# COMPARATIVE COGNITION
# BETWEEN SOCIAL AND NON-SOCIAL CICHLIDS

# COMPARATIVE COGNITION BETWEEN SOCIAL AND NON-SOCIAL CICHLIDS

By

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## ABSTRACT

Cooperation often draws on cognition (i.e. learning and memory) in order to track cooperative partners and their contributions, resolve conflict between partners, improve coordination between partners, and enhance strategic-decision making. Learning and memory are also vital to resolve what is frequently a spatial and temporal mismatch between performing a cooperative act and receiving any kind of benefit in return. In this thesis, I compared cognitive abilities between three cooperative and group-living cichlid fishes from the Lamprologini tribe of Lake Tanganyika *Neolamprologus pulcher*, *Neolamprologus multifasciatus*, and *Julidochromis ornatus* with three of their close relatives that are not cooperative and never form groups *Telmatochromis temporalis*, *Altolamprologus compressiceps*, and *Neolamprologus tretocephalus*. This thesis aims to address whether the evolution of cooperation coincided with the evolution of sophisticated cognition in these fishes. In Chapter 2, I present evidence that both cooperative and non-cooperative cichlids are able to recognize familiar individuals and have similar numerical abilities. In Chapter 3, I show that performance when learning to navigate a maze, arguably a more general cognitive ability, was equivalent across cooperative and non-cooperative species, with comparable scores in terms of time to maze completion, number of mistakes and inhibitory control. In Chapter 4, I report on the results of a literature review where I quantified the growing interest in the field of fish cognition research, outline the current practices and pitfalls (heavy use of captive bred individuals and a reliance on lab-based research) and suggest how to bring more ecological relevance to the field.

Taken together, the results of my thesis improve our understanding of how social factors like group-living and cooperation modulate cognitive abilities, and detail the current trajectory of the field of fish cognition.

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## THESIS ORGANIZATION AND FORMAT

This thesis is organized into five chapters. **Chapter 1** provides the theoretical background and motivation for this thesis, as well as outlines the research objectives. **Chapter 2** is a published paper in the *Canadian Journal of Experimental Psychology*. **Chapter 3** is in preparation for submission to *Animal Behaviour*. **Chapter 4** has been submitted and is currently under review with *Animal Cognition*. **Chapter 5** offers a synthesis and discussion of the results from these data chapters.

### CHAPTER 1: General introduction

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### CHAPTER 2: Social memory and quantity discrimination: a cross cichlid species comparison

*Authors*: Matthew G. Salena & Sigal Balshine

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*Comments*: MGS and SB conceived and designed the experiments. MGS collected the data, conducted the analysis, and wrote the first draft of the manuscript under the supervision of SB. Reprinted with permission.

**CHAPTER 3: Little difference in spatial learning between cooperative and non-cooperative cichlids**

*Authors:* Matthew G. Salena, Angad Singh, Olivia Weller, Xiang Xiang Fang & Sigal Balshine

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**CHAPTER 4: Understanding fish cognition: a review and appraisal of current practices**

*Authors*: Matthew G. Salena, Andy J. Turko, Angad Singh, Avani Pathak, Emily Hughes, Culum Brown & Sigal Balshine

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## Chapter 5: General Discussion

*Author:* Matthew G. Salena

# TABLE OF CONTENTS

## Chapter 1: General introduction

## Chapter 2: Social memory and quantity discrimination: a cross cichlid species comparison

**Chapter 4: Understanding fish cognition: a review and appraisal of current practices**

**Chapter 5: General Discussion**

## LIST OF FIGURES

## LIST OF TABLES

# Chapter 1:

# General introduction

Matthew G. Salena

## 1.1 Introduction

Cooperation allows animals to accomplish tasks insurmountable to the individual such as the collective construction of towering termite mounds or the subduing of large and dangerous prey by group hunting animals. In humans, our innate interest in the evolution of cooperation stems from the fact that cooperation has led to incredible societal and technological advancements, driving globalization and creating a more interconnected world. However, cooperation is not uniquely human and has evolved in a wide variety of taxa, from amoebas to primates (West et al., 2007). Cooperation poses a challenge to traditional evolutionary theory which is thought to favour self-interest. What is especially puzzling is that cooperation can range from an act of minimal effort, like the small donation of time or energy, all the way to self-sacrifice (Polischuk et al., 2001; Salomon et al., 2005; Wright, 1994). As such, the evolution of cooperation has been of keen interest to scientists for over a century (Axelrod and Hamilton, 1981). Despite recent progress towards uncovering the genetic underpinnings of cooperation, as well as the costs and benefits of cooperation—both providing a framework for determining when cooperation is likely to evolve—little is known about the cognitive traits that underly and facilitate cooperative behaviour (Griffin and West, 2003; Hamilton 1963, 1964; Kay et al., 2019).

It has been suggested that more sophisticated cognition and social intelligence may have developed as a response to the social challenges of group-living and cooperation (Dunbar, 1998; Dunbar and Shultz, 2007). Studies supportive of this theory frequently cite reports of impressive cognitive abilities in cooperative birds, primates and cetaceans (Brosnan et al., 2010; Burkart and van Schaik, 2016; Jerison, 1975; Thornton and McAuliffe, 2015). However, it remains unclear whether complex cognitive abilities were selected as a consequence of social challenges in cooperative vertebrates. My thesis aimed to address this issue using three comparative studies and, in this introductory chapter, I establish the background motivating each of these three studies.

## 1.2 Linking cognition and cooperation

Cognition is the processing of stimuli and downloading of experiences used to produce information that can inform future decisions i.e. learning and memory (Shettleworth, 2001). Cognition is not necessary for ‘simple’ cooperation (mutualisms) to occur, but it can support and enhance cooperation in two important ways (Brosnan et al., 2010). First, cognition can improve coordination between individuals and, second, cognition can improve strategic decision-making regarding the best response in a particular situation. An example of how cognition can enhance cooperation via better coordination comes from cooperative hunting, where success greatly depends on an individual’s ability to monitor the actions of partners and adjust behaviour accordingly (Boesch and Boesch, 1989). An example of how cognition can enhance strategic decision-making comes from partner switching after experiencing trade inequity; many animals will preferentially engage with partners that have a history of fair trade, thereby remembering and accounting for the past behaviour of others (Raihani et al., 2012).

In many social species, cooperation has a big impact on an individual's fitness, helping individuals find resources and protection from predation (Brosnan et al., 2010). In some cases, not performing cooperative duties may result in punishment or even ejection from a social group, which greatly reduces an individual's chance of survival and future reproductive success (Clutton-Brock and Parker, 1995; Faulkes and Bennett, 2001). Additionally, the ability to manipulate and deceive cooperative partners while avoiding detection should offer a variety of fitness benefits (e.g. reduced energy expenditure, risk and time investment; Mokkonen and Lindstedt, 2016). Therefore, cognitive abilities that enhance social competence ought to be under strong positive selection (Brosnan et al., 2010). Social factors such as group size have been linked to brain size in a plethora of social vertebrates (Barton and Dunbar, 1997; Byrne and Whiten, 1990; Dunbar and Shultz, 2007; Emery et al., 2007; West, 2014). For example, Shultz and Dunbar (2006) collected information on neocortex size in 38 species of ungulates and showed that relative neocortex size was associated with social factors such as group size, but not ecological factors like diet and habitat use (but see Powell et al., 2017 and DeCasien et al., 2017 for contrasting findings in primates). Furthermore, Burish et al (2004) analyzed brain and body weight measurements from 154 bird species and found that telencephalic volume fractions (forebrain volume divided by total brain volume) was strongly linked to their social complexity. In this case, social complexity was evaluated by sorting birds into categories primarily based on group size, because determining group size does not require the direct observations of social interactions. Although many studies have reported a relationship between social factors and brain size, some researchers have argued that the assumptions underlying these comparative works are erroneous (e.g. what brain size means, problems with data collection and combination; Healy and Rowe, 2006). Others have failed to find any relationship between social factors and brain size (Beauchamp and Fernández-Juricic, 2004; DeCasien et al., 2017; Reddon et al., 2016).

Dunbar (1998) observed a correlation between brain size and group size in anthropoid primates and coined the *Social Brain Hypothesis*, which presumes that social factors like group size are a key determinant in encephalization—the evolution of relatively enlarged brains. Since its inception, the *Social Brain Hypothesis* has been tested in a variety of highly social animals and little evidence supportive of this theory has been described outside the primate literature (Acedo-Carmona and Gomila, 2016; Heyes, 2012; Lihoreau et al., 2012). The *Social Intelligence Hypothesis*, a modification of the *Social Brain Hypothesis* (Kummer et al., 1997), postulates that brain size is not the only indicator of cognitive ability, and that cognitive differences between highly social and less social animals can manifest at finer levels of analyses (e.g. specific brain structures, neuronal density, synaptic connectivity; Ashton et al., 2018). The *Social Intelligence Hypothesis* has received greater empirical and comparative support (Holekamp, 2007); however, neither of these theories address exactly what defines a highly social animal and, therefore, it is unclear which social parameters scientists should investigate as potential drivers of brain size evolution and/or the evolution of cognitive complexity.

## 1.3 The cooperative breeding brain

Cooperative breeding represents an extreme form of cooperation in which group-members collectively care for offspring (allocare), and can be observed in numerous avian, mammalian and insect species (Bernasconi and Strassmann, 1999; Emlen, 1991). The *Cooperative Breeding Brain Hypothesis* is a further extension of the *Social Brain Hypothesis* and it posits that social challenges (e.g. group-member recognition, conflict resolution) are especially pronounced in cooperative breeding groups. Thus, cooperative breeders should have well-developed cognitive abilities compared to their non-cooperative congeners (Burkart and van Schaik, 2016; Thornton and McAuliffe, 2015). Unlike the *Social Brain Hypothesis* and the *Social Intelligence Hypothesis*, the *Cooperative Breeding Brain Hypothesis* provides a testable measure of social complexity. With the framework offered by the *Cooperative Breeding Brain Hypothesis*, instead of measuring social complexity as a function of highly variable and difficult to quantify parameters like group size, we can evaluate social complexity as the presence or absence of cooperative breeding. This approach permits the simplistic design of comparative studies to assess whether the evolution of cooperative breeding coincided with sophisticated cognition. Despite recent interest, studies addressing the validity of the *Cooperative Breeding Brain Hypothesis* have been mostly limited to primates and birds (Ben Mocha et al., 2019; Burkart et al., 2010; Iwaniuk and Arnold, 2004; Thornton and McAuliffe, 2015).

## 1.4 Study system: Lamprologini cichlids

With over 30,000 species, teleost fishes make convenient, perhaps even ideal organisms to study variation in cognitive and trait evolution between highly social and less social species. Teleosts demonstrate an incredible diversity of social behaviours (Powers, 1989; Roberts and Ormond, 1992; Tinbergen, 2013; Turner, 2007) and are capable of elaborate cognitive skills (Brown and Laland, 2003; Bshary et al., 2014; Swaney et al., 2001). Fish are adept at solving problems involving intricate coordination, memory and complex decision making, and apart from lacking a neocortex, the fish brain is remarkably similar to the brains of other vertebrates (Figure 1.1; Bshary et al., 2014).

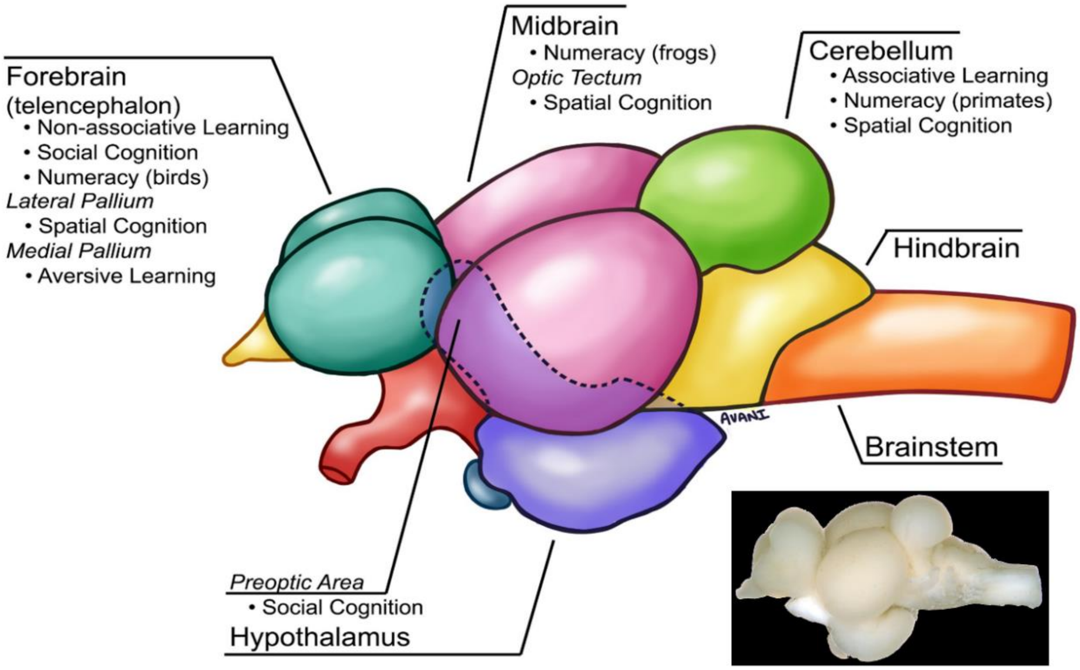

**Figure 1.1** A schematic depicting the fish brain and the regions implicated in various cognitive processes. Different parts of the brain will work in conjunction to control cognitive processes, and some links between cognitive abilities and particular brain regions remain somewhat undefined and/or debated. In fishes, the cerebellum is involved with associative learning, and the medial pallium plays a role in aversive learning. The telencephalon (forebrain) is thought to be primarily responsible for non-associative learning (Hurtado-Parrado, 2010). Numeracy has not been associated with a particular brain region in fishes, but has been linked to the cerebral cortex, subcortex and cerebellum in primates (Cantlon, 2012; Collins et al., 2017; Vandervert, 2017), to the telencephalon in birds (Ditz and Nieder, 2015), and to the midbrain in frogs (Edwards et al., 2002). Spatial cognition is thought to be centered in the lateral pallium of the telencephalon, a structure that is homologous to the hippocampus—the neural centre of cognitive maps in mammals and birds (Rodriguez et al., 2002). In addition, the cerebellum, the optic tectum and the medial pallium (regions outside of the forebrain) have also been implicated in spatial ability of fishes (Broglio et al., 2003). Socio-cognitive abilities are thought to be mediated by the preoptic area, hypothalamic area and dorsomedial and dorsolateral telencephalon of the fish brain (Godwin and Thompson, 2012; Maruska et al., 2019; O'Connor et al., 2016; Reddon et al., 2015; Reddon et al., 2017). Illustration by Avani Pathak.

Within teleost fishes, cichlids, a large freshwater tropical fish family, provide seemingly unlimited opportunities for the study of social behaviour and cognition (Rossiter, 1995; Turner, 2007). Not only are there many cichlid species, but there is considerable variation in social behaviour across cichlids and substantial knowledge of the genetic relationships among species (Keenleyside, 1991; Seehausen, 2006; Stauffer et al., 2002). While all cichlids provide parental care for their young, some cichlids live and care in groups, while others live more solitary lifestyles (Keenleyside, 1991). Moreover, rapid speciation and adaptive radiation of cichlid fishes has encouraged the creation and proliferation of phylogenies and molecular tools available for cichlids (Conte et al., 2019; Koblmüller et al., 2008; Kocher, 2004; Salzburger et al., 2005). These phylogenies and molecular tools are useful for grounding phylogenetically based comparative studies of social cognition and other traits. Within the cichlid family, one tribe, the Lamprologines of Lake Tanganyika, Africa, stand out as arguably the best candidates for such studies on the evolution of socially selected cognitive traits (Figure 1.2). Although cooperative breeding is rarely described in fishes (Reddon et al., 2017), there have been five independent evolutionary transitions to cooperative breeding in this group, and one transition away from cooperation (Dey et al., 2017). The Lamprologini cichlids include both non-cooperative more solitary species as well as closely related, obligately group-living, cooperatively breeding species, all of which are from a single lake, and experience similar abiotic and ecological regimes. Therefore, Lamprologini cichlids offer a powerful model to explore how the evolution of cooperation has shaped the brain and cognitive abilities.

Comparative studies to explore differences in cognitive abilities between cooperative breeding Lamprologines and their non-cooperative relatives would provide insight into the merit of the *Cooperative Breeding Brain Hypothesis* and help determine whether, and to what degree, cooperation shapes cognition.

**Figure 1.2** 69 of the 84 described Lamprologini cichlids of Lake Tanganyika, Africa. The species used in my research and discussed throughout this thesis are highlighted in white.

## 1.5 Aims and structure of the thesis

In this thesis, I employed a comparative approach to better understand the cognitive underpinnings of complex social behaviour. In my first data chapter (Chapter 2), I compared cognitive abilities of three cooperatively breeding cichlids (*Neolamprologus pulcher, Neolamprologus multifasciatus*, and *Julidochromis ornatus*) with three of their non-cooperative relatives (*Telmatochromis temporalis*, *Altolamprologus compressiceps*, and *Neolamprologus tretocephalus*). In this chapter (Chapter 2), which is now published, I assessed socio-cognitive abilities of these six cichlids, investigating social memory and quantity discrimination in a group-joining context. I predicted that the challenges of living in cooperatively breeding groups would select for an enhanced recognition and numerical capacity in cooperatively breeding species relative to their non-cooperative congeners. In Chapter 3, I describe a study in which I evaluated spatial learning abilities of these six cichlid species to see whether the challenges of social life have influenced the brain in such a way that benefits general cognitive traits like navigation. I predicted that the cognitive challenges of cooperative breeding would select for improved general cognitive traits and expected that the cooperatively breeding cichlids would outperform their non-cooperative relatives in this spatial navigation and learning task. Chapter 4 provides a review of current practices and pitfalls surrounding the fish cognition literature, and offers a set of recommendations for improving the ecological relevance and generalizability of findings. This last data chapter has been submitted for publication and is under review. In Chapter 5, I present a general discussion that aims to bring together my results and outline a number of future directions that naturally extend from my MSc research. In sum, my MSc thesis studies contribute to our understanding of how social factors and cooperation shape cognitive abilities. In particular, Chapter 2 and Chapter 3 evaluated whether the evolution of complex social conditions, such as those seen in cooperative breeding groups, coincided with the evolution of more sophisticated socio-cognitive and general cognitive abilities. The review also points out research trends that are currently hampering studies of fish cognition and offers suggestions on how to move the field forward.

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# Chapter 2:

# Social memory and quantity discrimination: a cross cichlid species comparison

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## 2.1 Abstract

Cooperation is a highly complex social interaction that often requires coordination and communication between two individuals. Reciprocity is one explanation for how cooperation evolves and is maintained; help now will eventually be repaid in kind. For reciprocity to work, individuals must be able to differentiate between those who helped previously versus those who cheated. However, there is little empirical evidence that cooperative species have an enhanced recognition capacity compared to non-cooperative species. Here, we conducted a comparative study to address this question using three cooperatively breeding cichlids and three of their close relatives that are not cooperative breeders, all from Lake Tanganyika. In a first experiment, we offered fish a choice between spending time with a familiar versus an unfamiliar conspecific and found that while cooperative cichlids spent more time with familiar individuals, the non-cooperative cichlids spent more time with unfamiliar individuals. In a second experiment, we provided a choice between affiliating with one versus three individuals (all unfamiliar) and found that 2/3 cooperative and 3/3 non-cooperative cichlids strongly preferred to affiliate with larger groups. Our results suggest that both cooperative and non-cooperative cichlids have evolved the ability to recognize familiar individuals and have affiliative preferences; however, the nature of these preferences differ.

## 2.2 Introduction

Cooperation is the act of working together towards a common goal and its evolution can be challenging to explain because one party often appears to benefit at the cost of another (Axelrod and Hamilton, 1981; Brosnan et al., 2010; Nowak, 2006). Kin selection (indirect benefit) has traditionally been used to explain cooperation; where donors of costly-help still benefit by having their relatives succeed (Hamilton, 1964; West et al., 2007). More recently, many researchers have shifted their focus from indirect to direct benefits to explain the evolution of cooperation, where enhanced resource acquisition, survival and reproduction are accrued via the cooperative act (Clutton-Brock, 2009; Leimar and Hammerstein, 2010; Taborsky et al., 2016).

Cooperative breeding represents a complex form of cooperation that is observed in numerous mammals, birds and insects (Arnold and Owens, 1999; Bernasconi and Strassmann, 1999; Koenig and Dickinson, 2004; Lacey and Sherman, 1997; Solomon and French, 1997). Cooperative breeding is characterized by alloparental care where individuals help raise young that are not their own (Cornwallis, 2018). Although group-living and social interaction are common in fishes (Brown et al., 2011), the Lamprologines of Lake Tanganyika, in Africa, are the only group of fishes that have evolved true cooperative breeding (Reddon et al., 2017; Taborsky, 2001; Tanaka et al., 2018). Within this tribe, cooperation has evolved independently, multiple times (Dey et al., 2017). The socio-cognitive challenges of living in a social group and cooperating to raise young are thought to have selected for particular cognitive abilities (Ashton et al., 2018; Croney and Newberry, 2007; Holekamp, 2007). For instance, living in a group and cooperating requires that individuals recognize, remember and respond appropriately to their own group members versus strangers and invaders (Reddon et al., 2016). They must also find ways to quickly settle disputes (Balshine et al., 2017). Thus, discrimination between familiar and unfamiliar individuals is requisite to the maintenance of group cohesion and facilitates complex cooperative acts between group members—however, the extent to which non-cooperative animals share this ability is still up for debate. This idea has not been tested in a comparative context and further study will provide a deeper understanding of how cognitive ability has been molded by social challenges and the evolution of cooperation.

The use of fishes as models for studies of social cognition is becoming increasingly popular (Brown and Laland, 2003; Bshary et al., 2014; Pouca and Brown, 2017). In this study, we examined differences in discrimination abilities (familiarity recognition and group-size assessment capability) between cooperative and non-cooperative Lamprologini cichlids from Lake Tanganyika. These fishes exhibit remarkable diversity in their social propensity and behaviour (O'Connor et al., 2015; Seehausen, 2006; Sturmbauer et al., 2010; Taborsky, 2016). In our first experiment, we tested social memory and predicted that cooperative species would have better developed social memory, since they must be able to distinguish group-members from non-members. We also predicted that, unlike the non-cooperative species, the cooperative species would treat familiar individuals differently from strangers. Further, based on results from two studies on Wistar rats (*Rattus norvegicus*) and zebrafish (*Danio rerio*), we predicted that cooperative cichlids would respond strongly to novelty, and spend more time investigating strangers versus familiar fish (Engelmann et al., 1995; Madeira and Oliveira, 2017). In our second experiment, we tested quantity discrimination ability and predicted that cooperative cichlids would have superior quantity assessment capacity and grouping tendencies and show stronger preferences to affiliate with groups compared to closely related non-cooperative cichlids. We expected cooperative species to outperform non-cooperative species in the quantity discrimination task and spend more time grouping when presented with cues of predation risk. Our prediction is based on the observation that cooperatively breeding species live in groups for their entire lives and must overcome socio-cognitive challenges, like moderating aggressive tendencies, to accept and tolerate other sexually mature adults in their territories. However, we were also aware that a number of other studies have found similar quantitative capacities in more social and less social animals (Agrillo et al., 2008; Agrillo et al., 2012; Vonk and Beran, 2012).

## 2.3 Materials and methods

### 2.3.1 Animals and housing conditions

Experiments were conducted between September 2018 and May 2019, using laboratory stocks of *Neolamprologus pulcher*, *Telmatochromis temporalis*, *Neolamprologus multifasciatus*, *Altolamprologus compressiceps*, *Julidochromis ornatus* and *Neolamprologus tretocephalus* housed at McMaster University in Hamilton, Ontario, Canada. *N. pulcher*, *N. multifasciatus* and *J. ornatus* are cooperative breeders while *T. temporalis*, *A. compressiceps* and *N. tretocephalus* are non-cooperative (Heg and Bachar, 2006; Heg et al., 2005; Mboko and Kohda, 1999; Nagoshi, 1983; Sefc, 2011; Taborsky et al., 2005). Each of these species are territorial (Awata et al., 2006; Dey et al., 2017; Hick et al., 2014; Nagoshi, 1983; Spreitzer et al., 2012; Suriyampola and Eason, 2015). *N. pulcher and T. temporalis* were laboratory-reared descendants of wild-caught fishes from Lake Tanganyika, Africa. *N. multifasciatus*, *A. compressiceps*, *J. ornatus*, and *N. tretocephalus* were commercially bred and purchased from a local fish supplier (Finatics, Mississauga, ON, Canada). Fish were held in mixed sex stock tanks fitted with filters, heaters, 2cm of coral sand substrate, terracotta flowerpot halves and opaque PVC tubes (10cm in diameter and 25cm in length) as shelter. Stock tanks were either 568L tanks with ~60 individuals/tank or 189L tanks with ~20 individuals/tank. These stock tanks and our experimental tanks (see below) were maintained at 25–28˚C and a 12L:12D photoperiod. Fish were fed six times per week *ad libitum* a diet of cichlid flakes and pellets.

