"S 35°49'01.2"E, precise coordinates unknown), and provided by Bangor University. All fish were housed in a recirculating aquarium at the University of Bristol. *O. amphimelas* were housed in three separate 90 L tanks and *O. niloticus* were housed in a single 90 L tank. Groups of *O. amphimelas* were always taken from the same housing tank, where they had been housed for at least 6 weeks prior to the experiment, to increase familiarity between conspecifics and reduce stress (Galhardo and Oliveira 2009). Both species were housed in mixed sex tanks, and because mature *O. amphimelas* males can become territorial, those showing early signs of male colouration were identified for analysis purposes. Lighting was maintained on a 12:12h light:dark cycle to mimic natural conditions in the tropics. Fish were fed *ad libitum* daily with a mixture of ZM Large Premium Granular feed (Tecniplast, London, UK), TetraMin flake (Tetra, Melle, Germany), frozen bloodworm (CC Moore & Co, Templecombe, UK) and Gamma™ Krill Pacifica, chopped prawn, Mysis Shrimp, Brineshrimp, and Vegetable Diet (Tropical Marine Centre, Chorleywood, UK). Fish were not fed for the 24 hours prior to an experimental day and were instead fed at the end of that day.

4.3.2 Experimental setup

Two 200 L experimental tanks (135 x 36 x 20 cm length x width x height) were filled with 100 L of water from the housing tanks (temp range 24° to 26°C). Shelters consisted of artificial plastic vegetation fixed to a white plastic board as in Champneys et al. (2020). Identical shelters were also placed in each of the housing tanks to improve welfare and increase familiarity with the shelter

resource. Shelters (30 x 12 cm length x width) were placed at both ends of the experimental tanks, and the centre was left as open arena (Figure 4.1).

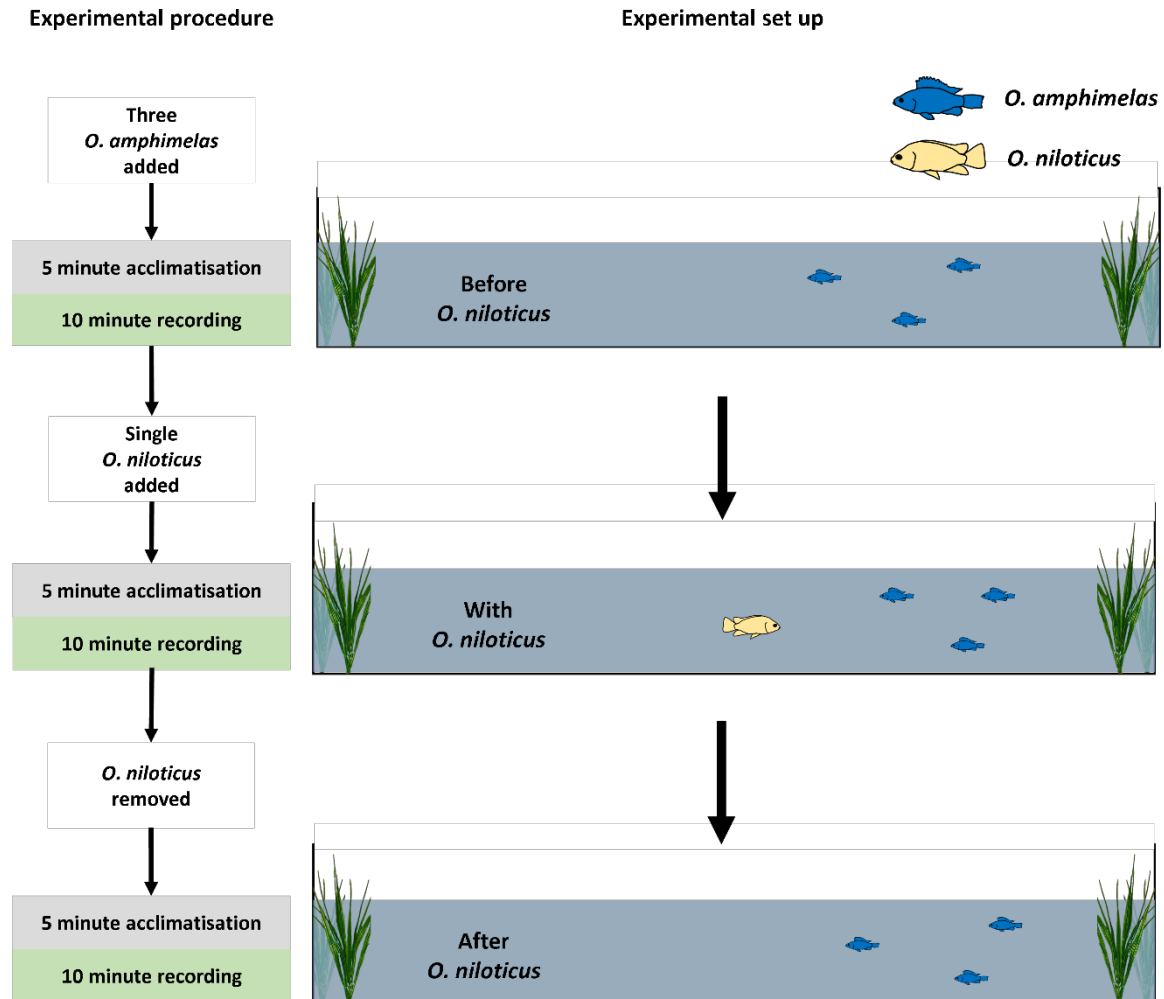


Figure 4.1 The experimental procedure and tank set up.

4.3.3 Experimental protocol

The experiment consisted of three distinct trial parts to examine the social behaviour of *O. amphimelas* with and without the presence of an unfamiliar heterospecific (Figure 4.1). At the start of each trial, one of the three *O. amphimelas* housing tanks was randomly chosen and three *O. amphimelas* were netted haphazardly from it and placed into a cylindrical transparent chamber (16 cm diameter × 25 cm high) in the centre of the experimental tank to allow for the tracking software to correctly identify each individual. At the start of the trial, the chamber was gently removed, and

the fish were able to move freely in the tank for 15 minutes. At the end of this period, a singular *O. niloticus* which had been netted haphazardly from its housing tank was placed into the centre of the experimental tank and left to move freely around the tank for the next 15 minutes. Following this, the *O. niloticus* was removed using a hand net and the *O. amphimelas* were able to move around the tank for a further 15 minutes. We note that the removal of the *O. niloticus* individual is likely to result in more disturbance than its addition into the tank, resulting in a confounding effect when considering trial part as an explanatory variable. Two identical experimental setups ran simultaneously. Following each trial, the standard length of individuals was measured using callipers and they were photographed on a polystyrene board next to a scale. *O. amphimelas* individuals were only tested once, whereas *O. niloticus* individuals were all tested once on each of the three experimental days. A combination of the total length, body depth, and distinctive markings allowed for individual identification of the 15 *O. niloticus* subjects, allowing for consistent individual differences between *O. niloticus* subjects to be investigated across the three trials. Five days elapsed between each experimental day to allow *O. niloticus* individuals time to recover following involvement in the experiment. The entire 45-minute trial of both experimental set ups was recorded from above using an overhead Panasonic HC-VX870 camcorder which was 123 cm above the experimental tanks recording in 4K (3840 x 2160 pixels) with a frame rate of 29.97 frames per second.

4.3.4 Data processing

The 45-minute recording was cut into three separate videos which were analysed individually. Part one “before *O. niloticus*” started five minutes after the *O. amphimelas* were placed into the tank and lasted 10 minutes, part two “with *O. niloticus*” started 5 minutes after the *O. niloticus* was added to the tank and lasted 10 minutes, part three “after *O. niloticus*” started five minutes after the *O. niloticus* was removed and lasted 10 minutes. Trajectory data for each fish were obtained from these three 10-minute periods using idTracker (version 2.1; Pérez-Escudero et al. 2014). These data consist of

x and y coordinate data for each individual, and the software maintains individual identities across the trial including when individuals entered then re-emerged from shelter. Thus, using individual coordinate data, it is possible to identify which individuals are in and out of shelter in a given frame. Minimum size was set to 150 pixels, the intensity threshold was set to 0.75, and the number of frames for reference was set to 5000. The shelter regions were excluded from tracking within the idTracker software. The boundaries of the shelter areas were later expanded and standardised based on defined coordinate limits. Coordinates within these defined limits were considered to be part of the shelter and replaced with NaN values. Pixels were converted to mm using a 50 mm scale present in all videos. Using ImageJ (version 1.53), three measurements in pixels of the 50 mm scale were taken from three videos per trial day and gave a mean of $81.74 \pm \text{SD } 0.62$ pixels. Given that the standard deviation was less than one pixel, a consistent pixel to mm ratio of 1:0.61 was applied to all videos.

For the analyses, we calculated the time spent in and out of shelter for each individual as a measure of refuge use and the distance travelled outside of the shelter in cm by each individual, which was divided by time out of shelter to provide mean speed in cm/s. We also recorded the number of leadership attempts from the shelters. These were defined as cases where one individual left a shelter which previously contained all individuals, i.e. the leader was initiating movement out of the shelter. Events where leaders returned to the shelter before another individual left were recorded as unsuccessful leadership attempts. The identity of the leader and the first fish to follow were recorded along with the time taken to be followed during a successful leadership attempt. In order to assess interference competition, the number of biting and chasing events was collected using BORIS version 7.13.5 by one reviewer to ensure consistency (Friard and Gamba 2016). We followed the ethogram outlined in Champneys et al. (2020), originally based on Alvarenga and Volpato (1995), where biting is defined as “the aggressor swims towards the opponent and bites” and chasing is defined as “the aggressor swims towards the opponent, while the opponent swims

away from the aggressor, without any physical contact”. For each interaction the species of the initiator and the recipient was identified, and this information was used to calculate the sum of conspecific aggressive interactions within the *O. amphimelas* group, the sum of heterospecific aggressive interactions directed by *O. amphimelas*, and the sum of heterospecific aggressive interactions directed by *O. niloticus*.

4.3.5 Statistical analysis: Changes in *O. amphimelas* behaviour across trial parts

All analyses were conducted using R version 4.2.1. In order to assess behavioural variation in *O. amphimelas* behaviour across the three trial parts, three response variables were used: mean time spent in the shelter by *O. amphimelas* individuals (seconds), mean speed of *O. amphimelas* individuals when outside the shelter (cm per second), and the total number of conspecific aggression events within the *O. amphimelas* group. For each of these three response variables, a set of mixed effects models was constructed based on *a priori* hypotheses. Every model contained the trial number nested in *O. niloticus* individual ID as the random effect as each *O. niloticus* was tested in three trials and each trial had three parts. The inclusion of these random effects allowed us to control for differences between trials and *O. niloticus* individuals that were not accounted for in the fixed effects. The mean standard length of *O. amphimelas* individuals in the trial and the time taken to catch the *O. niloticus* after part two, as a measure of experimental disturbance, were included as fixed effects in all models including the null model. Models without these effects were not included because the effect of these variables were not the hypotheses of interest in our study.

The explanatory variables included in the full model were chosen in order to test a number of *a priori* hypotheses. Another 34 models containing all possible combinations of the explanatory variables included in the full model, along with a null model, allowed us to infer which variables provided the best explanation for variation in the response variable. Models were compared based on the Akaike information criterion corrected for small sample sizes (AICc) using the *aictab* function in the package ‘*AICcmodavg*’ (Marc and Mazerolle 2020). AICc differences, referred to as

$\Delta AICc$, greater than two ($\Delta AICc > 2$) between two models indicates strong support for the model with the lower $AICc$. Models with stronger support are deemed to be more likely model given the data (Burnham and Anderson 2004).

Firstly, we predicted that the behaviour of *O. amphimelas* individuals would differ before, during and after interacting with an unfamiliar *O. niloticus*, the main hypothesis of this part of the study. Therefore, trial part (one, two or three) was included as a fixed effect. Sex differences in aggressive behaviour are often observed in *Oreochromis* species (Chifamba and Mauru 2017). Therefore, the fixed effect of the number of males in the *O. amphimelas* group was also included. We also hypothesised that a potential change in competitive motivation linked to the number of males in the group may change the response of *O. amphimelas* to *O. niloticus*, therefore, a trial part x number of males interaction term was included. We also hypothesised that certain behavioural and physical characteristics of the *O. niloticus* would affect the behavioural response of *O. amphimelas*. Aggression and size differences are known to affect the outcomes of competitive interactions between cichlid species, including *O. niloticus* (Sanches et al. 2012). Therefore, the fixed effects of the total number of aggressive interactions directed by the *O. niloticus* in part two of the trial (hereafter termed *O. niloticus* aggression) and the size difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial (hereafter termed size difference) were included. Given that in part one of the trial, *O. amphimelas* had not yet interacted with the *O. niloticus*, we expected any effect of *O. niloticus* aggression or size difference to be linked to the effect of trial part. Therefore, a trial part x *O. niloticus* aggression and trial part x size difference interaction term were included. In summary, the full model contained a trial part x number of males in the *O. amphimelas* group, a trial part x *O. niloticus* aggression, and a trial part x size difference interaction term, along with, included in all models, the fixed effects (the mean standard length of *O. amphimelas* individuals and the time taken to catch the *O. niloticus* after part two) and random effects (trial number nested within *O. niloticus* individual ID).

