

# Keeping it together: the effect of familiarity, personality, and active interactions on group coordination

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## Summary

**Thesis title:** Keeping it together: the effect of familiarity, personality, and active interactions on group coordination

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Group coordination is a universal feature of social life. Animals form social groups for a variety of reasons, including predator evasion and more efficient foraging, and individuals living in social groups must coordinate their activities in order for groups to function. Consequently, the factors that facilitate or impede group coordination are of great interest in understanding the lives of social animals. Familiarity between individuals has well-documented effects on group coordination, with familiar groups outperforming unfamiliar ones in predator evasion, foraging, and cohesion. Individuals also generally prefer to coordinate with familiar conspecifics over unfamiliar ones. Despite these advantages, the mechanisms through which familiarity might aid group coordination are poorly understood. Similarly, the individual personalities of group members have well-documented effects on group performance: bold individuals are more likely to be ‘leaders’ and determine the direction of group movements, and groups comprised of individuals of differing personalities outperform groups of all bold or all shy individuals. While the effect of individual personalities on group behaviour has been recorded extensively, the ways in which individuals affect each other’s behaviour are still poorly documented. In particular, active interactions where one individual can directly affect the behaviour of others have received limited attention, as it is difficult to distinguish such actions from passive effects.

I used two systems to investigate how individual behaviours can lead to group coordination. In three-spined sticklebacks (*Gasterosteus aculeatus*), well-established boldness assays allowed me to assess the effects of personality and familiarity on 1) coordination between pairs of fish; I found that individuals in unfamiliar pairs exhibited coordination patterns consistent with their individual personalities, with bold individuals more likely to leave cover independently, while in familiar pairs, individuals behaved in ways seemingly unrelated to their boldness scores. I also investigated how personality and familiarity affect 2) group coordination

and individual performance in a problem-solving paradigm. I found that familiarity, relative individual personality, and group mean personality interact to affect individual foraging success, and that group cohesion was affected by the interaction of group familiarity and group mean personality. These results suggest that individual characteristics can impact the behaviour of groups, and that the characteristics of an individual's group can in turn affect an individual's behaviour and success.

In the Bronze Cory catfish (*Corydoras aeneus*), I described a novel tactile interaction style termed 'nudging' that individuals use during group coordination. I investigated 3) the effect of familiarity on nudging and coordination in pairs and triplets. These results show that nudging can be used to overcome the disadvantages of familiarity, which has important implications for how communication can underlie group coordination in the absence of familiarity. I then investigated how 4) nudging affects group coordination following a flight response to a potential threat. I demonstrated that nudging leads to a higher likelihood of group cohesion and longer group flight times. This shows how active interactions can mediate group responses and affect the ecologically relevant scenario of predator evasion. Finally, I investigated 5) the development of this nudging behaviour. My results show that Bronze Cory catfish larvae develop toleration for tactile stimulation with age alongside their propensity to nudge conspecifics. This suggests that Bronze Cory catfish larvae require social feedback to develop appropriate responses to nudges from conspecifics and supports the important role of nudging in Bronze Cory catfish sociality. The presence of active interactions in the Bronze Cory catfish` modifies the way that social behaviour manifests in this species and has great potential for further questions about social behaviour and group functioning.

## **Preface**

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted or is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted or is being concurrently submitted for any such degree, diploma, or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.



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## **General Introduction**

### **INDIVIDUALS AND GROUP COORDINATION**

Coordination is a necessary feature of every life form. At the most basic level, cells require precise coordination of molecular processes to survive and replicate. Multicellular organisms require organization and coordination between cells, and more complex organisms require coordination amongst tissues and organs. The need for coordination extends beyond individual organisms in social animals, in which individuals must coordinate their activities with others to function effectively as a group.

The mechanisms by which biological entities achieve coordination differ greatly based on the level of organismal organization. Within cells, series of intermolecular hydrogen bonds knit the curved spines of DNA's double helix into a coherent molecule on which all cellular processes depend. Cells can coordinate directly with one another via junctions that physically connect one cell to another; cells can also broadcast to other cells in the body by releasing hormones that modify the processes of cells that receive the signal (Nussey and Whitehead, 2001). In the context of social interaction between organisms, coordination is the result of

behaviour. Group coordination can be very simple or extremely complicated, but it is never the result of chaos. Through simple or complex dynamics, individuals must navigate interactions with others. Individuals within groups can react to information about their group-mates through many different sensory modalities, but group members must modulate their behaviour in order to achieve a functioning group.

The huge diversity of taxa in which sociality has arisen, as well as the myriad types of animal aggregations that occur, provide strong evidence for the advantages of social living. For social animals, sociality is a fundamental level of biological organization, and understanding why groups form and how they function is vital for understanding this ubiquitous aspect of biology. In species across many taxa, individuals form groups for protection from predators. This occurs in invertebrates such as aphids (Turchin and Kareiva, 1989), in many fishes including minnows (Pitcher et al., 1986) and guppies (Magurran and Seghers, 1994), in reptiles such as iguanas (Greene et al., 1978), in many birds including cliff swallows (Brown, 1988) and ostriches (Bertram, 1980), and in many mammals including prairie dogs (Hoogland, 1981). Groups reduce the risk of predation through a number of mechanisms, notably the confusion effect (Landeau and Terborgh, 1986), as well as the pooling of defensive responses to increase the odds of driving off a predator, as in mobbing in birds such as chaffinches (Hinde, 1954) and group nest defence in great tits (Grabowska-Zhang et al., 2012a). In other species, groups form for the opposite reason, in order to increase the efficacy of predation efforts. Many mammals such as lions (Stander, 1992) and chimpanzees (Boesch, 1994), fish such as yellowtails (Schmitt and Strand, 1982), invertebrates such as social spiders (Yip et al., 2008), and birds such as Harris hawks (Bednarz, 1988) all hunt cooperatively, and individuals are far more likely to successfully find food as part of a coordinated group than if they were hunting on their own.

The advantages of group living can be achieved through a variety of coordination mechanisms, some of which rely on very simple individual behaviours. For example, complex mass movements, as in starling murmurations, can be achieved if each individual in the group follows simple rules about maintaining a specific distance from its nearest neighbours (Lukeman et al., 2010). In other systems, individuals coordinate their activities in more specific ways and occupy specific social roles that determine their behaviour within the group. Lionesses hunting in groups coordinate by taking on distinct roles for which different individuals showed marked preferences (Stander, 1992). Similarly, social spiders show specialization in task performance

(Settepani et al., 2013). The differentiation of social roles leads to more complex coordination schemes that depend on individual specialisation of behaviours.

It is very difficult to elucidate the social development of complex coordination schemes: for example, in lions, how and why individual lionesses chose their preferred roles is unknown, as is the effect of inter-individual interactions on individual role specialisation (Stander, 1992). In some systems, an individual can influence its group in ways that have an obvious impact on group coordination: for example, the use of alarm calls in meerkats (Clutton-Brock et al., 2002) and birds (Hollén et al., 2011). Explicit signals such as alarm calls are delivered by one individual to alert its group-mates about a predator, as in species such as vervet monkeys (Seyfarth et al., 1980) and birds such as black-capped chickadees (Templeton et al., 2005), and can encode specific information about the predator that is utilised by the caller's conspecifics. In other systems, including fathead minnows and zebrafish, injured individuals release an alarm pheromone that alerts conspecifics to danger, albeit without specific information about the predator (Brown et al., 2001; Stensmyr and Maderspacher, 2012). These types of broadcast defences (ie general and specific alarm calls or alarm pheromones) against predation are analogous to a cell releasing hormones to influence the other cells in the organism. They often follow simple rules (i.e. perceive a predator, begin alarm call), they are not targeted (i.e. they are intended for every conspecific in the vicinity), and they clearly elicit a group response.

However, the ways in which directed individual interactions among group-mates can shape group coordination are still poorly understood, even though it seems intuitive that interactions between individuals could have a strong influence on emergent group behaviour. It may be difficult to discern the effects of individual interactions because these are often inscrutable- it may be clear that individuals are coordinating with one another (as when a fish shoal all swims in the same direction), but discrete interactions between individuals may not be observable. Consequently, while coordination is certainly a universal feature of all social behaviour, and individuals must behave in ways that facilitate group coordination, unravelling just how individual interactions contribute to group coherence remains an important but unsolved problem in the study of social behaviour.

## **COORDINATION, PERSONALITY, AND FAMILIARITY**

To investigate the manifestation and development of coordination mechanisms, it is useful to examine perhaps the most straightforward and readily observable form of socially differentiated coordination in groups: leadership, in which one individual is on the forefront of the group's movement. The emergence of leaders can facilitate coordination, and leader and follower roles are expected to evolve if the cost of failing to synchronise is high (Johnstone and Manica, 2011).

Leadership tendencies in individuals are associated with many factors, in particular, personality and previous experience (Jolles et al., 2014). Boldness, a widely measured personality trait that assesses the willingness of an individual to take risks (e.g. foraging under threat of predation (Sloan Wilson et al., 1994)), is a major predictor of an individual's behaviour in group settings. Individuals of different boldness play different roles within social groups. Absolute and relative personality measures predict leadership in barnacle geese (Kurvers et al., 2009): bolder individuals are more likely to be 'leaders' and have lower social attraction and less responsiveness to shy partners during collective movements in many fishes (Harcourt et al., 2009a; Jolles et al., 2015). Boldness and leadership are particularly interesting social traits, as individuals who influence their group-mates could thereby have an effect on overall group decision-making and the fitness of other group members (Brown and Irving, 2014). For example, in guppies (*Poecilia reticulata*), shoals consisting solely of bold individuals solved a novel feeding task faster, gaining foraging advantages compared to groups of all shy individuals (Dyer et al., 2009). However, more individuals in mixed personality shoals were able to feed when compared to all bold or all shy shoals, showing that groups can benefit from bold leaders, but that an individual's success can vary in a more complex way depending on the boldness of its group-mates (Dyer et al., 2009). In this way it is clear that being bold is not necessarily better and having uniformly bold group-mates is not necessarily ideal.

Prior social interactions can also interact with personality variation to influence leadership behaviour (Jolles et al., 2014). In sticklebacks, shy fish paired first with a bold fish were less likely to lead with all subsequent partners, whereas bold fish did not seem to alter their behaviour as much from partner to partner (Jolles et al., 2014). Similarly, although social behaviour is dynamic, bold fish are less deterred by failure than shy fish, and leaders are less sensitive to their partner's movements than shy followers (Nakayama et al., 2012). Bold fish, and therefore likely leaders, seem much less attuned to the behaviour of their fellows. Therefore, it is

possible that bold fish are not necessarily better leaders, but merely are willing to initiate movements to a greater extent than shy fish. Generally speaking, understanding the dynamics of leadership and followership is challenging as it is often unclear whether or not individuals are actively trying to coordinate activities, or whether or not apparent leadership events occur spontaneously, without active coordination (King et al., 2009).

These factors mean that, although individual behaviours clearly must form the basis for group coordination, it is difficult to understand exactly how they do so. Furthermore, factors that impact an individual's social behaviour also impact a group's coordination, although it is sometimes unclear how they affect one another. In particular, familiarity, or sustained prior exposure to one or more conspecifics, has a marked effect on individual social behaviour and group coordination. Familiarity can have diverse effects depending on context (for example, many species seek out unfamiliar individuals for mating (Kelley et al., 1999)), and in this thesis I examine the essentially beneficial role of familiarity in group coordination.

Individuals of many species show a marked preference for familiar conspecifics over unfamiliar ones (Frommen et al., 2007; Griffiths and Magurran, 1997; Kohn et al., 2015) and familiar groups outperform unfamiliar ones in a variety of coordination measures, including greater cohesion, predator evasion, and foraging success (Chivers et al., 1995; Griffiths et al., 2004) as well as social learning (Kavaliers et al., 2005; Swaney et al., 2001). Given the advantages that familiarity offers to groups, it makes sense that the component individuals of a given group would prefer familiar group-mates. However, although many plausible explanations exist for why familiarity results in these advantages, it is unclear how familiarity benefits groups, and why unfamiliar groups do not find ways to compensate for their lack of familiarity. It could be that sustained exposure to others results in the acquisition of information about familiar group-mates' preferences and behavioural tendencies, allowing individuals to better adjust to familiar others. It may also be that species that do not have any sort of mitigating interactions, that is, a behavioural mechanism to convey or acquire information to or about group-mates, have no alternate method to overcome unfamiliarity. In any event, the mechanism through which familiarity provides such substantial benefits is unknown, as is the reason that unfamiliarity so often proves a profound disadvantage.

## **STUDY SYSTEMS**

In order to investigate the role of individual behaviours and familiarity on group cohesion and coordination, I used two systems with distinct characteristics. The first, three-spined sticklebacks (*Gasterosteus aculeatus*), was useful for answering questions about how group familiarity and individual personality affect group coordination. The second, the Bronze Cory catfish (*Corydoras aeneus*), allowed me to dig deeper into questions about the role of individuals in shaping the behaviour of their groups.

### **Three-spined sticklebacks**

Three-spined sticklebacks (*Gasterosteus aculeatus*) are a well-established system in behavioural biology. Subspecies inhabiting different aquatic and marine environments are common throughout the northern hemisphere, and stickleback husbandry and laboratory maintenance is well-known (Bell and Foster, 1994). Moreover, sticklebacks have been used as a model system to investigate questions related to coordination and behaviour. An individual preference for familiar shoals has already been demonstrated in sticklebacks (Frommen et al., 2007) and a reliable boldness assay has also been developed (Harcourt et al., 2009a).

These fish exhibit robust leadership and followership dynamics associated with boldness (Nakayama et al., 2012) as well as reduced aggression in familiar groups (Utne-Palm and Hart, 2000). Sticklebacks thus provide a system in which the effect of individual personality on group coordination can readily be investigated. However, although individuals clearly coordinate with one another, the mechanism by which individuals coordinate is not obvious or observable, and the most noticeable directed interactions between individuals are reproductive (under proper conditions) or aggressive. I was therefore limited in which questions I could use sticklebacks to answer, as it is difficult to understand the role of individuals in group coordination when leadership initiations are inscrutable, and leaders do not alter their behaviour in a discernible way that results in follower action.

### **Bronze Cory catfish**



Consequently, I developed a relatively new behavioural system that allowed me to investigate questions about the role of individual behaviours in the processes of group coordination. *Corydoras* is a genus of neotropical fish belonging to the armoured catfish family Callichthyidae. It is currently the most species-rich genus of catfish (Reis et al., 2003) and *Corydoras* diversity is so overwhelming that a system of “C-numbers” is in use to describe new species. They are perhaps known best for their prevalence in the aquarium trade and are of particular commercial interest in the Amazon River basin: individuals of various *Corydoras* species are among the most frequently exported aquarium fishes in the region (Prang, 2007).

Despite the popularity of *Corydoras* catfish in the aquarium hobby, the body of literature on *Corydoras* behaviour is not extensive. *Corydoras aeneus*, or the Bronze Cory catfish, is one of the most common aquarium fish in the world (Lambourne, 1995) and is extensively bred in captivity. In both captive-bred and wild fish, one of the most obvious features of Bronze Cory catfish behaviour is their sociality: they form large aggregations (Lambourne, 1995; Reis et al., 2003) that contain males, females, and juveniles (Nijssen in Sands, 1986). Due to their sociality and habitat preference, the sandy substrate of slow moving, shallow streams less than 2 meters in depth (Nijssen in Lambourne, 1995), they are often the most apparent fish in the small streams of the New World tropics. They live as social foragers and use their sensitive barbels to sift through the substrate for small invertebrates and detritus (Sands, 1986). Perhaps most important for their presence in the aquarium trade is their temperament. The Bronze Cory catfish is a very social, peaceable fish favoured for its gentle demeanour (Ghadially, 1969; Lambourne, 1995). They even negotiate reproductive competition peaceably- a laboratory investigation into the reproductive habits of Bronze Cory catfish found that males showed no aggressive behaviour to rivals, and females chose to mate with the males that exhibited the highest frequency of courtship interactions toward them (Kohda et al., 2002). Detailed information about wild Bronze Cory catfish reproduction is sparse, but they are known to be promiscuous fish that spawn during the wet season (Sands, 1986). They also exhibit a very unusual method of mating, that is, sperm drinking, in which females drink their mates’ seminal fluid during bouts of copulation (Kohda et al., 1995). This behaviour ensures that all eggs laid in a given clutch are full siblings.

Outside of these reproductive studies, very few in-depth studies on the behaviour or ecology of the Bronze Cory catfish have been performed, and the full life history of these fishes remains unknown. Some information has been gleaned from accounts of captive individuals in

the aquarium hobby. The Bronze Cory catfish has locking spines that increase its effective size and contain venom that can be injected in potential predators (Sands, 1986). These defences make this small catfish surprisingly resilient (Nijssen in Sands, 1986), and although they are small fish that measure less than 10 cm, their individual life histories are substantial. In the aquarium the Bronze Cory catfish is known for its longevity, often living 10-15 years, and one individual was said to have lived to the age of 34 years (Lambourne, 1995). Although these data from captive fish cannot be extrapolated directly onto wild populations, Bronze Cory catfish are not seasonal fish and may live for many years in the small bodies of water they inhabit. The long lifespan of these fish is remarkable, as is their substantial geographical range and social biology.