### 2.3.2 Experiment 1: Social discrimination task

Trials were conducted in 38L (50 x 25 x 31cm) aquaria, that were well lit, and fitted with a heater, a mechanical filter and 2cm of coral sand substrate. Each tank was lined with contact paper to minimize disturbance from neighbouring tanks. Tanks were divided into three compartments, by two fixed transparent barriers and two removable opaque barriers attached to a pulley system, allowing these barriers to be removed remotely (see Figure 2.1a).

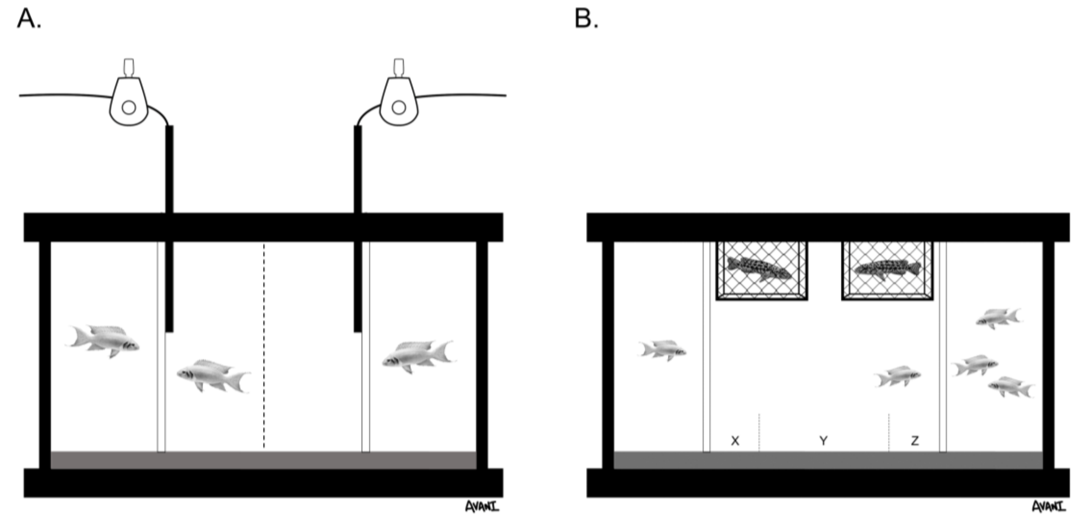

**Figure 2.1a. Experiment 1.** Schematic of the aquaria used for the social discrimination task. Pulleys allowed opaque barriers to be lifted remotely, then the fish could interact across the fixed transparent barriers. The *dashed line* represents the divide by which preference was assessed. **b. Experiment 2.** Schematic of the aquaria used for the quantity discrimination task. The *dashed lines* represent zones in the central compartment. The letters X and Z depict ‘choice’ zones while the letter Y depicts the neutral ‘no choice’ zone. Illustration by Avani Pathak.

*Day 1: Capture, measurement and habituation*

Focal fish were captured from a stock tank, sexed and measured (standard length and body mass) before being placed in the central compartment of an experimental tank. Then two size and sex matched unfamiliar conspecifics (see supplementary material for details) were selected from a different stock tank and were placed in opposite end compartments (Figure 2.1a). All three fish were provided their own refuge (PVC tube) and left overnight in their respective chambers with removable opaque barriers and fixed transparent barriers between them.

*Day 2: Initial preference test*

The next day, refuges were removed, and the fish left for 1h without shelter before the opaque partitions were lifted. Focal fish were then videotaped for 22-minutes and all interactions across the transparent barriers were recorded. After this period, opaque partitions were replaced, and one stimulus fish was randomly selected to remain in the test aquaria while the other was removed. The removed fish was replaced with another unfamiliar sex and size matched fish, again from a different stock tank, and all three fish had their refuges returned overnight.

*Day 3: Final test phase*

We repeated the identical procedure from Day 2 but now the focal fish was given an opportunity to interact with either a fish it had viewed the previous day (a familiar fish) or a fish it had never interacted with before (an unfamiliar fish). All interactions were videotaped for 22-minutes.

### 2.3.3 Experiment 2: Quantity discrimination task

Following Experiment 1, focal fish were guided into a start box attached to a pulley (a PVC tube closed off at one end, with a sliding door at the other end). Each focal fish was transported inside this start box and placed individually in the central compartment of a new 189L (89 x 50 x 50cm) aquarium. Fish in the start box were transported between tanks for Experiment 1 and Experiment 2 in a container of water (25 x 17 x 15cm), and were gently submerged into their new tank within this start box. The start box was placed so that the sliding door faced directly towards the camera, and away from each end chamber containing a stimulus. The central compartment (40 cm wide) of the test aquarium was separated by transparent partitions from two end chambers (each 23 cm wide, see Figure 2.1b). One end compartment contained three conspecifics, while the opposite end compartment contained a single conspecific. The stimuli fish were always placed in the test tank 10-minutes before the focal fish. Stimuli fish (the conspecifics in end chambers) always came from a different stock than the focal fish and hence were all unfamiliar to the focal individual. Stimuli fish were always selected randomly, varied in terms of sizes and sexes, and were changed across trials. The central chamber was further divided into three compartments labelled X, Y, Z. Compartments X and Z (both 12.5 cm wide) were our 'choice' zones; if the focal fish was in one of these zones it was considered to prefer one stimulus over the other. Compartment Y (17.5 cm wide) was labelled a 'neutral' zone, where the focal fish was considered to have no preference. Each focal fish was given 5-minutes to acclimate in the start box before the sliding door was removed remotely. Once the door of the start box was opened, fish were then given a maximum of 5-minutes to leave. After the fish had left the start box, or if the fish had not moved out of the start box after 5-minutes, the entire box was remotely removed from the tank. This removal forced fish to exit if they had not yet left the start box (forced exits occurred in: 0 of 25 *N. pulcher* trials, 7 of 25 *T. temporalis* trials, 8 of 24 *N. multifasciatus* trials, 7 of 24 *A. compressiceps* trials, 6 of 24 *J. ornatus* trials and 19 of 24 *N. tretocephalus* trials). From the moment the focal fish left the start box, they were recorded for another 20-minutes, moving around in the middle chamber and potentially interacting with conspecifics in both end chambers. Two predatory cichlids, *Lepidiolamprologus* *kendalli*, in mesh baskets were placed along the back wall of the central compartment to create a sense of predation risk and heighten the consequences of isolation.

### 2.3.4 Quantification of behaviour from the videos

Preferences of focal fish were recorded with Canon cameras (HF S200 and HF R80) placed in front of each tank 1h prior to the start of each trial. Experiment 1 employed a forced choice design; fish were always with one stimulus or the other and the focal fish was considered to be on a particular side (i.e. with a particular stimulus) if its head and gills were over the midway line. In Experiment 1, we recorded the time spent on each side of the tank (i.e. the time spent with each stimuli fish). Activity of stimuli fish was qualified by recording whether each fish was active or not every 2-minutes throughout the experiment. We got an activity rating every 2-minutes out of the 20-minute trial, for a total of 10 observations. Experiment 2 allowed fish to have a no choice option. If the focal fish was not in one of the 'choice' zones near the conspecifics (either the group or the single conspecific), then it was considered to have no preference at that time. Again, the focal fish was considered to be in a particular zone if its head and gills were over the line to that zone. In Experiment 2, we recorded the time spent in each zone of the tank (zone 'X' reflects time near the single conspecific; zone 'Y' reflects no choice; and zone 'Z' reflects time near the group of conspecifics). We scored videos using Behavioural Observation Research Interactive Software (BORIS), a behavioural scoring program (Friard and Gamba, 2016). Videos were always scored by an observer that was blind to treatment. In Experiment 1, we started recording behaviours after the first 2-minutes to account for the disturbance caused by lifting the partitions. In Experiment 2, we recorded all behaviour for 20-minutes after the fish had left the start box.

*Ethics statement*

The procedures used in this study were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol 18-04-16) and followed the guidelines established by the Canadian Council on Animal Care (CCAC) regarding the use of animals in research.

### 2.3.5 Statistical analyses

Analyses were performed using R (v.3.6.0, R Core Team, 2019) and a significance (α) of 0.05 was used for all tests. When data failed to meet the assumptions of normality and homogeneity, the equivalent non-parametric tests were used. In Experiment 1, to assess whether a particular species is able to discriminate between familiar and unfamiliar conspecifics and if any corresponding preference was associated with social breeding system, we created a generalized least square (pGLS) model, which accounts for phylogenetic relationships between species, using the package 'ape' (Paradis and Schliep, 2018). Branch lengths were estimated from the phylogeny presented by Day et al (2007), however, assuming the phylogenetic signal i.e. lambda (λ) equals 1 produced the same result. We then used Wilcoxon signed rank tests to assess whether the familiarity preference for each species differed significantly from zero. We conducted 140 Day 3 trials (see supplementary material), but 19 of these trials (5 *N. pulcher*, 1 *T. temporalis*, 7 *N. multifasciatus*, 5 *A. compressiceps*, 1

*J. ornatus*), were eliminated and excluded from analyses because one of the fish jumped across the barriers during the trial or one fish did not move during the trial.

In Experiment 2, we investigated species differences for grouping using a Kruskal-Wallis one-way analysis of variance test. To compare time spent with the single conspecific versus time spent with the group, across all species combined, we used a Wilcoxon matched pairs signed rank test. Next, to assess each species' individual preference (for the single conspecific or the group), we conducted a Wilcoxon matched pairs signed rank test on data for each species separately. Overall, our statistical analyses were based on 6 cichlid species and a total of 148 trials.

## 2.4 Results

### 2.4.1 Social Discrimination

On Day 2, there was no difference between cooperative and non-cooperative cichlids in the time spent with the unfamiliar conspecifics (pGLS: $\lambda = 0.18$, $t_{(1,5)} = 0.41$, $p = 0.71$). On Day 3, both cooperative and non-cooperative cichlids showed consistent preferences for one of the conspecifics suggesting that all 6 species are capable of social discrimination (pGLS: $\lambda = 0.18$, $t_{(1,5)} = 5.46$, $p < 0.01$). However, the direction of that preference varied between cooperative and non-cooperative species (Figure 2.2) with cooperative species showing a preference for *familiar* fish (Wilcoxon-test, $\mu = 0$: *N. pulcher*, $Z = 3.73$, $p < 0.001$; *N. multifasciatus*, $Z = 4.34$ , $p < 0.001$; *J. ornatus*, $Z = 4.53$, $p < 0.001$) and non-cooperative species preferring the *unknown or unfamiliar* individuals (Wilcoxon-test, $\mu = 0$: *T. temporalis*, $Z = 4.75$, $p <$

0.001; *A. compressiceps*, $Z = 3.75$, $p < 0.001$; *N. tretocephalus*, $Z = 4.61$, $p < 0.001$). There were no differences in activity levels between the two stimulus fish (Wilcoxon-test: $Z = 0.38$, $p = 0.35$).

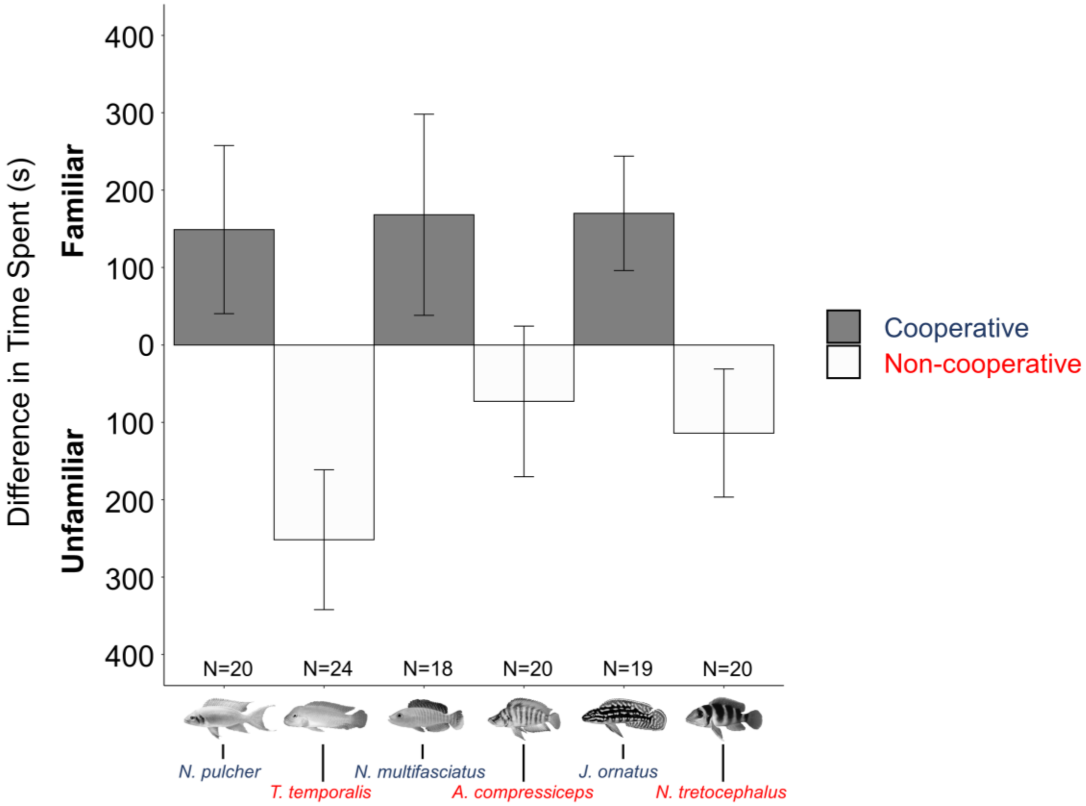

**Figure 2.2** Familiarity preferences of cooperative and non-cooperative cichlids. Social system predicted preference (pGLS: $\lambda = 0.18$, $t_{(1,5)} = 5.46$, $p < 0.01$). Values are means ± SEM.

### 2.4.2 Quantity discrimination

In this experiment, 5/6 species spent more time with the group of three fish versus the single fish. Although *N. multifasciatus* spent more time with the group than the single fish, this preference did not reach statistical significance (Wilcoxon-test: *N. pulcher*, $Z = 4.08$ , $p < 0.001$; *T. temporalis*, $Z = 3.66$, $p < 0.001$; *N. multifasciatus*, $Z = 1.67$, $p = 0.09$; *A. compressiceps*, $Z = 4.41$, $p < 0.001$; *J. ornatus*, $Z = 4.56$, $p < 0.001$; *N. tretocephalus*, $Z = 2.53$, $p = 0.012$, Figure 2.3). Males and females spent similar amounts of time with the group of three fish (LM: $\chi^2 = 1.54$, $p = 0.13$) and body mass of the focal fish did not influence the time spent with the group (LM: $\chi^2 = 0.56$, $p = 0.58$).

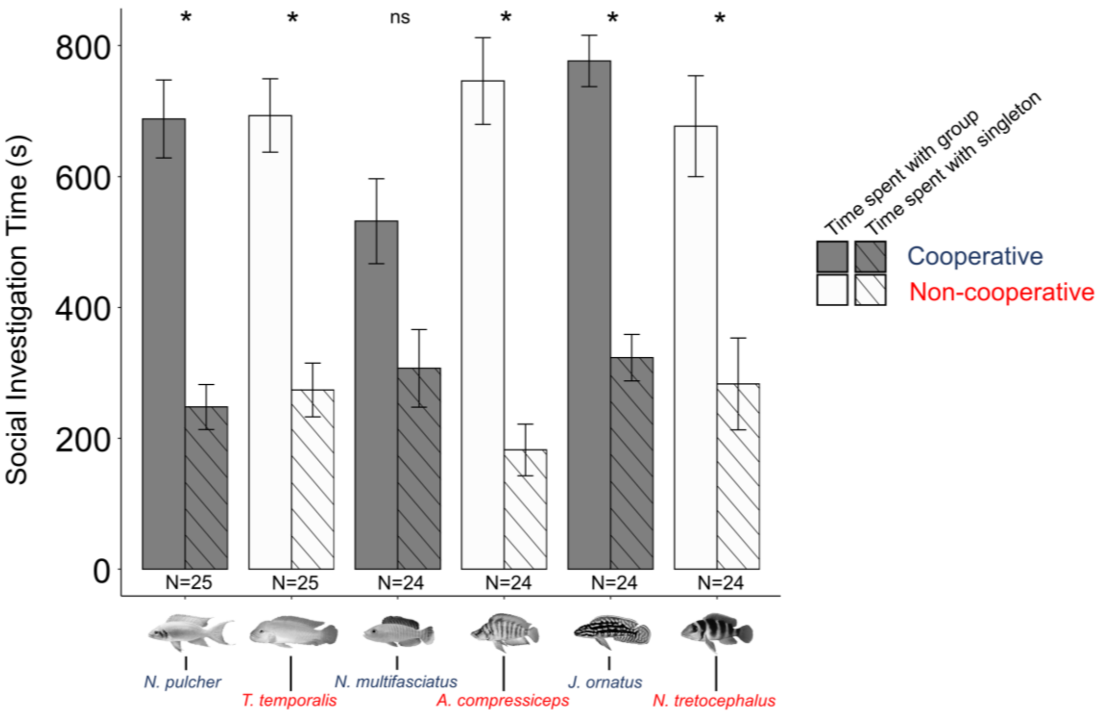

**Figure 2.3** Grouping preferences of cooperative and non-cooperative cichlids in the presence of predators. Overall, there was a strong preference for the group (Wilcoxon-test: $Z = 7.98$, $p < 0.001$), and there were no differences between species in the strength of this preference (KW: $\chi^2_{(1,5)} = 7.92$, p = 0.16). Values are means ± SEM.

## 2.5 Discussion

Familiarity plays a key role in social grouping decisions. For example, cattle (*Bos taurus*), red jungle fowl (*Gallus gallus*) and group-living predatory mites (*Phytoseiulus persimilis*) preferentially join familiar social groups (Muleta and Schausberger, 2013; Sato et al., 1987; Väisänen and Jensen, 2004). Similar results have been shown in guppies (*Poecilia reticulata*), minnows (*Phoxinus phoxinus*), sticklebacks (*Gasterosteus aculeatus*), rainbowfish (*Melanotaenia spp*.) and cichlids (*Pelvicachromis taeniatus* and *Neolamprologus pulcher*; Barber and Wright, 2001;

Brown, 2002; Frommen et al., 2007; Jordan et al., 2009; Lachlan et al., 1998; Thünken et al., 2015). In the chub (*Leuciscus cephalu*), even *familiar heterospecifics* are preferred over *unfamiliar conspecifics* (Ward et al., 2003). Preferentially shoaling with familiar individuals can lead to enhanced and more cooperative antipredator behaviour (as seen in fathead minnows *Pimephales promelas*; Chivers et al., 1995), and to more efficient prey location and consumption (as observed in three-spined sticklebacks *Gasterosteus aculeatus*; Ward and Hart, 2005). Then why did the non-cooperative cichlids prefer unfamiliar individuals while the cooperative and highly social cichlids preferred familiar individuals? More solitary animals could use different tactics to solve conflicts, and interest in unfamiliar fish or objects may be driven by a more general preference for novelty (Brown, 2002; Hick et al., 2014). Novelty seeking offers opportunity for social interaction with unknown individuals and groups and increased sampling of the environment (Greenberg, 2003). In contrast, cooperative species familiarity preference may make sense, as the social landscape is stable, and familiarity facilitates altruism or reciprocity, while dampening conflict in situations where interactions are regular. Familiarity allows better predictions about how a companion will respond in a variety of contexts (Brown, 2002). In many territorial species, like the cichlids used in this study, territory owners act less aggressively to neighbours versus strangers, a phenomenon known as the 'Dear Enemy' effect which requires recognition of familiar individuals (Jaeger, 1981; Sogawa and Kohda, 2018; Temeles, 1994). Thus, territoriality may be a good proxy for social recognition capacity (Saeki et al., 2018). In our study, the cooperative and non-cooperative species showed preferences for familiar and unfamiliar individuals respectively, suggesting that all 6 species were able to differentiate between them, significantly expanding the known examples of social recognition in fishes (Experiment 1). Contrary to our prediction, both cooperative and non-cooperative species demonstrated similar social recognition capacities and differential treatment of familiar versus unfamiliar individuals.

The ability to distinguish large from small or many from few can greatly impact an individual's fitness. Most fishes tested to date have shown preferences to join the larger of two shoals when provided an option (Agrillo and Dadda, 2007; Binoy and Thomas, 2004; Hoare et al., 2004; Krause and Godin, 1994; Pritchard et al., 2001). Larger groups offer a variety of fitness benefits such as protection from predators and the opportunity for social learning from more experienced individuals (Cresswell and Quinn, 2011; Laland and Williams, 1997; Mooring and Hart, 1992). In goldfish (*Carassius auratus*) and minnows (*Phoxinus phoxinus*), larger shoals find food faster (Pitcher et al., 1982). Moreover, female sand gobies (*Pomatoschistus minutus*) choose males with more eggs in their nest, thereby using social information to inform mate-choice decisions (Forsgren et al., 1996). Mosquitofish (*Gambusia affinis*) are even capable of discriminating between two shoals that differ in size by a single individual (Agrillo et al., 2008). In Experiment 2, cichlids were offered the choice between shoaling with a group of three or a single conspecific (accompanied by cues of predation risk) and 5/6 species spent more time with the group in this context. Albeit *N. multifasciatus* also spent more time with the group but this pattern did not reach statistical significance. The preference for the group in all cases reflects the direct benefits of grouping under threat of predation. We show that grouping is largely driven by threat of predation (Balshine et al., 2017), irrespective of the social breeding system exhibited by a species, and that both cooperative and non-cooperative species have similar group-size assessment capabilities.

Overall, our results underscore the evolutionary forces that drive socio-cognitive abilities and suggest that ecological challenges, encountered by both cooperative and non-cooperative animals, may play a greater role in shaping cognition than social challenges. Future experiments that manipulate familiarity in the context of shoal-choice and quantity discrimination would shed light on the relative importance of these factors in group-joining decisions. Moreover, assessing the nature of the social interactions and comparing other socio-cognitive traits, such as observational learning and collective decision-making between cooperative and non-cooperative animals, would provide insight into whether there have been consistent cognitive changes in response to an assortment of ecological and social challenges.

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## 2.8 Supplementary materials

**Supplementary Table 2.1** Morphological characteristics of the cichlid fishes used in Experiment 1. Day 3 data provided. Values are means ± SEM.

| Species | Males | Females | Focal Standard Length (mm) | Stimuli Standard Length (mm) |
|---|---|---|---|---|
| *N. pulcher* | 8 | 12 | 65.30 ± 1.65 | 64.72 ± 1.46 |
| *T. temporalis* | 12 | 12 | 53.87 ± 1.77 | 54.51 ± 1.59 |
| *N. multifasciatus* | 8 | 10 | 43.68 ± 0.54 | 43.64 ± 0.53 |
| *A. compressiceps* | 7 | 13 | 43.53 ± 0.91 | 44.70 ± 0.53 |
| *J. ornatus* | 8 | 11 | 50.13 ± 0.82 | 53.03 ± 0.52 |
| *N. tretocephalus* | 9 | 11 | 53.18 ± 0.88 | 54.15 ± 1.04 |

**Supplementary Table 2.2** Morphological characteristics of the focal fish used in Experiment 2. Values are means ± SEM.