We tested for correlation between the explanatory variables using the *chart.Correlation* function in the package ‘*PerformanceAnalytics*’ to identify any potential for multicollinearity in our models (Carl et al. 2010). We found no significant correlation between any of our explanatory variables. For each of the three response variables, a linear mixed-effect model (LMM) using the *lmer* function in the package ‘*lme4*’ was produced using the fixed and random effects from the global model for assumption testing (as recommended in Bates et al. 2015). The *simulateResiduals* function in the package ‘*DHARMa*’ was used to produce plots of observed vs expected residuals and residuals vs fitted values to identify any violations of the assumptions of normally distributed residuals and homogeneity of variance (Hartig 2020). For the mean time spent in the shelter by *O. amphimelas* individuals, the response variable was log transformed in order to meet these assumptions. For the mean speed of *O. amphimelas* individuals, the assumptions were met without the need for transformation. For the total number of conspecific aggression events within the *O. amphimelas* group, the assumptions of LMM’s were not met and negative binomial generalised linear mixed effect models (GLMM) were used using the *glmer.nb* function. Dispersion was tested using the *testDispersion* function in the package ‘*DHARMa*’ and there was no evidence of over or under dispersion. A *bobyqa* optimization algorithm in the ‘*lme4*’ package was applied to the second phase of optimisation for these models to improve convergence. All models were fit with maximum likelihood (ML) rather than restricted maximum likelihood (REML) because every model contained different fixed effects and the REML criterion changes based on the specification different fixed effects (Faraway 2016). As a result, when likelihood-based comparison methods (including AICc) are used to compare between models with different fixed effects, it is widely recommended that ML must be used and not REML (Gurka 2006; Zuur et al. 2009; Harrison et al. 2018).

4.3.6 Statistical analysis – Leading and following behaviour from shelter of *O. niloticus* and *O. amphimelas* in part two

To assess how leadership from shelter and following behaviour varied in part two of the trial, five response variables were used: the number of leadership from shelter attempts, the number of successful leadership from shelter attempts (attempts which resulted in at least one of fish following before the leading individual returned to shelter), the proportion of leadership attempts that were successful, the time taken to be followed during a successful leadership attempt, and the number of events where an individual was the first follower. For this analysis, the data from only part two of the experiment were used. For each of the five response variables, a set of mixed effects models was constructed based on *a priori* hypotheses. Every model contained the random effect of *O. niloticus* individual ID as each *O. niloticus* individual was used in three trials. The inclusion of this random effect allowed us to control for consistent differences in individual *O. niloticus* behaviour across the three trials in which they were tested, which could not be accounted for in the fixed effects. Given the potential for misclassifying successful leadership events (derived from the tracking data from idTracker) and events where one individual chased another from the shelter (derived from the event recording software BORIS), we tested for the correlation between the number of successful leadership attempts and the number of aggressive events within a trial using the *chart.Correlation* function. We found no correlation between counts of conspecific aggressive interactions within the *O. amphimelas* group, or aggressive interactions initiated by *O. niloticus*, with the number of successful leadership attempts ($r_s = -0.26$ and $R = 0.06$, respectively). This suggests that analysing variation in leadership events would not be confounded by chasing events, and instead provide insights into variation in leadership and following behaviour.

The explanatory variables included in the global model were chosen to test a number of *a priori* hypotheses. Another 12 models containing all possible combinations of the explanatory variables included in the full model, along with a null model which contained only the random effect, allowed us to infer which variables provided the best explanation for variation in the response variable. Models were compared using the same AICc comparison method detailed above.

Individual identities were randomly assigned by the idTracker software and the *O. niloticus* individual was manually identified in each video and assigned to the fourth individual identity of the trajectory files for consistency to facilitate data processing. Individual ID was included as a fixed effect to identify whether there were differences in leading and following behaviour between *O. amphimelas* (individual ID's 1, 2 and 3) and *O. niloticus* (individual ID 4), the main hypothesis of this part of the study. We hypothesised that leading and following may vary with the size difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial (size difference), and the total number of aggressive interactions directed by the *O. niloticus* (*O. niloticus* aggression). We also hypothesised that the relationship between individual ID and our response variables may depend on the size and aggressiveness of the *O. niloticus*. For example, *O. amphimelas* may preferentially follow conspecifics when the *O. niloticus* is aggressive. To test for such interactive effects, individual ID x size difference and individual ID x *O. niloticus* aggression interaction terms were included. In summary, the full model contained individual ID x size difference and individual ID x *O. niloticus* aggression interaction terms, along with the random effect (the individual ID of the *O. niloticus*) included in all models.

For each of the five response variables an initial LMM using the 'lmer' function in the package 'lme4' was produced using the fixed and random effects from the full model for assumption testing. The 'simulateResiduals' function in the package 'DHARMa' was used to produce plots of observed vs expected residuals and residuals vs fitted values from the full model to identify any violations of the assumptions of normally distributed residuals and homogeneity of variance. For the number of leadership from shelter attempts, the number of successful leadership from shelter attempts, and the number of first follow events, GLMM's with a Poisson family were run using the 'glmer' function. Dispersion was tested using the 'testDispersion' function in the package 'DHARMa' and there was no evidence of over or under dispersion. In all cases, the assumptions were met without need for transformation of the response variable. For the number of leadership

from shelter attempts, a ‘*bobyqa*’ optimizer was applied to the second phase of optimization to improve model convergence. To analyse the proportion of leadership attempts that were successful, the response variable was created using the ‘*cbind*’ function on the number of successful leadership attempts and the number of unsuccessful leadership attempts. GLMM’s with a binomial family were used for the analysis of this variable using the *glmer* function. For the time taken to be followed during a successful leadership event, LMM’s were used, and the response variable was log transformed in order to meet the normality assumption. As above, all models were fit with ML.

4.3.7 Statistical analysis – Repeatability of *O. niloticus* behaviour, and its impacts on *O. amphimelas*

Given that each *O. niloticus* individual was tested in three trials, we hypothesised that individual-level differences between the fifteen *O. niloticus* may result in repeatable behaviour with impacts on *O. amphimelas*. In order to test whether *O. niloticus* showed repeatable behaviour across the three trials, models were created for six response variables. These were the number of aggressive interactions initiated by *O. niloticus*, the time spent in shelter by *O. niloticus* (seconds), the speed of *O. niloticus* (cm/s), the number of leadership attempts made by *O. niloticus*, the number of successful leadership attempts made by *O. niloticus*, and the success rate of leadership attempts made by *O. niloticus*. In order to test whether *O. niloticus* individuals caused consistent behavioural effects on *O. amphimelas* individuals across the three trials in which they were tested, models were constructed for six response variables. These were the mean time spent in shelter by *O. amphimelas* individuals (s), mean speed of *O. amphimelas* individuals (cm/s), the sum of conspecific aggressive interactions within the *O. amphimelas* group, the number of leadership attempts made by *O. amphimelas*, the number of successful leadership attempts made by *O. amphimelas*, and the success rate of leadership attempts made by *O. amphimelas*. For all twelve response variables, two models were created, one with and one without the random effect of *O. niloticus* ID. The fixed effects in all models were *O.*

niloticus aggression and the size difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial as these were known characteristics of the *O. niloticus* individuals being tested. The only exception was when the response variable was the number of aggressive interactions initiated by *O. niloticus*, where the size of the *O. niloticus* individual, and the mean size of the *O. amphimelas* individuals within a trial, were included as separate fixed effects. This allowed us to assess whether *O. niloticus* showed repeatable levels of aggression across the three trials. In all other cases, support for the model containing the additional random effect of *O. niloticus* ID would suggest that their individual characteristics, not related to body size and aggression, were responsible for repeatable behaviours in *O. niloticus* or consistent behavioural changes in *O. amphimelas* across the three trials.

For the time spent in shelter by *O. niloticus*, the distance travelled by *O. niloticus*, the mean time spent in the shelter by *O. amphimelas* individuals, the mean speed of *O. amphimelas* individuals and the sum of conspecific aggressive interactions within the *O. amphimelas* group, LMM's were used. These response variables were log transformed to meet the normality and homogeneity of variance assumptions which were tested using the *simulateResiduals* function. For the number of aggressive interactions initiated by *O. niloticus*, the number of leadership attempts by *O. niloticus*, the number of successful leadership attempts by *O. niloticus*, the success rate of leadership attempts made by *O. niloticus*, the number of leadership attempts by *O. amphimelas*, the number of successful leadership attempts by *O. amphimelas*, and the success rate of leadership attempts made by *O. amphimelas*, GLMMs with the function *glmmTMB* in the package '*bbmle*' were used. This method allows for direct comparisons with and without random effects for models which do not satisfy the assumptions of a LMM. A negative binomial family was used for the number of aggressive interactions initiated by *O. niloticus*, the number of leadership attempts and the number of successful leadership attempts made by *O. niloticus*, and the number of leadership attempts and the number of successful leadership attempts made by *O. amphimelas*. A binomial family was used for

the success rate of leadership attempts made by *O. niloticus* and the success rate of leadership attempts made by *O. amphimelas*. Dispersion was tested using the *testDispersion* function, and there was no evidence of under or overdispersion. AICc values were used to compare the level of support for the two models within the model set, however the function *ICtab* in the package ‘*bblme*’ was used as it supports the comparison of models from different classes (e.g., ‘lmer’ and ‘lm’; Bolker 2022).

4.4 Results

4.4.1 *O. amphimelas* refuge use: mean time spent in shelter

ΔAICc values indicate that for the response variable mean time spent in shelter by *O. amphimelas*, the model containing the fixed effects of trial part and number of males is the most likely given the data (Table 4.1). The inclusion of trial part in the most likely model and all 27 models with ΔAICc scores > 2 units lower than the null model suggests that trial part is an important predictor of variation in shelter use by *O. amphimelas*. The model containing only trial part as a fixed effect has considerably more support than the null model providing further evidence for this. Plots of the predicted values of the trial part fixed effect from the global model reveal that *O. amphimelas* shelter use was higher in part three of the trial than in parts one and two (Figure 4.2). The number of males was also included in the most likely model and in 20 of the 27 models which had ΔAICc scores > 2 units lower than the null model. The model containing trial part and number of males was more likely than the model containing only trial part. This suggests that when the effect of trial part is controlled for, there is strong support for the inclusion of number of males. Plots of the predicted values of the number of males fixed effect from the global model reveal that *O. amphimelas* spent less time in the shelter when there were more males in the group (Figure 4.2). ΔAICc values indicate no evidence for an interaction between trial part and number of males, given that the inclusion of this interaction did not improve the likelihood of the model, when compared with the model containing only the fixed effects. The additional inclusion of *O. niloticus* aggression, size difference, or an interaction of these variables with trial part, did not improve the most likely models, suggesting these variables were not important predictors of variation in *O. amphimelas* shelter use.

Table 4.1 Model comparison for the LMM's used to analyse the mean time spent in shelter by the *O. amphimelas*. All models included the additional fixed effects of the mean standard length of *O. amphimelas* individuals and the time taken to catch the *O. niloticus* after part two. All models included the random effect of trial number nested within *O. niloticus* individual ID. Only models with greater than or equal support to the null model are included. *K* represents the estimated number of model parameters.

Model	Fixed effects	ΔAIC_c	K
25	Part + number of males	0.00	9
17	Part x number of males	0.41	11
22	Part + number of males + size diff	1.65	10
21	Part + number of males + <i>O. niloticus</i> aggression	2.08	10
12	Part x number of males + size diff	2.11	12
11	Part x number of males + <i>O. niloticus</i> aggression	2.57	12
31	Part	2.82	8
20	Part + number of males + <i>O. niloticus</i> aggression + size diff	3.58	11
27	Part + size diff	3.76	9
5	Part x number of males + <i>O. niloticus</i> aggression + size diff	4.11	13
26	Part + <i>O. niloticus</i> aggression	4.96	9
15	Number of males + part x size diff	5.05	12
23	Part + <i>O. niloticus</i> aggression + size diff	5.72	10
9	Part + number of males + part x size diff	5.83	14
13	Number of males + part x <i>O. niloticus</i> aggression	6.41	12
8	Part + number of males + part x <i>O. niloticus</i> aggression	6.93	14
7	Number of males + <i>O. niloticus</i> aggression + part x size diff	7.06	13
19	Part x size diff	7.13	11
3	Part + number of males + <i>O. niloticus</i> aggression + part x size diff	7.92	15
6	Number of males + part x <i>O. niloticus</i> aggression + size diff	7.98	13
2	Part + number of males + part x <i>O. niloticus</i> aggression + size diff	8.55	15
16	<i>O. niloticus</i> aggression + part x size diff	9.17	12
18	Part x <i>O. niloticus</i> aggression	9.24	11
14	Part x <i>O. niloticus</i> aggression + size diff	10.06	12
4	Number of males + part x <i>O. niloticus</i> aggression + part x size diff	11.30	15
1	Part x number of males + part x <i>O. niloticus</i> aggression + part x size diff	12.19	17
10	Part x number of males + part x size diff	13.34	14
32	Number of males	45.55	7
35	NULL	47.04	6

4.4.2 *O. amphimelas* activity: mean speed of *O. amphimelas*

ΔAICc values indicate that for the response variable mean speed of *O. amphimelas*, the model containing only trial part provided the most likely explanation of variation in *O. amphimelas* speed (Table 4.2). The inclusion of trial part in the most likely model, the strong support for the model containing only trial part as a fixed effect, and the inclusion of trial part in all of the 27 models which had ΔAICc scores > 2 units lower than the null model, suggests that trial part is an important predictor for variation in *O. amphimelas* speed. Plots of the predicted values of the trial part fixed effect from the global model reveal that *O. amphimelas* speed was higher in parts two and three of the trial than in part one (Figure 4.2). The additional inclusion of number of males, *O. niloticus* aggression, or an interaction of these variables with trial part, did not improve the most likely models, suggesting these variables were not important predictors of variation in *O. amphimelas* activity.