### **Bronze Cory catfish: field observations**

The social biology of the Bronze Cory catfish makes this species particularly suitable as a system for investigating how individuals influence group coordination. Specifically, these fish employ a unique tactile interaction style, which I initially observed in a wild population in the Peruvian Amazon. I conducted behavioural observations and experiments at Los Amigos Biological Field Station (often referred to by its Spanish acronym CICRA, or Centro de Investigación y Capacitación Río Los Amigos) during two field seasons in the summers of 2011 and 2013. During my first field season, I collected the data that formed the basis for my undergraduate project on social coordination in Bronze Cory catfish, and during my second field season I continued my behavioural observations and formally noted the tactile interaction behaviour that I sought to more fully characterize in this thesis. Here I will outline my experience in the field and the early stages of observing and describing this tactile interaction behaviour, which I will refer to as ‘nudging’.

CICRA is located in the Madre de Dios region of Peru near the confluence of the Madre de Dios and Los Amigos rivers. The Madre de Dios river is a tributary of the Amazon River, and CICRA resides in lowland tropical rainforest consisting of both floodplains, in which the forest floods during the wet season, and terra firma, which is at a slightly higher elevation such that the forest is never submerged. During my field seasons, I encountered two species of *Corydoras* catfish: the Bronze Cory catfish, which lived in streams on terra firma, and *Corydoras elegans*, which lived in streams in the floodplain. The streams in the floodplain were muddy and had poor

visibility, and I was unable to extensively observe *Corydoras elegans* in their natural habitat. Instead, I performed my observations of *Corydoras elegans* in the field station laboratory, where they behaved like a typical *Corydoras* species, eating food from the bottom of their enclosure and showing marked schooling behaviour. The Bronze Cory catfish, however, lived in a small stream with relatively clear water that allowed me to observe wild Bronze Cory catfish for substantial periods of time in their natural habitat. I first observed Cory catfish individuals utilizing a tactile modality to interact with one another while I was attempting to capture Bronze Cory catfish. If I attempted to catch an individual and did not succeed, I was able to observe the escaped individual approach its group-mates and physically nudge other individuals; this action resulted in the group fleeing to a less accessible part of the stream. I had many opportunities to observe this pattern of behaviour, and when I eventually caught and observed Bronze Cory catfish in the field station laboratory, I observed and filmed individuals nudging one another. Because I was able to perform observations on wild fish in their natural habitat, I focused my experiments on Bronze Cory catfish, although I observed *Corydoras elegans* utilizing this behaviour in the field station laboratory and even observed (and filmed) interspecific nudges between the two species in the context of my undergraduate project on mixed-species interactions (Riley, 2012). These observations suggest that nudging is a behaviour that occurs in many if not most *Corydoras* catfish and may be related to this group's great diversity.

Further observations on wild fish in the laboratory at CICRA and captive-bred fish in my laboratory in Cambridge allowed me to more fully describe this nudging behaviour and investigate how nudging affects group coordination. The general features of nudging are robust and involve one individual (the initiator) physically nudging another (the receiver). I have observed that these nudges frequently modify the behaviour of the receivers, most notably through a type of recruitment- an initiator will approach a receiver, deploy a nudge, and the receiver will subsequently follow the initiator in close proximity. The discrete, observable, easily quantifiable characteristics of this behaviour (we can generally see which individual initiates a nudge and how the receiver responds) opens new avenues of investigation with respect to the role of individuals in group coordination.

### **Bronze Cory catfish: potential for social biology research**

I developed a scoring system to quantify nudges and identify the initiator and receiver of nudges. By analysing how individuals utilise this behaviour, we can explore how nudges relate to group coordination characteristics such as leadership and how nudges affect group coordination in ecologically relevant scenarios such as predator evasion. We can also investigate whether nudging is a socially acquired behaviour that develops as the result of social experience. In many species, essential social behaviours require social exposure in order to properly develop, as in critical periods for language acquisition in humans (Kuhl et al., 2005) and singing ability in zebra finches (George et al., 1995). Nudges seem to be an important aspect of Bronze Cory catfish social behaviour and can modify individual social responses, and I was interested in how this nudging behaviour develops in the context of social exposure and the implications of nudging development for Bronze Cory catfish sociality.

## **THESIS AIMS**

In this thesis, I explore the interplay of familiarity, personality, and active interactions on group coordination.

I used three-spined sticklebacks to investigate how familiarity and personality affect social coordination and group performance. I first investigated pairs of sticklebacks to assess how individual personality and familiarity affect social coordination. I then used triplets of sticklebacks to assess how familiarity and individual personality interact to affect group performance on a foraging task.

I then used the Bronze Cory catfish to explore how active interactions can shape group coordination. I first assessed how nudging and coordination are linked. Next I studied how nudging affects familiarity and coordination in pairs and triplets. Subsequently I investigated the role of nudging in group coordination in the ecologically relevant setting of group flight responses from a potential threat. Finally, I performed a study to assess how nudging develops in larval Bronze Cory catfish, and whether social exposure is necessary for the development of nudging behaviour.

## **Chapter 1: Familiarity and coordination in three-spined sticklebacks**

*This chapter will be submitted as a first author manuscript with contributions from the following co-authors: Young Mi Kwon assisted with the experimental set-up, data analysis, and manuscript editing; Neeltje Boogert assisted with experimental design and data analysis; James Savage*

*assisted with data analysis and manuscript editing; Andrea Manica assisted with experimental design, data analysis, and manuscript editing.*

## **ABSTRACT**

All social animals must coordinate with the other individuals in their social environment. Many social and individual factors affect group coordination. Familiarity, or prior experience with conspecifics, leads to improved group coordination, and familiar groups outperform unfamiliar groups in foraging efficiency and predator evasion. Personality – consistent inter-individual differences in behaviour – affects the ways individuals coordinate with one another by predicting which individuals act as leaders and influence group movements and decisions. Boldness is a personality trait described in many species, and individuals that differ in boldness are affected by social conditions in different ways; individuals of differing boldness may also respond differently to familiarity. Here, I present a study assessing how familiarity differentially affects ‘bold’ and ‘shy’ individuals in pairs of three-spined sticklebacks (*Gasterosteus aculeatus*). My results show that familiarity changes the way individuals coordinate with their partners, and that leadership dynamics are determined by boldness levels in unfamiliar pairs, but not in familiar pairs. This suggests that familiarity with group-mates, an important part of an individual’s social environment, alters the leadership tendencies of individuals.

## **INTRODUCTION**

A social animal’s environment encompasses not only physical (temperature, light intensity, etc.), but also social (position, movements, and internal states of its near neighbours) parameters. In many social systems, individuals exhibit consistent differences in behaviour (i.e. personality characteristics such as boldness) and needs relative to others when operating within a group context. Previous studies suggest that leader and follower roles will evolve when the cost of failing to coordinate behaviours is high (Johnstone and Manica, 2011; Rands et al., 2003). Coordination of group behaviour was found to benefit both leaders and followers in fish shoals, with heterogeneous groups of bold leaders and shy followers feeding at higher frequencies than either exclusively bold or shy shoal groups (Dyer et al., 2009).

Personality is a key predictor of leadership behaviour, and in many species, individuals assayed as being bold are more likely to lead than their shy conspecifics when in a social context (Kurvers et al., 2009; Leblond and Reeb, 2006). Prior social interactions can also interact with personality variation to influence leadership behaviour, for example in three-spined sticklebacks (*Gasterosteus aculeatus*). In this species shy fish paired first with a bold fish were less likely to lead with all subsequent partners, whereas bold fish did not seem to alter their behaviour as much from partner to partner (Jolles et al., 2014). Similarly, although social behaviour is dynamic, bold fish are less deterred by failure than shy fish, and leaders are less sensitive to their partner's movements than shy followers (Nakayama et al., 2012). Bold fish, and therefore likely leaders, seem much less attuned to the behaviour of their fellows. Therefore, it is possible that bold fish are not necessarily better leaders but are simply more willing to initiate movements than shy fish regardless of their social conditions.

Given that personality can determine an individual's propensity to lead, and previous social experience impacts some personality types more than others, it seems that group composition and stability could have a strong impact on how effectively groups coordinate their activities. One measure of stability, familiarity (defined as previous exposure with other individual(s) over a period of time) can help mediate interactions between individuals in reliable proximity to one another. Familiarity can manifest in different ways, and there are various mechanisms individuals can use to recognize familiar others. Sticklebacks have been shown to use cues specific to habitat and diet as proxies for social familiarity (Ward et al., 2005), and habitat and diet experience (as mediated through olfaction) likely plays an important role in social recognition of familiar conspecifics (Ward et al., 2004a). Similarly, direct social familiarity, which I define as the direct recognition of specific individuals living in the same area, has been shown to influence aspects of social behaviour in sticklebacks, including aggression (Utne-Palm and Hart, 2000). For individuals housed under the same environmental conditions and fed the same diet, familiarity presumably occurs through direct social recognition (which may occur through visual or olfactory recognition, or a combination of the two).

In this study I was interested in the effect of social familiarity via direct social recognition on coordination between individuals who have experienced the same diet and habitat characteristics. Social familiarity has been shown to play an important role in coordination in many species. For example, in fathead minnows (*Pimephales promelas*), familiar groups

(composed of individuals drawn from the same shoal) exhibited greater shoal cohesion and anti-predator behaviours in the face of predation threats when compared to unfamiliar groups (Chivers et al., 1995). The same effect was found in familiar groups of juvenile trout (*Salmo trutta*), which responded significantly faster than unfamiliar groups to a predator attack; furthermore, the familiar trout were more successful foragers, feeding at a rate 2.6 times higher than unfamiliar groups (Griffiths et al., 2004). The value of familiarity to social animals appears in a variety of taxa, including birds (Senar et al., 1990) and a variety of schooling fishes (Ward and Hart, 2003), and the effects of familiarity in promoting the group's interest (in both evading predators and increasing foraging success) are well documented. Sticking with a familiar group, or, in the case of young or newly introduced individuals, developing familiarity, can be favourable in social animals, and individuals behave in ways to facilitate familiarity: individuals prefer to shoal with familiar individuals, even if there are opportunities to move to same-size shoals of unfamiliar individuals (Barber and Ruxton, 2000). Familiarity may even trump kinship in some species: guppies often form kin-based shoals, but were found to do so via familiarity preference and not a true preference for kin (Griffiths and Magurran, 1999).

However, it is difficult to pinpoint why familiarity offers this advantage. It is not fully known how groups of individuals settle into the behavioural patterns that allow groups to function, even though it seems intuitive that individuals that have prior experience with one another can form more effective groups. The behavioural mechanisms through which familiarity facilitates the emergence of social roles are of interest to help understand the ways that groups function, especially when individuals have different personalities. The personality and previous experience of individuals must interact both within and between group members to shape group behaviour, and the social precedents inherent in familiarity seem to help shape the ways individuals act on their preferences within groups. Familiarity likely affects individuals of different personalities in different ways as it changes how groups function in their physical environment. It seems likely that familiarity could offer groups an advantage in part by 'activating' shy individuals to have a greater influence on their groups, thus helping the group avoid predators. Bold individuals may be less susceptible to the effects of familiarity, but because group behaviour changes from familiarity benefit the entire group, bold individuals also stand to benefit from getting to know their fellow group members.



It is also important to consider how personality manifests in group settings. While boldness scores for stickleback individuals are robust and temporally consistent in fish housed in isolation, this consistency is not present in socially housed fish (Jolles et al., 2016). In addition, the effect of individual boldness disappears when groups of fish are placed in a novel foraging area, and group consensus drives a shoal's decision to take risks, not bold individuals' leadership (McDonald et al., 2016). Social feedback and experience likely have an important effect on an individual's social behaviour and may modulate the way individuals behave alongside their personality. Familiarity also has a substantial effect on social experience and may modify the effect of personality on individual behaviour. The social environment, which consists of an individual's group-mates and which is affected by familiarity, can have a substantial effect on an individual's apparent personality in social settings.

The behavioural mechanisms that cause personality and familiarity to impact individuals within a group are not well known. I designed a study to investigate how familiarity shapes the social behaviour of individuals of different personalities. I wanted to investigate how individuals of contrasting personalities respond differently to familiar and unfamiliar conspecifics, and I used pairs of fish to compare how individuals behave differently with familiar and unfamiliar conspecifics.

## **METHODS**

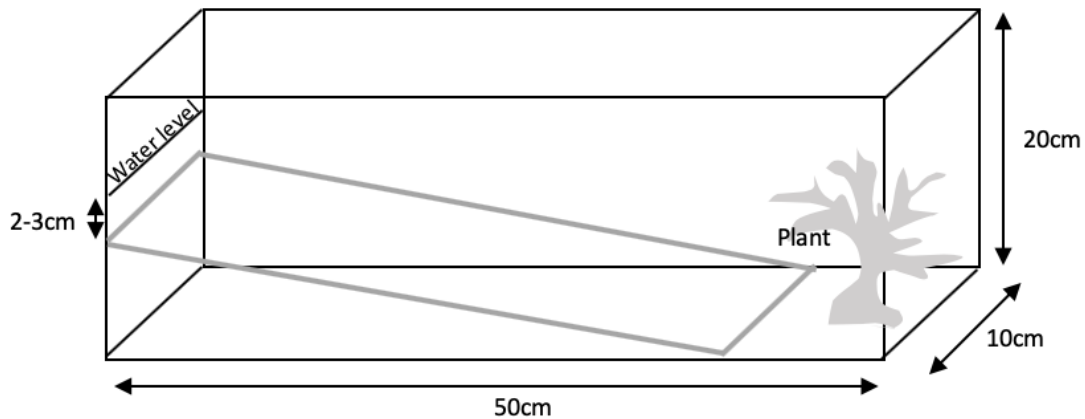
### **Husbandry**

I used wild three-spined sticklebacks, caught from a tributary off the River Cam, Cambridgeshire (UK) and housed in the University of Cambridge's Zoology Department for one year prior to the start of experiments. Fish were kept in a laboratory with a constant temperature ( $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) and light period (10L:14D) and were housed in three group-housing tanks ( $117 \times 45 \times 45\text{cm}$ ) containing gravel, artificial plants for cover, and power filtration. Fish were fed defrosted Chironomidae (bloodworm) larvae *ad libitum* once a day. As the temperature and photoperiod regime in the laboratory prevented the fish from entering breeding condition (Borg et al., 2004), I did not sex the fish.

I selected experimental subjects at random from two social housing tanks, ‘Tank A’ and ‘Tank B’. Fish in Tanks A and B had had no prior experience with one another for over a year. Fish from Tanks A and B were initially housed during the boldness testing phase in individual compartments in separate aquaria to ensure that fish from tank A would remain completely isolated from fish from tank B (and vice versa).

### **Boldness Testing**

I adopted the same boldness test protocol as that described in (Jolles et al., 2015; 2016): an individual’s boldness was quantified as the proportion of time it spent out of plant cover when placed in an opaque white Perspex test tank (50 x 10 x 20cm depth) for 30 min. The test tank contained a sloped floor connecting the tank’s darker deep end (13 cm water depth, 10 cm wide, 8 cm long) with a plastic plant, to the lighter, shallow and thus more “risky” end (2-3 cm water depth; figure 1). This light gradient was created using a lamp (Daffodil LEC200, 22 LED bulb) positioned behind the deep end of the tank and pointing upwards. There were eight such test tanks, each placed in a wooden box (60 × 50 × 50cm depth) to exclude external noise and additional light during testing. I tested eight fish at a time. I transferred fish using a dip net from their individual tanks to black holding cups positioned adjacent to each experimental tank. The fish were then poured into the deep end of the tank, after which recording started. Filming sessions lasted 30 minutes and were recorded with Raspberry Pi’s mounted in the roofs of the wooden test boxes. This procedure was conducted for all fish in a randomised order, between 9am and 6pm, and repeated three days later with a newly randomised order. Tank water was aerated with an air stone overnight prior to each boldness test day.



**Figure 1:** Schematic representation of a boldness test tank. Shy fish will spend more time in the shelter of the plant in the deepest area of the tank, while bold fish will spend more time exploring the increasingly shallow (and therefore more exposed) incline.

Fish in each boldness test video were tracked at 12 frames per second using automated tracking software written by Jolle W. Jolles in Python. The software tracked the number of frames that fish spent out of plant cover during the 30-minute boldness test trial. I calculated individuals' boldness scores as the mean number of frames out of cover during the two boldness trials divided by the total number of frames during the duration of the boldness test. I checked the automated tracking trajectories to correct any errors in the data where the software lost track of the fish due to shadows or debris in the water.