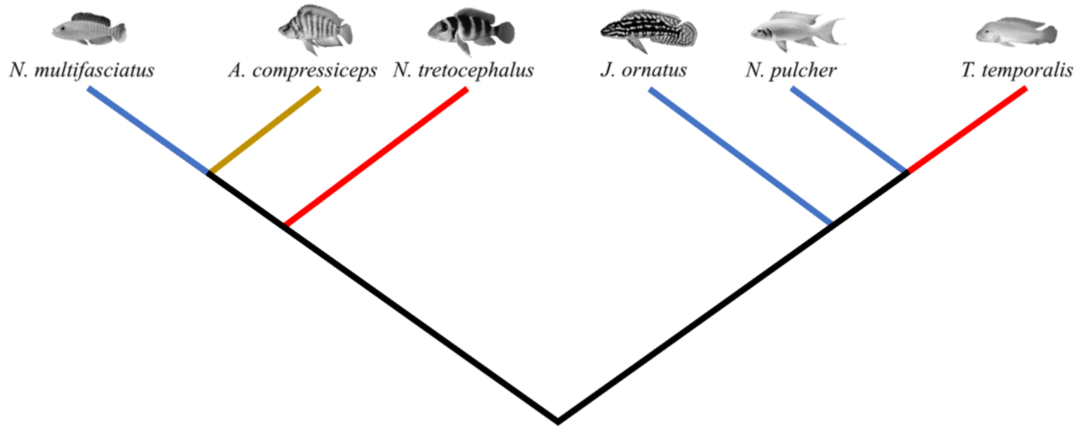
| Species | Males | Females | Standard Length (mm) |
|---|---|---|---|
| *N. pulcher* | 8 | 17 | 64.98 ± 1.41 |
| *T. temporalis* | 12 | 13 | 53.87 ± 1.72 |
| *N. multifasciatus* | 12 | 12 | 42.83 ± 0.60 |
| *A. compressiceps* | 8 | 16 | 43.97 ± 0.89 |
| *J. ornatus* | 9 | 15 | 50.10 ± 0.71 |
| *N. tretocephalus* | 10 | 14 | 51.39 ± 0.85 |

**Supplementary Table 2.3** Morphological characteristics of stimuli fishes used in Experiment 2. Values are means ± SEM.

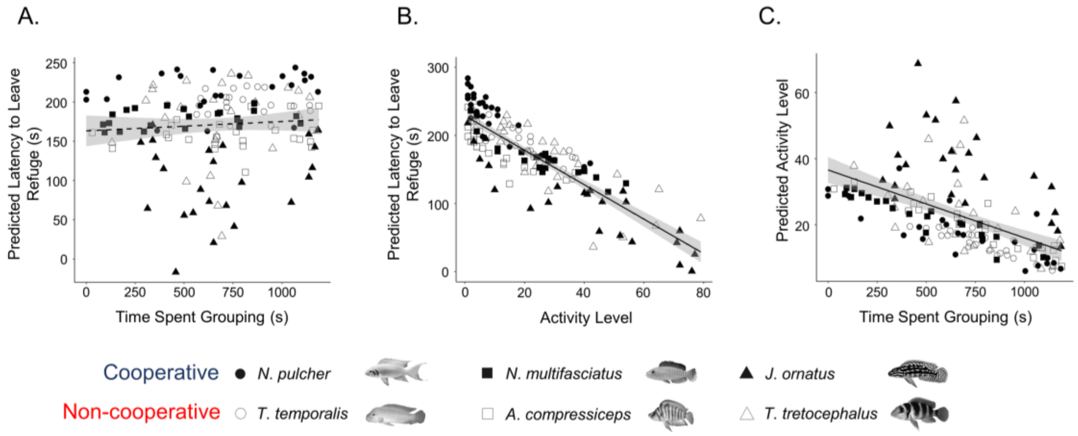
| Species | Standard Length (Single fish, in mm) | Standard Length (Group of fish, in mm) |
|---|---|---|
| *N. pulcher* | 62.44 ± 0.24 | 60.24 ± 1.08 |
| *T. temporalis* | 57.58 ± 1.71 | 56.51 ± 1.70 |
| *N. multifasciatus* | 43.44 ± 0.72 | 42.10 ± 0.71 |
| *A. compressiceps* | 45.51 ± 0.29 | 44.85 ± 0.59 |
| *J. ornatus* | 50.50 ± 0.37 | 51.43 ± 0.65 |
| *N. tretocephalus* | 57.43 ± 0.63 | 52.51 ± 0.93 |

**Supplementary Table 2.4** Type of care provided to broods by each species used in this study.

| Species | Type of Care Provided | Reference |
|---|---|---|
| *N. pulcher* | Alloparental | Taborsky et al., 2005 |
| *T. temporalis* | Biparental | Mboko & Kohda, 1999 |
| *N. multifasciatus* | Alloparental | Heg et al., 2005 |
| *A. compressiceps* | Female | Sefc, 2011 |
| *J. ornatus* | Alloparental | Heg & Bachar, 2006 |
| *N. tretocephalus* | Biparental | Nagoshi, 1983 |



**Supplementary Figure 2.1** A simplified phylogeny depicting the relationships among the cooperative and non-cooperative species used in this study. The phylogeny was recreated from a mitochondrial and nuclear phylogeny presented in Dey et al (2017). The colours denote the breeding system of each species; **blue** represents species with alloparental care (cooperative breeding), **red** represents species with biparental care (without cooperative breeding) and **gold** represents species with female care (without cooperative breeding).



**Supplementary Figure 2.2** Linear regressions depicting the relationship between the latency to leave refuge (boldness), grouping behaviour and activity level (number of entrances into a ‘choice’ zone). The dashed line in Figure **2.2a** represents a non-significant relationship whereas the bold lines in Figure **2.2b** and Figure **2.2c** represent significant relationships between variables.

## 2.9 Supplementary methods

*Quantity Discrimination: A behavioural syndrome?*

In Experiment 2, after the latency to leave the refuge was recorded, the activity of focal fish was scored by quantifying the total number of times a fish crossed into each ‘choice’ zone and this total number of crosses was used to estimate activity.

Relationships between the latency to leave the refuge (boldness), activity (number of entrances into a ‘choice’ zone), and grouping tendencies were assessed using simple linear regressions. In each model, species, sex and body mass were included as covariates because these variables are likely to impact grouping decisions (Reddon et al., 2011). Counts of focal fish activity were square root transformed to meet the assumptions of normality. Due to the additional disturbance caused by forcibly removing fish from the refuge, trials where this occurred were excluded from the correlations (see Experiment 2), although adding these trials back in did not change the pattern of the result.

We did not detect a relationship between latency to leave the refuge (boldness) and the strength of preference for the group i.e. grouping tendencies are a form of social refuge when faced with predation risk (LM: $\chi^2 = 0.19$, $p = 0.85$, Supplementary Figure 2.2a). We found a relationship between activity and latency to leave refuge; active fish left the refuge sooner (LM: $\chi^2 = 3.89$, $p < 0.001$, Supplementary Figure 2.2b). Finally, we found a relationship between activity and grouping; active fish spent less time grouping and more time exploring the other zones in the central compartment (LM: $\chi^2 = 4.78$, $p < 0.001$, Supplementary Figure 2.2c).

# Chapter 3:

# Little difference in spatial learning between cooperative and non-cooperative cichlids

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& Sigal Balshine

In preparation for: Animal Behaviour

## 3.1 Abstract

Although many factors shape cognition, three that are often cited are the number, duration and depth of social relationships that an individual maintains. To date, the link between social living and intelligence has been mainly supported by studies on primates, and far fewer tests connecting sociality to cognitive abilities have used other taxa. Here, we present the first comparative study in fishes that examines whether complex social living is linked to better performance on a cognitively demanding spatial task. Using three cooperative, group-living cichlid fishes and three of their non-cooperative, more solitary relatives, we studied maze learning and found that both the highly social, cooperative species and the more solitary, non-cooperative species took an equivalent time to complete the maze, made a comparable number of mistakes, and exhibited similar inhibitory control while in the maze. Both the cooperative and non-cooperative species completed the maze faster across subsequent trials, made fewer mistakes, and improved their inhibitory control. The fish did improve their performance over time, but we did not detect any differences in improvement between cooperative and non-cooperative species, or between males and females. Our results suggest that living and breeding in complex social groups does not necessarily imply better overall cognition nor, more specifically, an enhanced spatial learning capacity.

## 3.2 Introduction

The regularity and nature of social interactions vary across individuals, habitats, and species. Many animals are "social" for a short period of their lives (e.g. while mating or caring for young), while others spend their entire lives with the same relatively stable group of individuals (Kutsukake, 2009; Ward and Webster, 2016). The *Social Intelligence Hypothesis* posits that animals living in larger social groups must manage more relationships and, thus, have enhanced cognitive abilities to cope with the difficulties of social life (Byrne, 1994; Byrne and Whiten, 1988; Holekamp, 2007). Although the connection between social complexity and brain size evolution has been well-studied, the cognitive mechanisms that co-evolved with group-living and enable a highly social lifestyle are still strongly debated (Ashton et al., 2018; Johnson-Ulrich, 2017; Kummer et al., 1997; Reader and Laland, 2002).

Some of the most tightly knit and well-coordinated group-living species are cooperatively breeding animals. In cooperative breeding social groups, subordinate group members aid dominant group members in the care of the dominant's young, using behaviours collectively referred to as allocare (Solomon and French, 1997). Researchers have argued that cooperative breeding requires that individuals recognize their own group-members, remember past interactions with these individuals, and use this information to inform behaviour during future interactions (Iwaniuk and Arnold, 2004; Reddon et al., 2016; Thornton and McAuliffe, 2015). Hence, social memory and cheater detection are thought to be important cognitive traits in the evolution of cooperation (Burkart and Van Schaik, 2010; Dugatkin, 2002; West et al., 2007).

Cooperative breeding also requires the formation of strong and stable social bonds and the ability to resolve conflict within a group, while minimizing any associated costs (Balshine et al., 2017; Hick et al., 2014; Reddon et al., 2019). The *Cooperative Breeding Brain Hypothesis* suggests that social challenges are especially pronounced in cooperatively breeding groups, since these animals often live in strict hierarchical structures for which they must remember their relative rank, and monitor the rank and contributions of fellow group-members (Burkart and Van Schaik, 2010; Iwaniuk and Arnold, 2004; Thornton and McAuliffe, 2015). Thus, cooperative breeders ought to have highly developed socio-cognitive abilities. In this study, our aim was to address whether an enhanced socio-cognitive ability in highly social species also extends to other domains, giving these species an advantage when performing more general cognitive tasks.

Spatial navigation is one such cognitive task and is a key requirement for effective foraging, migration and predator avoidance; activities directly linked to fitness (Burns and Rodd, 2008; Fagan et al., 2013; Fukumori et al., 2010; Pravosudov and Roth, 2013). Underwater environments have three-dimensional accessibility and can be structurally complex, presenting pronounced spatial challenges for animals like fishes maneuvering in these habitats. The degree of habitat complexity that a fish needs to contend with can shape brain morphology (Carbia and Brown, 2019). For example, fishes inhabiting structurally complex environments have larger telencephalons in cichlids (Ectodini clade; Pollen et al., 2007), sunfish (*Lepomis gibbosus*; Axelrod et al., 2018), sticklebacks (*Pungitius pungitius*; Gonda et al., 2009) and guppies (*Poecilia* *reticulata*; Burns et al., 2009). The telencephalon is part of the teleost forebrain, and hosts the lateral telencephalic pallium, an area thought to represent the fish homologue of a hippocampus—the brain structure implicated in spatial learning and memory of mammals and birds (Durán et al., 2010; Rodriguez et al., 2002; Vargas et al., 2009). Studies that lesion or ablate part of the telencephalon in fishes confirm its prominent role in spatial learning (Broglio et al., 2003; Buechel et al., 2018; Riedel, 1998), but the telencephalon is also involved in the regulation and expression of social behaviour (Flood and Overmier, 1981; Scace et al., 2006). Although the role of habitat complexity in sculpting the fish brain is well established (Gonda et al., 2009; Strand et al., 2010; White and Brown, 2015), how the demands of complex group-living (i.e. social complexity) might shape general cognitive abilities, like spatial cognition, remains unclear.

Here, we describe a comparative study that assessed whether spatial learning and memory abilities differed between three cooperatively breeding cichlids and three of their non-cooperative relatives. Our study was conducted using Lamprologini cichlids, all from Lake Tanganyika in Africa. The Lamprologines are a tribe of closely related fishes (Day et al., 2007) that has evolved group-living and cooperative breeding on five separate occasions (Dey et al., 2017; Reddon et al., 2017). While many Lamprologini species live in social groups, rely on conspecific group members for protection and cooperate to raise young, other species rarely interact with conspecifics (other than their mated partners or during a territorial standoff). These less social species do not cooperate, and do not form permanent groups (Balshine et al., 2017;

Hick et al., 2014). We used a maze learning paradigm with repeated trials to assess spatial learning and memory for six territorial Lamprologini cichlids that vary in their social breeding system. Our study was conducted using the cooperatively breeding cichlid species *Neolamprologus pulcher*, *Neolamprologus multifasciatus* and *Julidochromis ornatus*, and the non-cooperative species *Telmatochromis temporalis*, *Altolamprologus compressiceps* and *Neolamprologus tretocephalus*. These fishes have comparable habitats and can all be found in shallow rocky areas of Lake Tanganyika (Barlow, 2008; Brichard, 1989; Konings, 1998). Therefore, they offer a powerful model system to explore how social living molds the brain and cognitive abilities.

We hypothesized that the cooperative species would outperform the non-cooperative species, initially, and over repeated trials, because their ability to cope with the cognitive demands of cooperative breeding might make them better problem solvers. We predicted that cooperative species would complete the maze faster, and show greater performance improvement compared to the non-cooperative cichlids. Second, in many cichlids including *N. pulcher*, males have larger home ranges in the wild and disperse sooner and faster than females (Stiver et al., 2007), *N. pulcher* also are often dominant in more than one social group and move large distances to travel between these groups (Desjardins et al., 2008; Wong et al., 2012 but see Schradin and Lamprecht, 2000 for evidence of female-biased immigration in *N. multifasciatus*). Therefore, we predicted that in *N. pulcher* and its relatives, males would complete the maze faster than females.

## 3.3 Methods

### 3.3.1 Animals and housing conditions

All fish were housed in the Aquatic Behavioural Ecology Laboratory at McMaster University, Hamilton, Ontario, Canada. Morphological information regarding our study specimens can be found in the supplementary material. *N. pulcher* and *T. temporalis* were the descendants of wild caught fishes from Lake Tanganyika, Africa, while *N. multifasciatus*, *A. compressiceps*, *J. ornatus* and *N. tretocephalus* were purchased from a commercial aquarist supplier (Finatics, Mississauga, Ontario, Canada). Prior to the experiment, fishes were held in mixed-sex stock tanks equipped with filters, heaters, coral sand substrate, terracotta flowerpot halves and opaque PVC tubes (10 cm in diameter and 25 cm in length) provided as shelter. *N. pulcher*, *T. temporalis, A. compressiceps*, and *J. ornatus* were held in 568L tanks containing approximately 40 individuals per tank and *N. multifasciatus* and *N. tretocephalus* were held in 189L tanks with approximately 20 individuals per tank. The stock tanks and our experimental tank (see below) were maintained at 25–28˚C and a 12L:12D photoperiod. Fish were fed a diet of Nutrafin basix flakes and Northfin floating pellets six times per week, with occasional supplementation of brine shrimp.

### 3.3.2 Spatial learning trials

In preparation for the experiment, each focal fish was captured from stock tanks with a hand net and gently guided into a start box. The start box was a PVC tube closed off permanently at one end, and a sliding door attached to a pulley at the other end. The focal fish in the start box was always placed at one end of the maze, in the start zone (Figure 3.1a). Three conspecifics from a different stock tank (and therefore unknown to the focal individual) were also captured and placed in a perforated transparent PVC tube (11 cm in diameter and 32 cm in length) at the opposite end of the maze, in the completion zone. The transparent PVC tube and its perforations allowed the exchange of water between the tube and the aquarium, and visual, olfactory and acoustic cues between the focal and stimulus fish. The fish were run repeatedly through the maze, a series of blind alleys and false openings or dead ends, which sat inside a 189L (89 x 50 x 50 cm) aquarium. The maze was constructed using both opaque and transparent PVC and contained a series of transparent PVC barriers (5 cm wide) running down in the middle section of the maze that allowed the stimulus group (in the completion zone) to be viewed by the focal fish in each section of the tank. The maze also had opaque PVC and one correct open route that led to the completion zone (Figure 3.1b). The maze contained two dead-end corridors made of opaque PVC (8 cm in width and 10 cm in length), and fish entering these corridors were considered to have made a wrong turn or a mistake. The maze had four compartments: a start zone, two intermediate zones (zones 1 and 2), and a completion zone. Each of these zones were identical in size (16 cm in length).

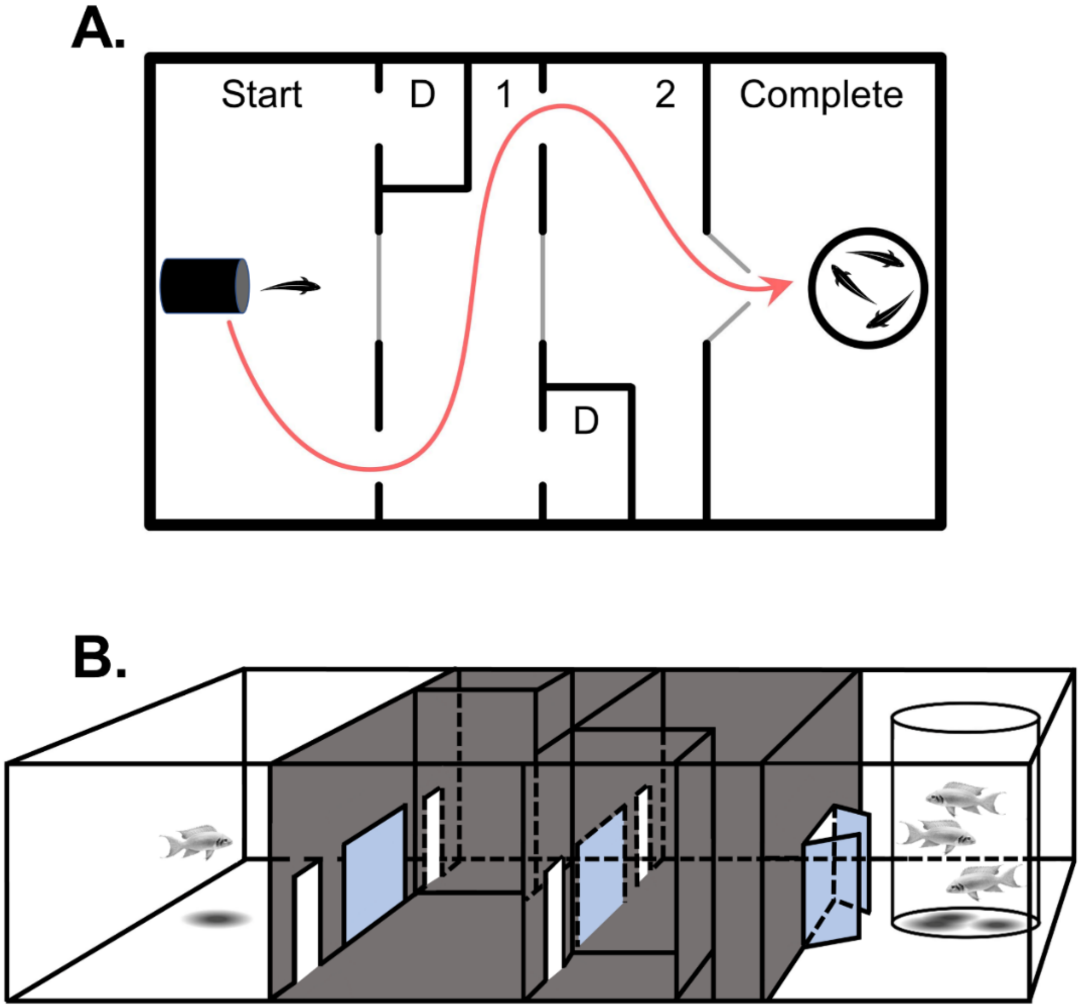

**Figure 3.1a.** Schematic of the experimental aquarium (bird's eye view) used for the maze learning experiment. The *black lines* represent opaque barriers and the *gray lines* represent transparent barriers. The 'Start' marks the starting zone and the 'Complete' marks the completion zone. The '1' marks zone 1 and the '2' marks zone 2. The 'D' represents dead-end corridors. To access the completion zone, the fish would need to navigate along the path illustrated above. **b.** Schematic of the experimental aquarium (eye level view). The blue panels represent transparent barriers through which the focal fish could see the stimulus group, and the gray panels represent opaque barriers. Illustration by Greaton Tan.

After being placed in the start zone, each focal fish was given 1-hour to recover from capture and transport, and to acclimate in the start box before the sliding front door was removed remotely. After the door had been removed, fish were then given a maximum of 5-minutes to leave the start box. If the fish had not left the start box after 5-minutes, the box was remotely lifted out of the tank from the back, and the fish were tipped out and forced to leave the start box. Once the focal fish left the start box, on its own volition or by being forced to exit when the box was removed, each fish then had a maximum of 2-hours to navigate the maze and reach the completion zone containing the group of conspecifics. Forced exits occurred in 2/15 *N. pulcher* trials, 2/11 *T. temporalis* trials, 12/23 *N. multifasciatus* trials, 7/18 *A. compressiceps* trials, 3/12 *J. ornatus* trials and 14/16 *N. tretocephalus* trials. Upon reaching the completion zone, re-entry into the maze was blocked off with an opaque barrier. The maze apparatus was then lifted and reversed in the tank, so that for the next trial, the maze was oriented in the opposite direction to how it had been on the previous trial. By reversing the maze apparatus, the focal fish could traverse the maze in the opposite direction while still experiencing the same layout as it had on the previous trial without requiring that it return to the original start zone. The cylinder containing the stimulus fish was lifted and placed at the opposite end (the previous start zone for this next trial was now the new completion zone). If after 2-hours (the trial maximum time) the fish had not yet completed the maze, the fish was gently guided to the completion zone using a hand net, and the barrier was placed and the maze direction was reversed as described above. Between trials 1–2 and 2–3, the focal fish was given 30-minutes to acclimate in its new start zone before the barrier was lifted and the fish were once again able to access the maze. Each focal fish had the opportunity to explore the maze three times in total.

### 3.3.3 Quantification of behaviour from videos

Trials were recorded from above the tank with a Canon HF R80 camera. The camcorder was accessed remotely, and the experimenter was able to monitor the progress of the focal fish without being physically present in the room to avoid disturbance. We scored the videos using BORIS, a behavioural observation scoring software (Friard and Gamba, 2016) and recorded the latency to leave the start box, as well as the time when the focal fish reached zones 1 and 2 and the completion zone. We also noted the number of times that each fish entered a dead-end corridor and classified each entry as a mistake. Finally, as a measure of inhibitory control, we recorded the number of times that each fish would swim directly into the transparent barriers, that ran down the middle of the maze. The term inhibitory control describes an individual's ability to resist an urge, particularly one that may be counterproductive, and is an increasingly common measure of cognitive ability (Bray et al., 2014). For each trial, we recorded and continually monitored location until the focal fish reached the completion zone or when a maximum of 2-hours had elapsed.

### 3.3.4 Statistical analysis

Individuals that did not complete the maze all three times, did not have the same learning opportunities or experiences as those that completed the maze three times. Therefore, data from these individuals who did not have three runs were removed from the dataset prior to analyses (0/15 *N. pulcher*, 9/23 *N. multifasciatus* [6F/2M], 3/12 *J. ornatus* [1F/2M], 3/11 *T. temporalis* [1F/2M], 2/18 *A. compressiceps* [1F/1M], and 13/16 *N. tretocephalus* [5F/8M]). We also re-ran the analyses with all of the data and individuals included (individuals who only completed the maze once, twice or three times) using the same statistical methods described below. The results showed the same general patterns when we used the more inclusive dataset (including all individuals) and these inclusive but less conservative results are included in the supplementary material.