Table 4.2 Model comparison for the LMM's used to analyse the mean speed of *O. amphimelas* individuals. All models included the additional fixed effects of the mean standard length of *O. amphimelas* individuals in the group and the time taken to catch the *O. niloticus* after part two. All models included the random effect of trial number nested within *O. niloticus* individual ID. Only models with greater than or equal support to the null model are included. *K* represents the estimated number of model parameters.

Model	Fixed effects	ΔAIC_c	K
31	Part	0.00	8
25	Part + number of males	0.20	9
19	Part x size diff	1.52	11
27	Part + size diff	1.97	9
15	Number of males + part x size diff	2.03	12
26	Part + <i>O. niloticus</i> aggression	2.27	9
22	Part + number of males + size diff	2.41	10
21	Part + number of males + <i>O. niloticus</i> aggression	2.47	10
17	Part x number of males	2.86	11
16	<i>O. niloticus</i> aggression + part x size diff	3.84	12
23	Part + <i>O. niloticus</i> aggression + size diff	4.22	10
7	Number of males + <i>O. niloticus</i> aggression + part x size diff	4.38	13
9	Part + number of males + part x size diff	4.65	14
20	Part + number of males + <i>O. niloticus</i> aggression + size diff	4.68	11
18	Part x <i>O. niloticus</i> aggression	4.81	11
10	Part x number of males + part x size diff	4.95	14
13	Number of males + part x <i>O. niloticus</i> aggression	5.09	12
12	Part x number of males + size diff	5.15	12
11	Part x number of males + <i>O. niloticus</i> aggression	5.21	12
4	Number of males + part x <i>O. niloticus</i> aggression + part x size diff	5.57	15
14	Part x <i>O. niloticus</i> aggression + size diff	6.83	12
3	Part + number of males + <i>O. niloticus</i> aggression + part x size diff	7.08	15
6	Number of males + part x <i>O. niloticus</i> aggression + size diff	7.37	13
5	Part x number of males + <i>O. niloticus</i> aggression + size diff	7.49	13
8	Part + number of males + part x <i>O. niloticus</i> aggression	8.00	14
1	Part x number of males + part x <i>O. niloticus</i> aggression + part x size diff	8.50	17
2	Part + number of males + part x <i>O. niloticus</i> aggression + size diff	10.36	15
35	NULL	93.01	6

4.4.3 Sum of conspecific aggressive interactions between *O. amphimelas*

ΔAICc values indicate that for the response variable sum of conspecific aggressive interactions between *O. amphimelas*, the model containing the fixed effects of trial part and size difference (the difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial) is the most likely given the data (Table 4.3). The inclusion of size difference in the most likely model, the strong support for the model containing only size difference as a fixed effect, and the inclusion of size difference in 20 of the 24 models which had ΔAICc scores > 2 units lower than the null model, suggests that size difference is an important predictor for conspecific aggression within the *O. amphimelas* group. Plots of the predicted values of the size difference fixed effect from the global model reveal that conspecific aggression within the *O. amphimelas* group increased as size difference increased (Figure 4.2). The model containing only trial part as a fixed effect was within $\Delta\text{AICc} < 2$ of the null model; however, the model containing trial part and size difference was the most likely model given the data. This suggests that when the effect of size difference is controlled for, there is strong support for the inclusion of trial part, but it has little explanatory power when size difference is not controlled for. Plots of the predicted values of the trial part fixed effect from the global model reveal a small increase in conspecific aggression within the *O. amphimelas* group in parts two and three of the trial compared to part one (Figure 4.2). ΔAICc values indicate no evidence for an interaction between trial part and size difference, given that the inclusion of this interaction did not improve the likelihood of the model when compared with the model containing only the main effects. The additional inclusion of number of males, *O. niloticus* aggression, or an interaction of these variables with trial part, did not improve the most likely models, suggesting these variables were not important predictors of conspecific aggression within the *O. amphimelas* group.

Table 4.3 Model comparison for the negative binomial GLMM's used to analyse the sum of conspecific aggressive interactions between *O. amphimelas*. All models included the additional fixed effects of the mean standard length of *O. amphimelas* individuals in the trial and the time taken to catch the *O. niloticus* after part two. All models included the random effect of trial number nested within *O. niloticus* individual ID. Only models with greater than or equal support compared to the null model are included. *K* represents the estimated number of model parameters.

Model	Fixed effects	ΔAIC_c	K
27	Part + size diff	0.00	9
34	Size diff	0.76	7
12	Part x number of males + size diff	0.77	12
19	Part x size diff	1.30	11
22	Part + number of males + size diff	2.05	10
23	Part + <i>O. niloticus</i> aggression + size diff	2.14	10
29	Number of males + size diff	2.77	8
30	<i>O. niloticus</i> aggression + size diff	2.88	8
5	Part x number of males + <i>O. niloticus</i> aggression + size diff	3.04	13
15	Number of males + part x size diff	3.42	12
16	<i>O. niloticus</i> aggression + part x size diff	3.45	12
14	Part x <i>O. niloticus</i> aggression + size diff	3.56	12
9	Part + number of males + part x size diff	3.63	14
20	Part + number of males + <i>O. niloticus</i> aggression + size diff	4.18	11
17	Part x number of males	4.74	11
24	Number of males + <i>O. niloticus</i> aggression + size diff	4.89	9
2	Part + number of males + part x <i>O. niloticus</i> aggression + size diff	5.55	15
7	Number of males + <i>O. niloticus</i> aggression + part x size diff	5.56	13
6	Number of males + part x <i>O. niloticus</i> aggression + size diff	5.69	13
31	Part	5.89	8
3	Part + number of males + <i>O. niloticus</i> aggression + part x size diff	5.95	15
10	Part x number of males + part x size diff	6.38	14
25	Part + number of males	6.42	9
35	NULL	6.57	6

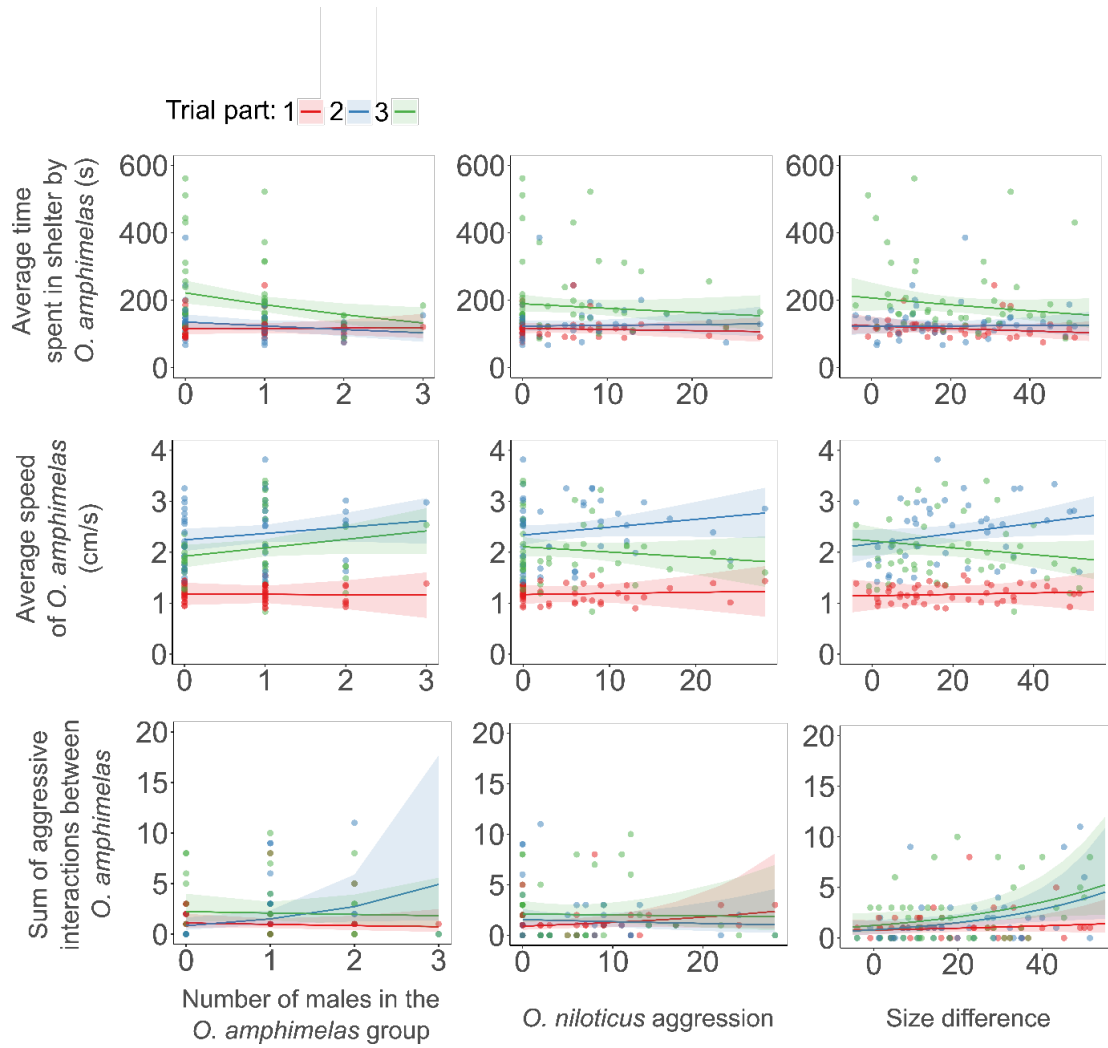


Figure 4.2 The mean time spent in shelter by the *O. amphimelas* (s; top row), the mean speed of *O. amphimelas* (cm/s; middle row) and the sum of aggressive interactions between *O. amphimelas* (bottom row) as a function of the number of males in the *O. amphimelas* group (first column), *O. niloticus* aggression (second column) and size difference (the difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial; third column) for trial parts one (red), two (blue) and three (green). Individual data points represent the predicted values from the full model for the three response variables, using the fixed effects of trial part and either number of males in the *O. amphimelas* group, *O. niloticus* aggression, or size difference. Fitted lines are calculated from LMM/GLMM fixed effect estimates from the full model and shaded areas represent 95% confidence intervals.

4.4.4 Leadership and following behaviour of *O. niloticus* and *O. amphimelas*

4.4.4.1 Number of leadership attempts

ΔAICc values indicate that for the response variable number of leadership attempts the model containing the individual ID (*O. amphimelas* 1, 2 or 3 or *O. niloticus* 4) x size difference interaction term is the most likely given the data (Table 4.4). Plots of the marginal effect of the interaction term reveal that leadership attempts were consistent between the four individuals unless there was a large size difference, which resulted in a higher number of leadership attempts by *O. niloticus* (Figure 4.3). All other models had ΔAICc scores within < 2 units of the null model or received less support than the null model.

Table 4.4 Model comparison for the Poisson GLMM's used to analyse the number of leadership attempts. All models included the random effect of *O. niloticus* individual ID. K represents the estimated number of model parameters.

Model	Fixed effects	ΔAICc	K
4	Individual ID x size diff	0.00	9
3	<i>O. niloticus</i> aggression + individual ID x size diff	1.36	10
1	Individual ID x <i>O. niloticus</i> aggression + individual ID x size diff	5.20	13
8	Individual ID + size diff	5.28	6
11	<i>O. niloticus</i> aggression	5.62	3
12	Size diff	5.97	5
13	NULL	6.37	2
6	Individual ID + <i>O. niloticus</i> aggression + size diff	6.66	7
9	<i>O. niloticus</i> aggression + size diff	6.93	4
7	Individual ID + <i>O. niloticus</i> aggression	7.59	6
10	Individual ID	7.92	3
2	Individual ID x <i>O. niloticus</i> aggression + size diff	11.98	10
5	Individual ID x <i>O. niloticus</i> aggression	12.80	9

4.4.4.2 Number of successful leadership attempts

When only successful leadership attempts (attempts where another individual left the shelter before the leader returned) were considered, ΔAICc values indicate near equivalent support for the model containing the individual ID x size difference interaction term, the model containing only the fixed effect of size difference, and the null model (Table 4.5). Plots of the predicted values of the interaction term reveal a similar pattern to the one observed from the model with most support for the number of leadership attempts (Figure 4.3). Namely, similar numbers of successful leadership attempts were exhibited by the four individuals when the size difference was small, but *O. niloticus* made relatively more successful leadership attempts when there was a large size difference. However, the strong support for the null model, which was not found when considering all leadership attempts, suggests that this interactive effect is weak when only successful leadership attempts are considered.