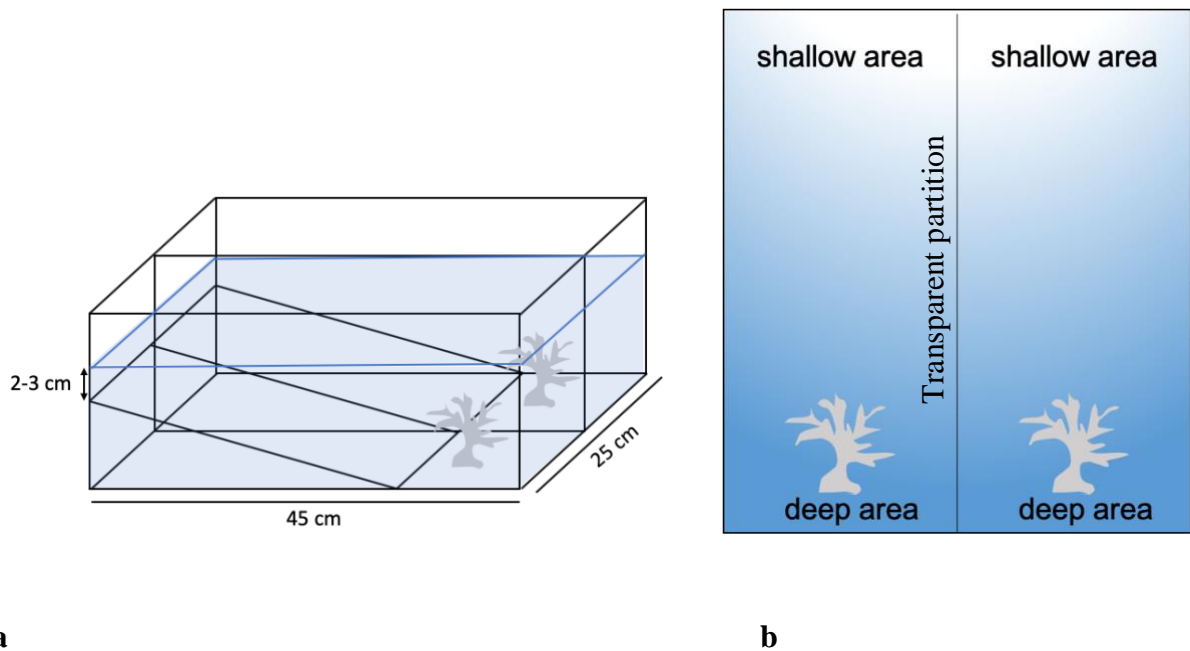
## Experimental procedure

After quantifying the boldness of each individual, I set up pairs of fish consisting of one Tank A fish and one Tank B fish. I only paired fish that differed by at least 0.10 in their boldness score, as this was the maximum boldness difference permitted by the distribution of boldness scores. Within each pair, I termed the individual with the higher score as 'bold' and then one with the lower score 'shy'. The mean boldness score of all individuals in this study was 0.268 (95% confidence interval: 0.208 - 0.329).

To ensure that initial pairs were familiar with one another and to control for acclimatization effects (Dyer et al., 2009; Griffiths and Magurran, 1997), I housed these initial

pairs together for four weeks prior to the experiments. Previous studies suggest that fish develop familiarity over about 12 days (Griffiths and Magurran, 1997). After familiarization, I randomly divided subjects into the control and experimental conditions. The control group remained with their familiar partner; the experimental group was placed with a new partner. Both the control and experimental groups were moved to a new enclosure with either their familiar or an unfamiliar partner. I constructed the experimental unfamiliar pairs by swapping partners between pairs of fish such that Tank A fish were always given new partners from Tank B, and vice versa. I recombined pairs on the first day of coordination testing and gave each recombined pair a new pair identification number.

To assess coordination, I used a standard coordination assay (as in (Harcourt et al., 2009a)). This coordination assay is similar to the assay used to assess boldness, with two lanes (12.5cm each) separated by a transparent partition through which individuals could interact (figure 2). Each lane consisted of a shadier, deeper area with cover, as well as a brighter, shallower area. Fish from the control group were placed in individual cups, and then placed in a test tank with their familiar partner in the other lane. Fish from the experimental group were placed in individual cups, and then placed in a test tank with an unfamiliar partner. Filming lasted for a total duration of one hour and began immediately before fish were transferred from individual cups to the test tanks. The first five minutes were not used for analysis to allow the fish to habituate to the new environment.



**Figure 2a-b:** Schematic representation of the coordination assay. a) Side angle view. Lanes were 12.5cm each and were separated by a transparent partition through which fish could visually interact. The depth of water at the shallowest point of the incline was 2-3cm. The coordination assay closely resembled two conjoined boldness assessment tanks. b) View from above. The deep area had a plant for cover. Lights were placed at the shallow end. The vertical line separating the lanes represents the transparent partition through which fish could visually interact.

## Data Collection

Videos were coded using BORIS, an open-source event logging software (Friard and Gamba, 2016). Each fish was scored as either being under cover or out of cover; I defined the threshold of a fish being out of cover if its entire body (including the tail) had crossed over to the incline near the base of the tank.

## Modelling of Movement Synchrony across between Familiarity Pairs

The data used for cross-correlation were binary (in or out of cover) time series of the entire observation period, minus the initial habituation period.

## **Modelling of Collective Movement**

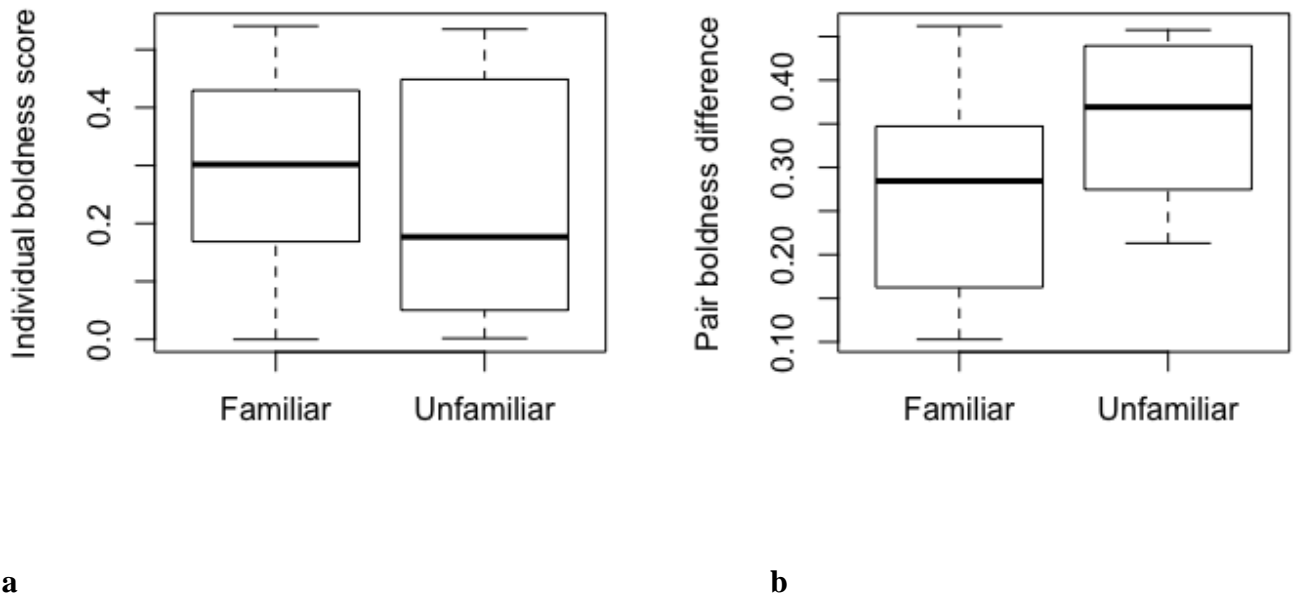
To investigate whether particular individuals tended to lead movements out of or into cover, I then the paired fish movements using a continuous time Markov model using the msm R package v1.6.6 (Jackson, 2011). As the fish were tested in bold-shy pairs, the system could be in four states based on the location and personality of the fish: (1) both fish under cover; (2) bold fish out of cover while shy fish is under cover; (3) shy fish out of cover while bold fish is under cover; and (4) both fish out of cover. I fit the model separately for data of familiar pairs and of unfamiliar pairs. The estimated transition intensities between these states in the fitted model allows us to compare the tendency of the bold fish to leave or return to cover relative to the shy fish, as well as the tendency of either fish to follow its partner in or out of cover. This elucidates who initiates trips (leave cover) in the pairs, whether the location of one fish influenced its partner location and movement, and if familiarity influences these effects.

Data were analysed in R (version R 3.5.1)

## **RESULTS**

### **Boldness scores and pair differences**

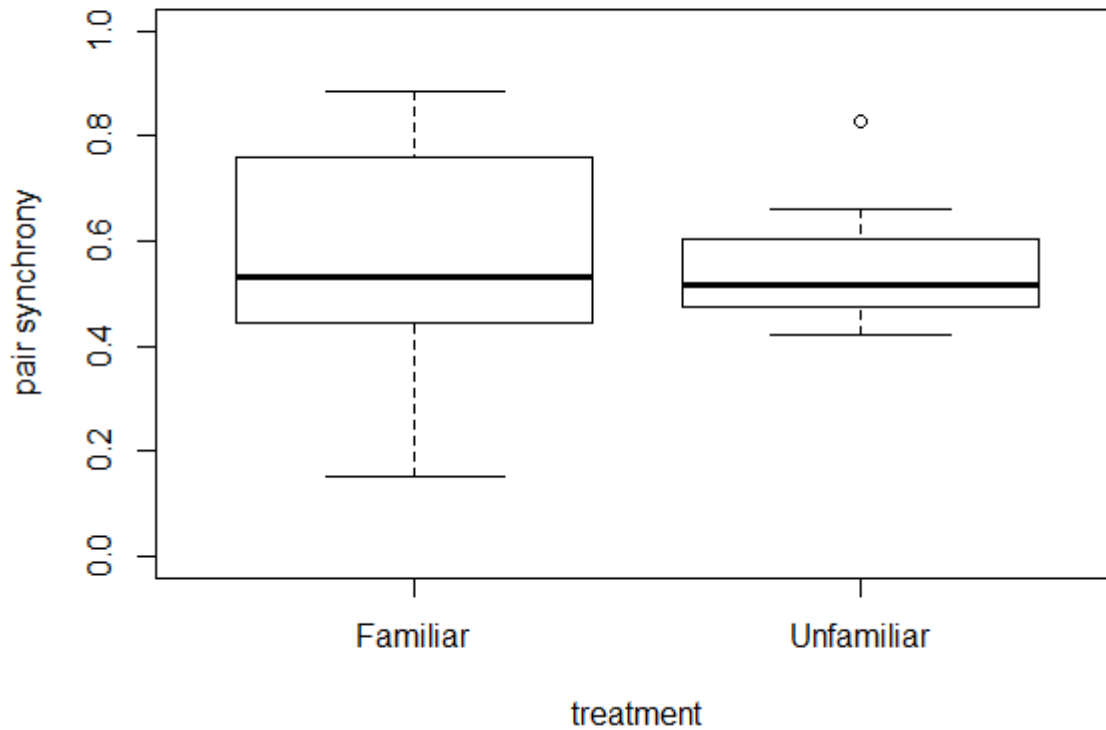
Boldness scores did not differ between individuals in familiar vs unfamiliar pairs ( $t_{34} = 0.89$ ,  $p=0.38$ , figure 3a). The boldness differences between individuals in pairs also did not differ based on familiarity ( $t_{16} = -1.48$ ,  $p= 0.16$ , figure 3b). This analysis implies that our results were not influenced by biases from boldness distribution.



**Figure 3a-b:** a) boldness scores in individuals in familiar and unfamiliar pairs b) difference between the shy and bold members of familiar and unfamiliar pairs. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers

## Synchrony

Both familiar and unfamiliar pairs exhibited a high degree of synchrony, or the degree to which both fish are either in cover or out of cover at the same time. The cross-correlation of pair locations in or out of cover was much higher than would be expected due to chance (one sample t-test,  $t_{19} = 14.37$ ,  $p < 0.0001$ ). Synchrony did not, however, differ between familiar and unfamiliar pairs (two sample t-test,  $t_{18} = 0.20$ ,  $p = 0.84$ , figure 4).

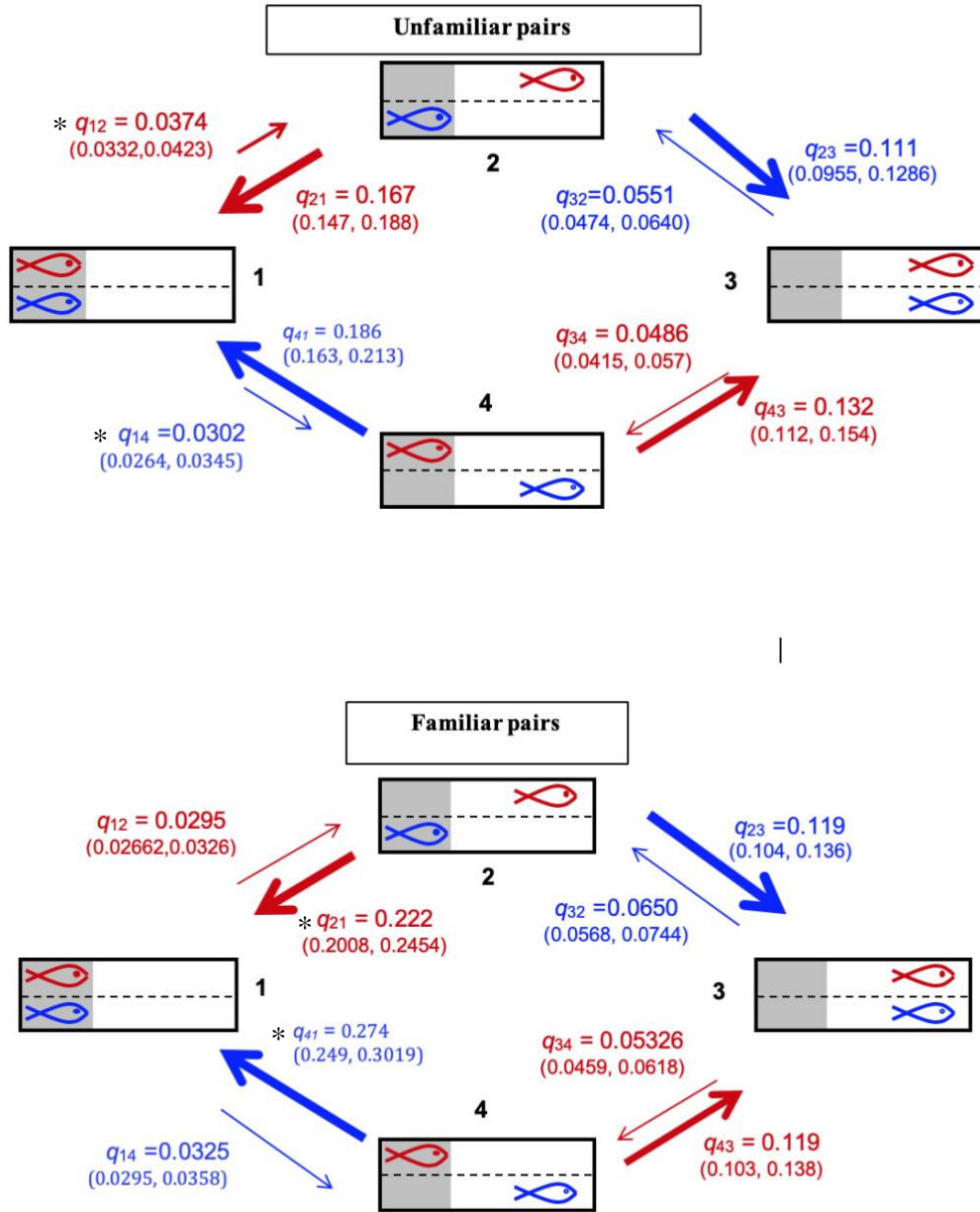


**Figure 4:** pair synchrony (cross-correlation of location) in familiar and unfamiliar pairs. Familiarity did not affect the degree of synchrony in pairs. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers

I found that both individuals in unfamiliar and familiar pairs seem to exhibit patterns of coordination and risk taking that represent a degree of suppression of personality-based behavioural tendencies; transition intensities for all state transitions in familiar and unfamiliar pairs are presented in figure 5. Bold and shy individuals were equally likely to leave cover to join their partner in unfamiliar ( $q_{23}/q_{43}=0.84$ , 95% confidence interval (0.68-1.05,  $p>0.05$ ) and familiar ( $q_{23}/q_{43}=0.99$ , 95% confidence interval (0.81-1.21)  $p>0.05$ ) pairs. Bold and shy individuals were also equally likely to terminate a joint trip in unfamiliar ( $q_{32}/q_{34}=1.13$ , 95% confidence interval (0.91-1.41,  $p>0.05$ ) and familiar ( $q_{32}/q_{34}=1.22$ , 95% confidence interval (1.00-1.49),  $p>0.05$ ) pairs.



Although the effect of personality on leadership dynamics seems to be dampened, personality does predict certain aspects of risk-taking or risk-avoiding behaviour that differ based on familiarity. In unfamiliar pairs, bold individuals are significantly more likely to leave cover on their own as compared to shy individuals ( $q_{12}/q_{14}=1.24$ , 95% confidence interval (1.04-1.49),  $p<0.05$ ); this effect is not present in familiar pairs ( $q_{12}/q_{14}=0.91$ , 95% confidence interval (0.79-1.04),  $p>0.05$ ). Individuals in familiar pairs, on the other hand, show personality-predictive tendencies to return to cover if they are out of cover alone. Shy individuals in familiar pairs are significantly more likely to abandon a solo trip and join their partners in cover ( $q_{41}/q_{21}=1.23$ , 95% confidence interval (1.07-1.42),  $p<0.05$ ); this effect is absent in unfamiliar pairs ( $q_{41}/q_{21}=1.12$ , 95% confidence interval (0.93-1.34),  $p>0.05$ ).