To analyze the relationship between time to maze completion and each of our predictor variables (social system, sex and trial number) we fitted linear mixed models (LMMs) using the "lme4" package (Bates et al., 2015). The time to completion data were log transformed prior to analyses to meet the assumptions of normality and homoskedasticity. We included each of our predictor variables and their interaction terms as fixed factors. Species and individual fish ID were also included as random effects in each model. The random effect of species was used as a phylogenetic control, but the true phylogenetically controlled models are reported and discussed in the supplementary materials. We constructed GLMMs (negative binomial family) to assess whether there were differences between cooperative and non-cooperative species, and between the sexes, in the number of mistakes (ie. dead-end corridor entry) and the degree of inhibitory control (i.e. the number of times that a fish swam directly at transparent barriers). All models used the same fixed and random effects. In order to assess whether the rates of improvement across trials differed between cooperative and non-cooperative species, and males and females, we analyzed the two-way interactions between social system and trial, and sex and trial. Assumptions for the linear mixed models were visually assessed using quantile-quantile and scale-location plots, while the assumptions for the generalized linear mixed models were assessed by plotting the simulated residuals with the “DHARMa package”. The effects of each model were evaluated using the Anova function from the “car” package. We analyzed pairwise differences for each performance metric between trials by incorporating successive differences contrasts into each model using the “emmeans” package. The results for each individual species are available in the supplementary material.

Phylogenetically controlled linear mixed models and phylogenetically controlled generalized linear mixed models were also constructed using methods described by Li and Bolker (2019). These methods manipulate the ‘lme4’ and ‘glmmTMB’ packages to include phylogenetic signal as a random effect term. The patterns of results for these phylogenetically controlled models were unchanged from those described herein and, thus, these phylogenetically controlled results are reported only in the supplementary materials. Overall, our analyses were conducted using data from 65 fishes and 195 trials. Analyses were performed with R (v.3.6.3, R Core Team, 2020) and a significance level (α) of 0.05 was used for all tests.

## 3.4 Results

### 3.4.1 Social system

The three cooperative species did not complete the maze significantly faster than the three non-cooperative species (Figure 3.2a; log-LMM: $\chi^2$ = 2.07, df = 1, $p$ = 0.15). Both cooperative and non-cooperative species completed the maze faster across subsequent trials (log-LMM: $\chi^2$ = 30.70, df = 2, $p < 0.001$; Figure 3.3a cooperative, 3.3d non-cooperative), and there was no difference in their rates of improvement i.e. in the reduction in time taken to complete the maze across trials (Social System*Trial Number, log-LMM: $\chi^2$ = 0.57, df = 2, $p$ = 0.75). Cooperative and non-cooperative species made a similar number of mistakes (GLMM: $\chi^2$ = 1.47, df = 1, $p$ = 0.23; Figure 3.2b) and both types of species made fewer mistakes with each subsequent trial (GLMM: $\chi^2$ = 35.87, df = 2, $p < 0.001$; Figure 3.3b cooperative, Figure 3.3e non-cooperative). There was no difference in the rate of improvement in terms of mistakes made between cooperative and non-cooperative species (Social System*Trial Number, GLMM: $\chi^2$ = 0.72, df = 2, $p$ = 0.70). Cooperative and non-cooperative species displayed similar degrees of inhibitory control and swam at the transparent barriers a comparable number of times (GLMM: $\chi^2$ = 0.21, df = 1, $p$ = 0.65; Figure 3.2c). Cooperative and non-cooperative species both improved with respect to their inhibitory control across trials (GLMM: $\chi^2$ = 50.74, df = 2, $p < 0.001$; Figure 3.3c cooperative, 3.3f non-cooperative), and there was no difference in their rates of improvement (Social System*Trial Number, GLMM: $\chi^2$ = 2.40, df = 2, $p$ = 0.30).

The patterns of these results were unchanged when we included data from all individuals and all trials, bar the time to completion, where including all data revealed a significant interaction between social system and trial number. In other words, when all data were analyzed together, cooperative species improved their time to completion more so than non-cooperative species over repeated trials, and this was also true for the models with phylogenetic control (see Supplementary Figure 3.2a).

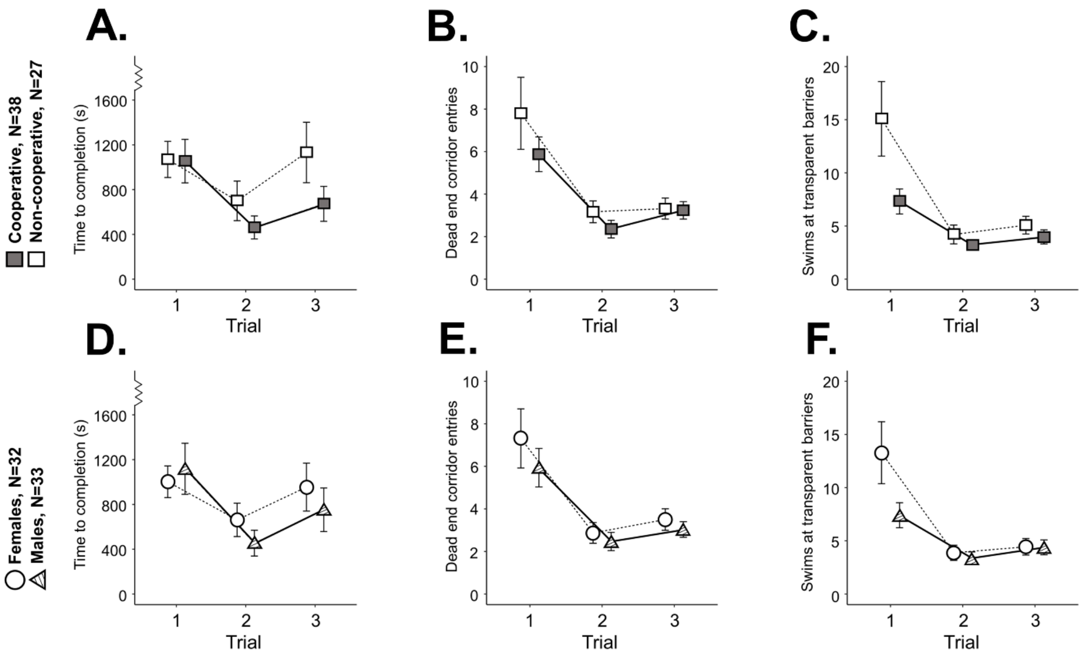

**Figure 3.2a.** The time taken to complete trials 1–3 for the cooperative and non-cooperative species. **b.** The number of times that cooperative and non-cooperative species entered a dead end corridor (mistakes). **c.** The number of swims at transparent barriers by cooperative and non-cooperative species (inhibitory control). **d.** The time taken to complete trials 1–3 for males and females. **e.** The number of times that males and females entered a dead end corridor. **f.** The number of swims at transparent barriers by males and females. All data removed for individuals that did not complete the maze on any of the three trials. Values are means ± SEM.

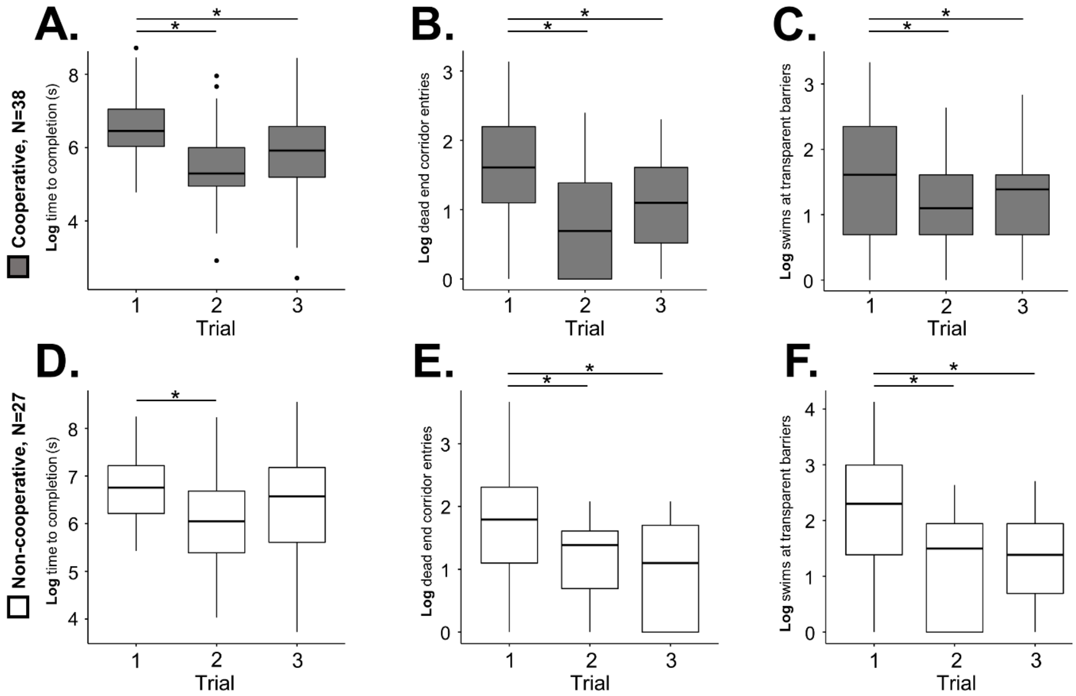

**Figure 3.3a.** The time taken for cooperative species to complete the maze on a given trial. **b.** The number of dead end corridor entries (mistakes) made by cooperative species. **c.** The number of swims at transparent barriers (inhibitory control) by cooperative species. **d.** The time taken for non-cooperative species to complete the maze. **e.** The number of dead end corridor entries made by non-cooperative species. **f.** The number of swims at transparent barriers by non-cooperative species. Values have been log-transformed for presentation. All data removed for individuals that did not complete the maze on any of the three trials. * denotes p < 0.05 as determined by successive differences contrasts.

### 3.4.2 Sex

Males and females did not differ in the time taken to complete the maze (log-LMM: $\chi^2 = 0.42$, df = 1, $p = 0.52$; Figure 3.2d). With each subsequent trial, both sexes completed the maze faster (log-LMM: $\chi^2 = 30.70$, df = 2, $p < 0.001$; Figure 3.4a males, 3.4d females), and males and females had similar rates of improvement in terms of the time taken to complete the maze (Sex*Trial Number, log-LMM: $\chi^2 = 2.29$, df = 2, $p$

= 0.32). Males and females also made a similar number of mistakes and entered the dead-end corridors at a comparable frequency (GLMM: $\chi^2$ = 0.67, df = 1, $p$ = 0.42; Figure 3.2e). Both males and females made fewer mistakes across trials (GLMM: $\chi^2$ = 35.87, df = 2, $p$ < 0.001; Figure 3.4b males, 3.4e females), and there was no sex difference in the rates of improvement (Sex*Trial Number, GLMM: $\chi^2$ = 0.10, df = 2, $p$ = 0.95). Males and females did not differ in their amount of inhibitory control displayed (GLMM: $\chi^2$ = 0.14, df = 1, $p$ = 0.71; Figure 3.2f), both improved their inhibitory control across trials (GLMM: $\chi^2$ = 50.74, df = 2, $p$ < 0.001; Figure 3.4c males, 3.4f females), and there was no difference in their rates of improvement (Sex*Trial Number, GLMM: $\chi^2$ = 1.62, df = 2, $p$ = 0.44). We did not observe any other significant effects or interactions. Again, the patterns of these results were unchanged when we included data from all individuals and all trials, and this was also true for the models with phylogenetic control (see supplementary materials).

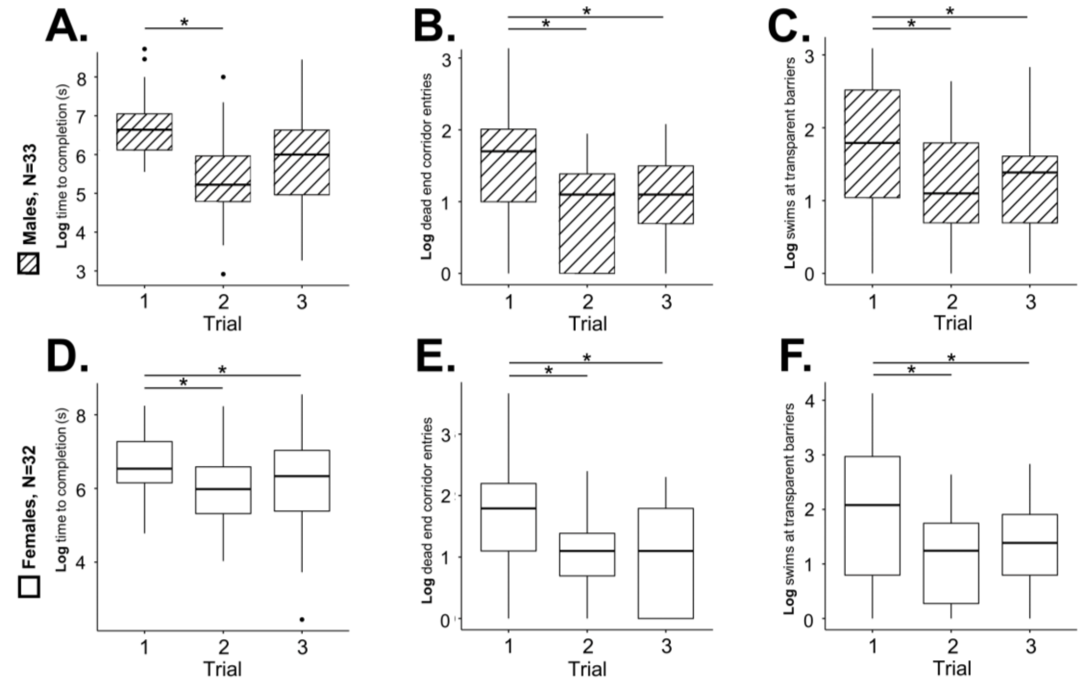

**Figure 3.4a.** The time taken for males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by males. **c.** The number of swims at transparent barriers by males. **d.** The time taken for females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by females. **f.** The number of swims at transparent barriers by females. Values have been log transformed for presentation. All data removed for individuals that did not complete the maze on any of the three trials. * denotes $p < 0.05$ as determined by successive differences contrasts.

## 3.5 Discussion

Contrary to our predictions, we did not find evidence that cooperative species outperformed their non-cooperative relatives in the spatial learning task. Both cooperative and non-cooperative cichlids took less time to complete the maze progressively over the three trials. Cooperative species did not display better inhibitory control or make fewer mistakes than their non-cooperative relatives. Cooperative and non-cooperative species also displayed similar improvement across trials in terms of time to complete the maze, the number of mistakes made and degree of inhibitory control. Despite our expectation to find sex differences, we also did not detect any major differences in maze performance between males and females. Overall, our results do not support the *Cooperative Breeding Brain Hypothesis*—cooperative and non-cooperative species performed comparably in all three spatial performance metrics, and improved similarly in these metrics.

Living in a social environment, forming and maintaining relationships, and working together to achieve shared goals are cognitively demanding challenges. However, what constitutes a social challenge and which challenges in particular are most influential as selective agents on brain evolution and cognition is unclear. In primates and ungulates, brain size is thought to be linked to group size (Dunbar and Shultz, 2007a; 2007b but see DeCasien et al., 2017 and Powell et al., 2017 for an opposing view). In contrast, flock size in birds does not correlate with brain size, likely because in large flocks there can be thousands of birds and strong social bonding does not occur (van Horik and Emery, 2011). In some insects, like paper wasps (Vespidae family), colony size is negatively related to brain size; wasp species that form larger groups have smaller brains because they can rely on information from their siblings and nestmates rather than produce this information for themselves (O'Donnell et al., 2015). Evidently, group size alone is an inadequate measure of social complexity, yet researchers have not agreed on a suitable alternative (Kappeler, 2019).

Rather than group size, it may be the depth and longevity of social interactions that have a more profound influence on the brain (van Horik and Emery, 2011), but it can be difficult to quantify the nature of social partnerships (e.g. number and quality of interactions). Also, intricate social relations are not exclusive to group-living animals. Territorial animals, for example, are likely to have regular and prolonged interactions with neighbouring territory owners, as well as other potential usurpers, which requires some of the same cognitive machinery as cooperation (e.g. remembering familiar individuals, detecting cheaters and resolving conflict). All six of the cichlid species used in this study face similar challenges of having to find, food and a mate, maintaining a pair bond, providing parental care, and acquiring, defending and maintaining a territory (reviewed by Sefc, 2011). It may be that it was these ecological and social challenges, and not the challenges posed by cooperative group living, that played a more prominent role in molding the cognitive abilities of these cichlids, including spatial cognition. Indeed, a definition of social complexity based entirely on the presence or absence of cooperative breeding may be overly simplistic, and could explain the similar spatial performance between cooperative and non-cooperative cichlids uncovered in our study. Supportive of this idea, previous research comparing the brains of cooperative and non-cooperative Lamprologines has found no differences in whole brain masses (Reddon et al., 2016). Yet, notable research on cichlids of the Ectodini clade (also from Lake Tanganyika) suggests that regional brain size differences are pervasive and dependent on social factors (mating system), warranting future research into evaluating which particular social challenges are most important in shaping the brain as a whole and its various regions and structures (Pollen et al., 2007).

Sex differences in the brain are well documented in mammals and birds (Rhodes and Rubin, 1999). It is theorized that gonadal steroids act early in development to organize neural substrate (the Organizational/Activational hypothesis; Becker et al., 2002; McCarthy et al., 2012) and have downstream implications for cognition. This theory has been substantiated with studies on rodents, such as rats (*Rattus norvegicus*; Raisman and Field, 1971, 1973) and hamsters (*Mesocricetus auratus*; Greenough and Carter, 1977), as well as various songbirds (Arnold et al., 1996; Wade and Arnold, 2004). There are many striking similarities between the fish brain and the brains of other animals (Bshary et al., 2014), but there are also some fundamental differences in neural architecture and organization which mean that sex differences in cognitive ability reported in other animals might not be as apparent in fishes (Ebbesson and Braithwaite, 2012). The current consensus is that the fish brain does not undergo sex-specific organization and remains neurally bisexual, because, unlike mammals and birds, the fish brain never becomes canalized in development. The dominant role of hormones in the developing fish brain is therefore activational, not organizational like in mammals, so sex differences should be less prominent (Zakon, 2000). We might still expect cognitive differences between male and female fishes if each sex faces unique challenges (e.g. differences in diet, or niche occupation; Lucon-Xiccato and Bisazza, 2017; Magurran and Garcia, 2000), but this is not the case for the cichlids used in this study where both sexes experience many of the same ecological and social challenges. We found that both sexes scored and improved similarly on a variety of spatial performance metrics. The comparable scores of males and females reported here do not provide support for the notion of sex-specific spatial abilities in this group of fishes. There is a great deal of overlap in terms of the cognitive challenges experienced by males and females, as well as a limited role of gonadal steroids in organizing the neural tissue associated with navigation in fishes compared to mammals and birds. We suggest that sex differences in locomotion and dispersal previously reported in *N. pulcher* (Stiver et al., 2006; Stiver et al., 2007), might reflect differences in the spatial distribution of social opportunities available to each sex, or motivation to move, rather than differences in spatial capacity.

The fish used in this study were captive bred. Future work could improve upon our study by using wild caught, rather than captive bred fishes. Artificial selection in captivity is known to affect cognitive performance (Doyle and Talbot, 1986; Huntingford, 2004; Huntingford et al., 1994) and changes can even occur within a single generation (Christie et al., 2012). We housed fishes in either 568L or 189L stock tanks containing approximately 20–40 individuals, conditions unnatural to each of these cichlid species in the wild. We also removed, reversed and replaced the maze back into the tank between each trial to avoid having to capture, handle and stress the focal individual. However, in tasking the fish to go through the maze repeatedly and in opposite directions, the extra-maze cues (outside the tank e.g. lights, distance to walls) were altered between trials. This could have made it more challenging for the fish to acquire and use the same cues in each trial. In the future, the fish should be able to run through the maze with all the internal and external cues being held constant. Lastly, motivational differences to reach the social stimulus at the completion zone of the maze may confound the results. Most fishes prefer to join larger groups (Ashley et al., 1993; Hager and Helfman, 1991; Keenleyside, 1955; Krause and Godin, 1994; Svensson et al., 2000), and previous research on Lamprologini cichlids suggests that all Lamprologines prefer to join the larger of two groups when threatened, regardless of their social breeding system (O'Connor et al., 2015; Salena and Balshine, 2020), and that in the absence of a threat, prosocial motivation differs between species (Balshine et al., 2017). In our study, the focal fish were netted, placed in a start box and inserted into an unfamiliar tank before the first trial. In contrast, between trials 1–2 and 2–3, the fish was not captured or handled. Therefore, the diminishment of perceived threat over subsequent trials may reveal underlying motivational differences to join a group under non-threatening conditions between cooperative and non-cooperative species, and indicates an area in need of future research.

Support for the *Cooperative Breeding Brain Hypothesis* outside the primate literature is equivocal. Although the cognitive challenges of social life may favour relatively larger neocortices in primates, it is unclear how these same challenges affect the brains of other highly social animals, and which social challenges in particular are most influential in shaping the brain and cognitive abilities. In our experiment, we did not find compelling evidence for differing spatial performance between cooperative and non-cooperative species, or males and females. We did, however, find that both cooperative and non-cooperative species, and males and females, took less time to complete the maze over repeated trials, made fewer mistakes and improved their inhibitory control, which suggests that the animals learnt and remembered the correct route. Our results contribute to the growing body of literature rebutting the *Cooperative Breeding Brain Hypothesis* and ascertain that an accurate assessment of social complexity requires the consideration of multiple social variables.

## 3.6 Acknowledgements

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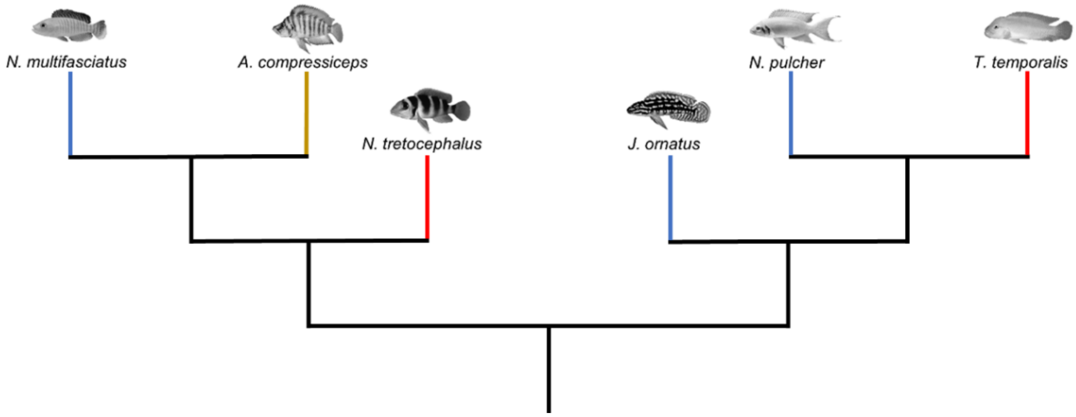
## 3.8 Supplementary materials

**Supplementary Table 3.1** Morphological characteristics of the focal fishes used in the spatial navigation experiment. Values are means ± SEM.

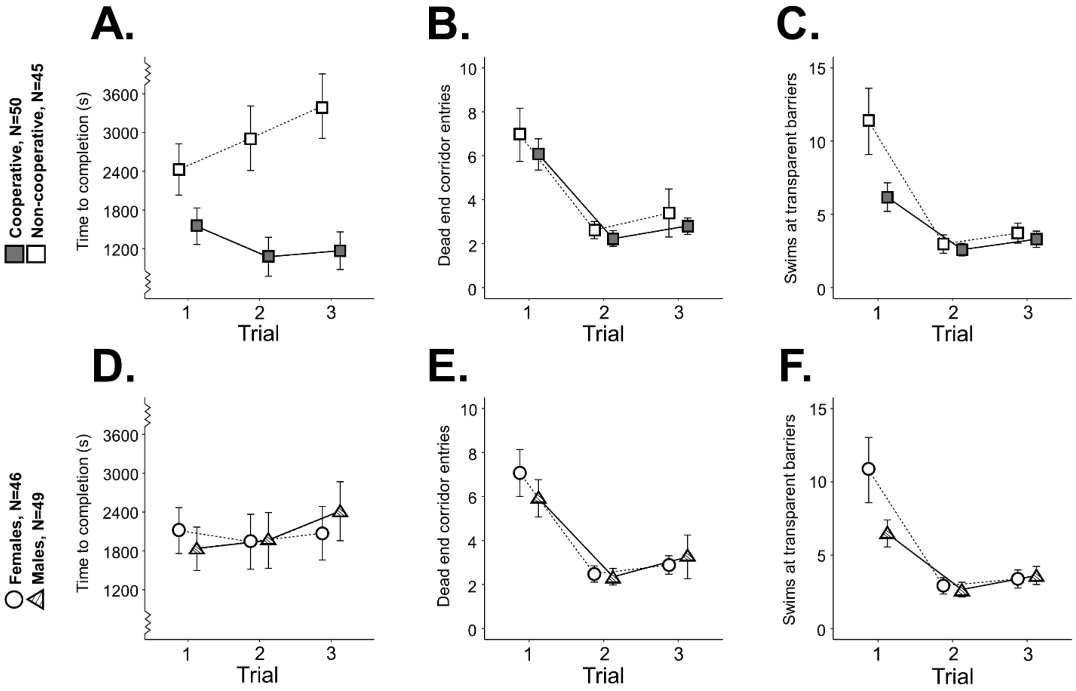
| Species | Males | Females | Standard Length (mm) |
|---|---|---|---|
| *N. pulcher* | 7 | 8 | 60.94 ± 1.39 |
| *N. multifasciatus* | 11 | 12 | 47.52 ± 0.80 |
| *J. ornatus* | 10 | 2 | 51.08 ± 1.83 |
| *T. temporalis* | 5 | 6 | 51.84 ± 0.94 |
| *A. compressiceps* | 5 | 13 | 52.34 ± 1.02 |
| *N. tretocephalus* | 10 | 6 | 57.98 ± 1.03 |

**Supplementary Table 3.2** Morphological characteristics of the stimuli fishes (chosen randomly) used in the spatial navigation experiment. Values are means ± SEM.