Table 4.5 Model comparison for the Poisson GLMMs used to analyse the number of successful leadership attempts. All models included the random effect of *O. niloticus* individual ID. K represents the estimated number of model parameters.

Model	Fixed effects	ΔAICc	df
13	NULL	0.00	2
12	Size diff	0.27	3
4	Individual ID x size diff	0.48	9
9	<i>O. niloticus</i> aggression + size diff	1.88	4
11	<i>O. niloticus</i> aggression	1.90	3
3	<i>O. niloticus</i> aggression + individual ID x size diff	2.08	10
10	Individual ID	3.51	5
8	Individual ID + size diff	3.86	6
1	Individual ID x <i>O. niloticus</i> aggression + individual ID x size diff	4.80	13
7	Individual ID + <i>O. niloticus</i> aggression	5.49	6
6	Individual ID + <i>O. niloticus</i> aggression + size diff	5.54	7
5	Individual ID x <i>O. niloticus</i> aggression	10.18	9
2	Individual ID x <i>O. niloticus</i> aggression + size diff	10.31	10

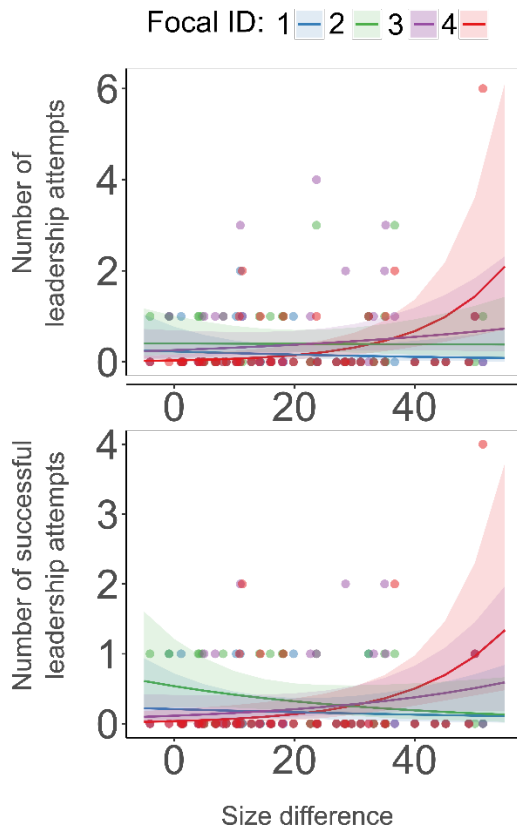


Figure 4.3 The number of leadership attempts (top) and the number of successful leadership attempts (bottom) as a function of the size difference between the *O. niloticus* and the mean size of *O. amphimelas* individuals (size difference) for the four individual ID's one (blue), two (green), three (purple) and four (red). Individuals one, two and three are *O. amphimelas* and four is the *O. niloticus*. Individual data points represent the predicted values from the full model for each response variable, using the fixed effects of individual ID and size difference. Fitted lines are calculated from LMM/GLMM fixed effect estimates and shaded areas represent 95% confidence intervals.

4.4.4.3 Leadership success rate

$\Delta AICc$ values indicate that for the response variable leadership success rate, the null model is the most likely given the data (Table 4.6). This suggests that the success rate of leadership attempts did not vary between individuals, meaning *O. amphimelas* individuals and the *O. niloticus* were equally likely to be followed during a leadership attempt from shelter. Thus, *O. amphimelas* were as likely to follow *O. niloticus* as they were a conspecific following a leadership attempt from either species.

Table 4.6 Model comparison for the binomial GLMM's used to analyse the leadership success rate. All models included the random effect of *O. niloticus* individual ID. K represents the estimated number of model parameters.

Model	Fixed effects	$\Delta AICc$	df
13	NULL	0.00	2
12	Size diff	1.00	3
11	<i>O. niloticus</i> aggression	2.02	3
9	<i>O. niloticus</i> aggression + size diff	3.07	4
4	Individual ID x size diff	4.06	9
10	Individual ID	4.21	5
8	Individual ID + size diff	5.23	6
3	<i>O. niloticus</i> aggression + individual ID x size diff	6.11	10
7	Individual ID + <i>O. niloticus</i> aggression	6.17	6
6	Individual ID + <i>O. niloticus</i> aggression + size diff	7.29	7
5	Individual ID x <i>O. niloticus</i> aggression	8.75	9
1	Individual ID x <i>O. niloticus</i> aggression + individual ID x size diff	8.94	13
2	Individual ID x <i>O. niloticus</i> aggression + size diff	9.85	10

4.4.4.4 Time taken to be followed during a successful leadership attempt

ΔAICc values indicate that for the response variable time taken to be followed during a successful leadership attempt, the model containing *O. niloticus* aggression as a fixed effect is the most likely given the data (Table 4.7). Plots of the predicted values from the model with most support reveal that individuals were followed faster when *O. niloticus* aggression was high (Figure 4.4). Models containing the additional effect of individual ID and size diff were not more likely than the model containing only *O. niloticus* aggression. Support for these models is likely a result of the inclusion of *O. niloticus* aggression, which is an important predictor of variation in the time taken for individuals to be followed. All other models were had ΔAICc scores within < 2 of the null model or received less support than the null model.

Table 4.7 Model comparison for the LMM's used to analyse the time taken to be followed during a successful leadership attempt. All models included the random effect of *O. niloticus* individual ID. K represents the estimated number of model parameters.

Model	Fixed effects	ΔAICc	K
11	<i>O. niloticus</i> aggression	0.00	4
7	Individual ID + <i>O. niloticus</i> aggression	1.16	7
9	<i>O. niloticus</i> aggression + size diff	2.18	5
6	Individual ID + <i>O. niloticus</i> aggression + size diff	3.48	8
5	Individual ID x <i>O. niloticus</i> aggression	5.44	10
13	NULL	6.14	3
12	Size diff	8.06	4
2	Individual ID x <i>O. niloticus</i> aggression + size diff	8.30	11
10	Individual ID	8.93	6
8	Individual ID + size diff	10.79	7
3	<i>O. niloticus</i> aggression + Individual ID x size diff	10.88	11
4	Individual ID x size diff	18.10	10
1	Individual ID x <i>O. niloticus</i> aggression + Individual ID x size diff	18.72	14

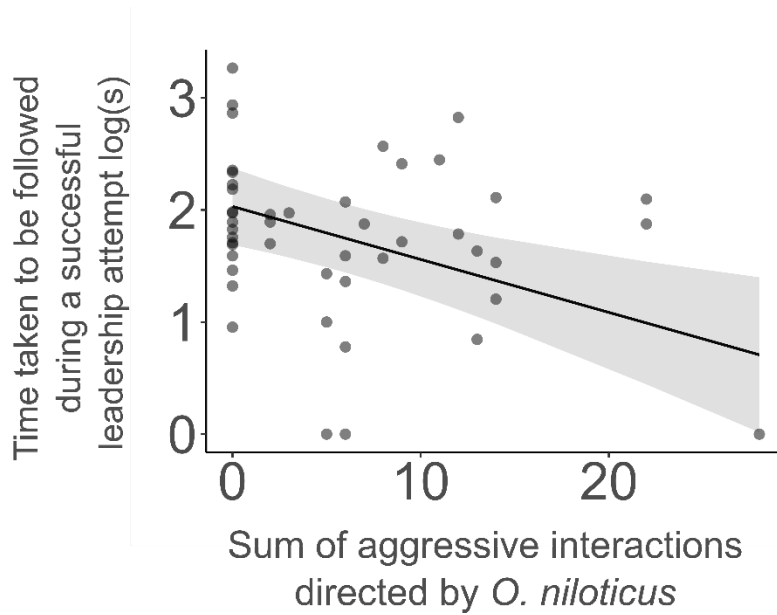


Figure 4.4 The time taken to be followed during a successful leadership attempt (log(s)) as a function of the sum of aggressive interactions directed by *O. niloticus*. Individual data points represent the predicted values from the model with the most support using the fixed effect of *O. niloticus* aggression. The shaded area represents 95% confidence intervals.

4.4.4.5 Number of first follow events

$\Delta AICc$ values indicate that for the response variable number of first follow events (the number of events where an individual left the shelter following a leadership attempt, before the leader returned to the shelter) the null model was most likely given the data (Table 4.8). This suggests that the number of follow events did not vary between *O. amphimelas* individuals and the *O. niloticus* individual, i.e. they made an equivalent amount of first follow events.

Table 4.8 Model comparison for the Poisson GLMM's used to analyse the number of first follow events. All models included the random effect of *O. niloticus* individual ID. *K* represents the estimated number of model parameters.

Model	Fixed effects	$\Delta AICc$	df
13	NULL	0.00	2
12	Size diff	0.27	3
9	<i>O. niloticus</i> aggression + size diff	1.88	4
11	<i>O. niloticus</i> aggression	1.90	3
10	Individual ID	4.07	5
8	Individual ID + size diff	4.41	6
4	Individual ID x size diff	5.48	9
7	Individual ID + <i>O. niloticus</i> aggression	6.04	6
6	Individual ID + <i>O. niloticus</i> aggression + size diff	6.10	7
3	<i>O. niloticus</i> aggression + Individual ID x size diff	7.15	10
5	Individual ID x <i>O. niloticus</i> aggression	12.46	9
2	Individual ID x <i>O. niloticus</i> aggression + size diff	12.59	10
1	Individual ID x <i>O. niloticus</i> aggression + Individual ID x size diff	13.18	13

4.4.5 Repeatability of *O. niloticus* behaviour, and its impacts on *O. amphimelas* groups

4.4.5.1 Repeatability in *O. niloticus* behaviour

For the number of aggressive interactions directed by *O. niloticus*, the time spent in shelter by the *O. niloticus*, the mean speed *O. niloticus*, the number of leadership attempts made by *O. niloticus*, the number of successful leadership attempts made by *O. niloticus*, and the success rate of leadership attempts made by *O. niloticus*, $\Delta AICc$ values indicate strong support for the models not containing the random effect of *O. niloticus* ID (Table 4.9). *O. niloticus* showed no consistent inter-individual variation in aggression across the three trials they were tested in while controlling for body size and mean *O. amphimelas* size. *O. niloticus* also showed no consistent inter-individual variation in the time spent in shelter, speed, leadership behaviour or likelihood of being followed during a leadership attempt across the three trials they were tested in, while controlling for the effects of size difference (the difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial) and *O. niloticus* aggression.

Table 4.9 Model comparison for the Generalised Linear and Linear Mixed-Effects Models used to analyse the six response variables used to test the repeatability of *O. niloticus* behaviour across the three trials they were tested in. The df represents the number of model parameters.

Response variable	Fixed effects	Random effect	ΔAIC_c	df
Number of aggressive interactions directed by <i>O. niloticus</i>	<i>O. niloticus</i> size + mean <i>O. amphimelas</i> size	NONE	0	4
	<i>O. niloticus</i> size + mean <i>O. amphimelas</i> size	<i>O. niloticus</i> ID	2.5	5
Time spent in shelter by the <i>O. niloticus</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.5	5
Mean distance travelled by the <i>O. niloticus</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	1.9	5
Number of leadership attempts by <i>O. niloticus</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.2	5
Number of successful leadership attempts by <i>O. niloticus</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.3	5
<i>O. niloticus</i> leadership success rate	<i>O. niloticus</i> aggression + size difference	NONE	0	3
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.4	4

4.4.5.2 Repeatable impacts of individual *O. niloticus* on *O. amphimelas*

For the mean time spent in the shelter by *O. amphimelas*, mean speed of *O. amphimelas*, and the sum of conspecific aggressive interactions between *O. amphimelas*, ΔAICc values indicate strong support for the models not containing the random effect of *O. niloticus* ID (Table 4.10). In all cases the model including the random effect had ΔAICc scores > 2 higher than the model without the random effect. This suggests that while controlling for *O. niloticus* size and aggression, individual *O. niloticus* had no consistent effects on the shelter use, activity, or conspecific aggression of *O. amphimelas* across the three trials in which they were tested. For the number of leadership attempts made by *O. amphimelas*, the number of successful leadership attempts made by *O. amphimelas*, and the success rate of leadership attempts made by *O. amphimelas*, ΔAICc values also indicate strong support for the models not containing the random effect of *O. niloticus* ID (Table 4.10). In all cases, the model including the random effect had ΔAICc scores > 2 higher than the model without the random effect. This suggests that while controlling for *O. niloticus* size and aggression, individual *O. niloticus* had no consistent effects on the leadership behaviour of *O. amphimelas* across the three trials in which they were tested.