**Figure 5:** transition intensities in unfamiliar and familiar pairs. Red individuals represent bold individuals, blue individuals represent shy. The size of the arrows represents a larger or smaller relative transition intensity between paired state transitions for visual reference. Asterisks denote significant comparisons.

## DISCUSSION

Although pairs exhibited synchronised movements into and out of cover, familiarity did not seem to play a role in defining the extent of coordination in pairs of fish, and individuals aligned their risk taking with their partner to the same degree whether their partner was familiar or unfamiliar. This is somewhat surprising, as the literature suggests that familiarity improves group coordination (Chivers et al., 1995) and that individuals tend to prefer familiar conspecifics (Griffiths and Magurran, 1997). However, for a true preference to develop, individuals need to have a choice, and in this experiment, they did not have one- they were forced to coordinate with the partner they had. In addition, coordination may pose a simpler challenge for pairs rather than groups. In this way, familiarity may not directly affect aspects of coordination in pairs under constrained circumstances, as were the pairs in this study.

The pairs in this study were also constrained in the ways they could interact in the coordination assay, and these conditions may have influenced the way that individuals could respond to their partner. The coordination assay used in this study allowed individuals to visually interact with their partner, but water flow between the two lanes was very limited, which may have prevented individuals from responding to olfactory cues. This could have the effect of limiting an individual's ability to recognize and respond to a familiar partner. Similarly, as individuals were transported to the coordination assay in a small cup of water from their home tanks, individuals may have been exposed to olfactory cues from their home tanks when visually interacting with an unfamiliar fish. This may have reduced the difference in coordination between familiar and unfamiliar pairs.

As with familiarity, my results show that the effect of personality in pairs was dampened. This is surprising, given that personality predicts aspects of social behaviour in other species, as in barnacle geese (Kruvers et al., 2009). In fact, personality was shown to predict leadership tendencies in three-spined sticklebacks, but in that study, fish were housed alone prior to exposure to a partner (Jolles et al., 2014). All of the pairs in our study were housed with a partner prior to the start of the experiment, and my results suggested that social housing, in this case just one group-mate, generally dampened the effect of personality on individual social behaviour. Individual personality failed to predict leadership dynamics, and shy and bold individuals joined their partner on trips with equal likelihood. They were also equally likely to terminate a joint trip.

This is consistent with other studies on three-spined sticklebacks showing that individual boldness scores are not consistent when individuals are housed socially (Jolles et al., 2016), and that individual boldness fails to predict individual risk-taking when individuals are tested in groups (McDonald et al., 2016).

The effect of personality, while muted, was not entirely absent in either familiar or unfamiliar pairs, although it manifested differently based on familiarity. In unfamiliar pairs, individual risk-taking behaviour was consistent with individual boldness scores, with bolder individuals more likely to leave cover on their own. In familiar pairs, this difference was not present, and both individuals were equally likely to leave cover on their own. In familiar pairs, individual risk-avoidance behaviour was consistent with individual boldness scores, with shy individuals more likely to terminate a lone trip out of cover and return to safety. This difference was not significant in unfamiliar pairs.

The overall reduction in the effect of individual personality on leadership dynamics emphasizes the importance of the social environment, and social experience more generally, on individual social behaviour. It is clear that consistent interindividual differences in personality, and specifically boldness, occur in many animals (Sloan Wilson et al., 1994), and sticklebacks are consistent in their boldness scores when tested in isolation (Jolles et al., 2014). However, the connection between boldness and leadership may be more complicated in wild populations, where individual sticklebacks seldom if ever live alone. In particular, across studies of various species that connect boldness and leadership, causality is never clear. In barnacle geese, dominant individuals tended to have higher boldness scores, but it was not obvious whether individuals were dominant because they were bold, or bold because they were dominant (Kurvers et al., 2009). Similarly, leader and follower roles were found to develop based on personality in sticklebacks (Harcourt et al., 2009a), but these fish were housed in isolation and tested in a similar assay to the one I used for this experiment, such that fish had visual access to one another but could not directly interact. In this way, the ecologically inevitable effect of social living (which sticklebacks always experience) was absent in this study.

In this way, although boldness and leadership are intertwined, it does not seem that individual boldness necessarily leads individuals to become leaders. Humans provide an interesting example to support the complexity underlying the relationship between boldness and leadership- humans that were prompted to behave in ways reflective of leadership and power

(through ‘power posing’, or adopting an open stance that expresses dominance) subsequently exhibited elevated tolerance for risk taking (Carney et al., 2010). Similarly, it does not seem that sticklebacks (and certainly humans) provide a simple mechanism for the development of leaders- leadership is not determined simply, or even largely, by an individual’s behavioural tendencies when alone. It seems instead that leaders, instead of being born, are made through interactions between an individual’s personality and social environment. This may explain the differences in coordination based on personality I observed in familiar and unfamiliar groups. Individuals in unfamiliar pairs did exhibit a type of risk-taking behaviour consistent with their individual personalities, and it may be that individuals in unfamiliar groups may default to their asocial behavioural tendencies until they acquire experience and information about their group-mates which they can use to more effectively coordinate in groups.

The interplay of personality, social experience, and environment have important implications for understanding how groups function, and how social species with different characteristics approach the challenge of social coordination. My results suggest that individual social behaviour is malleable and responds to social experience, and information about what individuals do in isolation does not necessarily translate to how they behave socially. The ability of social animals to adjust their behaviour to social conditions is an important part of many species’ sociality, as many individuals experience drastically different social environments when they are young and small versus mature and full-grown. Investigating how individuals make social decisions based on their own tendencies and external conditions is an important step to understanding how individual behaviours lead to group functioning.

## **Chapter 2: Familiarity, personality, and problem solving in three-spined sticklebacks**

*This chapter will be prepared as a first author submission with contributions as follows. Neeltje Boogert assisted with experimental design and data analysis; Beth Gillie assisted with experimental design, execution, data analysis, manuscript input, and editing; Andrea Manica assisted with experimental design, data analysis, and manuscript editing.*

### **ABSTRACT**

Animals can gain large benefits from living in groups, however, in order to do so, individuals must coordinate with their group-mates. Consistent individual differences in behaviour, particularly boldness, and familiarity, sustained prior social experience with group-mates, have been shown to profoundly affect how individuals interact with their group-mates. However, the interplay between these two factors is so far unexplored. Here I describe how familiarity and boldness affect both individual performance and group coordination in groups of three three-spined sticklebacks (*Gasterosteus aculeatus*) solving a novel foraging task. Familiarity affected shy and bold individuals differently depending on group characteristics: the shyest group member was most successful in relatively shy shoals with familiar group-mates; on the contrary, the boldest group member was most successful in relatively bold groups with familiar group-mates. In addition, a group's mean boldness score was negatively correlated with group cohesion in familiar, but not unfamiliar, groups. These results suggest that both boldness and familiarity are important for establishing group behaviour and coordination, and that consistent inter-individual differences in behaviour may primarily impact group coordination after familiarity has been established.

### **INTRODUCTION**

The benefits of social living have been well-documented in a variety of taxa. Social living can reduce an individual's chances of predation (Delm, 1990; Ward and Webster, 2016) as well as increase foraging success (Chivers et al., 1995; Coolen et al., 2003). However, in order to reap these benefits, individuals must coordinate with their group-mates and make group decisions (Conradt and Roper, 2005). Furthermore, individuals in groups face challenges (e.g. increased competition over food, more exposure to disease, and greater likelihood of attracting predators) and must navigate changeable environments (Chivers et al., 1995; Conradt and Roper, 2005).

Group coordination can be facilitated through consistent individual differences in behaviour, or personalities. Boldness, a widely measured personality trait that assesses the willingness of an individual to take risks (e.g. foraging under threat of predation (Sloan Wilson et al., 1994)), is a major predictor of an individual's behaviour in group settings (Harcourt et al., 2009a; Jolles et al., 2015; Kurvers et al., 2009). Individuals of different boldness can play different roles within social groups: high absolute and relative boldness measures predict leadership in barnacle geese (*Branta leucopsis*) (Kurvers et al., 2009), and bolder individuals are more likely to be 'leaders' and have lower social attraction and less responsiveness to shy partners during collective movements in three-spined sticklebacks (*Gasterosteus aculeatus*) (Harcourt et al., 2009; Jolles et al., 2015). The link between boldness and leadership is important as individuals who influence their group-mates could determine overall group decision-making and the fitness of other group members (Brown and Irving, 2014). In guppies (*Poecilia reticulata*), for example, shoals consisting solely of bold individuals solved a novel feeding task faster than groups of all shy individuals (Dyer et al., 2009). However, more of the individuals in mixed personality shoals were able to feed when compared to the individuals in all bold or all shy shoals, showing that groups benefit from bold leaders, but that individual success can vary depending on the boldness of group-mates (Dyer et al., 2009). In addition, individual behaviours depend not just on an individual's personality, but also on its group-mates' composition of personalities and the interactions between the individual's own personality and those of its group-mates (Jolles et al., 2018, 2017).

Familiarity, defined as individuals having had prior experience with one another, has also been shown to improve individual and group performance in a variety of taxa (Chivers et al., 1995; Grabowska-Zhang et al., 2012b; Griffiths et al., 2004). For example, in minnows

(*Pimephales promelas*), familiar groups are more cohesive and perform more anti-predator behaviours than unfamiliar ones (Chivers et al., 1995). Familiar groups of brown trout (*Salmo trutta*) respond faster than unfamiliar groups to a predator attack and are also more successful foragers, feeding 2.6 times the rate of unfamiliar groups (Griffiths et al., 2004). Familiarity can also have an effect on an individual's reproductive success. Breeding great tits (*Parus major*) with a higher proportion of familiar neighbours experienced increased breeding success (Grabowska-Zhang et al., 2012b). Great tits are also more likely to help defend the nests of familiar conspecifics (Grabowska-Zhang et al., 2012a). In guppies, familiarity facilitates social learning: individuals can learn to navigate a maze to reach a food reward more quickly from shoaling with a familiar rather than with an unfamiliar demonstrator (Swaney et al., 2001). Familiarity also facilitates social learning in deer mice (*Peromyscus maniculatus*), in which observers exhibit more robust learning from familiar demonstrators (Kavaliers et al., 2005). In addition, individuals exhibit a preference to associate with familiar conspecifics in many species. In shoaling groups of European minnows, individuals preferred to join groups of familiar fish over unfamiliar shoals (Barber and Wright, 2001; Chivers et al., 1995), and a similar preference is present in guppies (Griffiths and Magurran, 1999) and three-spined sticklebacks (Barber and Ruxton, 2000).

While a body of literature shows how familiarity and boldness affect group behaviour, the way these two factors interact to affect individual and group behaviour has rarely been investigated. I first wanted to see how group mean personality and familiarity were associated with group performance in a problem-solving task; I predicted that familiar groups would solve the task more quickly. I then wanted to investigate how familiarity and personality (both group mean personality and an individual's personality relative to its group) affect group coordination. I examined how group mean personality and familiarity affect cohesion of individuals within a group. I expected that bolder groups would be less cohesive (as bolder individuals are less sociable (Jolles et al., 2015)) and familiar groups would tend to be more cohesive. I then investigated whether the foraging success of individual three-spined sticklebacks (which I defined as the number of bloodworms each individual ate) in a food-rewarded maze is affected by an individual's boldness, the boldness of their group-mates, and familiarity of individuals within the group. I predicted that familiarity would affect individuals differently based on boldness, with shy fish tending to benefit from familiarity. Finally, I analysed how an



individual's boldness relative to its group-mates affects its position in the group and likelihood of being at the forefront of the group's movement. I expected that shy individuals would tend to be closer to the front of the group's movement in familiar groups.

## **METHODS**

### **Study Species and Housing**

I used wild three-spined sticklebacks, caught from a tributary off the River Cam, Cambridgeshire (UK) and housed in the fish laboratory of the University of Cambridge's Zoology Department for 6 months before experiments started. These fish were caught in 2016 and were different individuals than those used in Chapter 1. The fish were kept in three group-housing tanks (117cm × 45cm × 45cm depth) containing gravel, water plants, a water filtration system and air stone, at 15°C ± 1°C on a 10L:14D light period and fed defrosted *Chironomidae* (bloodworm) larvae *ad libitum* once a day. 32 individuals were randomly selected from each housing tank (96 subjects in total) and transferred to individual-compartment tanks (18.5cm × 9.5cm × 18cm depth). In the latter tanks, fish were kept in rows of eight, separated by transparent, perforated Perspex partitions for visual contact and water flow between neighbours, to minimise stress potentially induced by housing in social isolation. Each row of eight compartments contained a water filtration system and an air stone, and each individual compartment was lined with gravel and contained a plastic plant. Each subject was fed two to four defrosted *Chironomidae* (bloodworm) larvae at the end of each test day.

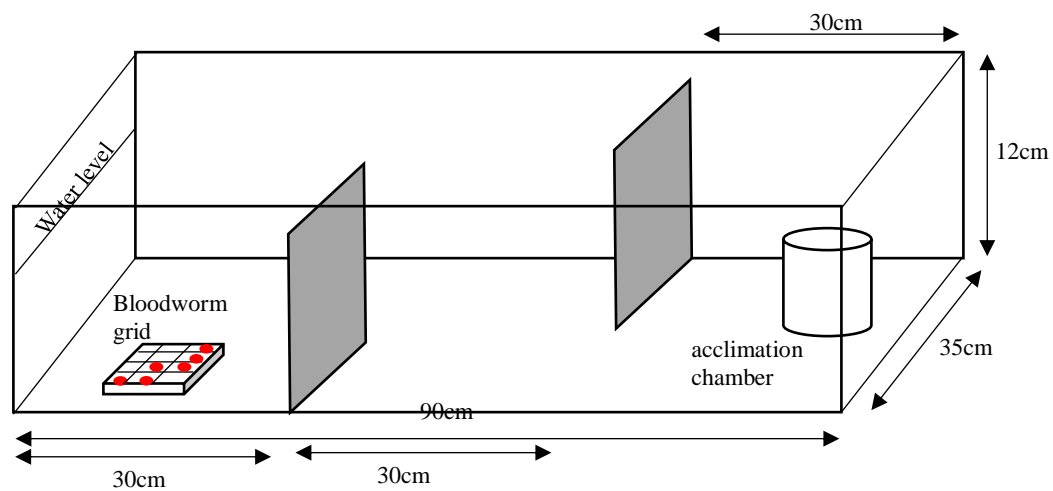
### **Group Composition and Individual Tagging**

Boldness testing was performed with the same equipment and procedure as Chapter 1, and videos were scored and boldness scores assessed using the same criteria. I assigned each test subject to one of 30 groups that consisted of one shy, one intermediate and one bold individual, where the minimum relative difference in boldness scores between group members was 10% (as with Chapter 1). Groups were composed such that individuals could be distinguished from each other by body size. This allowed for identification of individuals at close range while applying

tags. Groups were housed together for 16 days in group housing tanks ( $30 \times 30 \times 20$  cm) that contained aquarium gravel, air stones, and artificial plants. Groups were fed defrosted *Chironomidae* (bloodworm) larvae *ad libitum* each day. Two days before I started the maze task, fish were tagged following the procedure described by (Webster and Laland, 2009). In short, this involved handling each fish for 30-60 seconds out of water to attach a 5mm diameter circular disc of electrical tape to the third dorsal spine. The three fish in each shoal were tagged with tags of different colours (green, black, blue, brown, yellow, and light green). Fish were given two days to acclimatise to their tags in the group tanks. To create unfamiliar groups, 15 of the original 30 groups were mixed when put into the acclimation chamber in the maze task. These ‘unfamiliar’ groups had the same personality composition as the ‘familiar’ groups and thus consisted of one bold, one intermediate and one shy fish, again with a minimum relative difference in boldness scores of 10%. I ensured that the three fish in these newly formed ‘unfamiliar’ shoals each came from different housing tanks, to avoid any effects of previous familiarity.

## **Maze Task**

Familiar groups and unfamiliar groups were tested within a white opaque Perspex “maze” ( $89 \times 35$  cm  $\times$  12 cm depth; figure 1) surrounded by white cloth hung around a 1 m<sup>3</sup> metal frame, and illuminated with one lamp (Daffodil brand, model LEC200) suspended above the maze to improve video visibility. Before the start of each maze trial, I put six bloodworms in a feeding grid at one end of the maze. I transferred a test group from its holding tank into a transparent Perspex cylinder (9cm diameter) that served as an “acclimatisation chamber” at the tank end opposite the end where the food was located. After one minute of acclimatisation, I lifted and removed the cylinder, thus releasing the fish into the maze. Fish were filmed from above for a maximum of 30 minutes using a Raspberry Pi. Each group was tested once. I tested half of the groups on one day and the second half the following day, between 9am and 6pm. The maze water was changed after each test day and aerated with an air stone overnight between test days. Due to an equipment error, some videos from the first day of filming had an obscured view and could not be used. Therefore, 11 familiar and 11 unfamiliar groups were scored.