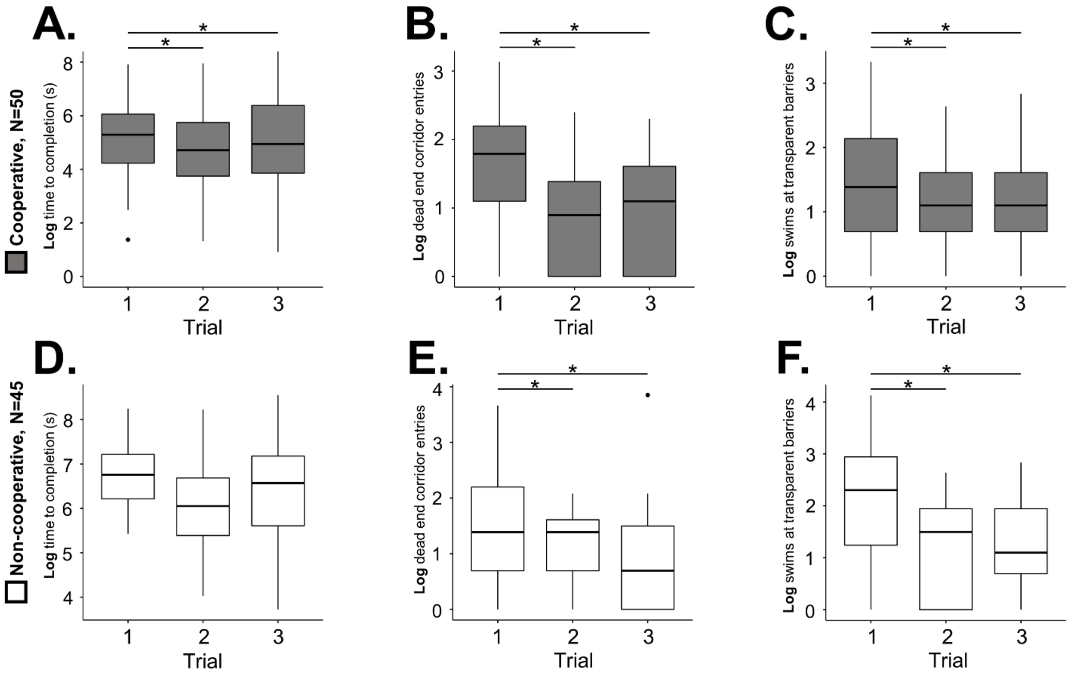
| Species | Males | Females | Standard Length (mm) |
|---|---|---|---|
| *N. pulcher* | 14 | 31 | 58.82 ± 1.12 |
| *N. multifasciatus* | 36 | 33 | 46.74 ± 0.84 |
| *J. ornatus* | 24 | 12 | 53.53 ± 0.50 |
| *T. temporalis* | 13 | 20 | 49.90 ± 0.93 |
| *A. compressiceps* | 15 | 39 | 50.65 ± 1.25 |
| *N. tretocephalus* | 29 | 19 | 54.58 ± 2.95 |



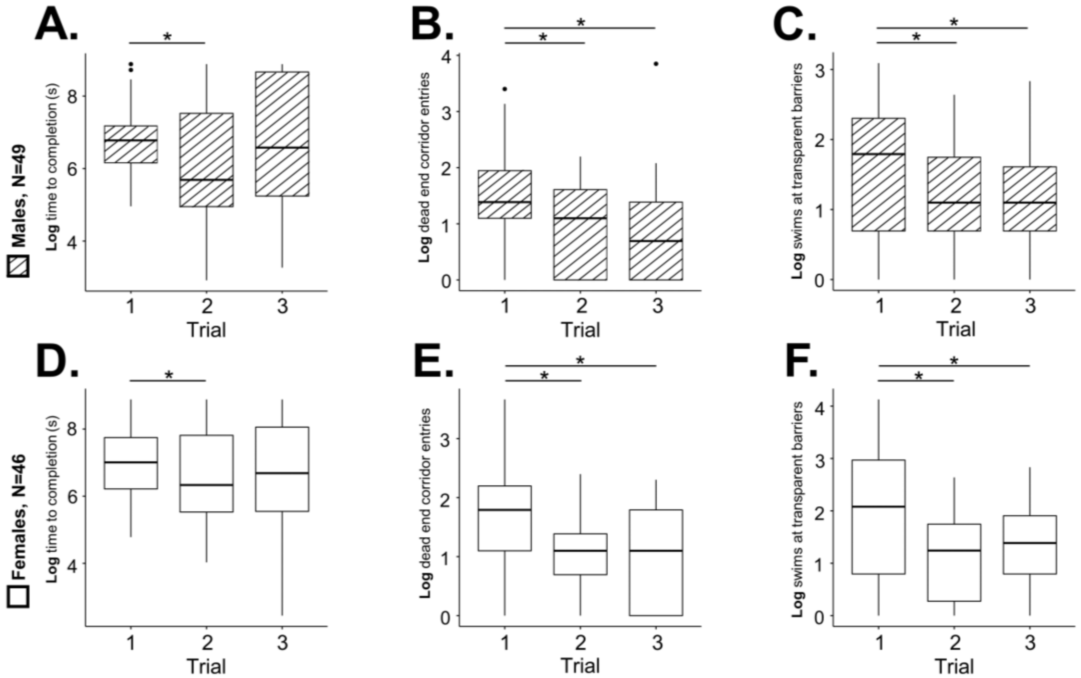
**Supplementary Figure 3.1** A simplified phylogeny depicting the relationships among the cooperative and non-cooperative species used in this study. The phylogeny was recreated from a mitochondrial and nuclear phylogeny presented in Dey et al (2017). The colours denote the breeding system of each species; **blue** represents species with alloparental care (cooperative breeding), **red** represents species with biparental care (without cooperative breeding) and **gold** represents species with female care (without cooperative breeding).



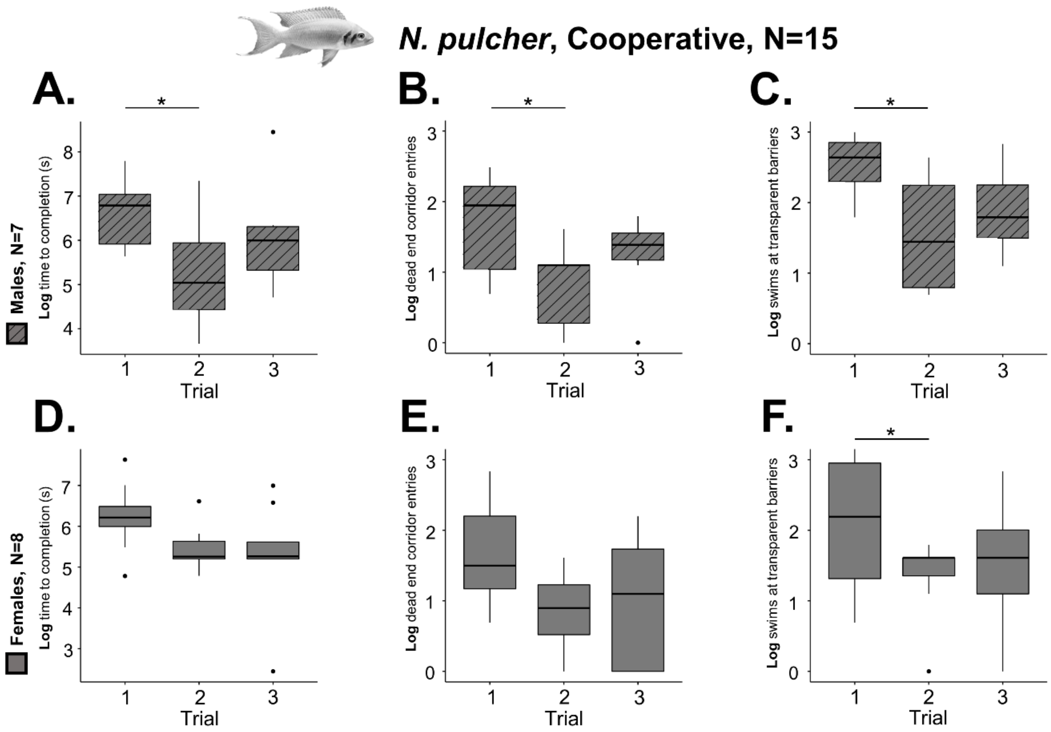
**Supplementary Figure 3.2a.** The time taken to complete trials 1–3 for all individuals of both the cooperative and non-cooperative species. **b.** The number of times that cooperative and non-cooperative species entered a dead end corridor (mistakes). **c.** The number of swims at transparent barriers by cooperative and non-cooperative species (inhibitory control). **d.** The time taken to complete trials 1–3 for males and females. **e.** The number of times that males and females entered a dead end corridor. **f.** The number of swims at transparent barriers by males and females. Values are means ± SEM and include all observations, regardless of whether the individuals completed the maze in the allowed time.



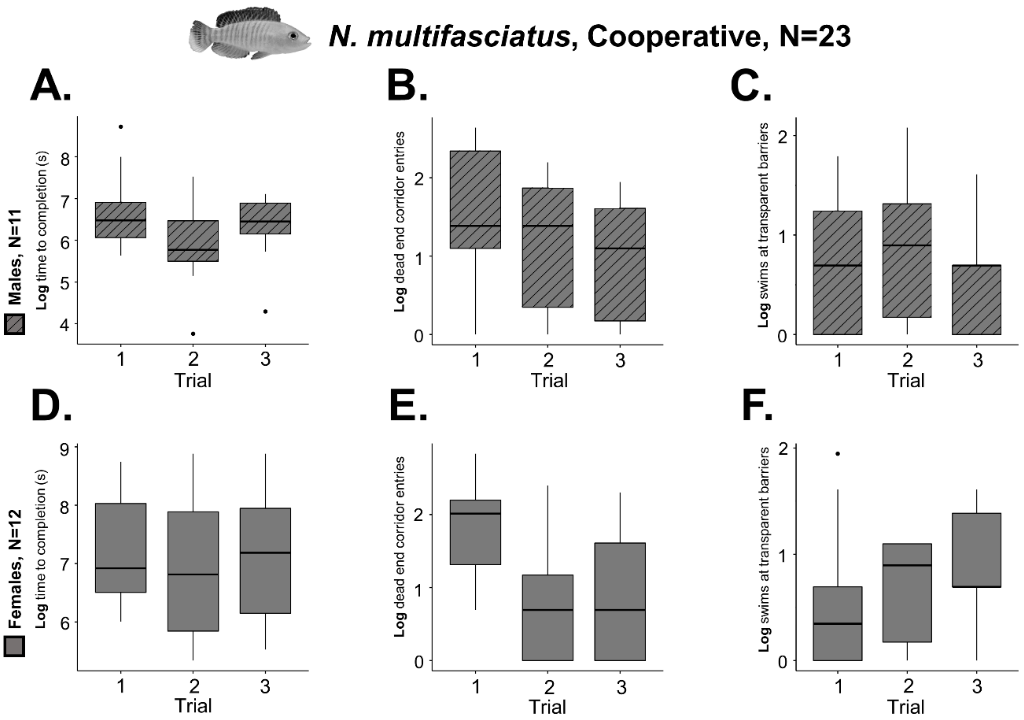
**Supplementary Figure 3.3a.** The time taken for cooperative species to complete the maze on a given trial. **b.** The number of dead end corridor entries (mistakes) made by cooperative species. **c.** The number of swims at transparent barriers (inhibitory control) by cooperative species. **d.** The time taken for non-cooperative species to complete the maze. **e.** The number of dead end corridor entries made by non-cooperative species. **f.** The number of swims at transparent barriers by non-cooperative species. Values have been log-transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



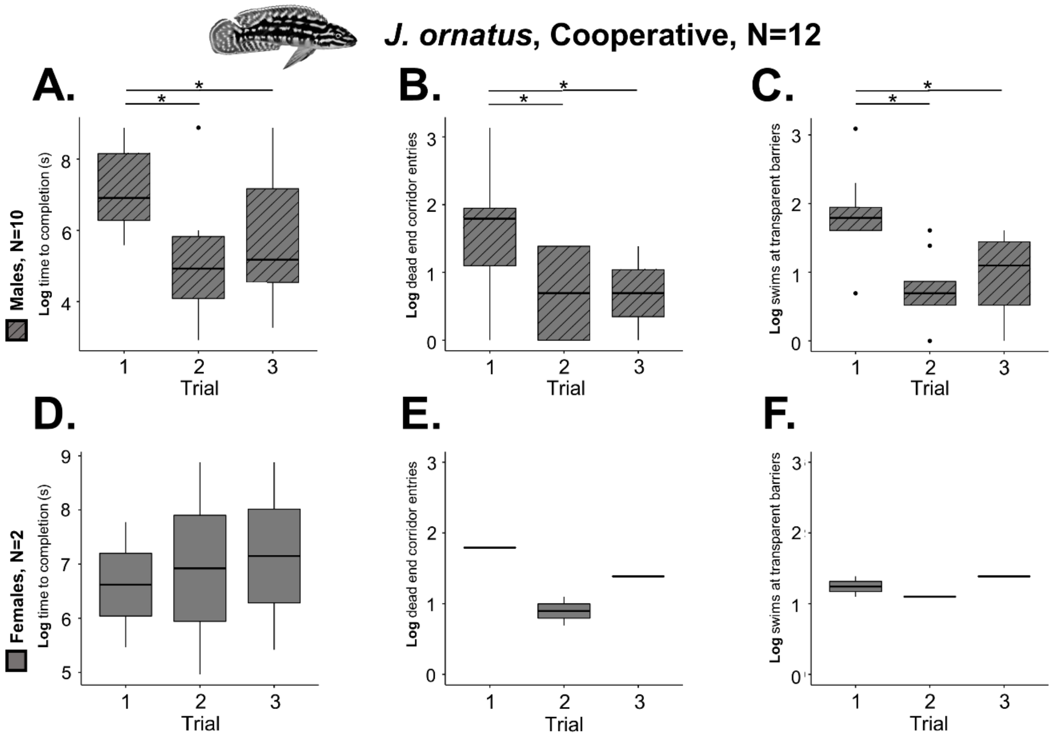
**Supplementary Figure 3.4a.** The time taken for males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by males. **c.** The number of swims at transparent barriers by males. **d.** The time taken for females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by females. **f.** The number of swims at transparent barriers by females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



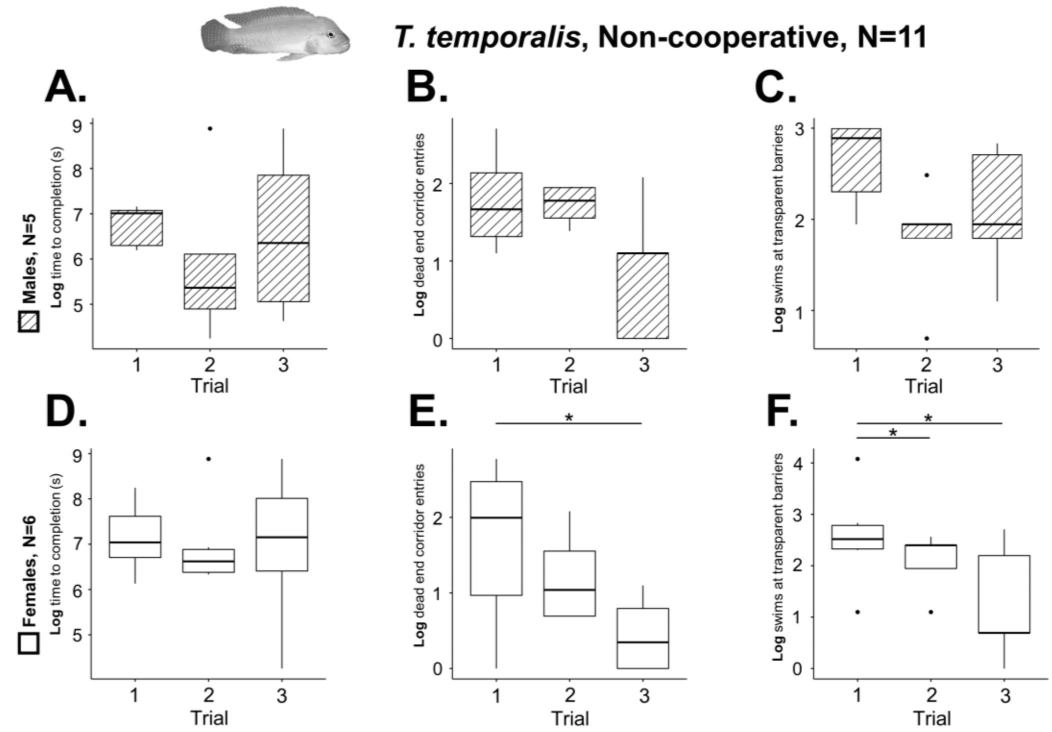
**Supplementary Figure 3.5a.** The time taken for *N. pulcher* males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by *N. pulcher* males. **c.** The number of swims at transparent barriers by *N. pulcher* males. **d.** The time taken for *N. pulcher* females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by *N. pulcher* females. **f.** The number of swims at transparent barriers by *N. pulcher* females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



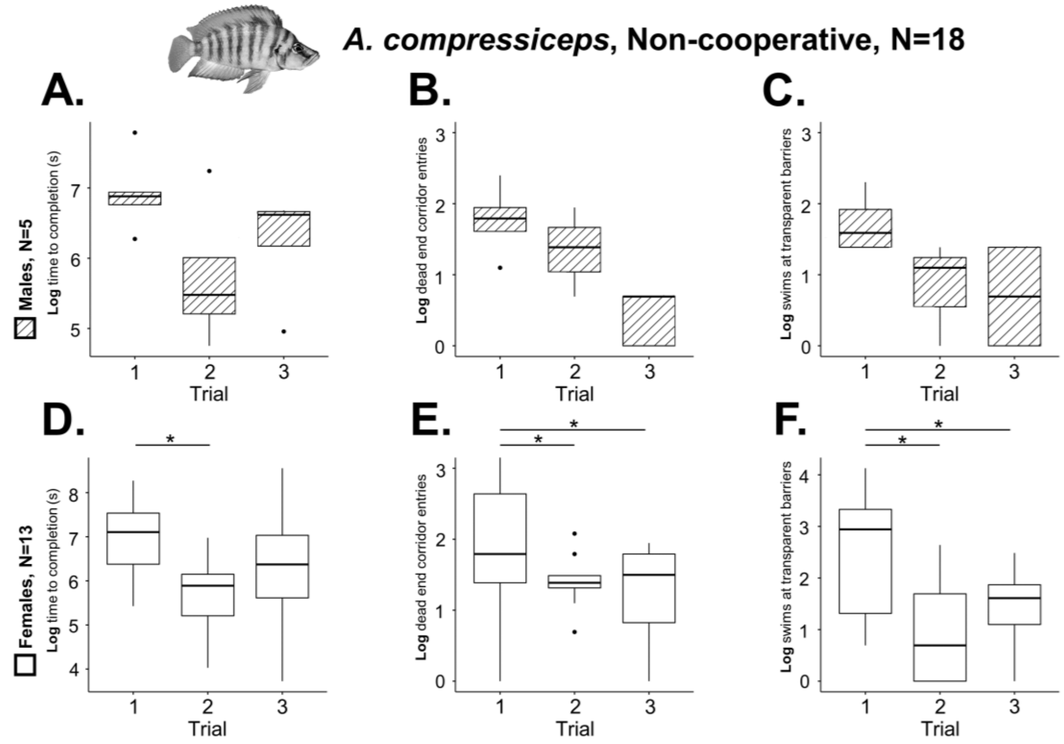
**Supplementary Figure 3.6a.** The time taken for *N. multifasciatus* males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by *N. multifasciatus* males. **c.** The number of swims at transparent barriers by *N. multifasciatus* males. **d.** The time taken for *N. multifasciatus* females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by *N. multifasciatus* females. **f.** The number of swims at transparent barriers by *N. multifasciatus* females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



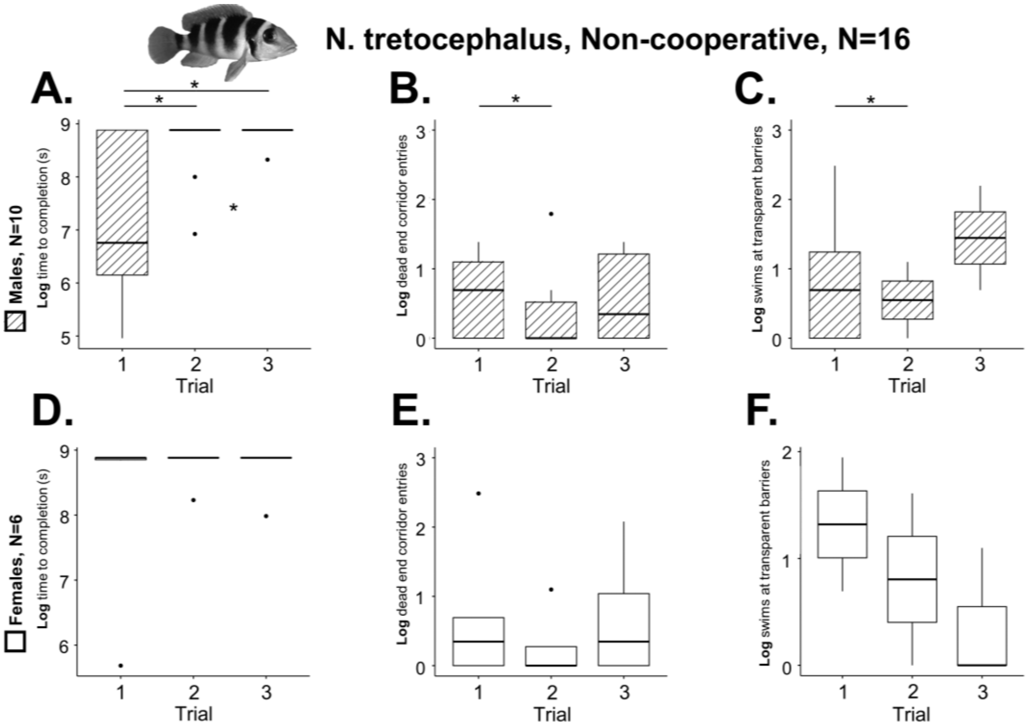
**Supplementary Figure 3.7a.** The time taken for *J. ornatus* males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by *J. ornatus* males. **c.** The number of swims at transparent barriers by *J. ornatus* males. **d.** The time taken for *J. ornatus* females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by *J. ornatus* females. **f.** The number of swims at transparent barriers by *J. ornatus* females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