Table 4.10 Model comparison for the Generalised Linear and Linear Mixed-Effects Models used to analyse the six response variables used to test for repeatable effects of *O. niloticus* behaviour on the *O. amphimelas* group. The df represents the number of model parameters.

Response variable	Fixed effects	Random effect	ΔAICc	df
Mean time spent in shelter by the <i>O. amphimelas</i> group	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.5	5
Average speed of <i>O. amphimelas</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.5	5
Sum of conspecific aggressive interactions between <i>O. amphimelas</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.6	5
Number of leadership attempts by <i>O. amphimelas</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.2	5
Number of successful leadership attempts by <i>O. amphimelas</i>	<i>O. niloticus</i> aggression + size difference	NONE	0	4
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.2	5
<i>O. amphimelas</i> leadership success rate	<i>O. niloticus</i> aggression + size difference	NONE	0	3
	<i>O. niloticus</i> aggression + size difference	<i>O. niloticus</i> ID	2.1	4

4.5 Discussion

Our results show that *O. amphimelas* changed their shelter use, swimming speed and conspecific aggression level across the three parts of the trial: before, during and after the introduction of a singular *O. niloticus* to the group. Shelter use was highest in part three of the trial, following the removal of *O. niloticus* but remained stable following its introduction. Shelter use was higher when there were three males in the *O. amphimelas* group, suggesting sex specific effects on the shelter use of *O. amphimelas*. *O. amphimelas* swimming speed increased following the introduction, and subsequent removal of the *O. niloticus* individual. Aggression between *O. amphimelas* depended on the difference between the mean standard length of *O. amphimelas* individuals and the *O. niloticus* in the trial, with larger size differences resulting in more conspecific aggression. These results highlight that the behavioural mechanisms which underpin the impact of introduced species can vary based on individual-level characteristics such as body-size. We found no evidence of repeatability in the behaviour of individual *O. niloticus* and observed no repeatable impacts of *O. niloticus* on the behaviour of *O. amphimelas* across the three trials in which they were tested.

There was no difference in the number of leadership attempts made by the four individuals in part two of the trial unless there was a large size difference between the average length of *O. amphimelas* and the *O. niloticus*. When there was a large size difference *O. niloticus* made more leadership attempts relative to the three *O. amphimelas*. However, when only successful leadership attempts were considered this effect of size difference was not found suggesting that larger *O. niloticus* showed a propensity for leadership attempts above that of *O. amphimelas* within the group, but they were not followed more than the other *O. amphimelas* attempting to lead. The success rate of leadership attempts did not vary between individuals, suggesting that *O. amphimelas* were just as likely to follow an unfamiliar *O. niloticus* as a familiar conspecific. Similarly, the number of first

follower events (i.e. the identity of the second individual to leave the shelter during a successful leadership attempt) did not vary between individuals.

In part two of the experiment, all individuals were followed faster when the *O. niloticus* was more aggressive. We hypothesise that this may result from increased cohesion within the group when *O. niloticus* were more aggressive, resulting in shorter follow times. However, the results could also be explained by a number of alternative hypotheses. Firstly, individuals could have been followed faster when the *O. niloticus* was more aggressive because chase events by *O. niloticus* were being mistaken for leadership events. If this were the case then evidence for an interaction between *O. niloticus* aggression and individual ID would be expected, with *O. amphimelas* being followed faster than *O. niloticus* on average when *O. niloticus* were aggressive. Additionally, an interaction between *O. niloticus* aggression and individual ID would be expected for the number of first follower events. We found no evidence for either of these interactions in our results. Secondly, *O. amphimelas* aggression may have increased when *O. niloticus* were more aggressive, and chase events by *O. amphimelas* were being mistaken for leadership events. However, *O. niloticus* aggression had no effect on conspecific aggression within the *O. amphimelas* group in any part of the trial suggesting this was not the case. In general, we found no correlation between *O. niloticus* aggression or *O. amphimelas* aggression with the number of leadership attempts, suggesting that mistaken cases of leadership which were actually chase events from shelter did not affect our analyses. Finally, higher activity levels when *O. niloticus* were more aggressive could explain a reduction in follow times. However, we found no effect of *O. niloticus* aggression on *O. amphimelas* activity (speed) in any part of the trial, including part two, suggesting this was not the case.

We therefore infer that the shorter follow latencies when *O. niloticus* were more aggressive were as a result of increased cohesion within the group when *O. niloticus* were more aggressive. The lack of evidence for an interaction between *O. niloticus* aggression and individual ID also suggests that this

increased cohesion included cohesion with and by the *O. niloticus*. While aggression can lead to avoidance behaviour, dominance hierarchies with more aggressive individuals at the top and less aggressive individuals below them commonly form within animal groups (Dehnen et al. 2022). However it is unclear whether the relatively short trial length is sufficiently long to allow the formation of a stable dominance hierarchy following high levels of aggression from *O. niloticus*. Another explanation is that *O. amphimelas* began to display an anti-predator response, through increased group cohesion, in the face of sufficient aggression. The long-term outcome of either strategy is not measurable in our experiment, but future work investigating the long-term implications of *O. niloticus* aggression on native tilapia may provide useful insights. We acknowledge that currently our inferences surrounding increased group cohesion in this experiment are largely assumed based on a lack of evidence for other theories and further work would be needed to confirm this result.

The willingness for *O. amphimelas* to follow *O. niloticus* at the same rate as familiar conspecifics suggests that *O. niloticus* can successfully integrate into groups of unfamiliar native species over short time frames. There are a number of clear benefits that introduced species can gain from grouping with native species with similar niches (Camacho-Cervantes et al. 2014a). Access to social information from individuals with evolutionary experience of the local environment could allow them to make better decisions related to habitat use, foraging and predator avoidance (Camacho-Cervantes et al. 2014a). In our experiment, when *O. amphimelas* attempted to lead, they were as likely to be followed by an *O. niloticus* as an *O. amphimelas*. This suggests that *O. niloticus* were able to access the social cues of unfamiliar *O. amphimelas* which could benefit them when naïve to a novel environment following introduction. Additionally, introduced populations of non-native species typically suffer from Allee effects, due to the relatively small numbers of individuals which are typically introduced at a given time (Taylor and Hastings 2005). Indeed, the best predictor of invasion outcome across taxonomic groups is propagule pressure, which is defined as the number

of introduced individuals (Colautti et al. 2006). Therefore, increasing group size by integrating with native species could have a number of benefits for introduced species. Increased group size has been linked to improved foraging and reduced predation pressure (Hamilton 1971; Pavlov and Kasumyan 2000; Couzin and Krause 2003), which could benefit the establishment of introduced *O. niloticus*.

It is less clear whether native species are likely to benefit from grouping with introduced species. While similar benefits may be gained by native species at the individual level (i.e. larger group sizes reducing predation risk), there are potential species level consequences by improving the fitness of an introduced species which exploits similar resources. As population sizes of introduced species expand, there is a likely to be an increased competition for resources which is one of the key costs of living in groups (Ward and Webster 2016). Experimental trials have shown *O. niloticus* dominate *O. amphimelas* in interference competition (Champneys et al. 2020), which could restrict access to resources in *O. amphimelas* once populations of *O. niloticus* reach a certain threshold. Exploitative competition with introduced *O. niloticus* has also been shown to reduce the growth rate of native species during mesocosm experiments (Gu et al. 2015). We also observed changes in the social behaviour of *O. amphimelas* following the introduction and subsequent removal of *O. niloticus* across all of our recorded behavioural parameters (shelter use, swimming speed, and conspecific aggression). It is apparent that introducing an additional individual to a group of conspecifics is likely to result in changes regardless of species. However, the resulting shifts in habitat, energy use and inter-individual interactions that we observed when *O. niloticus* joined the group of *O. amphimelas* could have consequences for the fitness of individuals. Further, *O. amphimelas* were just as likely to follow the leadership attempts of an unfamiliar *O. niloticus* as a familiar conspecific. Introduced species have relatively little information about their environment following introduction compared to native species (Camacho-Cervantes et al. 2014a), therefore readily following them during leadership attempts is likely to be maladaptive for *O. amphimelas*.

In conclusion, our results reveal that *O. niloticus* may derive benefits from grouping with native species with similar niches, while the same native species may incur costs from grouping with *O. niloticus*. In Tanzania, invasive *O. niloticus* populations are close to their native range and therefore occur in sympatry with a number of closely related, functionally similar congeners. The propensity shown by *O. niloticus* in this experiment to group with native species could present a mechanism which may explain their successful establishment as an invasive species and the negative impacts that they have on native species across their non-native range.

5 | Rapid growth of a locally-endemic tilapia may contribute to its persistence in an African lake invaded by Nile tilapia



Korogwe tilapia caught during fieldwork sampling

5.1 Abstract

The introduction of non-native species can lead to competition with native species for key resources, driving the decline and extinction of endemic biodiversity. Recently, a newly discovered and evolutionarily distinct lineage of Korogwe tilapia (*Oreochromis korogwe*) was reported from small lakes in Southern Tanzania. This small-bodied lineage is potentially threatened by introduced *O. niloticus* (*Oreochromis niloticus*), an invasive large-bodied congeneric with a pan-tropical non-native distribution. *O. niloticus* is known to dominate ecologically similar native tilapia in competitive interactions, preventing access to resources such as food and shelter. We therefore hypothesised that competition between *O. niloticus* and *O. korogwe* could limit access to resources by the native species, reducing growth rate, a key determinant of fitness. In this study, tilapia were collected from Lake Rutamba in two field seasons, and individuals were classified as *O. niloticus*, *O. korogwe* or interspecific hybrids using microsatellite genotypes. Recent growth rate of these individuals was determined by measuring the distance between scale circuli. We found that at small sizes, the two species and their hybrids had equivalent fitness. However, and in contrast to expectations, we found that at larger body sizes native *O. korogwe* had a faster growth rate than the invasive *O. niloticus*, with hybrids showing growth rates more similar to *O. korogwe*. We propose that in Lake Rutamba the persistence of the small-bodied *O. korogwe* could be partially enabled by a faster growth rate than the large-bodied invasive *O. niloticus* however our results remain highly speculative in the absence of more detailed population data over time. Based on these results, we suggest that predictions of the effects of invasive species may benefit from information on relative fitness across life stages, as well as ecological niche overlap.

5.2 Introduction

Fish populations are typically subject to high mortality rates at juvenile stages, with relatively few individuals surviving to breeding age (Sogard 1997). Factors affecting the fitness of fish species at juvenile stages are therefore especially important in determining population dynamics. Often, key resources necessary for juvenile survival such as food and shelter are limited, and thus interspecific competition can determine access to these resources (Chase et al. 2016). Individuals which are at a disadvantage in either exploitative or interference competition will have restricted access to food or shelter, increasing the likelihood of predation, starvation or reduced growth rate (Martin et al. 2010).

The introduction of non-native species can increase competition for key resources, especially when the introduced species occupies a similar niche to native species (Britton et al. 2011; Pacioglu et al. 2019). Many studies have highlighted how competition with non-native species can result in the decline and in some cases extinction of native populations (Human and Gordon 1996; Case et al. 2016). The theoretical principle of competitive exclusion predicts that inferior competitors will go extinct if they are unable to shift niches to avoid competition with the superior species (Hardin 1960; Bohn et al. 2008). Thus, competition with non-native species poses a severe potential threat to native fish populations.

Oreochromis niloticus is a freshwater cichlid fish, with a pan-tropical non-native distribution. Declines in native species following the introduction of *O. niloticus* have been reported in many ecosystems, however the mechanisms that drive these declines are not always known (Canonico et al. 2005). The vast majority of non-native *O. niloticus* populations are descendants of fish selected for aquaculture or capture fisheries improvement, and therefore possess phenotypes leading to high production yields, including fast growth rates and large body sizes. It is therefore often predicted

that introduced *O. niloticus* may outcompete native species, limiting their access to resources (Canonico et al. 2005). Studies have demonstrated that under experimental conditions *O. niloticus* forage more efficiently than native species (Gu et al. 2015), including other tilapia (Wing et al. 2021). Such competitive advantages over shared food resources could result in a reduced growth rate, which is a crucial determinant of fitness. Additionally, *O. niloticus* have been shown to dominate aggressive interactions (Chifamba and Mauru 2017), including outcompeting native cichlids over access to shelter resources (Martin et al. 2010; Champneys et al. 2020). This could increase the likelihood of predation *in situ*, resulting in a lower survival rate, and a reduced opportunity to reach breeding age. Male-male interference competition over lekking spaces could also reduce breeding output in subordinate species, with further consequences for population fitness. Given the current evidence of competition-induced effects by *O. niloticus* on native populations *in situ* (Chifamba and Videler 2014; Bradbeer et al. 2020), research into their impact on potentially affected species appears warranted.