**Figure 1:** Schematic representation of maze task. Fish had to find their way to the food reward by going around two white opaque Perspex partitions that occluded their view of the bloodworm-baited foraging grid at the opposite end of the test tank

## Video Analysis

I virtually divided the maze into three ‘chambers’ defined by Perspex partitions within the maze apparatus. For each group, I recorded the time to the first bite of bloodworm (bloodworms were generally all eaten within a few seconds once located by one of the fish), the identity of the first individual to eat, and the number of bloodworms eaten by each fish.

I also assessed group cohesion by analysing the movement of each individual within the maze beginning from release and ending when all of the bloodworms were consumed. I

manually recorded the position of each individual at five second intervals during the trial. I calculated the mean pairwise distance between each pair over the course of the video, and averaged these amongst all pairs in a group to calculate mean group cohesion. I also analysed the relative distance of each individual from the feeding grid to assess which individual was closest to the feeding grid during the trial and therefore at the forefront of the group's movement.

## **Data Analysis**

For my sample size of 11 familiar and 11 unfamiliar groups, I analysed how the group mean personality (the mean of the boldness score of the three fish) and familiarity (a binary factor) affected the log-transformed time to first bite (i.e. time to find the food in the maze) using a linear model, allowing for an interaction of the two predictors.

I used a linear model to assess the effect between group mean personality and familiarity on group mean cohesion, allowing for an interaction of the two predictors. The main effects of the predictors were tested if the interaction was not significant.

I then analysed the performance of individuals. I tested the effect of group mean personality and familiarity, as well as their interaction, on the number of blood worms eaten by each of the bold, intermediate and shy fish in each group. For each personality type, I used a GLM with a Poisson distributed error structure to test if group mean personality and familiarity were associated with the number of blood worms that an individual consumed.

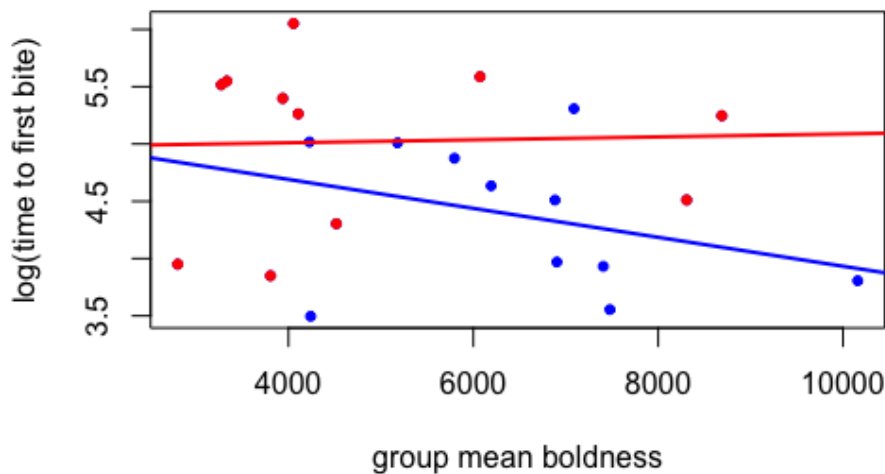
I tested the effect of group mean personality and familiarity, as well as their interaction, on the tendency of individuals to be in front of the group movement (i.e. the mean relative distance of an individual to the feeding grid from release until first bite); I used a linear model using familiarity and an individual's boldness relative to its group-mates as predictors of the log of the individual's relative distance from the feeding grid relative to its group-mates. For each personality type, I fitted a separate linear model with relative distance to the feeding grid as the response variable.

Data were analysed in R version 3.3.1 (Team 2016).

## **RESULTS**

## Group performance

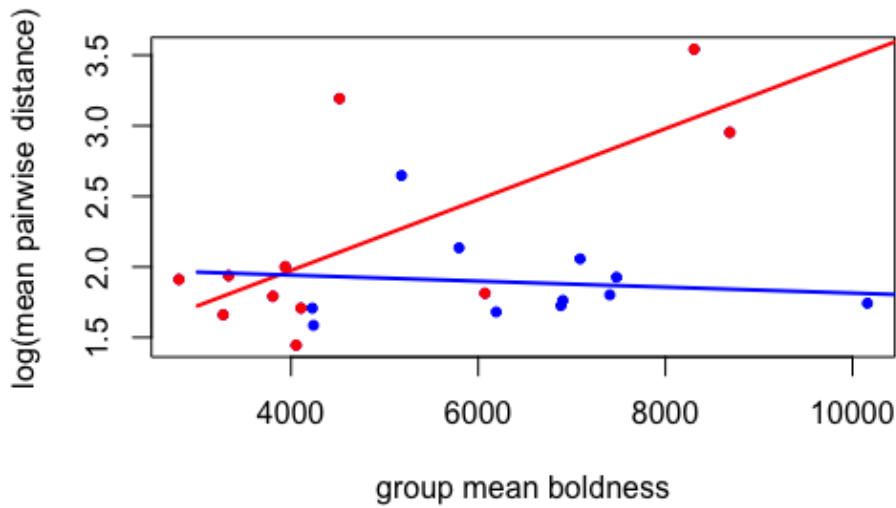
Groups exhibited different patterns of behaviour and performance based on familiarity. Contrary to my expectations, familiar groups did not outperform unfamiliar groups (figure 2) and familiarity, group mean boldness, and the interaction between those factors had no effect on the log-transformed time to first bite (Effect of familiarity:  $F_{1,19} = -1.71$ ,  $p=0.10$ ; effect of group mean boldness:  $F_{1,19} = 0.53$ ,  $p=0.61$ ; effect of interaction between familiarity and group mean boldness:  $F_{1,18} = 0.083$ ,  $p= 0.78$ ).



**Figure 2:** log-transformed time to first bite (seconds) as a function of group mean boldness in familiar (red) and unfamiliar (blue) groups. Neither familiarity, group mean boldness, or their interaction was a significant predictor of the group's time to first bite.

## Group cohesion

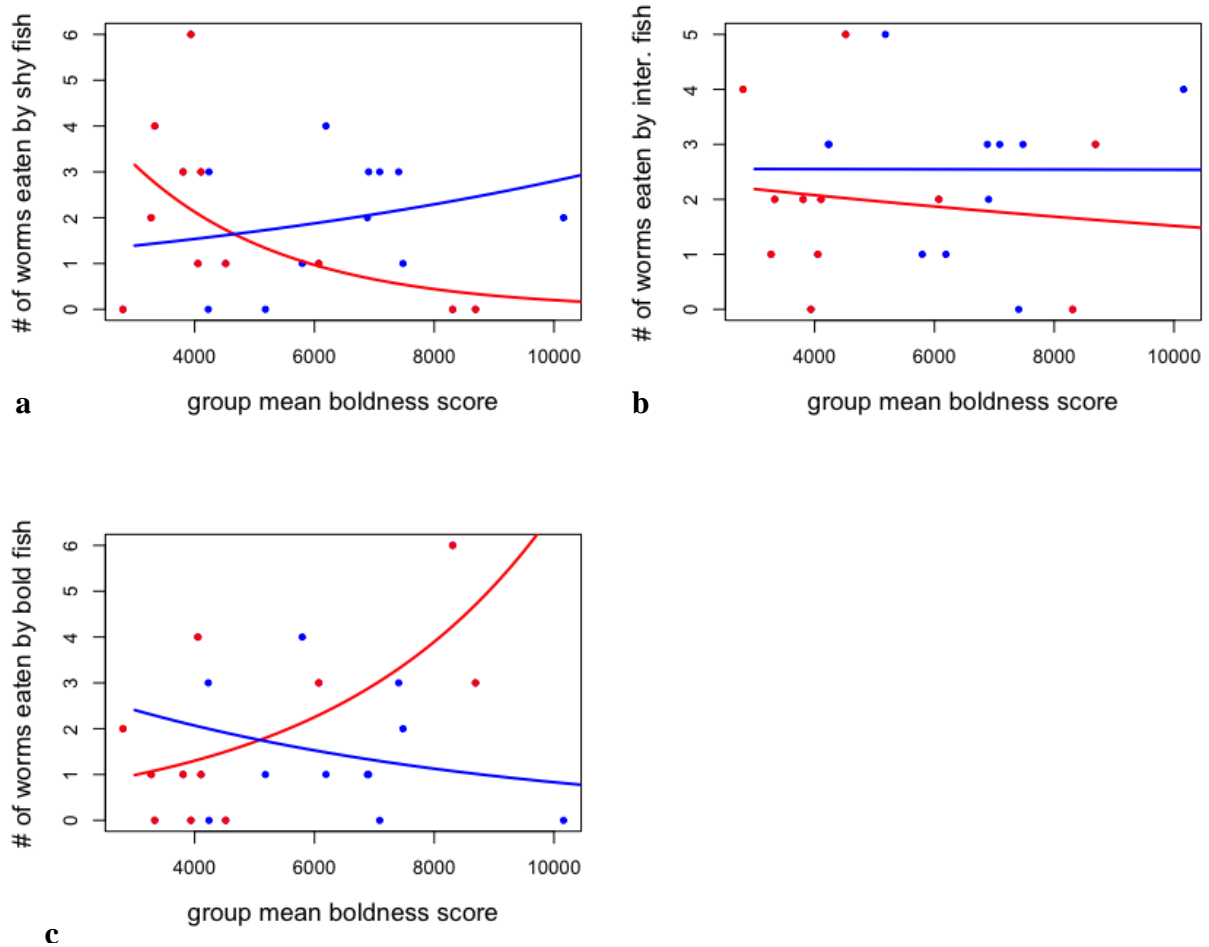
Group cohesion decreased with increasing mean group boldness, but this effect was only present in familiar groups; fish in unfamiliar groups tended to stay close together irrespective of their group mean boldness (interaction between personality and familiarity  $F_{1,18}=6.7$ ,  $p=0.018$ , figure 3).



**Figure 3:** log-transformed mean pairwise distance between individuals in a group vs group mean boldness, for familiar (red) and unfamiliar (blue) groups. The interaction between familiarity and group mean boldness was significant, and familiar groups show a relationship between group mean boldness and cohesion.

### Individual feeding success

Like the groups they comprise, individuals differed in their feeding success based on boldness and familiarity. For the shy fish, feeding success was affected by both group mean boldness and familiarity (interaction in GLM:  $\chi^2 = 5.42$ ,  $df = 1$ ,  $P = 0.033$ , Figure 4a): shy individuals ate fewer bloodworms in bolder groups when group-mates were familiar, but group mean boldness had a minimal effect on the number of worms eaten by shy fish in unfamiliar groups. The foraging success of bold fish was also affected by group mean boldness in familiar groups (interaction in GLM:  $\chi^2 = 5.2$ ,  $df = 1$ ,  $P = 0.022$ , Figure 4c), but in the opposite direction: bold fish ate more worms in bolder groups of familiar fish. However, similarly to the shy fish, there was little effect of group mean boldness on foraging success in unfamiliar groups. Finally, the number of worms eaten by fish of intermediate boldness was not affected by group familiarity ( $\chi^2 = 0.82$ ,  $df = 1$ ,  $P = 0.36$ ), group mean personality ( $\chi^2 = 0.11$ ,  $df = 1$ ,  $P = 0.74$ ) or their interaction ( $\chi^2 = 0.096$ ,  $df = 1$ ,  $P = 0.76$ ; Figure 4b).

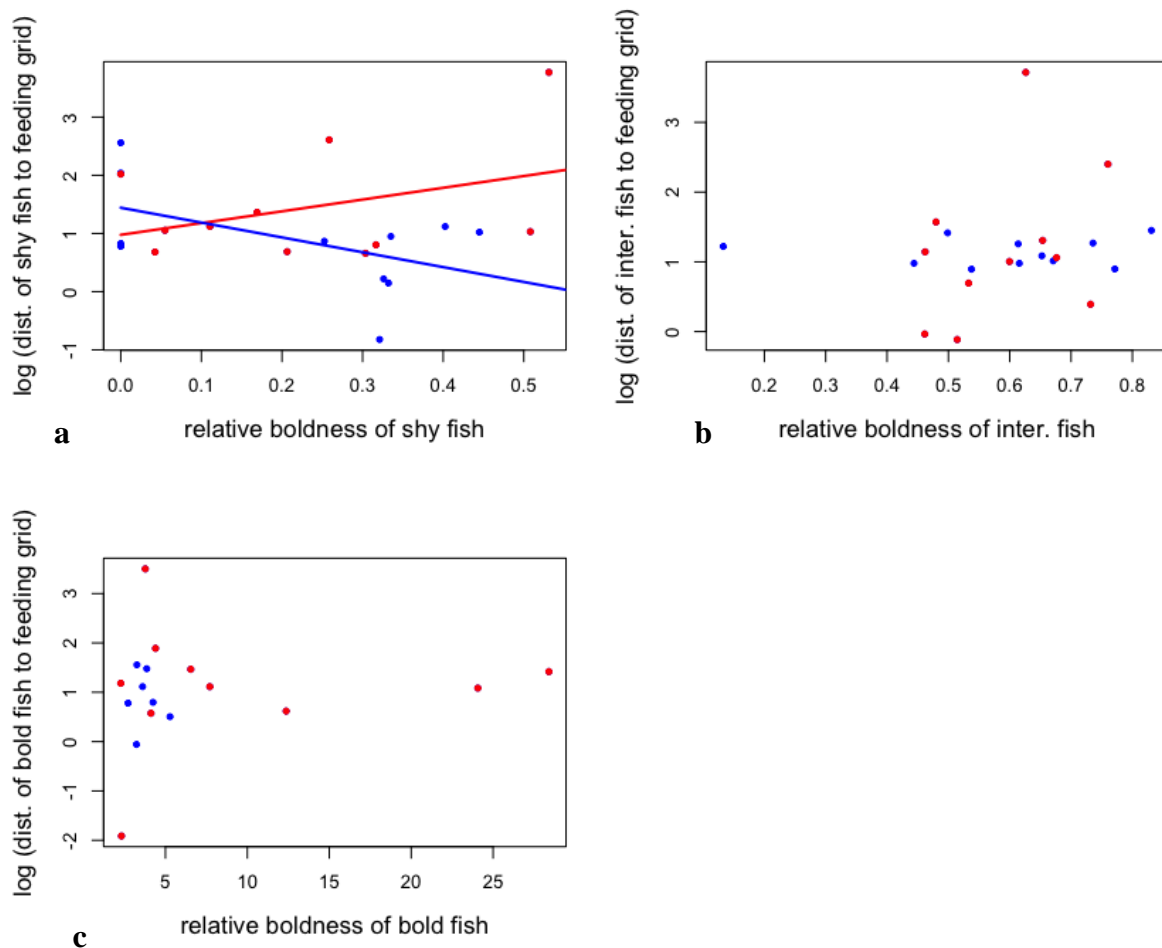


**Figure 4a-c:** Relationship between group mean boldness score and the number of bloodworms eaten by the a) shy, b) intermediate, and c) bold individuals in unfamiliar (blue) and familiar (red) treatments. The interaction between group mean boldness and familiarity was significant for the number of eaten bloodworms eaten by the shy and bold fish; this relationship was not significant for the intermediate fish.

### Relative distance to grid

The relative spatial position of shy individuals within a group tended to be affected by the shy fish's boldness relative to their group-mates' and familiarity (interaction in GLM:  $F_{1,18}=4.2$ ,  $p=0.055$ , figure 5a). Relatively less shy fish in familiar groups were more likely to be further from the feeding grid as compared to their group-mates (and thus in the back of the group).

Relatively less shy fish in unfamiliar groups, however, were more likely to be closer to the feeding grid (and thus in the front of the group). However, there was no significant effect of individuals' boldness relative to that of their group-mates on group position in either bold ( $F_{1,18}=2.6$ ,  $p=0.13$ , figure 5c) or intermediate ( $F_{1,18}=2.03$ ,  $p=0.17$ , figure 5b) individuals.



**Figure 5a-c:** Mean relative distance from the feeding grid as a function of the relative boldness (as compared to the group-mates' boldness) of a) shy fish b) intermediate fish and c) bold fish, in familiar (red) and unfamiliar (blue) shoals. The interaction between relative boldness and log-transformed distance to the feeding grid was significant for the shyest group member, but not for the intermediate or bold group members.

## DISCUSSION



Our study found no effect of familiarity on group foraging performance- familiar groups did not solve the maze more quickly than unfamiliar groups. This result is at odds with previous work suggesting that familiar groups of minnows and trout forage and explore more effectively than unfamiliar ones (Chivers et al., 1995; Griffiths et al., 2004). It seems possible that the maze assay used was too simple to uncover the performance-enhancing effects of familiarity. Alternately, in sticklebacks, cues specific to habitat and diet are important for recognition of familiar conspecifics (Ward et al., 2005), and all the individuals in this study were kept in identical enclosures and fed the same food. This may have dampened the effect of familiarity on the level of group coordination, even as an individual's boldness still seems to impact individual success based on familiarity.