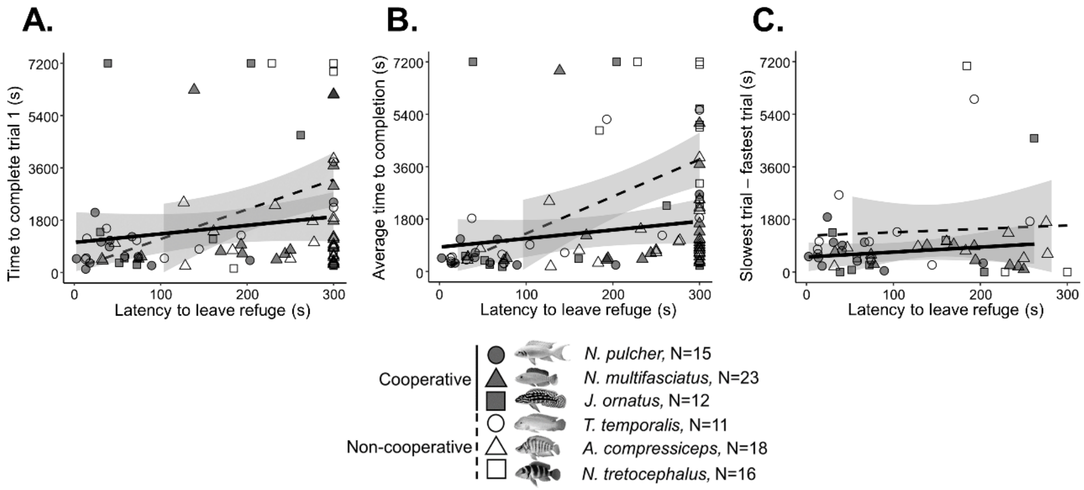
**Supplementary Figure 3.8a.** The time taken for *T. temporalis* males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by *T. temporalis* males. **c.** The number of swims at transparent barriers by *T. temporalis* males. **d.** The time taken for *T. temporalis* females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by *T. temporalis* females. **f.** The number of swims at transparent barriers by *T. temporalis* females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



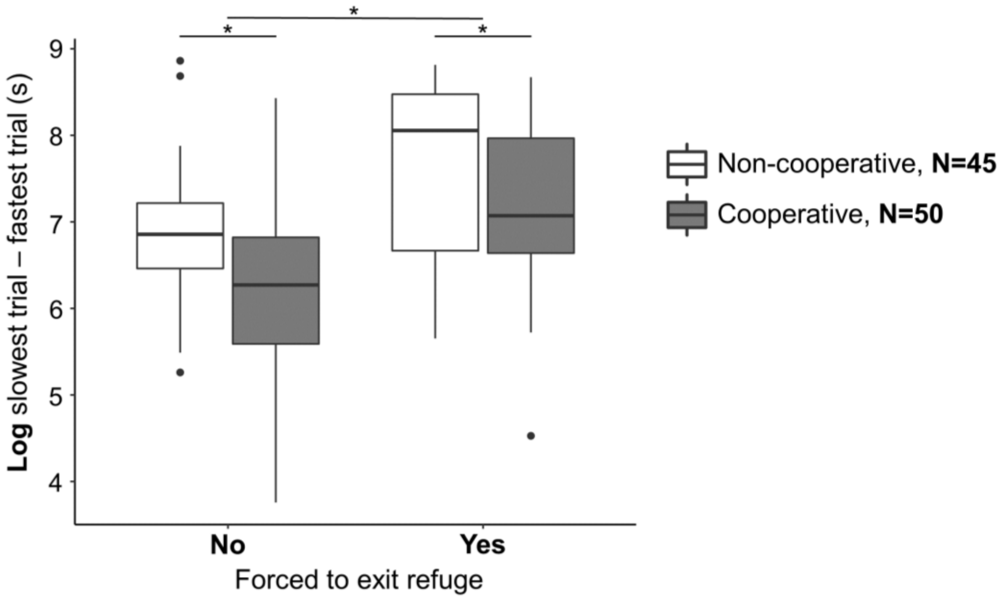
**Supplementary Figure 3.9a.** The time taken for *A. compressiceps* males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by *A. compressiceps* males. **c.** The number of swims at transparent barriers by *A. compressiceps* males. **d.** The time taken for *A. compressiceps* females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by *A. compressiceps* females. **f.** The number of swims at transparent barriers by *A. compressiceps* females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



**Supplementary Figure 3.10a.** The time taken for *N. tretocephalus* males to complete the maze on a given trial. **b.** The number of dead end corridor entries made by *N. tretocephalus* males. **c.** The number of swims at transparent barriers by *N. tretocephalus* males. **d.** The time taken for *N. tretocephalus* females to complete the maze on a given trial. **e.** The number of dead end corridor entries made by *N. tretocephalus* females. **f.** The number of swims at transparent barriers by *N. tretocephalus* females. Values have been log transformed for presentation, and include all observations, regardless of whether the individuals completed the maze in the allowed time. * denotes $p < 0.05$ as determined by successive differences contrasts.



**Supplementary Figure 3.11a.** The time taken to complete trial 1 versus the latency to leave the start box at the onset of the first trial (a measure of exploration). **b.** The average time taken to complete a trial (all three trials included) versus the latency to leave the start box. **c.** The difference in time taken between the slowest trial and the fastest trial versus the latency to leave the start box (data removed for individuals that were forced to exit the start box). The shaded bands depict 95% confidence intervals on the fitted values for each line. The solid line represents the relationship for the cooperative species, and the dashed line represents the same relationship for the non-cooperative species.



**Supplementary Figure 3.12** The difference in time taken to complete the maze from the fastest trial to the slowest trial, distinguished by whether or not the fish was forced to exit the refuge or left of their own volition, and by whether or not they are classified as cooperative breeders or non-cooperative. Values have been log transformed for presentation.

## 3.9 Supplementary methods

*Social system (all individuals and trials)*

The cooperative species did not complete the maze significantly faster than the non-cooperative species (log-LMM: $\chi^2 = 1.47$, df = 1, $p = 0.23$; Supplementary Figure 3.2a). Overall, the cichlids took less time to complete the maze across trials (log-LMM: $\chi^2 = 16.81$, df = 2, $p < 0.001$; Supplementary Figure 3.3a cooperative, 3.3d non-cooperative), but cooperative species improved their time taken to complete the maze considerably, and the non-cooperative species did not (Social System*Trial Number, log-LMM: $\chi^2 = 6.28$, df = 2, $p = 0.04$). Cooperative and non-cooperative species did not differ in their average number of mistakes (GLMM: $\chi^2 = 0.24$, df = 1, $p = 0.62$; Supplementary Figure 3.2b), both made fewer mistakes across trials (GLMM: $\chi^2 = 52.70$, df = 2, $p < 0.001$; Supplementary Figure 3.3b cooperative, 3.3e non-cooperative), and there was no difference in their rates of improvement regarding the number of mistakes (Social System*Trial Number, GLMM: $\chi^2 = 0.06$, df = 2, $p = 0.97$). Cooperative and non-cooperative species displayed similar degrees of inhibitory control and swam at the transparent barriers a comparable number of times (GLMM: $\chi^2 = 0.04$, df = 1, $p = 0.84$; Supplementary Figure 3.2c). Cooperative and non-cooperative species both improved their inhibitory control across trials (GLMM: $\chi^2 = 69.53$, df = 2, $p < 0.001$; Supplementary Figure 3.3c cooperative, 3.3f non-cooperative), and there was no difference in their rates of improvement for inhibitory control (Social System*Trial Number, GLMM: $\chi^2 = 3.95$, df = 2, $p = 0.14$).

*Sex (all individuals and trials)*

Males and females did not differ in the time taken to complete the maze (log-LMM: $\chi^2 = 2.55$, df = 1, $p = 0.11$; Supplementary Figure 3.2d). Males and females took less time to complete the maze across trials (log-LMM: $\chi^2 = 16.81$, df = 2, $p < 0.001$; Supplementary Figure 3.4a males, 3.4d females), and there was no difference in their rates of improvement for the time taken to complete the maze (Sex*Trial Number, log-LMM: $\chi^2 = 1.92$, df = 2, $p = 0.38$). There was no difference in dead-end corridor entries (mistakes) between males and females (GLMM: $\chi^2 = 0.10$, df = 1, $p = 0.75$; Supplementary Figure 3.2e), both made fewer mistakes across trials (GLMM: $\chi^2 = 52.70$, df = 2, $p < 0.001$; Supplementary Figure 3.4b males, 3.4e females), and there was no difference in their rates of improvement regarding the number of mistakes (Sex*Trial Number, GLMM: $\chi^2 = 0.36$, df = 2, $p = 0.83$). Males and females did not differ in their inhibitory control (GLMM: $\chi^2 = 0.09$, df = 1, $p = 0.76$; Supplementary Figure 3.2f), both improved their inhibitory control across trials (GLMM: $\chi^2 = 69.53$, df = 2, $p < 0.001$; Supplementary Figure 3.4c males, 3.4f females), and there was no difference in their rates of improvement for inhibitory control (Sex*Trial Number, GLMM: $\chi^2 = 0.83$, df = 2, $p = 0.66$).

*Phylogenetically controlled models*

We constructed phylogenetically controlled linear mixed models and phylogenetically controlled generalized linear mixed models (negative binomial family) using the ‘lme4’ and ‘glmmTMB’ packages which can be manipulated to include phylogeny as a random effect term (Li and Bolker, 2019). Branch lengths were estimated from the phylogeny presented in Day et al (2007); however, manipulating the branch lengths manually had no effect on model output. Each model included fixed effects of social system, sex and trial number, as well as the random effects of phylogenetic signal, tip variation (to address the multiple observations per species in our model) and standard residual variation. The effects of each factor were determined with the Anova function from the 'car' package.

*Social system with phylogenetic control (all individuals and trials)*

Cooperative and non-cooperative species had similar times to complete the maze (log-phyloLMM: $\chi^2 = 1.75$, df = 1, $p = 0.19$). Overall, they got faster over repeated trials (log-phyloLMM: $\chi^2 = 17.35$, df = 2, $p < 0.001$); however, cooperative species significantly improved their time to completion and non-cooperative species did not (Social System*Trial Number, log-phyloLMM: $\chi^2 = 6.56$, df = 2, $p = 0.04$). Cooperative and non-cooperative species made a similar number of mistakes (phyloGLMM: $\chi^2 = 0.13$, df = 1, $p = 0.72$). Both cooperative and non-cooperative species made fewer mistakes over repeated trials (phyloGLMM: $\chi^2 = 57.51$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement regarding the number of mistakes (Social System*Trial Number, phyloGLMM: $\chi^2 = 0.17$, df = 2, $p = 0.92$). Cooperative and non-cooperative species displayed similar degrees of inhibitory control (phyloGLMM: $\chi^2 = 0.53$, df = 1, $p = 0.47$). Both cooperative and non-cooperative species improved their inhibitory control over repeated trials (phyloGLMM: $\chi^2 = 75.56$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for inhibitory control (Social System*Trial Number, phyloGLMM: $\chi^2 = 2.22$, df = 2, $p = 0.33$).

*Sex with phylogenetic control (all individuals and trials)*

Males and females had similar times to complete the maze (log-phyloLMM: $\chi^2 = 2.38$, df = 1, $p = 0.12$). Both males and females got faster over repeated trials (log-phyloLMM: $\chi^2 = 17.35$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for time taken to complete the maze (Sex*Trial Number, log-phyloLMM: $\chi^2 = 1.99$, df = 2, $p = 0.37$). Males and females made a similar number of mistakes (phyloGLMM: $\chi^2 = 0.10$, df = 1, $p = 0.75$). Both males and females made fewer mistakes over repeated trials (phyloGLMM: $\chi^2 = 57.51$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement regarding the number of mistakes (Sex*Trial Number, phyloGLMM: $\chi^2 = 0.15$, df = 2, $p = 0.93$). Males and females displayed similar degrees of inhibitory control (phyloGLMM: $\chi^2 = 0.20$, df = 1, $p = 0.66$). Both males and females improved their inhibitory control over repeated trials (phyloGLMM: $\chi^2 = 75.56$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for inhibitory control (Sex*Trial Number, phyloGLMM: $\chi^2 = 1.35$, df = 2, $p = 0.51$).

*Social system with phylogenetic control (data removed for individuals that did not complete the maze on any of the three trials)*

Cooperative and non-cooperative species had similar times to complete the maze (log-phyloLMM: $\chi^2 = 1.92$, df = 1, $p = 0.17$). Both cooperative and non-cooperative species got faster over repeated trials (log-phyloLMM: $\chi^2 = 30.67$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for the time taken to complete the maze (Social System*Trial Number, log-phyloLMM: $\chi^2 = 0.66$, df =

2, $p = 0.72$). Cooperative and non-cooperative species made a similar number of mistakes (phyloGLMM: $\chi^2 = 1.51$, df = 1, $p = 0.22$). Both cooperative and non-cooperative species made fewer mistakes over repeated trials (phyloGLMM: $\chi^2 = 36.16$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement regarding the number of mistakes (Social System*Trial Number, phyloGLMM: $\chi^2 = 0.78$, df = 2, $p = 0.68$). Cooperative and non-cooperative species displayed similar degrees of inhibitory control (phyloGLMM: $\chi^2 = 1.74$, df = 1, $p = 0.19$). Both cooperative and non-cooperative species improved their inhibitory control over repeated trials (phyloGLMM: $\chi^2 = 50.60$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for inhibitory control (Social System*Trial Number, phyloGLMM: $\chi^2 = 2.02$, df = 2, $p = 0.37$).

*Sex with phylogenetic control (data removed for individuals that did not complete the maze on any of the three trials)*

Males and females had similar times to complete the maze (log-phyloLMM: $\chi^2 = 0.27$, df = 1, $p = 0.60$). Both males and females got faster over repeated trials (log-phyloLMM: $\chi^2 = 30.67$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for the time taken to complete the maze (Sex*Trial Number, log-phyloLMM: $\chi^2 = 2.01$, df = 2, $p = 0.37$). Males and females made a similar number of mistakes (phyloGLMM: $\chi^2 = 0.84$, df = 1, $p = 0.36$). Both males and females made fewer mistakes over repeated trials (phyloGLMM: $\chi^2 = 36.16$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement regarding the number of mistakes (Sex*Trial Number, phyloGLMM: $\chi^2 = 0.09$, df = 2, $p = 0.96$). Males and females displayed similar degrees of inhibitory control (phyloGLMM: $\chi^2 = 0.08$, df = 1, $p = 0.78$). Both males and females improved their inhibitory control over repeated trials (phyloGLMM: $\chi^2 = 50.60$, df = 2, $p < 0.001$), and there was no difference in their rates of improvement for inhibitory control (Sex*Trial Number, phyloGLMM: $\chi^2 = 1.51$, df = 2, $p = 0.47$).

*Correlation between maze performance and exploration tendencies (all individuals)*

We assessed the relationship between the latency to leave the start box at the start of trial 1 (exploration) and time to complete trial 1 with an LMM, after inverse transforming the latency data to meet the assumptions of parametric tests. We assessed the relationship between the latency to leave the start box and the average time to complete all three trials with an inverse transformed LMM. We also examined the relationship between the latency to leave the start box and the difference between the fastest and slowest trials, again with an LMM, after inverse transforming the latency data.

More exploratory fish (those that left the start box more quickly) did not complete the maze any faster on trial 1 (inverse-LMM: $\chi^2 = 0.67$, df = 1, $p = 0.41$; Supplementary Figure 3.11a), and did not have a faster average time to complete the maze (inverse-LMM: $\chi^2 = 1.22$, df = 1, $p = 0.27$; Supplementary Figure 3.11b). We removed all data from individuals that were forced to exit the refuge, and did not find a relationship between exploratory tendencies and the scale of improvement between the slowest trial and the fastest trial (inverse-LMM: $\chi^2 = 0.09$, df = 1, $p = 0.76$; Supplementary Figure 3.11c). We did not detect any differences in these measures between cooperative and non-cooperative species nor between males and females. We noted that 14/16 *N. tretocephalus* took the maximum time of 300s to leave the start box, so we performed the same analyses with data for *N. tretocephalus* removed and the patterns of these results were unchanged. We did not detect any other significant effects or interactions.

To examine whether the fish that left the start box on their own volition improved their time to completion more so than those that were forced out, we performed the following test. We first subtracted the time to complete the fastest trial from the time to complete the slowest trial, log-transformed this new variable to meet the assumptions of parametric tests, and included this measure as a dependent variable in an LMM. We included the fixed effects of social system, sex, and a binary variable describing whether or not the fish was forced to exit the start box, as well as their interaction terms. We also included a random effect of species. Surprisingly, we found that fish that were forced to exit the start box had greater improvement from their slowest trial to their fastest trial (log-LMM: $\chi^2 = 13.09$, df = 1, $p < 0.001$), and that non-cooperative species showed greater improvement from their slowest trial to their fastest trial compared to cooperative species (log-LMM: $\chi^2 = 7.59$, df = 1, $p = 0.01$; Supplementary Figure 3.12). These results suggest that removing the start box and forcing fish to exit prematurely is likely to result in a slow trial, and this is especially true in non-cooperative species which had a greater number of forced exits (forced exits occurred in 17/50 or 34% of trials for cooperative species and 23/45 or 51% of trials for non-cooperative species).

### *Species improvement*

To assess how each species individually improved across trials, we created new models and incorporated successive differences contrasts into these models. The time to completion data was analyzed for each species with an LMM, after log-transforming the data, and included the predictor variables sex and trial. Individual fish ID was also included as a random effect. The number of mistakes and number of swims at transparent barriers were analyzed with the same fixed and random effects, using GLMMs fitted to a negative binomial distribution. The results of these contrasts are presented in Supplementary Figures 3.5–3.10.

# Chapter 4:

# Understanding fish cognition: a review and appraisal of current practices

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Culum Brown & Sigal Balshine

Under review for: Animal Cognition

## 4.1 Abstract

With over 30,000 recognized species, fishes exhibit an extraordinary variety of morphological, behavioural and life-history traits. The field of fish cognition has grown markedly, with numerous studies on fish spatial navigation, numeracy, learning, decision making and even theory of mind. However, most cognitive research on fishes takes place in a highly controlled laboratory environment, and it can therefore be difficult to determine whether findings generalize to the ecology of wild fishes. Here, we summarize four prominent research areas in fish cognition, highlighting some of the recent advances and key findings. Next, we survey the literature, targeting these four areas, and quantify the nearly ubiquitous use of captive bred individuals and a heavy reliance on lab-based research. We then discuss common practices that occur prior to experimentation and within experiments that could hinder our ability to make more general conclusions about fish cognition, and suggest possible solutions. Only by complementing ecologically relevant laboratory-based studies with *in situ* cognitive tests will we truly unravel how fishes learn and make decisions about food, mates and territories.

## 4.2 Introduction

Over the last few decades, interest in the fields of animal cognition and cognitive ecology have increased dramatically (Brown et al., 2011; Bshary and Brown, 2014; Dukas, 1998; Dukas and Ratcliffe, 2009). Studies of animal cognition aim to understand the processes that help animals make decisions (e.g. perception, learning and memory; Ebbesson and Braithwaite, 2012). Fishes are well suited for such cognitive studies and have become regular experimental subjects in cognitive research. The more than 30,000 species of fishes provide valuable subjects for cognitive studies because of their taxonomic diversity, variety of habitats and range of life-history strategies (Patton and Braithwaite, 2015). Despite the growing interest in the cognitive ecology of fishes, studies to date have focused on standard laboratory models, and only a handful of fish cognition studies have been conducted in the field or on wild fish. Captivity can have severe impacts on cognition both due to plasticity during an individual's lifetime and via artificial selection over multiple generations in the laboratory. Thus, the reliance on captive-bred fishes may limit our understanding of fish cognition in nature. Noting this strong laboratory bias and the accompanying gap in our knowledge, we embarked on this systematic survey and commentary of fish cognition research. Our objective is to bring attention to the biases present in the literature and to encourage the thoughtful design of ecologically relevant experiments. Before discussing the findings of our literature survey, we first provide a brief synopsis describing our current understanding of fish cognition.

## 4.3 What we know about fish cognition

The field of fish cognition has a reasonably long history, as comparative psychologists have studied goldfish (*Carassius auratus*) alongside rats and pigeons for over 100 years (Churchill Jr, 1916). In the last decade, several review papers have been published on the topic of fish cognition (see Brown, 2015; Bshary et al., 2014; Patton and Braithwaite, 2015; Pouca and Brown, 2018; Sneddon and Brown, 2020). The research has mainly focused on four areas: 1) simple learning, 2) numeracy, 3) spatial cognition and 4) social cognition of fishes. For the purpose of this paper, we also focus on these four areas of fish cognition, summarizing current research in each area and then organizing our survey such that our literature search targeted the practices in each area.

### 4.3.1 Simple learning

Learning describes an animal's ability to use information from past experiences to inform future behaviour (Cauchoix and Chaine, 2016). Simple learning includes non-associative forms of learning, such as habituation and sensitization. It also includes associative forms of learning, in which connections are made either between unconditioned and conditioned stimuli (classical conditioning) or between stimuli and a certain behaviour (operant conditioning).

In fishes, simple learning can be rapid and long-lasting (Brown et al., 2011). For example, goldfish learned to avoid an area of a tank after a single electric shock (Riege and Cherkin, 1971). Similarly, zebrafish (*Danio rerio*) learned which colours predicted electric shocks with 89% accuracy after only two hours of training (or 20 trials; Aoki et al., 2015). Crimson spotted rainbowfish (*Melanotaenia duboulayi*) greatly improved their escape response to a novel trawl apparatus over 5 trials, and they highlight the longevity that such associations can be retained, remembering learned escape-techniques for up to 11 months (Brown, 2001). For many fishes, simple learning also begins early in life. Zebrafish, for example, can learn basic classical and operant conditioning tasks from as early as four weeks of age (Valente et al., 2012). Simple learning in fishes is pertinent to survival-related tasks, like predator avoidance and foraging (Kieffer and Colgan, 1992).

### 4.3.2 Numerical cognition

Numerical cognition refers to the ability to discriminate between two different discrete or continuous quantities (Agrillo et al., 2011). The ability to discern quantities is widespread among vertebrates and some invertebrates, while abstract numerical representation (counting) is considered a more demanding cognitive process and has only seldom been demonstrated in fishes (Agrillo et al., 2009; Davis and Memmott, 1982). Many fishes use quantity assessment to inform ecologically important behavioural decisions (e.g. what shoal to join, where to forage, or what mating tactic to use; reviewed by Agrillo et al., 2017). For example, fishes often choose to affiliate with larger groups when given a choice between two different shoal sizes, and there are numerous fitness benefits for doing so, such as improved foraging and predator defense, increased vigilance, predator confusion and dilution of risk (Agrillo et al., 2017). However, assessing the extent of more complex numerical abilities, such as counting, requires complex experimental protocols. In mosquitofish (*Gambusia holbrooki*), individuals showed a preference for larger shoals even when a series of baffles meant that only one conspecific could be viewed at any given time; thus, the focal ‘choosing’ fish needed to count how many individuals were at each end of the arena (Dadda et al., 2009). It seems that true numerical representation by fishes is largely limited to numbers no greater than 4 or 5, while ratios are typically used to compare larger quantities, consistent with many mammalian studies (Agrillo et al., 2017). For instance, mosquitofish discriminated between two shoals that differed in number by a single individual when each shoal had fewer than 5 fish, but discrimination between larger shoals was only possible if the bigger of the two had twice as many individuals or more (Agrillo et al., 2008).

### 4.3.3 Spatial cognition

Spatial cognition is the ability to acquire and reorganize spatial information to make sense of an environment (Poucet, 1993). Spatial cognition plays a role in many behavioural processes including foraging, mating, predator avoidance, and migration (Fukumori et al., 2010). Animals can navigate space by using orientation (egocentric) or mapping (allocentric) strategies and some fishes, such as goldfish, rely on both, using internal egocentric cues (such as recalling motion patterns) and external allocentric cues (such as landmarks) when navigating a maze (Rodriguez et al., 1994). Other species, like the weakly electric fish *Gnathonemus petersii*, preferentially use egocentric cues during maze learning experiments (Schumacher et al., 2017). In contrast, intertidal gobies (*Bathygobius soporator*) create cognitive maps of the shoreline (thereby relying primarily on allocentric cues) and use these maps to jump between nearby tide pools when threatened and then can return to their home pool quickly (Aronson, 1951; Jorge et al., 2012; White and Brown, 2013). To construct and use a cognitive map (i.e. a mental representation of an environment), an animal needs to: (i) encode information about an object relative to other landmarks, (ii) integrate newly acquired information into the map, and (iii) use the map to come up with novel movement strategies (Poucet, 1993).