The highly biodiverse freshwater habitats of Tanzania are home to a large number of tilapia species of the genus *Oreochromis*, many of which are endemic to the region (Darwall et al. 2015; Shechonge et al. 2019). Native tilapia play an important role in capture fisheries (Lind et al. 2012), ecosystem functioning (Lévêque 1995), and provide valuable genetic resources from which to develop novel aquacultural strains (Eknath and Hulata 2009; Lind et al. 2012). Thus, the preservation of these species has been highlighted as an important conservation goal (Lind et al. 2012). Between 2013 and 2016, an evolutionarily unique lineage of a small-bodied species, the *O. korogwe* (*Oreochromis korogwe*), was discovered in the Rutamba lakes (Lakes Rutamba, Nambawala and Mitupa) near Lindi in Southern Tanzania (Figure 5.1a; Blackwell et al. 2021). Living in sympatry with the *O. korogwe* is an introduced population of non-native large-bodied *O. niloticus*, and the two species are known to be hybridizing (Blackwell et al. 2021). Hybridization with invasive species can have irreversible impacts on the genetic diversity of native species, but the extent of the threat depends in part on

the relative fitness of these hybrids compared to parental species (Dudgeon et al. 2006). *O. niloticus* is known to hybridise with a number of other *Oreochromis* species across its introduced range, and investigating the outcome of hybridization in the Rutamba lakes could help to more clearly define its impact as an invasive species (Blackwell et al. 2021).

O. niloticus and *O. korogwe* are closely related, fully sympatric and both are omnivorous - feeding primarily on macrophytes, phytoplankton, and detritus of vegetation (M. Genner and Z. Lyu *pers obs.*). We therefore hypothesised that this ecological similarity may drive competition between the two species, causing a discrepancy in access to food and shelter. To test our hypothesis, we collected individuals from both species in Lake Rutamba, the largest of the three lakes in which they co-occur. Specimens were genotyped using microsatellite markers, enabling classification as either *O. niloticus*, *O. korogwe*, or their hybrids. We then measured the growth rate of specimens using data from scale circuli. Growth rate is a crucial determinant of fitness in fish and reduced individual growth rate can provide evidence of competition induced restriction to accessing food resources (Diehl and Eklov 1995; Bohn et al. 2008). Our comparisons of differences in growth rate between species, and between purebreds and hybrids, provide insight into the relative fitness of the populations. These results are discussed with reference to mechanisms that may enable the small-bodied native *O. korogwe* to persist in Lake Rutamba, despite hybridizing and sharing resources with the large-bodied *O. niloticus*.

5.3 Methods

5.3.1 Study site and sample collection

Sampling was conducted at Lake Rutamba (~10°01'52" S, ~39°27'44" E; Figure 5.1) near Lindi in Tanzania during two field sampling events (2-4 May 2015; 1-2 November 2019). Lake Rutamba is a turbid soft-bottomed lake, measuring approximately 2 km x 1 km, and with an approximate depth of 2.5 m. Beds of *Arundo donax* surround the lake but during the summer months the water level drops and recedes away from the littoral vegetation reducing the potential for fish to shelter in reeds. Fish predators observed to be associated with the lake include sharp tooth catfish (*Clarias gariepinus*), Nile crocodile (*Crocodylus niloticus*) and birds (*Mycteria ibis*, *Microcarbo africanus*, *Ardea alba*), suggesting competition over shelter habitats may to be relevant to survival. The lake is also heavily exploited as a capture fishery, with approximately 25 active fishers being recorded daily during the 2019 sampling season, using gill nets.

O. niloticus, *O. korogwe* and their hybrids were purchased from local fishers using gill nets (2016 and 2019) or collected using a survey seine net (30 m x 1.5 m, 25.4 mm mesh, fine mesh cod-end) (2019). Individuals were selected for retention based on phenotypes. All individual retained were greater than 35 mm. Samples collected using the survey seine net were euthanised using an overdose of anaesthetic (clove oil). Specimens were pinned to a polystyrene board, photographed, labelled, and stored individually in 100% ethanol before transport. Long-term storage was in 70% ethanol.

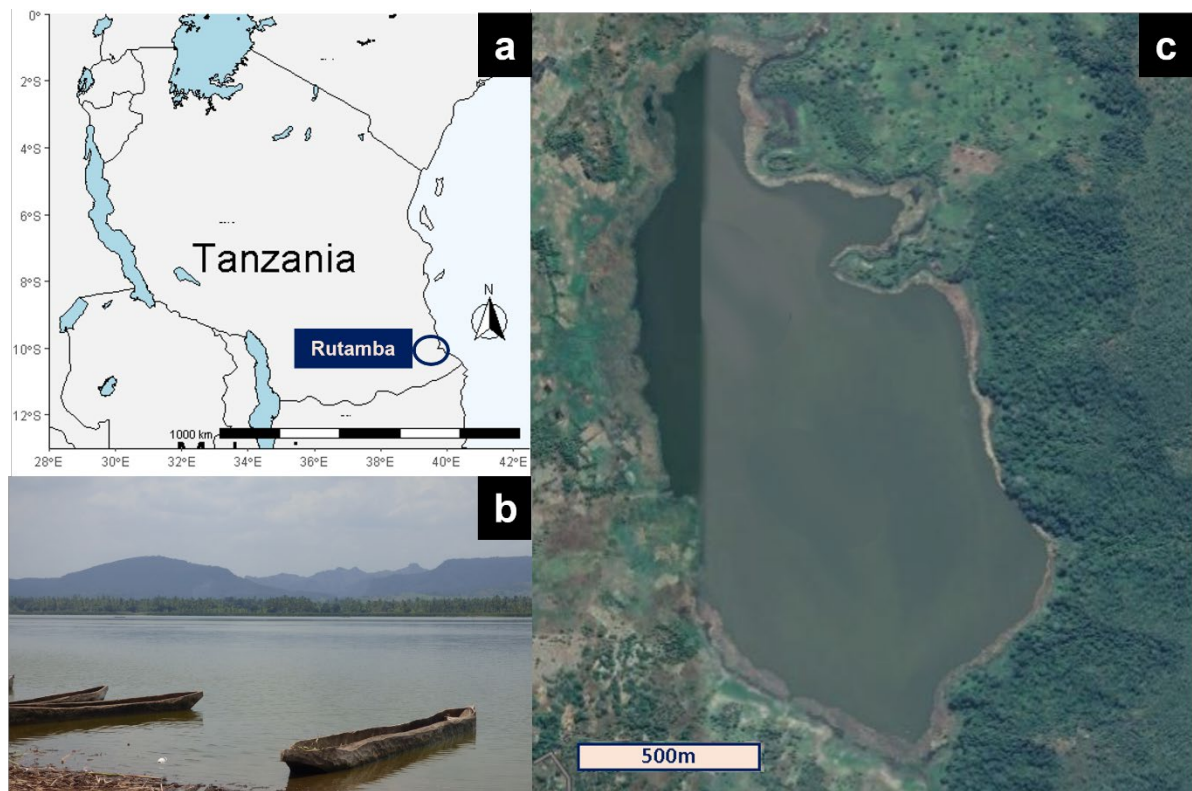


Figure 5.1 a) Tanzania showing the location of Lake Rutamba. b) Western shore of Lake Rutamba with fishing canoes. c) Satellite image of Lake Rutamba (Google Earth, image dated March 3 2017).

Table 5.1 Sampling dates and samples sizes of each species, as resolved through microsatellite genotypes, used in the final analysis.

Date	<i>O. korogwe</i>	Hybrid	<i>O. niloticus</i>
22/10/2016	17	1	10
23/10/2016	11	2	8
24/10/2016	10	2	0
01/11/2019	4	4	12
02/11/2019	27	4	19
	87	13	49

5.3.2 Microsatellite genotyping

We determined the genetic composition of sampled individuals using microsatellite genotypes. DNA was extracted from fin clips following the Wizard Genomic DNA Purification Kit protocol (Promega, WI). DNA concentrations were measured using an N60 Touch NanoPhotometer (Implen, München, Germany), and diluted to 50ng/μl. For the assay we selected six microsatellite loci (OMO219, OMO229, OMO391, OMO337, OMO129, OMO043) from Saju et al. (2010) and Liu et al. (2013), previously used to classify individuals as *O. korogwe*, *O. niloticus* or their hybrids (Blackwell et al. 2021). Polymerase chain reactions were performed using 10 μl solutions comprised of: 1μl DNA, 0.2 μl of the six forward primers (each 10μM), 0.2 μl of the six reverse primers (each 10μM), 5 μl of Multiplex PCR Master Mix (Qiagen, Hilden, Germany) and 1.6 μl of distilled water. PCR conditions were an initial denaturation at 95°C for 60s, followed by 35 cycles of 94°C for 30s, 57°C for 90s and 72°C for 60s, before a final extension stage at 60°C for 30 minutes. PCRs were conducted on a 3PRIMEX/02 thermal cycler (Techne, Staffordshire, UK). Amplicons were genotyped using an 3500 Genetic Analyser (Applied Biosystems, MA) with a LIZ500 size standard, and scoring was conducted using Genemapper 4.1 (Applied Biosystems, MA). Amplification of one locus (OMO043) was unsuccessful, so analyses were conducted on the five remaining loci. Genetic compositions of individuals were estimated using the admixture model in Structure 2.3.4 (Pritchard et al. 2000), assuming two populations ($K = 2$; *O. korogwe* or *O. niloticus*), in 10 separate runs of 100,000 steps following a 100,000 burn-in. This resulted in an assignment probability of between 0 and 1 for each specimen. Following Blackwell et al. (2019) Individuals with < 0.1 were deemed to be *O. korogwe*, individuals > 0.9 *O. niloticus*, and individuals >0.1 and < 0.9 hybrids.

5.3.3 Growth rate scale measurement

To assess the recent growth rate of the *Oreochromis* specimens using scale circuli, we followed methods outlined in Martin (2012), validated in experimental trials on two African cichlid species (*Oreochromis mossambicus*; *Hemichromis bimaculatus*), and previously used to compare relative growth

of invasive *O. niloticus* and indigenous Tanzanian tilapia species (Bradbeer et al. 2020). These studies show that the growth rate to scale diameter ratios is equivalent in species within the same genus as those studied here, and our assumption is that this will also be true when comparing *O. niloticus* and *O. kribia*, however this is not guaranteed and experimental trials in the lab using these species would be necessary to confirm our assumptions. Firstly, three scales were collected from the right side of each specimen, from the first scale row dorsal to the lateral line and posterior to the pelvic girdle. To ensure consistency in scale type, scales were removed sequentially until three fully formed scales with tight foci were obtained (Figure 5.2a). Scales were submerged in water and excess skin and debris were removed using forceps to ensure individual circuli were visible. Scales were then dried, coated with glycerol on a microscope slide and covered with a glass coverslip. Images of individual scales were then taken using a M205c stereo microscope (Leica Microsystems, Wetzlar, Germany) with a GXCAM HICHROME MET-M camera attachment (GT Vision, Newmarket, UK). Five measurements were recorded from each scale in micrometres (μm). Firstly, the total width of the scale at its widest point ($0.78 \times$ magnification), and secondly, four measurements of the distance between the five outermost circuli on four primary radii ($5 \times$ magnification; Figure 5.2b). Measurements, calibrated using an image of a graticule taken at the same magnification, were made using Image-J 1.3.3, (Schneider et al. 2012).

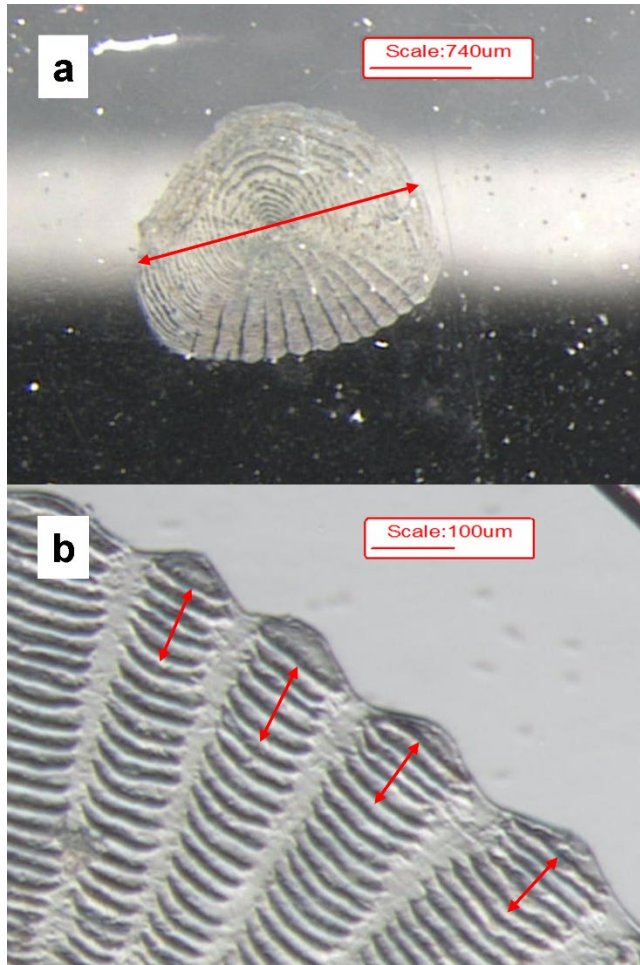


Figure 5.2 Stereo microscope image examples of a) whole scale image and b) outer scale circuli on primary radii. Double sided red arrows represent the measurements made in ImageJ and scale bars are shown for each image as calculated via a graticule.