With respect to cohesion, my result that bolder groups of familiar fish tended to be less cohesive than shyer groups is consistent with previous work showing that bold three-spined sticklebacks were less sociable (Jolles et al., 2015). However, this effect of group mean boldness on cohesion was not present in unfamiliar groups. Familiarity has been shown to lead to more stable dominance hierarchies (Croft et al., 2005; Jordan et al., 2010), and it seems that previous experience and social information about an individual's group-mates may inform an individual's expression of its own personality tendencies. This implies that the ways that individual personalities manifest in groups may be facilitated by familiarity.

Our results suggest that familiarity also mediates the effect of an individual's relative boldness (i.e. whether it is the shyest, intermediate, or boldest group member) and its group's mean boldness on its foraging success. My results show that, when an individual is the shyest member of its group, it will have the highest foraging success with a relatively shy, familiar group. For the boldest group member, however, the effect was reversed, and the boldest individual had the highest foraging success in a relatively bold, unfamiliar group. These results suggest that it might be beneficial for individuals to preferentially associate with familiar conspecifics with specific personality traits. Some studies have suggested that higher boldness is generally preferred in group-mates. In three-spined sticklebacks, for example, both shy and bold individuals preferred to associate with a bold shoal instead of a shy shoal (Harcourt et al., 2009b). However, individuals in that study had only visual access to both shoals, and bold shoals were consistently more active than shy shoals, which likely made them more visually apparent

(Harcourt et al., 2009b). When individuals had the chance to directly interact with group-mates, shy sticklebacks preferred to follow shy leaders, while bold fish had no such assortative preference (Nakayama et al., 2016). It may be that familiarity allows individuals to interact with group-mates in specific ways based on personality; this may primarily affect shy individuals, which tend to be more sociable (Jolles et al., 2015) and which prefer to follow shyer leaders (Nakayama et al., 2016). A shy individual's preference to follow a shy leader may seem surprising, as all-bold and mixed-boldness groups of guppies found food faster than all-shy groups (Dyer et al., 2009), but in the context of foraging, the performance of the group as a whole does not necessarily equate to individual success. All individuals were more likely to feed in a mixed-boldness shoal (Dyer et al., 2009), which implies that shy fish may not necessarily be incentivized to choose the group that finds food fastest. Rather, in the case of group foraging, if an individual is familiar with a group in which its individual foraging success is low, it may prefer an unfamiliar group where it may have a higher chance of success.

This difference in foraging performance based on the interactions between familiarity and personality may also reflect differences in group dynamics that occur through the direct effects of boldness and familiarity. In particular, higher boldness is associated with several other factors, including higher feeding rates and more successful competition in foraging (Ward et al., 2004b), as well as increased aggression (Bell and Stamps, 2004). Familiarity, on the other hand, is associated with lowered aggression and competition (Utne-Palm and Hart, 2000). Therefore, the shyest fish in a familiar group of low mean boldness will have experienced the lowest level of aggression and competitive interactions from its group-mates, which might allow for increased foraging performance and increased leadership tendencies in the shyest group-member.

Given that individuals experienced differing foraging successes based on their boldness, their group-mates' boldness, and familiarity, I assessed how individuals were positioned as the group navigated the problem-solving task, and whether positional differences could help explain the foraging success results. Individuals who tend to be closest to a desired resource, and thus at the forefront of group movement, can have a larger influence on the group's movements than individuals in the back of the group. This has been shown in golden shiners, in which informed individuals lead uninformed conspecifics from cover to a foraging patch (Reebs, 2000). My results suggest that familiarity caused the shyest member in groups of different mean boldness to take on different roles. In familiar groups, the shyest fish was relatively closer to the

grid with increasing group mean boldness. In unfamiliar groups, however, the trend seemed to be reversed, with the shyest group member further back in the group with increasing mean group boldness. The absence of any effect in the intermediate and boldest group members suggest that the propensity of shy group members to lead their groups is more heavily influenced by the social environment and group's characteristics, while bolder fish seem less sensitive to their social environment. This is consistent with previous work suggesting the shy fish are more susceptible to social feedback and social conditions (Nakayama et al., 2012). The result that shy fish tend to be relatively close to the feeding grid in familiar groups of high mean group boldness is interesting, as the shyest group member in familiar, relatively bold groups tended to experience lower foraging success. The fact that the shy individual's position within the group was uncorrelated with its apparent foraging success implies that group foraging can involve interactions between individuals other than leading and following. This is consistent with results from other species, including pigeons, in which group structures differ during foraging and group movement (Nagy et al., 2013). More specifically to our study, the measure of personality used in this study, boldness, was tested using a risk-taking paradigm where fish had the choice to either remain in cover or venture into an exposed area. The maze assay used, however, was an exploration paradigm in which fish could not take shelter under cover, but rather were required to explore a new area. This alteration in paradigm might lead to unexpected dynamics in groups consisting of a diversity of personalities and familiar or unfamiliar conspecifics.

These results suggest that familiarity may have a more complicated effect on groups than simply improving coordination. Similarly, familiarity may have a more complicated effect on individuals than preference for familiar conspecifics. Although my study suggests important interactions between familiarity and personality, I did not test how individual behaviours vary across different contexts. There is substantial variation in the ways shy fish respond to conspecifics during group coordination, and shy fish are more sensitive to previous social experience than bolder conspecifics (Nakayama et al., 2016, 2012). It would be interesting to assess whether or not a shy individual is more likely to forage successfully and exert influence on group-mates (e.g. lead others) under different social conditions, given the lack of connection between cohesion, leadership, and personality in unfamiliar groups.

The familiarity and boldness-based variation in foraging success evident from my results suggest that group performance and dynamics are fundamentally affected by group-level

features as well as the characteristics of the individuals that form groups. This is consistent with theoretical and empirical work that shows that individual differences can drive group coordination (Jolles et al., 2017), and highlights the importance of group-level (familiarity) and individual-level (personality) characteristics in influencing individual and group performance. If some individuals are responsive to specific social conditions, it may be that groups with those specific social conditions activate these individuals, leading to different group dynamics with different benefits. It is possible that there may be mechanisms that allow individuals to form groups that maximise their individual success. However, important among the social conditions that affect individual and group characteristics is familiarity, which can only be established through time. It may be that stable, individual driven differences in group structure and function require a degree of familiarity (or, potentially, other social information) to become established.



## **Chapter 3:** Coping with strangers: how familiarity and active interactions shape group coordination in *Corydoras aeneus*

*This chapter will be submitted as a first author manuscript with contributions from the following co-authors. Beth Gillie assisted with experimental design, execution, and video scoring; Neeltje Boogert assisted with experimental design and analysis; Rufus Johnstone assisted with data analysis and manuscript editing; Andrea Manica assisted with experimental design, data analysis, and manuscript editing.*

### **ABSTRACT**

Social groups whose members have had sustained prior experience with each other frequently exhibit improved coordination and outperform groups whose members are unfamiliar with one another. The mechanisms by which familiarity assists coordination are not well known. Prior social experience may simply allow individuals to learn the behavioural tendencies of familiar group-mates and coordinate accordingly. In the absence of prior social experience, it would be adaptive for individuals to develop strategies for coping with unfamiliar others to minimise the disadvantage of unfamiliarity. To explore the dynamics of familiarity in shaping group behaviours, I used a highly social catfish, *Corydoras aeneus*, that utilises a distinctive, observable tactile interactions. Here I describe this tactile interaction behaviour, physical “nudges” that are deployed to initiate group movements and maintain contact with group-mates during group movements. I then report the results of two experiments exploring the relationship between nudges and coordination. First, within triplets of two familiar and one unfamiliar individual, I found no individual differences in nudging rate based on familiarity. Despite all individuals interacting at similar rates, however, unfamiliar individuals failed to coordinate as well as their familiar group-mates and were more frequently absent from group movements.

Second, when comparing pairs of familiar with pairs of unfamiliar fish, there was no difference in the level of coordination between pairs. Instead, I found that unfamiliar pairs exhibited significantly higher nudging rates, suggesting that unfamiliar pairs could compensate for their unfamiliarity by nudging more frequently. In contrast, familiar individuals coordinated with comparatively little nudging, presumably because they were experienced with each other. Overall, these results suggest that nudges can be used to improve coordination of group activities, but that their usage is reduced in the case of familiar individuals, implying that these potential signals may be costly.

## INTRODUCTION

Animals can gain great benefits from living in groups. Through group coordination, individuals can increase the likelihood of evading predators and improve foraging success (Chivers et al., 1995; Griffiths et al., 2004). Familiarity, defined as previous experience with a given other individual, has been shown to increase coordination in a variety of taxa, including birds (Senar et al., 1990) and schooling fishes (Ward and Hart, 2003). For example, great tits show increased anti-predator defences within groups based on familiarity, with previous experience of nest-site neighbours leading to a greater likelihood of a familiar neighbour joining in to defend a conspecific's nest (Grabowska-Zhang et al., 2012a). In fathead minnows, familiar groups exhibited greater shoal cohesion and more effective anti-predator behaviours (i.e. predator inspection) in the face of predation threats when compared to unfamiliar groups (Chivers et al., 1995). The same effect was found in familiar groups of juvenile trout, which responded significantly faster than unfamiliar groups to a predator attack and were more successful foragers (Griffiths et al., 2004).

Given the benefits of grouping with familiar individuals, it is not surprising that individuals tend to associate preferentially with familiar over unfamiliar individuals in a number of species, including cowbirds (Kohn et al., 2015) and guppies (Griffiths and Magurran, 1997). For example, female cowbirds preferentially associate with familiar group-mates when put into a group with familiar and unfamiliar conspecifics (Kohn et al., 2015). In sticklebacks, the preference for familiar group mates is so strong that individuals tend to prefer smaller groups of

familiar individuals to large groups of unfamiliar individuals, even though they generally prefer larger groups of familiar individuals to smaller groups (Barber and Ruxton, 2000).

Despite familiarity's many benefits, the mechanisms by which familiarity improves coordination have rarely been investigated. It seems likely that familiar individuals are better informed about each other's preferences and characteristics, and thus respond more promptly or appropriately to a partner's actions. However, quantifying such responses is challenging, as it is often unclear whether individuals are actively trying to coordinate activities or not (King et al., 2009). In this paper, I study movement coordination in a species of fish, *Corydoras aeneus* (the Bronze Cory catfish), which exhibits an unusual behaviour during coordinated activities. In this highly social neotropical catfish, individuals often nudge each other. This nudging behaviour occurs during both foraging and group flight responses to potential threats (Chapter 4), both when initiating and during joint movements, thus providing an easily scored behaviour that might affect coordination.

I first assessed pairs of individuals to investigate how individuals interacted and coordinated their behaviour when they were not given a choice of partner. I noted for how much time a given individual was at the front of coordinated movement, or the 'front fish' in a directed pair movement. I also assessed the connection between nudging and coordinated movements. I tested whether pairs' nudging rates were higher during coordinated movements than when partners were close together but not engaged in coordinated movements. I also investigated whether the amount of time an individual spent as the 'front fish' in a coordinated movement was related to the rate at which they nudged their partner.

I then examined both familiar and unfamiliar pairs to test how coordination, cohesion, and the use of nudging varied based on familiarity. I predicted that unfamiliar pairs would require a higher nudging rate to achieve the same level of coordination as familiar pairs.

Finally, I observed the movements of triplets of fish composed of two familiar individuals and one unfamiliar one. I tested whether familiar individuals spent more time close to each other (i.e. coordinated their movements better) than unfamiliar individuals. I also measured the rate at which individuals nudge each other during joint movements, to test whether familiarity affected an individual's use of this behaviour. I predicted that individuals would coordinate better with familiar group-mates.



## METHODS

### *Corydoras aeneus*

*Corydoras* is a highly social genus of neotropical catfish widely used in the aquarium trade. In captivity, they have lifespans from 10-15 years (Lambourne, 1995), but their life histories in the wild are not fully known. *Corydoras* are generally benthic fish that prefer slow moving, shallow water; they are known for their marked sociality and shoaling behaviour (Nijssen in Lambourne, 1995). In the wild, *Corydoras aeneus*, commonly known as Bronze Cory catfish, are social foragers that live in mixed groups of males, females, and juveniles (Nijssen in Sands, 1986). *Corydoras aeneus* has a slight sexual dimorphism, with females being larger and thicker-bodied than males (Kohda et al., 2002). I have observed that captive-bred individuals exhibit an unusual tactile interaction behaviour during coordinated activities. Wild fish were also observed utilizing this behaviour in several small streams in the Madre de Dios locality of the Peruvian Amazon (Riley, personal observation.).

### **Social housing husbandry**

I obtained Bronze Cory catfish from three local pet shops in Cambridgeshire: Maidenhead Aquatics Cambridge, Pet Paks LTD, and Ely Aquatics and Reptiles. All fish used in both experiments were at least 24 weeks of age and had been housed in the lab for at least six weeks prior to the start of experiments. I maintained the fish on reverse osmosis (RO) water purified to 15 or less total dissolved solids (TDS) and re-mineralised to 105-110ppm TDS using a commercially prepared RO re-mineralizing mix (Tropic Marin Re-mineral Tropic). The fish lived on a 12:12 light:dark cycle at a temperature of  $23 \pm 1$  °C. Prior to the start of the experiment, I housed the fish in mixed-sex social housing tanks (60cm x 30 cm x 34 cm) of 6-10 fish. The tanks were equipped with four Interpet Mini internal filters and an air stone. I fed the fish daily on a varied diet of alternating Hikari wafers (Hikari brand, USA), Tetra Prima granules (Tetra brand, Germany), and thawed frozen bloodworms (SuperFish, UK). The group composition of social housing tanks was stable for at least six weeks prior to experiment, and

unfamiliar fish had not been exposed to each other for at least six months prior to the experiment, if at all. At the conclusion of each experiment, all fish were returned to the social housing tanks.

### **Pair study experimental procedure**

I investigated the behaviour of familiar and unfamiliar pairs of fish; these trials were completed in three batches. I analysed 27 pairs for a total of 54 individuals. Each batch consisted of 3-6 familiar pairs, and 3-6 unfamiliar pairs. Experimental batches were tested in October 2016, November 2016, and February 2017 to allow new fish to habituate to the laboratory environment. I formed experimental pairs by randomly assigning individuals to ‘familiar’ or ‘unfamiliar’ treatments. Individuals in the ‘familiar’ condition were paired with an individual from their same social housing tank; unfamiliar individuals were paired with an individual from a different social housing tank (i.e. had not been exposed to each other for at least six months, if ever). Individuals in pairs were visually distinguishable from each other and could be identified based on size, coloration, and markings. I paired individuals in both treatments with same-sex partners to avoid courtship interactions, and fish were not fed prior to the trial to encourage exploratory movement of the tank in search of food

Pairs were placed into one of two filming tanks, which had dimensions (45.5 cm x 25 cm x 21cm) and a sand substrate. Each filming tank had two small plastic plants in one corner of the tank to provide cover. During each session, one filming tank contained a familiar pair while the other contained an unfamiliar pair. Pairs were filmed in random order and were assigned to a filming tank randomly. Pairs were not fed prior to filming. I filmed each pair with a Toshiba Camileo x100 video camera for one-hour.

### **Triplet study experiment procedure**

I investigated the behaviour of familiar and unfamiliar individuals in triplets over three weeks in May-June 2017. I analysed 18 triplets for a total of 54 individuals. Each triplet consisted of two familiar individuals taken from the same social housing tank and an unfamiliar individual taken from a different tank. I could visually distinguish the individuals in triplets (as with pairs), and I recorded the physical characteristics of all group members for video scoring

purposes. Triplets were composed of same-sex individuals to avoid courtship interactions, and fish were not fed prior to the trial (as with pairs). I placed each triplet in one of two testing arenas with a thin layer of aquarium sand as substrate (to reflect the Bronze Cory catfish's natural habitat). Each arena (47cm x 30 cm x 29 cm) was constructed by partitioning a larger aquarium with a fitted opaque plexiglass sheet. I used a GOPRO HERO 3 camera to film each triplet for 30 minutes from above.

## **Video scoring**

Triplet and pair videos were scored using the same criteria, except where noted. Video scoring commenced at the first joint trip, which I defined to be a directional group movement (in which each fish was within two body lengths of another individual, and all members of the triplet or pair were moving in the same direction) lasting at least five seconds. Videos were scored by Beth Gillie and Riva Riley, and pair videos were scored blind (i.e. without knowing if any given pair were familiar or unfamiliar). On average, 40% of videos (randomly determined) were scored by both scorers to ensure consistency (for details, please see appendix). The remaining videos were scored by one scorer. I scored triplet videos for five minutes and pair videos for 10 minutes following the first joint trip.

I quantified cohesion by estimating the amount of time two individuals spent in proximity to one another, defined as within 7 cm (roughly two body lengths of an average sized fish). I also recorded “nudges” (tactile interactions), which include any time fish touch one another while in motion, while one is at rest, or when fish are resting close to one another and one individual starts to move in a way that causes it to brush against its partner. For each interaction, I identified the actor and the recipient of the interaction. I also noted whether the interaction was delivered by the forward part of the initiator's body (i.e. the head region) or by the initiator's tail. Since interactions can only occur when fish are in proximity to one other, I focus on the rate of tactile interactions delivered by one fish to another whilst in proximity (i.e. within 7 cm). I define this ‘nudging rate’ as the number of nudges initiated by an individual divided by the number of seconds the pair spend in proximity (i.e. time together). For pairs, it was possible to classify which individual was in front during coordinated movements (defined as both fish moving in the same direction with one individual at least one half of a body length in front of the other for at

least three seconds). This is essentially a measure of leader-directed pair movements, as movements with a clear front fish and back fish represent periods where pairs are actively coordinating using leader/follower dynamics, a well-established coordination scheme (as in Rands, 2003). A detailed ethogram fully explaining our video scoring criteria and consistency checks is provided in the appendix for this chapter.