### 4.3.4 Social cognition

Social cognition describes an animal's ability to recognize, react to, and predict the actions of other individuals (Shettleworth, 2010). This form of cognition includes social recognition, social learning, conflict resolution, collective decision making and cooperation—skills often considered to be highly complex (Bshary et al., 2014, Bshary et al., 2006; Grosenick et al., 2007; Grutter, 1999).

Social recognition, or the ability to recognize familiar individuals, has been demonstrated in many fishes (reviewed by Griffiths, 2003). Some fishes also use information from conspecifics to inform their decisions concerning mate choice, foraging locations and antipredator behaviour (i.e. social learning; reviewed by Brown and Laland, 2003). Intraspecific cooperation, that is cooperation between individuals of the same species, has been shown in a variety of fishes (Balshine and Buston, 2008; Brown and Laland, 2003; Lindeyer and Reader, 2010; Reader et al., 2003) while interspecific cooperation, cooperation between individuals of different species, has also been observed between the grouper *Plectropomus pessuliferus*, and the giant moray eel *Gymnothorax javanicus*. These species communicate intentions to jointly hunt when the grouper approaches an eel and performs a distinct head-shake or when the grouper points its body directly at a prey item that has escaped into a crevice, signaling for assistance. By indicating prey location, groupers attempt to recruit nearby giant moray eels, and if successful, together they flush prey out of these crevices and hiding areas (Bshary et al., 2006).

## 4.4 Literature survey of fish cognition studies

While surveying the fish cognition literature on these four key topics, we noticed that most studies were laboratory-based, and conducted on a small number of model species (e.g. goldfish, zebrafish, and guppies). To quantify the extent of this laboratory and species bias, we conducted a systematic literature survey on fish cognition (Figure 4.1). We first carried out a PubMed subject search using seven search terms specific to each of the four cognitive areas described above. Our search was conducted on August 28th, 2019 and based on search terms present in the Article Title, Abstract, or Keywords (a full list of search terms used is available in Table 4.1). This search yielded a total of 2019 results. We then carried out the same search in ISI Web of Science and this search yielded an additional 449 results. After removing duplicates (i.e. articles that appeared in both our PubMed and ISI Web of Science searches), we scrutinized each article, identified a total of 608 studies relevant to fish cognition, and extracted information from each.

**Table 4.1** Keywords used in PubMed and ISI Web of Science literature reviews.

| *Simple Learning* | *Numeracy* | *Social Cognition* | *Spatial Cognition* |
|---|---|---|---|
| Simple Learning | Numeracy | Social Cognition | Spatial Cognition |
| Habituation | Quantity Discrimination | Social Learning | Navigation |
| Classical Conditioning | Numerical Representation | Group Decision Making | Orientation |
| Instrumental Conditioning | Number System | Individual Recognition | Maze Learning |
| Associative Learning | Numerical System | Social Recognition | Spatial Memory |
| Avoidance Learning | Continuous Quantities | Social Memory | Spatial Learning |
| Aversive Learning | Discrete Quantities | Familiarity | Spatial Perception |

We found a dramatic increase in the number of fish cognition studies over the last decade (68% of the total studies identified were published between 2010–2019, Figure 4.1a). Cyprinids (e.g. minnows, goldfish) were the most studied fish order (31% of all studies; Table 4.2). Spatial ability was the most studied of the various areas of fish cognition, while numeracy is the least studied (Figure 4.1a). We found that most studies (69%) used captive bred rather than wild fishes (Figure 4.1b). We also found that the majority of studies (52%) conducted with sexually mature individuals did not identify the sex of their study specimens (Supplementary Figure 4.1). Extremely few studies were conducted in the field; 91% of fish cognition studies took place in a laboratory (Figure 4.1c).

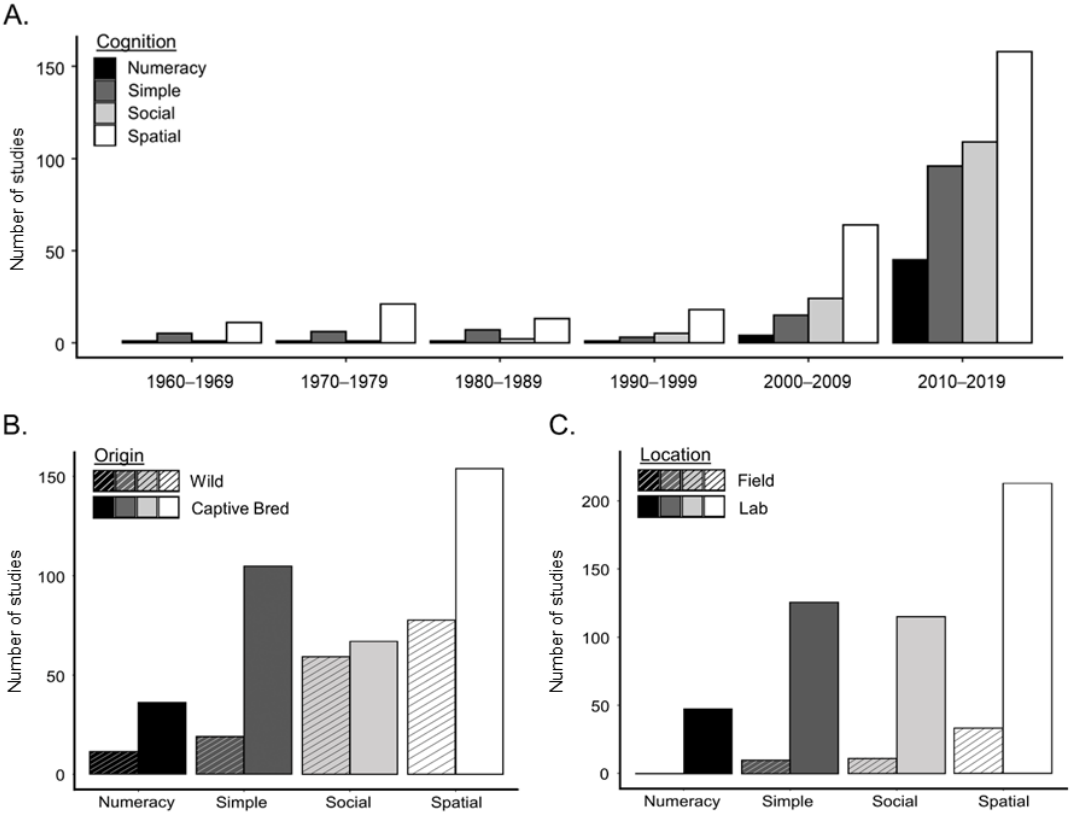

**Figure 4.1a.** Peer reviewed publications since 1960 on fish simple learning as well as numerical, social and spatial fish cognition**. b.** Use of wild versus captive bred fishes in cognitive studies. **c.** Field versus lab-based studies for each type of fish cognition. All figures are based on our PubMed and ISI Web of Science systematic literature survey. Search parameters used to locate papers can be found in Table 4.1.

**Table 4.2** Fish orders as represented in the cognition literature. Papers located were based on PubMed and ISI Web of Science systematic literature review (n=608). Review articles (n=72) were removed and studies focusing on more than one fish order (n=12) were counted multiple times to cover all the orders represented. The top 5 most prevalent orders in fish cognition research are identified in **bold** font.

| *Order* | *Simple* | *Numeracy* | *Social* | *Spatial* | *Total* | *Percent of Grand Total* |
|---|---|---|---|---|---|---|
| Anabantiformes | 6 |  | 2 | 3 | 11 | 2.0 |
| Anguilliformes |  |  |  | 7 | 7 | 1.2 |
| Atheriniformes | 2 |  | 1 |  | 3 | 0.5 |
| Beloniformes | 1 |  | 6 | 2 | 9 | 1.6 |
| Blenniiformes |  |  |  | 2 | 2 | 0.4 |
| Carcharhiniformes | 1 |  | 2 | 10 | 13 | 2.3 |
| Characiformes |  | 1 |  | 9 | 10 | 1.8 |
| **Cichliformes** | **8** | **9** | **20** | **8** | **45** | **8.0** |
| **Cypriniformes** | **72** | **5** | **21** | **76** | **174** | **30.8** |
| **Cyprinodontiformes** | **13** | **27** | **27** | **24** | **91** | **16.1** |
| Gadiformes |  |  | 1 | 1 | 2 | 0.4 |
| Gasterosteiformes | 1 | 3 | 17 | 3 | 24 | 4.3 |
| Gobiiformes |  |  | 2 | 4 | 6 | 1.1 |
| Gymnotiformes | 1 |  |  | 9 | 10 | 1.8 |
| Heterodontiformes | 2 |  |  |  | 2 | 0.4 |
| Kurtiformes |  |  | 1 | 5 | 6 | 1.1 |
| Labriformes |  |  |  | 2 | 2 | 0.4 |
| Myliobatiformes | 1 |  | 2 | 4 | 7 | 1.2 |
| Orectolobiformes | 3 |  |  | 7 | 10 | 1.8 |
| Osmeriformes |  |  |  | 1 | 1 | 0.2 |
| Osteoglossiformes |  |  | 2 | 11 | 13 | 2.3 |
| **Perciformes** | **15** | **2** | **23** | **25** | **65** | **11.5** |
| Petromyzontiformes |  |  |  | 3 | 3 | 0.5 |
| Pleuronectiformes |  |  |  | 5 | 5 | 0.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Salmoniformes** | **6** | | **4** | **21** | **31** | **5.5** |
| Scorpaeniformes | | | | 5 | 5 | 0.9 |
| Siluriformes | | | 1 | 7 | 8 | 1.4 |
| *Grand Total* | 132 | 47 | 132 | 254 | 565 | 100 |

## 4.5 Factors compromising our assessment of fish cognition

Our survey results confirmed that most fish cognition studies are performed in the lab, use lab-reared animals, and focus on only a few species. There is evidence that these practices can negatively impact our understanding of fish cognition. Next, we summarize this evidence and outline considerations for designing more ecologically relevant studies of fish cognition. The considerations comprise two categories or types of experimental issues: pre-experimental factors and within-experimental factors (Figure 4.2). We argue that researchers should consider these factors when conducting future cognitive studies on fishes. Further, we suggest that conducting fish cognition research in the wild would solve many of these issues, and acknowledge that the combination of laboratory controlled experiments and field based studies is likely to be the most powerful approach to fully understand fish cognitive abilities.

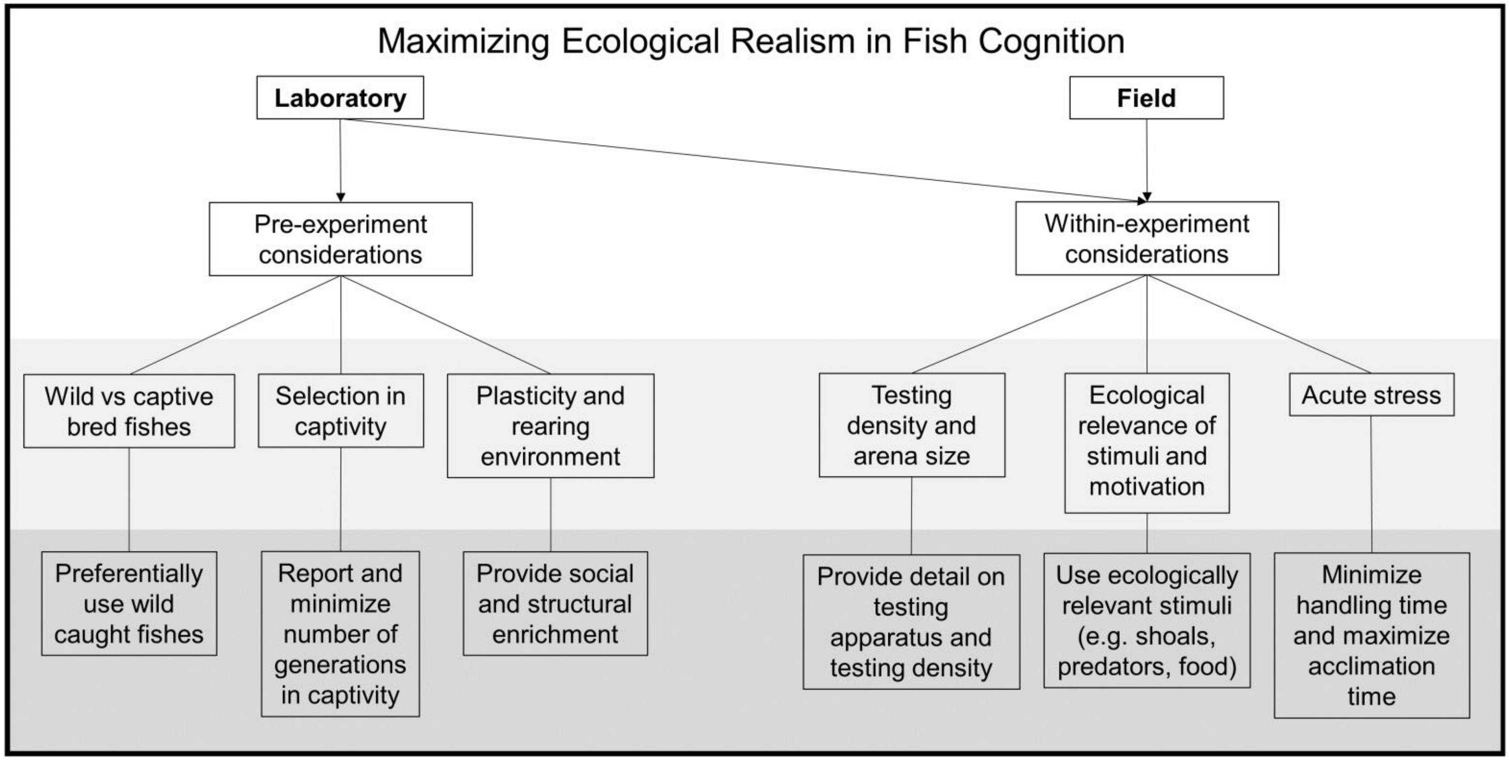

**Figure 4.2** A concept map depicting the pre- and within-experiment considerations that must be made when designing studies of fish cognition for the laboratory or the field.

### 4.5.1 Pre-experiment factors that influence cognition

*Selection in captivity*

Natural selection operates differently in the lab than in the wild. Captive rearing often provides a benign environment and limits the strength of natural selection, thus allowing a wider range of phenotypes to persist than would occur in nature. In some cases, traits suited for life in the wild can be maladaptive in captivity (Courtney Jones et al., 2018; Garner, 2005; McDougall et al., 2006). In aquaculture, for example, there may be positive selection on traits such as boldness and aggressive feeding due to high rearing densities, while in nature these traits would expose individuals to high predation risk (Johnsson et al., 2014; Tave and Hutson, 2019). These changes can occur even within a single generation (Christie et al., 2012). Artificial selection in captivity can also affect cognitive performance (Doyle and Talbot, 1986; Huntingford, 2004; Huntingford et al., 1994). In some cases, this may result from an energy allocation trade-off between brain development versus digestive tract production. Growing quickly makes individuals more competitive in gaining access to food resources (Doyle and Talbot, 1986) but can come at the expense of cognitive investment (Stamps, 2007; Tsuboi et al., 2015). For example, artificial selection for large brains in guppies, which has been linked to improved cognition (Kotrschal et al., 2013; Kotrschal et al., 2015a), also results in slower growth rates (Kotrschal et al., 2015b) and reduced gut size (Kotrschal et al., 2013). Thus, it seems plausible that inadvertent artificial selection for fast growth rates in captive fishes could also cause declines in cognitive performance, though this possibility has not been well investigated.

### *Plasticity and the rearing environment*

The rearing environment can impact fish behaviour long before an experiment begins. Fish cognition is widely thought to be most dramatically influenced by environmental conditions during early development; however, recent evidence suggests that fish brains remain responsive across all life stages (Ebbesson and Braithwaite, 2012; Näslund et al., 2012). Both the social and physical conditions that a fish experiences are known to impact neural development and cognitive abilities.

Chronic exposure to social stress has been shown to decrease neural proliferation (Johansen et al., 2012; Sørensen et al., 2013). While the consequences of long-term social stress on cognitive performance in fishes have not been well studied, most studies to date suggest that social stress impairs learning (Laudien et al., 1986; Olla and Davis, 1989; Sørensen et al., 2013). In the cichlid *Cichlasoma paranaense*, isolation decreased performance on an associative learning task (Brandão et al., 2015). Juvenile guppies reared under stressful, crowded conditions were worse at learning from experienced conspecifics how to navigate a maze compared to conspecifics raised at lower densities (Chapman et al., 2008). Cognitive abilities can also be influenced by the conditions experienced early in development or even the conditions experienced by parents (Eriksen et al., 2011; Moore et al., 2019). While no fish studies have directly linked maternal investment to offspring cognitive abilities, there is evidence in salmonids that cognitively demanding tasks like foraging (Leblanc et al., 2011) and schooling (Tierney et al., 2009) are affected by maternal condition and egg investment. In other animals, such as honeybees *Apis mellifera*, increased maternal investment in workers improved their performance in a later associative learning experiment (Scheiner, 2012). In general, our assessment of cognitive abilities in laboratory-raised fishes may be biased by the quality of care received by parents and the conditions experienced during rearing (reviewed by Jonsson and Jonsson, 2014; Sørensen et al., 2013). Responses may also be species and/or context specific (Ghio et al., 2016).

The degree of environmental enrichment is considered the primary mechanism for differences in cognitive capacity between captive and wild fishes (reviewed by Näslund and Johnsson, 2016). Generally, increased complexity results in enhanced brain growth (Kihslinger and Nevitt, 2006) and faster rates of neural proliferation (Dunlap et al., 2011; Salvanes et al., 2013; von Krogh et al., 2010). For instance, adult zebrafish kept in isolation in structurally enriched environments showed increased telencephalic cell proliferation after only one week (von Krogh et al., 2010). These neuro-anatomical differences have been linked to improvements in several aspects of cognition, including foraging on novel prey (Brown and Laland, 2003), hiding from predators (Salvanes and Braithwaite, 2005), and spatial learning (Salvanes et al., 2013). Interestingly, *Simochromis pleurospilus* cichlids that received variable, sometimes low-ration diets were cognitively superior to those fed a constant, high-ration amount, suggesting that the cognitive benefits from environmental variability occur even when they result in objective decreases in environmental quality (Kotrschal and Taborsky, 2010). While variability in food provisioning might benefit cognition, positive effects of variability are not universal. Thermally stressed female rainbow trout produce offspring with impaired spatial learning abilities and brains with differential expression of important genes for neural development (Colson et al., 2019). Evidence from guppies suggests that even maternal exposure to mild intermittent stress from routine laboratory maintenance procedures, such as twice weekly water changes, can negatively impact associative learning ability in their offspring (Eaton et al., 2015).

The cognitive benefits of enrichment are neither guaranteed nor permanent. In zebrafish, regular exposure to novel objects increased brain growth and associative learning performance, but this effect disappeared in the presence of a mild chasing stressor (DePasquale et al., 2016). Social rearing in captivity increased neural proliferation in the electric fish *Brachyhypopomus gauderio*, but only in brain regions associated with communication, and rates of neural proliferation were still far below those in wild fish (Dunlap et al., 2011). In juvenile Atlantic salmon *Salmo salar*, increases in brain size caused by environmental enrichment were quickly reversed (one month) when fish were later transferred to bare tanks. In addition, captured wild salmon developed smaller brains in captivity than their wild relatives (Näslund et al., 2012). Thus, habitat complexity seems to matter for cognitive function in both developing and adult fish. To assess ecologically relevant cognitive abilities of fishes in the laboratory, providing environmental enrichment is therefore important when working with both captive-reared and wild-caught animals.

### 4.5.2 Within-experiment factors that influence cognition

*Testing densities and arena size*

Wild fishes almost always experience a bigger social and physical world than those in the laboratory. Generally, fish perform better in the presence of other conspecifics (dampening social isolation stress) and with experienced demonstrators (Brown and Warburton, 1999; Lindeyer and Reader, 2010; Reader et al., 2003); however, the vast majority of fish used in cognitive studies are tested in isolation. Isolation is known to impair learning (Brandão et al., 2015; Laudien et al., 1986). To induce 'more natural' behaviour, researchers will sometimes test dyads or trios (Culbert et al., 2019; Silk, 2007a; Silk, 2007b) and, to reduce social isolation stress, other fish or social companions are often placed behind partitions (Jones and Godin, 2009). Despite these practices, it remains unclear whether is it more appropriate to test fish in isolation or test several fish together on any given trial and at the same time, which highlights the difficulty of teasing apart individual from social learning (Brown et al., 2011). Outlining the appropriate social conditions for testing is especially difficult, considering that the effect of social stimulation on performance differs between species. For instance, guppies improved their ability to differentiate quantities when tested in a dyad versus when tested as a singleton (Bisazza et al., 2014), but there was no such improvement in quantity differentiation in dyads versus singleton grass carp *Ctenopharyngodon della* or Chinese bream *Parabramis pekinensis* (Bai et al., 2019).

Another issue with laboratory-based fish cognition experiments is that these studies are conducted using a wide variety of tank sizes and shapes. In some rodents and birds, individuals can transfer their place-finding ability (spatial cognition) to novel enclosures of different shapes, sizes and colours (Tommasi and Thinus-Blanc, 2004), suggesting that their learning abilities depend on abstract geometric rules rather than the exact structure of the testing apparatus. Fishes also use geometry when solving spatial tasks, and manipulation of the rearing environment can affect spatial performance (Brown et al., 2007; Carbia and Brown, 2018). For example, convict cichlids *Archocentrus nigrofasciatus* reared in a circular tank rather than a rectangular tank rely less on angular geometric cues and more on featural navigation cues (Brown et al., 2007). We therefore recommend thoughtful consideration and careful reporting of rearing and testing conditions (e.g. social density, tank size, tank shape and structural enrichment) in future studies of fish cognition.

*Ecological validity of stimuli and motivation*

Animals will respond to stimuli most naturally, and learn associations most easily, when these stimuli have evolutionary and ecological relevance (Dukas, 1998; Dukas and Ratcliffe, 2009; Garcia and Koelling, 1966). However, many fish cognition studies rely on simplistic or unnatural stimuli (e.g. plastic blocks or electrical shocks; Dunlop et al., 2006; May et al., 2016). Stimuli also can vary in terms of their attractiveness and saliency (Millsopp and Laming, 2008) and, thus, it is important before starting experiments to determine if the test fish have a pre-existing bias for the stimuli because of its particular colour, smell, pattern or shape (Basolo, 1995; Endler and Basolo, 1998; Rodd et al., 2002). Another drawback of laboratory experimentation is that stimuli (e.g. food, shelter, predator, competitor, or a potential mate) are typically placed in clear containers or behind glass. Both the focal and stimulus fish often quickly habituate to such barriers, dampening their drive to reach the reward or avoid the unpleasant stimulus (Peeke and Herz, 1973). Barriers also reduce the ability to learn by limiting the exploration, sampling and interaction that can occur (Peeke and Herz, 1973).

### *Acute stress*

During most laboratory experiments, researchers stress their animal subjects by capturing, transporting, handling, and/or confining the animal, or by simply placing individuals where they can detect a predator or a competitor. Despite the ubiquitous stress experienced in most experiments, there is little research about how different stressors influence individual learning and how this effect varies among species, sexes, ages and individuals. In rainbow trout, simulated semi-acute stress (achieved via cortisol implantation) impairs learning and memory (Barreto et al., 2006). Furthermore, when rainbow trout were selected for consistently high or low cortisol responses to stress, the low-responsive group had a longer retention of a conditioned response (Øverli et al., 2004). Individual differences in stress reactivity also impact learning; in many species, bolder individuals learn faster (Mamuneas et al., 2014;

Trompf and Brown, 2014), perhaps because these individuals will explore and sample more frequently. Precisely how individual differences in stress responsiveness influences fish spatial, numerical and social cognition remains to be explored. Despite this uncertainty, we suggest that methodological steps that can reduce stress (e.g. placing cognitive testing apparatus in the home/rearing tank to eliminate handling stress, netting/transferring fish underwater to eliminate air exposure) should be employed whenever possible.

## 4.6 The steps towards more ecological relevance in fish cognition

Over the past decade, there has been a dramatic increase in the number of studies on fish cognition and, in tandem, a recognition that environmental and developmental factors shape cognition. Both the pre- and within-experiment issues affecting fish cognition studies can be minimized or eliminated by using wild-caught fish, by raising fish in enriched environments and by using more ecologically relevant stimuli (Figure 4.2). Embedding ecology into cognitive research is a necessary next step to understand the evolution and function of fish cognitive abilities because organisms' neural processes have developed in, and are adapted to, their wild environments.