5.3.4 Statistical analyses

All analyses were performed in R 3.6.1 (R Core Team 2019). To investigate variation in growth rate between *O. niloticus*, *O. korogwe* and their hybrids a linear model as was constructed with growth increment (average distance in μm between the five outer circuli from four primary radii on three separate scales) as the dependent variable. We included species (*O. korogwe*, *O. niloticus*, *O. korogwe* x *O. niloticus* hybrids) and mean scale diameter (μm) as fixed factors. We also included the interaction term of species x mean scale diameter, to investigate whether the association between growth increment and mean scale diameter varied among the groups of individuals. In a second

linear model, also including growth increment as the dependent variable, we included species, standard length and an interaction between these variables to assess how mean scale diameter and standard length compared as predictors of growth increment, we expected these variables to be highly correlated. The *simulate.Residuals* function in the package DHARMA (Hartig 2020) was used to produce a Q-Q plot of observed vs expected residuals, and residuals vs fitted values, to identify any violations of the assumptions of normally distributed residuals and homogeneity of variance. The dependent variable was \log_{10} transformed following evidence of non-normally distributed residuals and mean scale diameter and standard length were \log_{10} transformed to ensure a linear relationship between predictor and response. The function *Anova* in the package car (Fox et al. 2013) was used to test for the significance of the fixed effects, employing a type II model due to unequal sample sizes across the three groups of individuals.

5.4 Results

Across all samples, *O. niloticus* ranged from 42.9 to 107.3 mm SL, *O. korogwe* from 33.6 to 102.3 mm SL, and hybrids from 34.7 to 90 mm SL (Figure 5.3a). When analysing growth increment as the dependent variable, there was a significant interaction between species and mean scale diameter ($F_{2,x} = 9.46$, $P < 0.001$; Table 5.2; Figure 5.3b), suggesting that while controlling for body size, growth increment, a key determinant of fitness, differed between the species. The same result was found when mean standard length was used rather than mean scale diameter in an otherwise equivalent analysis; scale diameter and standard length are strongly correlated (Table 5.2, Figure 5.3c-d). Plots of the predicted values reveal that at small body sizes (33 - 50 mm SL) there was little difference in fitness between the species (Figure 5.3b-c). By contrast at larger body sizes (50 - 110 mm SL), *O. korogwe* had significantly higher growth rate than *O. niloticus*. *O. korogwe* x *O. niloticus* hybrids had fitness values closer to *O. korogwe* (Figure 5.3b-c).

Table 5.2 Linear models quantifying variation in growth increments in relation to fish size (measured using scale diameter and standard length) and species (*O. kornogwe*, *O. niloticus* and interspecific hybrids)

	Predictor variables	Sum of squares	df	F value	P value
Model A	log ₁₀ mean scale diameter	0.3	1	137.9	<0.001
	species	0.05	2	12.6	<0.001
	log ₁₀ mean diameter x Species	0.04	2	9.5	<0.001
	residuals	0.31	143		
Model B	log ₁₀ standard length	0.27	1	117.1	<0.001
	species	0.06	2	13.6	<0.001
	log ₁₀ standard length x species	0.04	2	8.1	<0.001
	residuals	0.31	143		

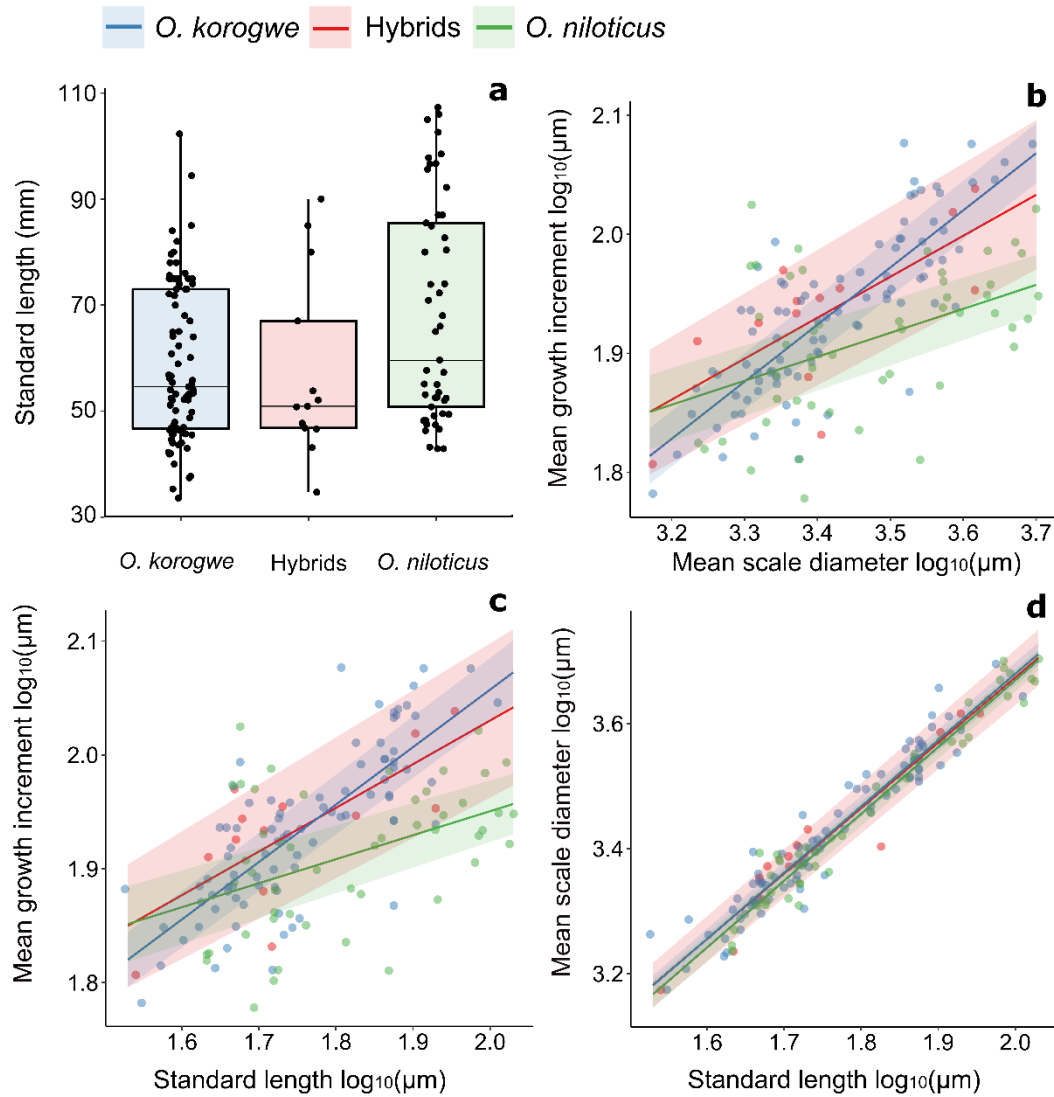


Figure 5.3 a) Standard length of analysed *O. korogwe*, *O. niloticus* and *O. korogwe* x *O. niloticus* hybrids. b) Growth increment as a function of mean scale diameter (μm). c) Growth increment as a function of standard length (mm). d) Mean scale diameter as a function of standard length. In b-d, individual data points represent the predicted values from the linear model, fitted lines are calculated from fixed effect estimates and shaded areas represent 95% confidence intervals.

5.5 Discussion

5.5.1 The relative growth of *O. korogwe* and *O. niloticus*

O. niloticus is a large-bodied tilapia species widely used in aquaculture due to a relatively fast growth rate, and in natural water bodies, higher growth rates than native *Oreochromis* species have been observed (Chifamba and Videler 2014; Bradbeer et al. 2020). However, within Lake Rutamba, we found that at larger body sizes, *O. korogwe* had higher growth rates than *O. niloticus*. This result contrasts with our expectations that the large bodied and fast-growing *O. niloticus* would have a greater growth rate than the native species (Bradbeer et al. 2020). Growth rate is a crucial determinant of fitness in fish as it allows them to quickly bypass the most vulnerable stages of their lifespan where mortality is highest (Sutherland 1996). Thus, we consider that the population dynamics within this lake and the survival and fitness of *O. korogwe* in the face of *O. niloticus* introduction could be linked to this increased growth rate and below we explore this further. It is important to note that in the absence of validation in experimental trials with these two species that our measure of relative growth rate cannot be linked to a clear effect size in growth rate over time, for example standard length increase in mm per week. Without such information it is hard to make more accurate predictions about the likely impact of the observed reduced growth rate in *O. niloticus* in this system.

It is important to note that numerous factors are likely to drive the population dynamics within this system and in the absence of information about the relative population sizes of *O. korogwe* and *O. niloticus* over an extended period, we have little certainty in our predictions that growth rate is indeed a contributing factor to the impact of introduced *O. niloticus* in Lake Rutamba. Below we discuss how growth rate could lead to persistence in the face of *O. niloticus*, a large bodied competitor, however it is not clear whether population sizes of *O. korogwe* have indeed remained stable following *O. niloticus* introduction and thus our discussion of the results of this experiment must be deemed highly speculative in regard to this lake specifically and are more appropriate as

a discussion of the mechanisms which could moderate the impact of *O. niloticus* on native tilapia more generally.

5.5.2 How might *O. korogwe* persist in the face of invasion by a large bodied competitor?

O. niloticus has been widely introduced to natural water bodies across Tanzania since the 1950s, but the precise timing of the introduction into Lake Rutamba is unclear. We know that *O. niloticus* were fully established in the lake in 2013 (Blackwell et al. 2021). We also know that samples of *O. korogwe* from Lake Rutamba were accessioned to the Natural History Museum in London in 1982 (as *Sarotherodon ruvumae*), alongside *Coptodon rendalli* (as *Tilapia rendalli*), but not *O. niloticus*. This is consistent with *O. niloticus* being absent from Lake Rutamba in the early 1980s. We therefore estimate that *O. niloticus* were introduced between to the lake between the early 1980s and the early 2010s. Evidence of the negative effects of *O. niloticus* across its native range (Canonico et al. 2005), including the extinction of a native tilapia species from the Hombolo reservoir in Tanzania (Turner et al. 2019), has led to predictions that *O. niloticus* pose a major threat to native tilapia in East African freshwaters. Thus, the ability of *O. korogwe* to persist in the face of *O. niloticus* introduction for numerous generations, is contrary to expectation, and suggests that this species may possess traits which predispose them for resilience to *O. niloticus* invasion.

Higher growth rates leading to increased body size are linked to several competitive advantages. These include performance during interference competition for shelter, greater efficiency during exploitative competition for food resources, increased reproductive output in mature females, and an enhanced probability of success during interference competition for lekking spaces in males (Chifamba and Videler 2014; Barneche et al. 2018; Bradbeer et al. 2020). Previous studies have shown that *O. niloticus* is an aggressive competitor that can dominate competitive interactions and prevent access to shelter in subordinate species (Martin et al. 2010; Champneys et al. 2020). Typically, fish are most vulnerable to predation during juvenile stages (Sogard 1997), and in Lake

Rutamba, survival is likely to depend on access to shelter resources such as the large reed beds located in the littoral regions. Elevated growth rates leading to increased body size could enable *O. korogwe* to avoid competitive dominance by *O. niloticus* and access shelter resources and preferred habitats despite competition. During sampling in 2019, *O. niloticus* and *O. korogwe* were both found in all four seine locations in the littoral areas of the lake, suggesting strong habitat overlap and likely competition over preferred habitats. However, more accurate information about the habitat use, and niche overlap of the two species within the lake would lead to more accurate predictions about the likely prevalence and outcomes of competition between the species over shared resources.

5.5.3 How does *O. korogwe* achieve higher growth rates?

Given that introduced *O. niloticus* are typically descendants of fish selected for high production yields, including fast growth rates, the increased growth rate observed in the native *O. korogwe* contrasts with our expectations. One explanation is that introduced *O. niloticus* are relatively poorly adapted to the local environment in comparison to the native *O. korogwe*. Unlike native species, which have a long evolutionary timeframe in which to adapt to environmental conditions, introduced species are faced with novel conditions to which they must rapidly adapt in order to become established (Flores-Moreno et al. 2015). Studies have shown that introduced species can adapt quickly to novel environments through behavioural, phenological and morphological changes (Thompson 1998; Lambrinos 2004). However, such rapid adaptation is not ubiquitous, and slow rates of phenotypic evolution following introduction are also reported (Mooney and Cleland 2001; Sakai et al. 2008). Growth rate is crucially influenced by access to food resources and a relatively poor ability to locate and consume food items could result in higher growth rates in *O. korogwe*, which may be better adapted to exploiting these resources.