In familiar and unfamiliar pairs, I investigated whether coordinated movements were associated with a higher nudging rate when compared to individuals close to one another but not engaged in coordinated movements. I also tested whether individuals that initiate more nudges tend to spend more time in front during coordinated movements. I performed these analyses using the full data set, and the subset of the data that excludes nudges initiated by contact from the tail.

I compared the amount of time in proximity between pairs of familiar and unfamiliar fish (proportion of time together,  $\arcsin(\sqrt{\cdot})$  transformed), and their rates of tactile interactions (nudges) using two-sample *t*-tests. As some values seemed to deviate from the assumption of normality, I confirmed these results also by running a Wilcoxon test. Finally, I tested for a link between proportion of time spent in front ( $\arcsin(\sqrt{\cdot})$  transformed) and nudging rate with a linear model with an interaction between familiarity and initiation rate, with pair ID as a blocking factor.

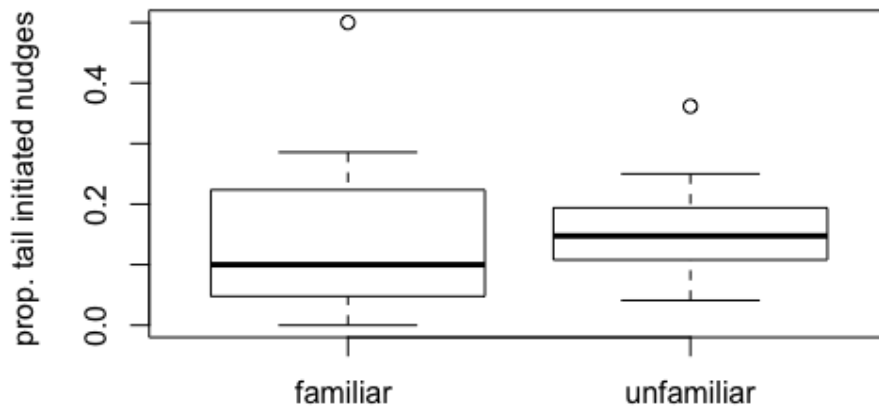
Similarly, for triplets I used an ANOVA test for differences between familiar and unfamiliar fish in the proportion of total time they spent in proximity to one another (I defined proportion of time together as (time together / total time), which was  $\arcsin(\sqrt{\cdot})$  transformed), and the rates of initiating and receiving tactile interactions, using triplet ID as a blocking factor. As with pairs, I performed these analyses using both the full data set and with the subset of data that excludes nudges initiated by contact from the tail.

Data were analysed in R (R version 3.2.2).

## **RESULTS**

### **Nudge initiation patterns**

Overall, 15.1% of total nudges were initiated by the initiator's tail (95% confidence interval: 10.4%-19.8%). The proportion of nudges initiated by tail contact did not significantly differ in familiar and unfamiliar groups (two sample t-test,  $t_{24} = -0.33$ ,  $p = 0.742$ , figure 1). Overall, the majority (84.9%) of nudges were initiated through contact from the initiator's head.

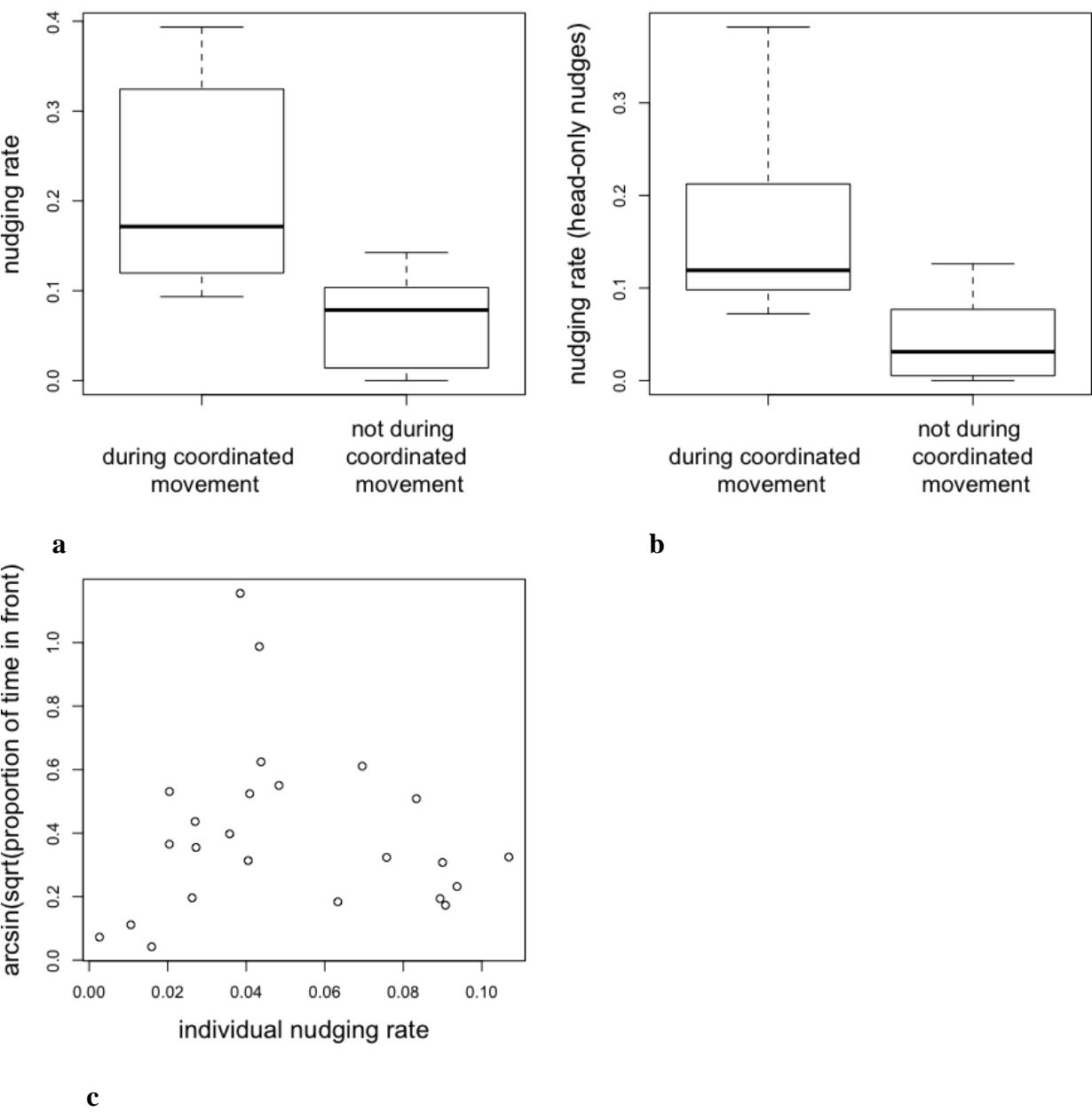


**Figure 1:** proportion of nudges initiated by the tail in familiar and unfamiliar pairs. Overall, 15.1% of all nudges were initiated by tail contact, and familiarity did not impact the proportion of nudges initiated through the tail.

### Nudges and coordination in pairs

Nudging was associated with coordinated movements in familiar and unfamiliar pairs: nudging rates during coordinated movements were significantly higher than rates when fish were in proximity with one other but were not coordinating their movements in familiar pairs (Paired Wilcoxon signed rank test,  $V=120$ ,  $df=14$ ,  $p<0.001$ ), unfamiliar pairs (Paired Wilcoxon signed rank test,  $df=11$ ,  $V=66$ ,  $p<0.001$ ), and overall (Paired Wilcoxon signed rank test,  $df=25$ ,  $V = 351$ ,  $p<0.001$ , figure 2a). This pattern remains robust when nudges initiated through tail contact were removed and only head-initiated nudges were included (Paired Wilcoxon signed rank test,  $V=350$ ,  $df=25$ ,  $p<0.001$ , figure 2b). There was no association between the amount of time an

individual spent in front and its initiating nudges (Pearson’s correlation,  $t = -0.08$ ,  $df = 22$ ,  $p$ -value = 0.939, figure 2c).

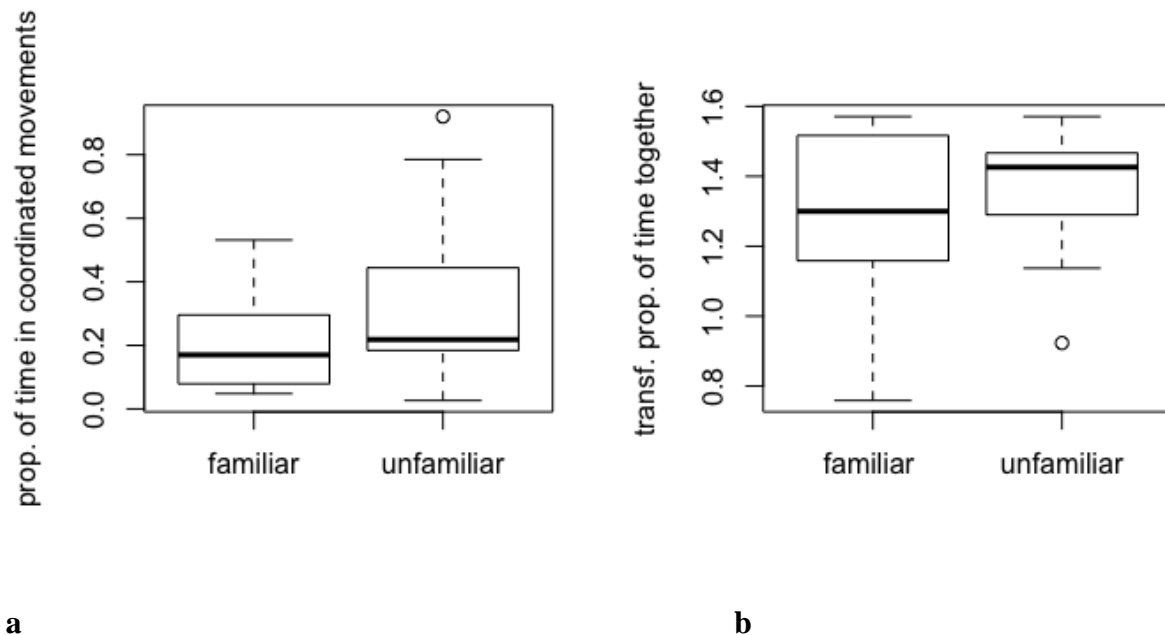


**Figure 2:** a) nudging rates during and outside of coordinated movements in pairs; b) head-only nudging rates during and outside of coordinated movements in pairs; c) proportion of time in front (arcsin(sqrt) transformed) vs rate of nudges initiated by the individual in front in unfamiliar pairs. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range,

whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers. Nudging rates are significantly higher during coordinated movements than when individuals are together but not engaged in a coordinated movement, but this increase does not seem to be primarily linked to nudges from the front fish.

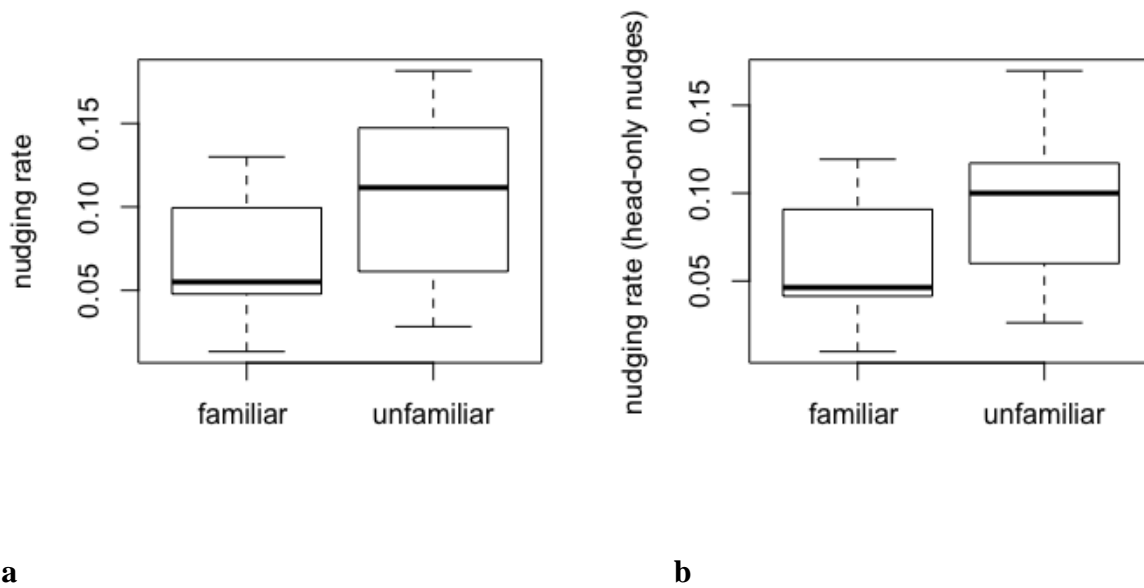
### Comparing coordination and cohesion between familiar and unfamiliar pairs

Familiarity had no effect on the level of coordination or cohesion in pairs of fish. Familiar and unfamiliar pairs spent the same proportion of time engaged in coordinated movements (two sample t-test,  $t_{25} = 1.3$ ,  $p = 0.367$ , figure 3a) and the same proportion of time in proximity to one another as unfamiliar pairs (two sample t-test,  $t_{25} = -0.93$ ,  $p = 0.36$ , figure 3b)



**Figure 3:** a) proportion of time in coordinated movements in familiar and unfamiliar pairs; b) arcsin(sqrt) transformed proportion of time together in familiar and unfamiliar pairs. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers. Familiarity did not have an effect on the proportion of time pairs spent in coordinated movements or in the amount of time individuals spent in proximity to one another.

While patterns of coordination and nudging were similar in familiar and unfamiliar pairs, there was a significant difference in familiar and unfamiliar pairs in the rate of nudging, with individuals in unfamiliar pairs nudging each other more frequently than in familiar pairs (two-sample  $t$ -test:  $t_{25} = -2.18$ ,  $p = 0.039$ , figure 4a). This pattern is consistent and slightly more robust when only nudges initiated through contact from the head are considered (two-sample  $t$ -test,  $t_{25} = -2.31$ ,  $p$ -value = 0.0297, figure 4b).

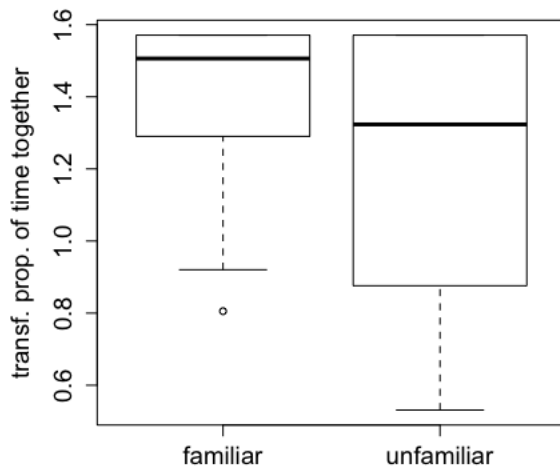


**Figure 4:** nudging rate in familiar and unfamiliar pairs; a) overall nudging rate; b) head-only nudging rate. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5$ IQR. Unfamiliar pairs exhibited a significantly higher nudging rate than familiar pairs, and this relationship is more robust when only nudges initiated through contact from the head are considered.

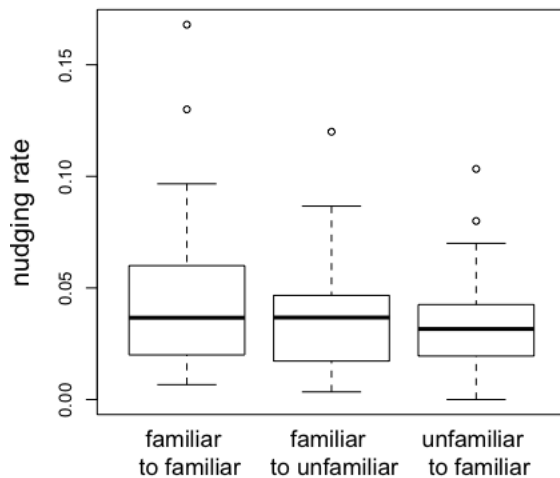
## Triplets



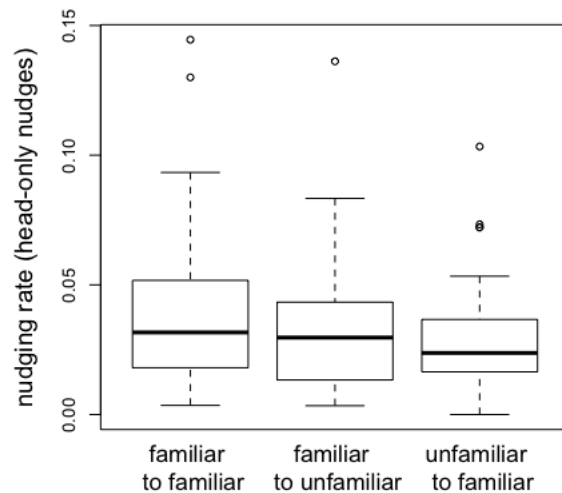
Within triplets, familiarity was associated with higher levels of coordination: the two familiar fish spent a higher proportion of time in proximity to one another than they did near the unfamiliar fish (ANOVA,  $F_{1,18}=14.1$ ,  $P=0.0006$ , figure 5a; removing the outlier does not impact the result). However, when fish were in proximity to one another, there was no effect of familiarity on the rate at which they nudged each other (ANOVA,  $F_{2,18}=2.08$ ,  $p=0.131$ , figure 5b). Removing the nudges initiated by contact from the tail does not impact these results (ANOVA,  $F_{2,18}=1.91$ ,  $p=0.153$ , figure 5c).