In addition to performing more ecologically relevant fish cognition studies in the laboratory, we can maximize ecological realism by conducting some experiments in the field. Although field work can present additional challenges like long-distance travel, unpredictable weather, and limited control over exogenous factors, there are many benefits. Conducting cognitive research in the wild ensures the use of wild fishes and nullifies selection in captivity. There are also certain behaviours that are best expressed, and therefore best studied, in complex environmental contexts (e.g. problem solving; MacDonald and Ritvo, 2016). Technological advances in telemetry, acoustic receivers and underwater cameras offer researchers a number of potentially fruitful avenues to explore spatial navigation and social cognition remotely (Ebner and Thiem, 2009; Lucas and Baras, 2000; Mourier et al., 2017). The manipulation of landmarks and rewards (number or position) is widely used in other taxa (especially with birds and insects) to test both numeric and spatial cognition in the wild (Chittka and Geiger, 1995; Helfman and Schultz, 1984; Pritchard et al., 2018; Pritchard and Healy, 2018; Reese, 1989) and shows promise for fish research (see Helfman and Schultz, 1984 for a nice example with fish).

As fishes are the most speciose group of extant vertebrates, it would be inappropriate to suggest universal rules for designing ecologically relevant cognitive studies. Rather, the goal of this paper is to emphasize the complexity of designing high-quality cognitive studies in fishes and clearly label the factors that should be considered when planning such research. Our survey demonstrates the paucity of fish cognition field studies (<10% of studies to date), including their near complete absence from some sub-fields such as numeracy. Our results underscore an overwhelming reliance on lab-based research, and potential confounds spanning from long-term, pre-experimental holding practices to the within-experiment or immediate effects of ecologically irrelevant tasks. Further studies are necessary to clarify the full extent of fish cognitive abilities, and we hope that these studies will seriously consider their ecological applicability—as well as the complementary nature of laboratory and field work—and take steps to study fish cognition in more natural settings.

## 4.7 Acknowledgements

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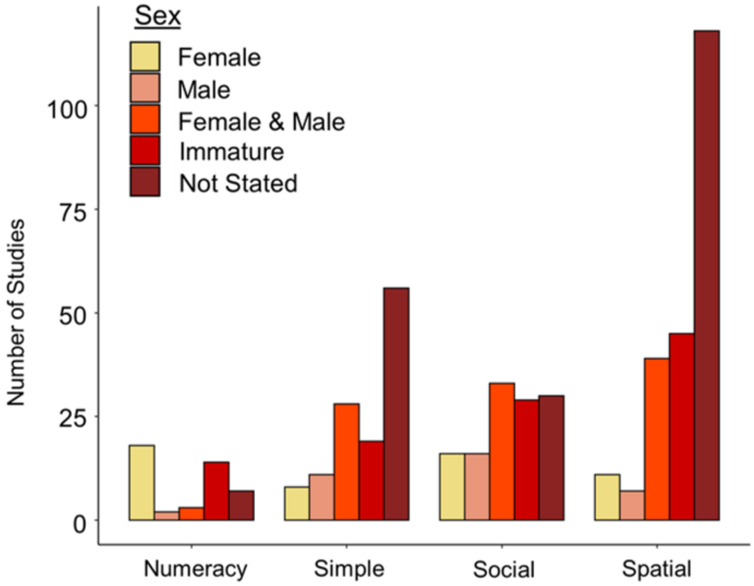
## 4.9 Supplementary materials



**Supplementary Figure 4.1** The sex of study specimens used in fish cognition research, as reported by each article. This figure is based on our PubMed and ISI Web of Science systematic literature survey. Search parameters used to locate papers can be found in Table 4.1.

# Chapter 5:

# General discussion

Matthew G. Salena

## 5.1 Overview

In this thesis, I sought to uncover whether there are cognitive differences between cooperative and non-cooperative cichlid fishes. I predicted that the social challenges of living and breeding in a cooperative group would select for enhanced social and general cognitive abilities in cooperative Lamprologini cichlids; however, I did not find evidence for any substantial cognitive differences between cooperative and non-cooperative species. In Chapter 2, I compared social memory and social recognition between three cooperative and three non-cooperative species. Although I predicted that cooperative species would have an enhanced recognition capacity, I found that all cichlids, regardless of their social system classification, appear to recognize familiar individuals. In a related study, I investigated quantity discrimination abilities in a group-choice paradigm with cues of heightened predation risk. I predicted cooperative species to have an enhanced group-size assessment capacity, but I found that both cooperative cichlids and their non-cooperative relatives strongly preferred to affiliate with larger groups, suggesting that both are capable of making basic numerical judgements.

In Chapter 3, I assessed the spatial learning abilities of cooperative and non-cooperative cichlids in a maze learning paradigm. I predicted that the cooperative species would complete the maze faster, make fewer mistakes, display better inhibitory control, and improve their scores more readily than their non-cooperative relatives. I found that both cooperative and non-cooperative species had comparable scores for these three performance measures and showed similar performance improvement. Furthermore, I predicted that males of the six cichlid species would outperform females since male cichlids typically have larger territories. There is also considerable evidence that males of the cooperative species *Neolamprologus pulcher* are often dominant in more than one social group and have to travel longer distance to move between these groups. I expected this pattern would hold true in *N. pulcher* and their relatives. Yet, I found little evidence for overall sex differences in spatial performance, and males and females exhibited similar performance improvement over consecutive trials.

In Chapter 4, I discuss a number of common practices in studies of fish cognition that limit the generalizability of results, and identify ways to improve experimental designs and create more ecologically relevant studies of fish cognition. I suggest that fishes must be provided socially and structurally enriched (physically complex) environments in captivity to ensure cognitive development remains similar to their wild counterparts (efforts should be made to create the same environmental conditions fishes experience in nature, in the lab). I also address the need for more ecologically relevant experimental conditions and motivational stimuli, if researchers wish to make broader generalizations regarding how fish cognition operates in the wild.

In this general discussion chapter, I connect my primary research findings to the broader literature on fish cognition and expand upon how my results contribute to our understanding of the social brain and cooperation. Further, I identify crucial considerations for designing experiments of fish cognition and recommend avenues for future research.

## 5.2 True individual recognition in cichlids

In Chapter 2, I asked whether cooperative fishes have an enhanced social memory/recognition capacity, since they must be able to distinguish group members from non-group members, and between different members within their group. But both the cooperative and non-cooperative fishes used in this study are territorial and must keep track of their neighbours' identities, and this requires recognizing and remembering different individuals and their residency status. I found evidence that both cooperative and non-cooperative cichlids are able to recognize familiar individuals; however, their affiliative preferences differed based on their social system classification, with cooperative cichlids spending more time interacting with familiar individuals and non-cooperative cichlids spending more time interacting with unfamiliar individuals. My results align with previous studies on *N. pulcher*, one of the cooperative fishes included in my research, that indicates territorial cichlids can recognize familiar individuals (Sogawa et al., 2016). For instance, Sogawa et al (2016) found that fish established in an experimental tank (territory) were initially very aggressive towards a neighbour, but the aggression quickly decreased within four days. When the researchers moved the neighbour to the opposite side, the focal fish was more aggressive than the previous day yet exhibited far less aggression towards this familiar fish than towards an unfamiliar fish placed on the shifted side. This differential treatment suggests that the focal fish recognized the familiar neighbour.

Furthermore, Saeki et al (2018) demonstrated that *N. pulcher* are capable of 'true individual recognition' in a territory conflict paradigm. True individual recognition, the ability to distinguish multiple individuals by their unique features, is more elaborate than class-level recognition in which individuals are categorized, and these categories are used for differential treatment. Saeki et al (2018) used one-way mirrors to quantify aggression of a focal fish towards two neighbours, $N_A$ and $N_B$. Focal fish displayed limited aggression towards these two familiar neighbours, but aggression towards $N_B$ quickly increased when $N_A$ neighbour was removed and $N_B$ was switched to the opposite side of the tank. However, aggression towards the shifted $N_B$ decreased within one minute, which contrasts with the lengthy and more frequent aggression received by unfamiliar individuals. These results suggest that the focal fish differentiated $N_A$ from $N_B$ and likely tried to punish $N_B$ for moving beyond its territory. The authors indicate that true individual recognition may be widespread in territorial animals that exhibit a dear enemy effect, where territorial animals are often less aggressive towards neighbouring residents than non-residents. There is also considerable evidence for the 'face-specific hypothesis' in *N. pulcher* and the closely related cichlids *Neolamprologus brichardi* and *Julidochromis transcriptus*, which argues that these animals can recognize conspecifics using facial patterns alone (Hotta et al., 2017; 2019; Kohda et al., 2015). Moreover, a study by Awata et al (2012) conducted in Lake Tanganyika using SCUBA showed that another territorial cichlid, *Variabilichromis moorii*, are even capable of recognizing familiar individuals of the species *Neolamprologus mustax*. The authors caught *N. mustax* in their territories, released them at a distance from their territory and followed them to observe any interactions with *V. moorii*. The displaced *N. mustax* received far more aggression from the territorial *V. moorii* than did *N. mustax* residents. The results from these studies, in combination with my own, indicate that social memory—and perhaps true individual recognition—may be necessary for conflict avoidance in territorial cichlids, regardless of their social proclivity.

## 5.3 Cichlid social preferences

Interestingly, I found that *N. pulcher* spent more time associating with familiar individuals which is contrary to results of a prior study by Frostman and Sherman (2004) that found *N. pulcher* spent more time near the territorial boundaries of unfamiliar neighbours than familiar neighbours. That being said, Frostman and Sherman (2004) only performed experiments on males, had a small sample size (N = 12), and differed further in that the focal fish had 72 hours to view the 'familiar neighbour' in a specific section of the experimental tank. In our study, focal fish were allowed only 22-minutes to view and familiarize themselves with the (soon to be) 'familiar neighbour'. These differing familiarization periods could have influenced the territorial threat response of the focal fish, since fish that are established in neighbouring territories often receive reduced aggression (Dear Enemy Effect; Saeki et al., 2018). Focal fish in my study may not have had sufficient time to learn that the neighbouring fish owns a particular section of the tank or territory (i.e. Frostman and Sherman allowed *N. pulcher* to view a conspecific in the same spot for 72 hours and I allowed 22-minutes). The neighbour that was viewed for only 22-minutes, in my study, might appear as a familiar, non-resident. A familiar individual without a resident territory may be a lesser threat to the focal fish, which might encourage more affiliation between the two. It is well established that territory owners or residents fight more intensely over their space than non-residents because the value of the territory is greater to them (Alcock and Bailey, 1997; Davies, 1978; Parker, 1974). Typically, animals that have held territories for longer, have invested considerable time learning the area and becoming comfortable in that space, and having increased knowledge of a territory often leads to a more tenacious territorial defence. This is why in studies of territorial conflict, long-term territory residents are unlikely to be ousted by an intruder (da Silva Nunes and Jaeger, 1989; Mendiola-Islas et al., 2016). The threat of a hard-fought defence from long-term territory residents may play a role in driving social preferences.

## 5.4 Social memory and territoriality

Many of the other Lamprologini cichlid species used in my thesis research are unstudied in terms of behavioural research, except for *N. multifasciatus* and *Julidochromis* spp. This is surprising since the Lamprologines are also the only group of fishes known to have evolved true cooperative breeding (Reddon et al., 2017), although spontaneous alloparental care in the absence of biological parents has recently been observed in the anemonefish *Amphiprion ocellaris* (Phillips et al., 2020). Social memory is well supported in other territorial animals (mammals, birds, and invertebrates; Tibbets et al., 2007), but up until the mid-1990s relatively few studies investigating the link between social memory and territoriality had been conducted on fishes (Temeles, 1994). Fishes are worth studying because they represent the most speciose group of extant vertebrates and exhibit a remarkable diversity of social behaviours. By neglecting research on fishes, we disregard approximately half of all living vertebrates. In the last quarter-century, this deficit in taxonomic diversity has improved considerably. To date, the social memory studies that have been conducted on fishes still have a heavy focus on cichlids and zebrafish *Danio rerio* compared to other fishes, which suggests a need for future research to expand their scope of model fish species (Aires et al., 2015; Kohda et al., 2015; Lehtonen et al., 2010; Madeira and Oliveira, 2017). My research provides further evidence supportive of social memory in *N. pulcher* and *Julidochromis* spp. and is the first to investigate and find evidence for these same abilities in their close relatives. Overall, my results indicate that like other animals, social memory ability may be widespread in territorial fishes.

## 5.5 Cichlid numeracy and relevance to sociality

I also predicted that cooperative species would have enhanced numerical abilities because cooperative fishes might need to distinguish between small and large groups when moving between groups, and choosing the optimal group to join. When I offered fishes the choice between joining a group of three conspecifics vs one conspecific in the presence of predators, both cooperative and non-cooperative cichlids showed a strong preference for the group of three. Given that grouping offers safety in numbers (Hager and Helfman, 1991), individuals must be able to differentiate three (the group) from one (the single conspecific) to reap the benefits of this social refuge. Although I expected cooperative species to have an enhanced group-size assessment capacity and spend more time with the group than the single conspecific, my task may have been overly simple. There is extensive evidence to suggest that many animals, even insects, are able to successfully discriminate between small numbers (e.g. less than 4 or 5; Pahl et al., 2013; Petrazzini, 2014; Rugani et al., 2008; Ward and Smuts, 2007). There is even some evidence in fishes that highly social and less social species have similar small number discrimination abilities. A study by Agrillo et al (2012) compared quantitative abilities of five teleosts: redtail splitfin (*Xenotoca eiseni*), guppies (*Poecilia reticulata*), zebrafish (*Danio rerio*), Siamese fighting fish (*Betta splendens*), and angelfish (*Pterophyllum scalare*), some of which differ dramatically in their social behaviour. The authors found evidence for equally impressive quantitative abilities in each species, except zebrafish (considered to be highly sociable), which had difficulty learning the procedure. The procedure required fishes to discriminate between two sets of geometrical figures using a food reward. Since highly social, schooling fish such as zebrafish are usually surrounded by many conspecifics, perhaps they seldom need to differentiate between small numbers, and geometric figures may not be particularly engaging.

Impressive numerical abilities have been reported in a wide variety of taxa including mammals, birds, amphibians, and fishes which has sparked debate about whether all species share the same innate quantitative processing mechanisms (Al Aïn et al., 2009; Gómez-Laplaza and Gerlai, 2011; Krusche et al., 2010; Perdue et al., 2012; Ward and Smuts, 2007). Building off of my research, future work that manipulates the size of each stimulus group, and the familiarity of individuals to the stimuli group, would help us to identify the limitations, quantitative abilities and the key drivers of group-joining decisions in these cichlid fishes. Moreover, manipulating the type of quantitative stimulus (shapes, food, etc.) used would offer further insight into the numerical abilities of these cichlids while negating any potential differences in prosocial motivation between cooperative and non-cooperative species. Recent work has employed the use of non-social stimuli in numerical cognition studies such as geometric shapes, colour and food to address how fishes use quantitative reasoning outside of group-joining contexts (Agrillo et al., 2017; Gómez-Laplaza and Gerlai, 2020; Lucon-Xiccato et al., 2019; Oliveira et al., 2015). In my research, I used conspecifics as opposed to shapes, colours, or food to evaluate numeracy because this social stimulus offers a visually salient and ecologically relevant motivator. I suggest that similar studies using non-social stimuli in Lamprologini cichlids would strengthen our understanding of how these fishes use numerical judgements in non-social contexts and would provide greater control over potential prosocial motivational differences between cooperative and non-cooperative species.

## 5.6 Spatial cognition in cichlids

In Chapter 3, I compared spatial performance and learning across Lamprologini cichlids and did not find evidence that spatial learning capacities differed between cooperative and non-cooperative species. Both cooperative and non-cooperative species took comparable times to complete the maze, made a similar number of wrong turns or mistakes, and displayed similar degrees of inhibitory control. Overall, the cichlids completed the maze faster, made fewer mistakes and improved their inhibitory control across trials. I also did not find evidence for sex-specific performance in this group of fishes, despite previous research indicating that male cichlids typically hold larger territories, and in one of the species used, the male *N. pulcher* typically navigate larger distances across their home range and move more between different social groups (Desjardins et al., 2008; Stiver et al., 2007; Wong et al., 2012 but see Schradin and Lamprecht, 2000 for evidence of female-biased immigration in *N. multifasciatus*). I expected that holding larger territories and travelling longer distances to cover the span of those territories would necessitate bettered spatial cognition in males. However, I entertain the possibility that there exists a trade-off between the ability to form detailed localized maps versus larger coarser maps, which might offer females an advantage in our small-scale spatial study. If this is the case, even though males might have an edge when it comes to spatial learning tasks, this male advantage would have been masked by the small-scale design of the maze, and such a trade-off would help explain the comparable results observed between the sexes. Whether any type of spatial learning trade-off exists between small scale vs large scale spatial abilities has not yet been addressed.

Spatial cognition is the longest and most intensely studied cognitive trait in fishes (Vila Pouca and Brown, 2017), and maze learning experiments have frequently been used to investigate spatial abilities of fishes alongside many other animals. Unfortunately, trying to draw comparisons to the results of similar studies is particularly difficult since maze apparatuses across studies almost always differ in their design, and scientists differ in their choice of motivators or stimuli, their chosen performance metrics and methods by which they conduct trials (e.g. sets of trials on a single day versus one trial per day over multiple days). This lack of standardization causes problems for researchers seeking to compare their findings about spatial ability to those uncovered in other species (Lee et al., 2012). I recommend that future studies aim to review methods from published works on maze learning and outline the optimal conditions for promoting, assessing and comparing spatial cognition in fishes and other animals. A road map detailing the available options for designing a maze, how to approach training, and highlighting the different ways maze paradigms can and have been used to test behaviour and cognition (e.g. anxiety, lateralization, learning) would be an incredibly useful starting point for researchers seeking to perform similar experiments.

## 5.7 Evaluating cognition in the laboratory

In Chapters 2 and 3, I report on the results of lab-based experiments, and lab work may not capture what wild animals are truly capable of (Webster and Rutz, 2020). In my studies, *N. pulcher* and *T. temporalis* were the descendants of wild caught fishes, but they have been reared in the laboratory for multiple generations. The other species were purchased from a commercial supplier, and presumably have been bred in captivity for multiple generations or at the very least, have spent much of their lives in captivity. Rearing and keeping fishes in captive, simplistic environments has been shown to cause a decrease in brain size over generations and even within a lifetime (wild fishes raised in captivity have smaller brains; Burns et al., 2009; Marchetti and Nevitt, 2003). Each of the fishes used in my study were kept in artificially constructed environments, with social and structural conditions that are unrealistic in the wild (e.g. both cooperative and non-cooperative fishes were kept in stock tanks housing many individuals, they were also provided relatively simplistic structural conditions and limited space). Hence, the true cognitive abilities and behavioural repertoire of these fishes may be clouded by the problems of artificially imposed social conditions and a lack of structural enrichment in the laboratory, which is a common issue in most studies of fish cognition. Evidence for artificial selection in captivity is vast, especially in fishes (Johnsson et al., 2014; Näslund and Johnsson, 2016; Salvanes et al., 2013), and it is unclear whether findings from the lab generalize to what is happening in the wild. Therefore, in Chapter 4, I evaluated current practices in fish cognition research, and offered recommendations to improve the generalizability of findings. I surveyed the literature for studies relevant to four commonly studied areas of fish cognition: simple learning, numeracy, social cognition and spatial cognition. I quantified the growing interest in the field of fish cognition, the extensive use of captive-bred animals, and a reliance on lab-based research. I also highlight common issues in experimental design and outline how they could influence our understanding of fish cognition. Chapter 4 outlines a path towards more ecologically relevant experimental designs and a list of considerations that can help improve the generalizability of findings (Figure 5.1).

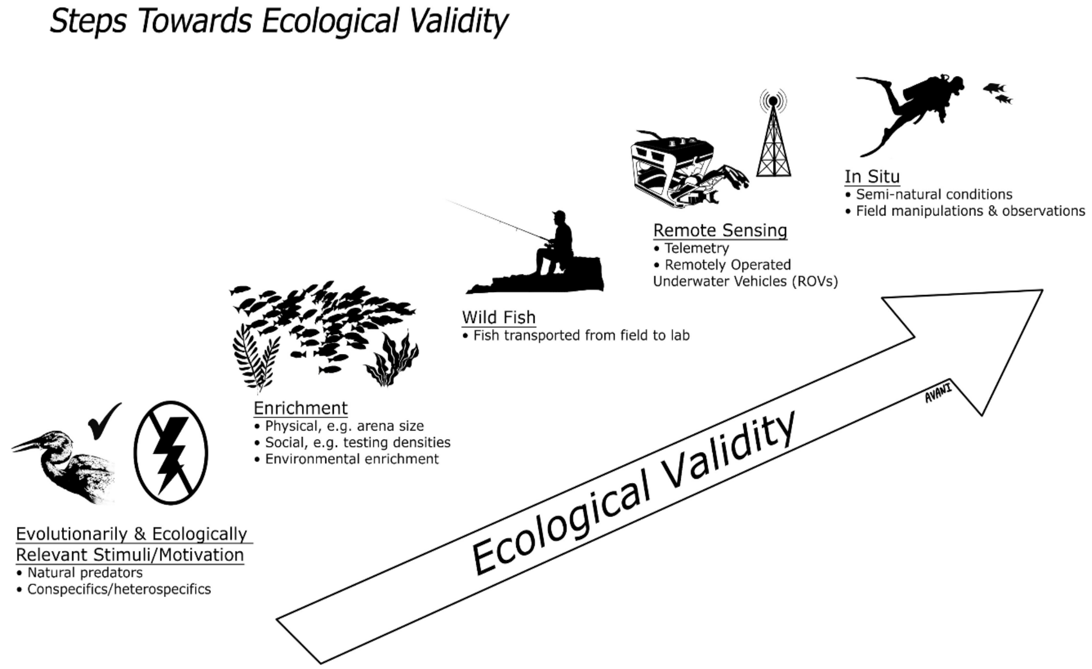


**Figure 5.1** Considerations for designing and implementing more ecologically valid fish cognition studies, with *in situ* cognitive studies being the most valid and difficult to conduct. Illustration by Avani Pathak.

## 5.8 Summary

One of the most important questions in animal behaviour is understanding how cooperative behaviour evolves (Kennedy and Norman, 2005). Living in groups and cooperating with group-members produce cognitive challenges and requires social skills, such as recognition and conflict resolution abilities, which should draw on cognitive resources. With cichlid fishes, I sought to answer whether the evolution of cooperative breeding coincided with the evolution of more sophisticated social and general cognitive abilities. Using a comparative approach, I assessed cognitive differences between cooperative Lamprologini cichlids and their non-cooperative relatives. In a series of assays placed in a comparative context, my results underscore the many cognitive similarities between cooperative cichlids and their non-cooperative relatives, and highlight that the evolution of cooperative breeding does not appear to drive enhanced cognitive abilities in this group of fishes.

As I was performing these laboratory studies of fish cognition, I began thinking deeply about the trade-offs between conducting studies in the lab versus in the field. Laboratory-based studies of fish cognition often use captive-bred animals that have been raised in simplified environments, and these conditions have a profound impact on cognitive development and the expression of social traits (Salvanes et al., 2013) This presents an important methodological issue that has not received much attention in the cognition literature, so I set out to quantify these patterns and bring them to light in a collaborative, systematic review. Additionally, few cognition studies conduct multi-species comparisons because of the difficulties controlling for life history variables between species. My research is fairly unique in that I conducted comparative cognition studies with six species, but by using closely related fishes from the same lake, I have addressed many of the problems with previous works in this field which typically compare animals from more dissimilar environments. Furthermore, many comparative cognition studies only focus on two species. My choice of animal model and multi-species design therefore offers a more reasonable comparison than most comparative cognition studies published to date.

In sum, Chapter 2 and Chapter 3 do not provide support to the notion that complex social conditions, such as those found in cooperative breeding groups, are the primary driver of cognitive abilities of these fishes. Chapter 4 provides a framework for tackling cognitive research in fishes and identifies methods to ensure ecological validity is put at the top of the priority list. Overall, these works contribute to our understanding of how the social challenges of group-living and cooperation shape cognitive abilities and help to elucidate the social and physical challenges that select for sophisticated cognition.

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