Given that the increased growth rate was only observed in larger individuals, another explanation is that the two species diverge in their feeding strategy as they grow. Fish commonly shift their diet upon reaching larger sizes (Juncos et al. 2015), and while both species are microphagous generalists (M. Genner pers obs.), offshore habitats could support, for example, both a planktivorous and detritivorous feeding strategy, with one providing nutritional properties which facilitate higher growth rates. Such a separation of dietary niches may not be feasible in inshore nursery habitats, and this could explain the size-dependent differences in growth rate between the two species. Further investigation into the dietary overlap of the two species using gut content or stable isotope analysis may help to clarify dietary niche use of these species, while surveys of distributions of the species in the lake will provide corresponding insight into any differences in habitat use.

5.5.4 The effect of fishing pressure impacts of invasive *O. niloticus* in Lake Rutamba

Fishing pressure is high within Lake Rutamba, with 25 fishers active during the 2019 sampling period. Fishers were primarily using gill nets which target larger individuals in the central areas of the lake. During both sampling periods, catches were observed to be dominated by *O. niloticus*, which suggests that fishing pressure may be reducing the population of *O. niloticus* disproportionately more than *O. korogwe*, potentially removing breeding adults from the population. This effect appears to evidence by our sampling records within which the largest *O. niloticus* was only 23cm total length roughly half the maximum size of *O. niloticus* observed in Tanzania (~45cm total length; Genner et al. 2018). By reducing population sizes of *O. niloticus*, fishing likely reduces the competition for resources, and in turn potentially prevents the formation of interspecific dominance hierarchies over shared resources. When ecosystems are affected by multiple stressors there can be antagonistic interactions between them, with one stressor offsetting the other (Berlarde et al. 2016). Few studies have considered how other stressors such as fishing pressure in this example, can interact with the impacts of invasive species to exacerbate or reduce

their negative effects. Further research may be able to confirm if there is an antagonistic interaction between fishing pressure and the invasive *O. niloticus*. Which, combined with the fast growth of *O. korogwe*, may contribute to the persistence of this native species in a heavily modified environment.

5.5.5 Hybridisation and growth rate

We found clear evidence of hybridisation in our specimens, in accordance with the findings of Blackwell et al. (2021), and the hybrid individuals within our sample were of a range of body sizes and showed a growth rate closer to the faster growing *O. korogwe*. This result suggests that there is no strong ecological selection against hybrid genotypes, and hybrids may persist within the population. Considering our findings, it is possible that hybrids possessing a faster growing phenotype may have competitive advantages over the parental species, if the elevated growth rates of *O. korogwe* are combined with traits which may benefit *O. niloticus* such as a maximum large body size. Parental species are thought to be most threatened when hybrids possess fitness advantages. Where such heterosis is present genetic swamping can lead to the formation of hybrid swarms (Hwang et al. 2012). Further work is needed on reproductive preferences of parental species and their hybrids. Moreover, information on the abundance and habitat use of purebreds and hybrids within this population, which we were unable to ascertain from our sampling protocol, would also be useful for accurate prediction of the consequences of hybridization for the fish populations of Lake Rutamba. In the absence of this information our conclusions on hybrid selection within the lake remain highly speculative.

6 | General Discussion



Lake Nambawala, Tanzania

6.1 Thesis summary

The aim of the research presented in this thesis was to explore the role of behaviour in the success and impact of *O. niloticus* in Tanzania. Each of the four data chapters addressed unique hypotheses related to this aim. When combined, the results provide us with a better understanding of two key questions related to the invasion of freshwater systems by *O. niloticus*:

- 1) What are the likely outcomes and expected impacts of competition over shared resources between *O. niloticus* and native tilapia?
- 2) How might early interactions between naïve native tilapia and *O. niloticus* affect the establishment and impact of introduced *O. niloticus*?

I explore each of these questions in turn by discussing the results and the limitations of the four data chapters included in this thesis. Additionally, I will explore how future work might build on the research conducted in this thesis to a) improve our understanding of the impacts of introduced *O. niloticus* in Tanzania and b) to design experimental approaches which may improve our understanding of the role of behaviour in biological invasions more broadly.

6.2 What are the outcomes and subsequent impacts of competition over shared resources between *O. niloticus* and native tilapia?

Several studies have suggested that competitive dominance of shared resources is a mechanism underpinning the negative impacts of introduced *O. niloticus* (Canonico et al. 2005; Martin et al. 2010). Based on these findings we hypothesised that in Tanzania competition with native species might be especially relevant. This is because *O. niloticus* is close to its native range and lives in sympatry with a number of closely related and functionally similar native congeners. In **Chapter two** we showed that juvenile *O. niloticus* dominate size matched *O. amphimelas* in dyadic competitive

interactions over shelter under laboratory conditions. The ability for juvenile tilapia to survive and reach maturity is likely to depend on their ability to use shelter resources which limit predation threat. Our shelters were designed to mimic the reed beds commonly found in aquatic habitats in Tanzania, and we would therefore expect that competitive dominance of these habitats would likely increase predation and affect the fitness of native species.

In **chapter five** I conducted a field-based investigation into of the relative fitness of native tilapia and introduced *O. niloticus*. Behavioural observations in a field setting posed a number of logistical challenges and my initial attempts to do so using *in situ* camera set-ups proved to be infeasible due to the highly turbid water. Instead, I measured the growth rate of wild-caught specimens, expecting, based on the results of **chapter one**, that *O. niloticus* may dominate competitive interactions with sympatric populations of native *O. korogwe*, with the higher fitness *O. niloticus* being reflected in a higher growth rate. In contrast to our expectations, we found that it was *O. korogwe* with the higher growth rate at larger body sizes, a trait which may facilitate the persistence of this native species in the face of invasion by a large-bodied competitor.

It is also possible that fishing pressure within Lake Rutamba, which appears to disproportionately target *O. niloticus* (T. Champneys and M. Genner, pers. obs.), may further enable the persistence of this species. The importance of considering interactions between multiple stressors when measuring anthropogenic impacts on freshwater ecosystems is becoming increasingly recognised (Ormerod et al. 2010). For example, anthropogenic driven turbidity appears to favour the foraging of *O. niloticus* over native tilapia in experimental trials (Wing et al. 2021). Future work into how other anthropogenic stressors interact with species introduction is recommended for both *O. niloticus* and other widespread introduced species.

The results of **chapters two** and **five** reveal that the mechanisms underpinning the impact of *O. niloticus* are likely to vary based on the environmental context, including the species they are interacting with and the presence of other anthropogenic stressors such as fishing pressure. In the research for this thesis I was unable to compare the results of laboratory and field investigations on the same native species; *O. amphimelas* were used in the laboratory and *O. korogwe* were collected from the field. The advantage of laboratory studies is that they allow a high degree of control over environmental variables and behaviours can be measured at extremely high resolution through video tracking software. Conversely, investigation of invasive species mechanisms *in situ* incorporates the ‘true’ environmental context, a known regulator of invasive species impacts. Therefore, future work investigating behavioural interactions using a combined laboratory and field approach is likely to lead to the most accurate predictions about the impact of *O. niloticus* on a given native species. Behavioural trials in the laboratory using wild caught specimens would likely lead to the most accurate predictions, as invasion stages are known to act as selective filters which result in differences in the expression of behavioural traits across the various stages (Chapple et al. 2022).

In **chapter two** *O. niloticus* showed high levels of aggression towards a native *O. amphimelas* in competition over shelter. However, when two of the same shelter resources were present in **chapter four**, aggression levels were lower. We expect that this is a result of two key differences between the experimental designs. Firstly, the individuals in **chapter two** were size matched while in **chapter four** there was typically a large discrepancy in body size between *O. amphimelas* and *O. niloticus*. Secondly, the presence of multiple *O. amphimelas* may be the driver of this change in aggression. Grouping with native species may have benefits for the survival of *O. niloticus* following introduction and despite naïvete *O. niloticus* appears to readily access social information. Interestingly, in **chapter four** we found that follow latencies during leadership attempts were faster when *O. niloticus* were more aggressive and this was found to be evidence of increased group

cohesion. Thus, aggression by *O. niloticus* appears to differ depending on environmental context but drives potential fitness advantages in both dyadic and group interactions. This flexibility in expression of aggressive behaviour may be a key mechanism underpinning the successful establishment of this species. Behavioural plasticity in foraging behaviour has been linked to invasion success in birds (Sol et al. 2002), but how plasticity in other traits and in other widespread invasive species, affects invasion success is unclear and research into this issue appears warranted based on our results. Future work investigating within-individual variation in aggressive behaviour in *O. niloticus* across several environmental contexts (i.e. with different group sizes of con/heterospecific individuals) would provide necessary empirical evidence to these predictions.

6.3 How might early interactions between naïve native tilapia and *O. niloticus* affect the establishment and impact of introduced *O. niloticus*?

The precise timings of *O. niloticus* introductions are often unknown, yet the early stages of invasion are known to be particularly important in defining invasion outcome. Therefore, laboratory studies investigating behavioural interactions between naïve individuals can lend insights into the mechanisms which underpin successful establishment. In **chapter three**, we investigated how naïve native tilapia (*O. amphimelas*) and *O. niloticus* responded to one another in experimental trials. We found that both species readily approached the stimulus chamber containing the unfamiliar heterospecific more than in control trials, a behavioural response which would increase the prevalence of behavioural interactions between the species during early encounters. In **chapter four**, during leadership attempts from shelter, we found that *O. amphimelas* were just as likely to follow *O. niloticus* as they were to follow a conspecific. Given that *O. niloticus* would be naïve to their environment during such encounters *in situ* this could be a maladaptive behavioural response. Together, the results of **chapters three and four** highlight how naivete can result in maladaptive responses to invasive species.

In **chapter four** I investigated how the social behaviour of groups of native *O. amphimelas* changed before, during and after the introduction of a singular *O. niloticus* individual. In accordance with the results from **chapter three** I found that *O. niloticus* readily grouped with this unfamiliar heterospecific and this grouping resulted in a change in the social behaviour of native tilapia across three behavioural metrics: activity, speed and aggression. Access to social information from native tilapia which are well-adapted to their environment could provide fitness benefits to *O. niloticus* and *O. niloticus* were shown to readily follow *O. amphimelas*, suggesting a readiness to access this social information. Additionally, group living may enhance survival in *O. niloticus* by avoiding the increased predation threat resulting in living in small groups. The results of **chapter two** highlight how *O. niloticus* may avoid predation through competitive dominance of shelter resources and combined with grouping with native species outside of shelter, this could be an anti-predatory strategy which greatly enables their establishment in novel environments.

To better understand the behavioural responses of native species to introduced *O. niloticus*, field-based research is necessary. *O. niloticus* introductions are still taking place, and knowledge of a recent release could provide a study system in which to test whether predictions from our experimental trials hold up *in situ* and are consistent across different native species. For example, future work, investigating whether *O. niloticus* are readily grouping with native species trapping or remote underwater cameras would be useful to build on our results (Clark and Ioannou 2022; Glassman et al. 2022). To some extent our results in **chapter five**, namely that *O. niloticus* and *O. koroque* were found sympatrically in all of our 2019 seine drags, suggest that heterospecific grouping between native tilapia and *O. niloticus* may be occurring *in situ*. However, the fine scale make-up of groups within this habitat is still unknown, and the invasion of the Lake Rutamba and other nearby lakes is thought to have been ongoing for several decades, limiting insights from these results about the mechanisms underpinning initial establishment by *O. niloticus*.

6.4 *O. niloticus* in Tanzania: the costs and benefits of introduction

A central goal in invasion biology is understanding the impact of an introduced species on a recipient ecosystem (Parker et al. 1999). Based on an understanding of these impacts, ecologists and policy makers are faced with a decision about the costs and benefits of management interventions (Hanley and Roberts 2019). In the case of *O. niloticus* there are two conflicting forces at play. Unlike many non-native species, *O. niloticus* form an intrinsic part of the diet of local human populations and are often introduced for the purpose of improving capture fisheries (Canonico et al. 2005). Conversely, native species also form an important component of food security in Tanzania through capture fisheries, and the development of novel aquacultural strains (FAO 2018). *O. niloticus* are known to have negative impacts on native tilapia and may even contribute to the extinction of local tilapia species, as observed in Hombolo reservoir (Turner et al. 2019). In other countries these negative impacts have been shown to effect whole ecosystems (Starling et al. 2002) which could ultimately lead to lower yields from capture fisheries. To effectively mitigate the negative impacts of introduced *O. niloticus*, while optimising food security and the aims of local communities, a strong understanding of the impact of *O. niloticus* on native tilapia in Tanzania is necessary.

The results presented in this thesis contribute insights into the biology of a key invasive species of tropical freshwaters. Future work based on our findings may further strengthen our ability to a) understand and mitigate the impacts of previously introduced *O. niloticus* on native species, and b) predict the outcome of future introductions and prevent introductions into vulnerable areas. It is increasingly recognised that local knowledge and cultural memory are an essential component of conservation efforts which have too often been overlooked (Nazarea 2006; Shukla and Gardner 2006; Brook and McLachlan 2008). Ongoing collaborations with the Tanzanian Fisheries Research Institute and links to sources of local community knowledge, will be paramount to achieving sustainable food production in the face of multiple anthropogenic stressors to the biodiverse freshwater ecosystems of Tanzania.

7 | References

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