**a**



**b**



**c**

**Figure 5:** a) arcsin(sqrt) transformed time together for individuals in triplets; b) pairwise comparisons of full nudging rates within triplets; c) pairwise comparison of head-only nudging rates in triplets. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated. Unfamiliar group members tended to spend more time alone, but familiarity did not impact nudging rates.

## DISCUSSION

The presence of easily identifiable nudges in Bronze Cory catfish allows us to quantify the link between coordination and interactions among group members (even though I note that nudges are only one method of interacting). Specifically, nudging rates are significantly higher during coordinated movements than when pairs are not engaged in a coordinated movement. The fact that an individual's nudge initiation rates are not correlated with the individual's time in front suggests that nudging is not used exclusively by leaders for recruitment, but by both the 'front fish' and the 'back fish' to maintain coordination during joint movements. This suggests that nudging is a behaviour that Bronze Cory catfish individuals utilise while coordinating with others both during group flight responses (Chapter 4) and while exploring a new area, as the pairs and triplets in this study were obliged to do. This is consistent with other systems in which communication behaviours regulate group coordination, as in mouse lemurs, which use olfactory signals to regulate inter-group spatial coordination and acoustic signals to regulate intra-group cohesion and coordination (Braune et al., 2005). Similar examples exist in birds, for example, in green woodhoopoes, vocalizations are used to maintain group cohesion while moving to new territory (Radford, 2004). The use of tactile interactions by Bronze Cory catfish introduces interactions that utilise a different sensory modality than these examples, and which are strongly associated with coordinated movements directed by a front fish. Consequently, I used nudging as one metric to assess how familiarity affects coordination in triplets and pairs of Bronze Cory catfish. The consistency of my results when only head-initiated nudges are considered further supports the importance of this behaviour. Furthermore, this additional analysis strengthens the assessment protocols I developed to quantify nudging behaviour and investigate its functions and consequences.

I found that familiarity affected individuals differently based on group size. Individuals in pairs do not have a choice of group-mates, and without this choice, familiar and unfamiliar pairs

spent similar amounts of time together and exhibited similar patterns of nudging and coordination. However, it appears that unfamiliar pairs had to engage in a significantly higher nudging rate in order to achieve the same degree of coordination as familiar pairs. In triplets, however, these results were consistent with the established literature on the effect of familiarity on group coordination. Individuals in a triplet with one familiar group-mate and one unfamiliar group-mate spent more time in proximity with their familiar group-mate, although they nudged both group-mates at similar rates. The effect of cohesion on familiarity is in line with my expectations, and similar results obtained in a number of other species (Chivers et al., 1995; Griffiths and Magurran, 1997). This difference between the two experiments reveals that Bronze Cory catfish have the ability to coordinate effectively with unfamiliar individuals, but their failure to do so when given a choice suggests that coordination with unfamiliar individuals is likely to carry some cost (Bronze Cory catfish are highly social, and rarely forage in isolation). Thus, it seems that individuals are willing to pay such a cost only when they have to, as when evading potential threats, a situation in which prior familiarity did not affect cohesion or nudging tendencies (Chapter 4).

The higher nudging rates in unfamiliar compared to familiar pairs suggest that these nudges might play a role in aiding coordination between individuals without prior experience (and therefore without social information) about one another. The finding that increased use of nudges occurred only when individuals were forced to coordinate with an unfamiliar partner suggests that there might be some cost associated with this behaviour. Cory catfish rely on camouflage to avoid predators and freeze when threatened. Thus, it seems likely that individuals might avoid excessive use of nudges, as this is likely to make them more conspicuous to predators. It is somewhat surprising that unfamiliar fish in the triplet experiment did not attempt to increase their interaction rates to help them coordinate with the other two shoal mates; however, it might be the case that, since familiar fish preferentially spent time together, the unfamiliar fish had limited opportunities to interact extensively with them and preferred instead to rely on being still and inconspicuous. Thus, an increased level of nudging was only present in the pair setting, in the absence of a more desirable, and possibly more receptive, partner.

Because unfamiliar pairs coordinate just as effectively as familiar pairs, but require more nudges, while familiar individuals in groups coordinate more effectively with familiar group-mates despite having similar nudging rates with familiar and unfamiliar group-mates, it seems

that familiarity may reduce the level of interaction necessary to achieve effective coordination. This suggests that familiar individuals in groups may be able to achieve greater levels of coordination because they have had more chances to interact with one another previously and can respond to one another more effectively. Individuals can then initiate and respond to nudges more effectively based on the previous interactions they have had with their familiar group-mates.

Given the fact that the pairs in this study could achieve the same level of coordination via either familiarity or increased nudging, it is interesting to consider the effect of previous interactions on an individual's response to conspecifics. Evidence from guppies and deer mice suggests that familiar groups are capable of social learning at a faster rate than unfamiliar ones (Kavaliers et al., 2005; Swaney et al., 2001), which could be due to increased cohesion and individuals' (both demonstrator and follower) greater receptiveness to familiar group-mates. In addition, familiarity reduces aggression in many species, with an individual less likely to display aggressive behaviours toward an individual with which it has prior experience (Utne-Palm and Hart, 2000; Ward and Hart, 2003). It would be intriguing to investigate how familiarity leads to such outcomes. Familiarity may lead to greater sensitivity to others, which in turn increases social learning potential and reduces the risks of competitive interactions.

Finally, this study highlights the wider importance of exploring active responses to unfamiliarity. There is substantial literature exploring the negative effects of unfamiliarity on groups, but many animals can use tactics to ameliorate these effects. These results suggest that species that can actively coordinate with potential group-mates will selectively employ such tactics to obviate the disadvantages of unfamiliarity- individuals will do so only when the costs of poor coordination associated with unfamiliarity outweigh the costs of active mediation to coordinate with unfamiliar conspecifics. The costliness of unfamiliarity (likewise, the benefits of familiarity), as well as the necessity for animals to coordinate with others, are a potential selection pressure for the evolution of efficient communication systems.

## Chapter 3 Appendix

### DESCRIPTION OF SCORING PROTOCOL

Each tactile interaction consisted of two fish making visible physical contact in the video. We only scored interactions that occurred when both fish were on the bottom of the filming tank, because when fish were higher in the water column it was impossible to tell if fish were actually touching or if one was merely above the other.

Each tactile interaction has an initiator and a receiver:

- An initiator is the individual whose movement resulted in the nudge
  - ie if a two fish are in proximity at rest, and Fish I begins to swim and makes contact with Fish II as a result, Fish I has initiated the interaction
- A receiver is the individual who was touched by the movement of the receiver.
- We noted the region of the body that the initiator made contact with and the region of the body on which the receiver was contacted

A receiver can ignore the interaction.

- ‘Ignoring’ an interaction occurs when the receiver is initially at rest prior to the interaction, and does not move (beyond physical recoil from the nudge itself) within three seconds following the interaction
- ‘Ignoring’ interactions were not counted in the total number of interactions, or in the individual initiations
- there were few instances of ‘ignoring’ reactions in the videos

A mutual interaction is one which both fish are moving toward one another and make contact. Thus both ‘initiate’ and ‘receive’ the interaction.

- Mutual interactions counted toward the total number of interactions, but were not included in individual counts

Total interaction counts include: interactions initiated by fish I + interactions initiated by fish II + mutual interactions

Fish were defined as ‘together’ if they were within two body lengths (7cm) of another fish, and apart if they were further than two body lengths from their group-mate(s).

Interaction rates are defined as: number of interactions initiated by fish I divided by the number of seconds fish I spent together with a given groupmate.

We defined 'time in front' as a measure of leadership. We only defined this measure for pairs. We defined an individual as being 'in front' if both members of the pair were swimming in the same direction and the individual was at least one half of a body length in front of its partner for at least three seconds.

We scored each instance that each individual was in front, noting start and end times, and took the sum of the number of seconds each individual spent in front.

Total time in front is defined as: (Fish I time in front) + (Fish II time in front)

## **ASSESSMENT OF CONSISTENCY OF THE SCORING PROTOCOL**

### **For pairs**

For the first two batches of pair videos we used a set of one to three randomly selected videos as a training set. The training set was scored by both Riva Riley and Beth Gillie, and scores were compared and inconsistencies resolved by referring to the scoring protocol and reaching a consensus about each interaction. After the training set, an additional comparison set of videos was scored by both scorers, and the scores for each measure (number of interactions initiated by each fish, mutual, number of interactions ignored, amount of time spent together, amount of time each individual spent as front fish) compared. The comparison set was also consensus scored in the process of comparison. If all measures were within 85% for both scorers, scores were deemed consistent. If any measure was not within 85% for both scores, an additional training set was completed by both scorers, followed by an additional comparison set. For the first batch of videos we completed two training sets and two comparison sets- by the second comparison set, the two scorers were consistent by at least 85%. The rest of the videos were scored individually by either Beth Gillie or Riva Riley. For the second batch of videos, we completed one training set and one comparison set, which was found to be at least 85% consistent. The remainder of the videos from the second batch were scored individually by either Riva Riley or Beth Gillie. For the third batch, a comparison set was completed by both scorers, and was consensus scored in the process of comparison- both scores for all videos were at least 85% consistent. The remainder of the videos for the third batch was scored individually by Riva Riley or Beth Gillie.

### **For triplets**

We used a set of one to three randomly selected videos as a training set. The training set was scored by both Riva Riley and Beth Gillie, and scores were compared and inconsistencies resolved by referring to the scoring protocol and reaching a consensus about each interaction. After the training set, an additional set of comparison videos were scored by both scorers, and each measure (number of interactions initiated by each fish, mutual, number of interactions ignored, amount of time spent together) was compared across both scorers. If all measures were within 85% for both scorers, scores were deemed consistent. The comparison set was found to be consistent by this definition, and was also consensus scored in the process of comparison. The rest of the videos were scored by Riva Riley.

**Other scoring notes:**

Only pairs and triplets consisting of same-sex individuals were scored. It was clear if opposite-sex pairs or triplet groups occurred because vigorous courtship interactions ensued. These groups (three pairs and one triplet) were excluded from analysis. Therefore 30 pairs and 19 triplets were initially tested, for 60 individuals participating in the pair experiment and 57 individuals in the triplet experiment.

## **Chapter 4:** The role of tactile interactions in flight responses in the Bronze Cory catfish (*Corydoras aeneus*)

*This chapter will be submitted as a first author manuscript with contributions from the following co-authors. Beth Gillie assisted with experimental design, execution, and video scoring; Neeltje Boogert assisted with experimental design and analysis; Arne Jungwirth assisted with data analysis and manuscript editing; James Savage assisted with data analysis and manuscript editing; Andrea Manica assisted with experimental design, data analysis, and manuscript editing.*

### **ABSTRACT**

One of the primary functions of animal aggregations is defence against predators. Many social animals enjoy reduced predation risk as a result of grouping, and individuals within groups can benefit from information transferred by their group-mates about a potential predator. I present evidence that a tactile interaction behaviour I term ‘nudging’ modified group responses to a potential threat in a highly social catfish species, *Corydoras aeneus*. These catfish deployed nudges during flight responses, and these nudges were associated with a greater likelihood of group cohesion following a threat event. Increased nudging behaviour resulted in longer flight responses. In addition, individuals that perceived the threat first were more likely to initiate nudges, implying that nudges could be used to alert group-mates to the presence of a threat.



Taken together, my results suggest that tactile communication plays an important role in gaining anti-predator benefits from sociality in these fish.

## INTRODUCTION

Animal aggregations occur across the animal kingdom, with this ubiquitous sociality likely arising through the profound advantages group living can offer. Among the most evident of these benefits is reducing the risk of predation (Major, 1978; Neill and Cullen, 1974). Examples of aggregative behaviours reducing predation risk occur in invertebrates such as aphids (Turchin and Kareiva, 1989), in many fishes including minnows (Pitcher et al., 1986) and guppies (Magurran and Seghers, 1994), in reptiles such as iguanas (Greene et al., 1978), in many birds including cliff swallows (Brown, 1988) and ostriches (Bertram, 1980), and in many mammals including prairie dogs (Hoogland, 1981). Predation risk also increases cohesion in many species, including walleye (Sogard and Olla, 1997) and fiddler crabs in the context of a ‘selfish herd’ response (Viscido and Wetthey, 2002). By living in close proximity to others, individuals gain the benefit of their conspecifics’ perception and attention and can dedicate less time to predator vigilance while still being more likely to escape an attack (Bertram, 1980; Hoogland, 1981).

In addition to the benefit of seeing through their group-mates’ eyes, individuals also avoid predators due to the active spread of information about potential threats through groups. In some species, explicit signals such as alarm calls are delivered by one individual to alert its conspecifics about a predator, as in primates such as vervet monkeys (Seyfarth et al., 1980) and birds such as black-capped chickadees (Templeton et al., 2005). In the prior two examples, alarm calls encode specific information about the predator that is utilised by the caller’s conspecifics. In other systems, including fathead minnows and zebrafish, injured individuals release an alarm pheromone that alerts conspecifics to danger, albeit without specific information about the predator (Brown et al., 2001; Stensmyr and Maderspacher, 2012). Predator inspection is another behaviour that occurs in many taxa, including birds (Hinde, 1954) and fishes (Pitcher et al., 1986), and is performed by individuals or sub-groups but has consequences for the entire group. For example, in minnows a small contingent of a much larger shoal will inspect a potential predator at great potential risk, and if they perceive that the predator is a threat, they return to the

group instead of hiding immediately, after which their group-mates alter their behaviour (Pitcher et al., 1986). This implies that information about the predator was transferred, although it seems that individuals rely mostly on personal information to ignite a flight response (Magurran and Higham, 1988).

The acquisition of information from conspecifics that is potentially costly to obtain personally is certainly beneficial to individuals, and many species have evolved social behaviours that allow individuals to convey information about predators to their group-mates or otherwise influence their group-mates' behaviour in mutually beneficial ways. Understanding how an individual's behaviour can impact the coordination of its group is of particular importance to understanding how groups function, and how group living provides the myriad of advantages seen across taxa. One factor that strongly affects group coordination is familiarity, defined as previous experience with a given other individual. Familiarity leads to improved coordination in a variety of taxa, including birds (Senar et al., 1990) and schooling fishes (Ward and Hart, 2003). In particular, familiarity improves a group's anti-predator defences, such as in great tits, in which previous experience with nest-site neighbours results in a higher probability of a familiar neighbour contributing to the defence of a conspecific's nest (Grabowska-Zhang et al., 2012b). In fathead minnows, cohesion is greater and anti-predator behaviours (i.e. predator inspection) more effective in familiar groups when compared to unfamiliar groups (Chivers et al., 1995), and the same effect has been observed in juvenile trout (Griffiths et al., 2004). Given the advantages of familiarity, it makes sense that individuals often prefer familiar individuals over unfamiliar ones in a number of species, including cowbirds (Kohn et al., 2015) and guppies (Griffiths and Magurran, 1997).

This study investigated how individual *Corydoras aeneus*, the Bronze Cory catfish, can initiate or mediate a coordinated group response to a potential predator attack, how familiarity affects interactions during a group response to a predator attack, and how individuals can maximize group coordination under stressful circumstances. The Bronze Cory catfish is a highly social neotropical species (Lambourne, 1995) that uses an unusual tactile interaction behaviour in which individuals physically nudge one another during coordinated movements (Chapter 3). To investigate how individuals use nudges in response to a potential threat and how nudges affect group coordination, I scrutinised this behaviour in a controlled laboratory setting. I predicted that familiarity will impact nudging tendencies, and individuals will be more likely to

both deliver nudges and successfully recruit their familiar partner over their unfamiliar partner. I also predicted that nudging may serve an important function for both the spread of information about a potential predator, as well as maintaining proximity between group members following a flight response.

## **METHODS**

### **Study species**

In the wild, *Corydoras aeneus* are social foragers that live in groups of variable size consisting of males, females, and juveniles (Lambourne, 1995). Because they are bottom-dwelling they shoal in two dimensions and their social behaviour can be accurately and reliably recorded from above. I have observed that both wild-caught and captive-bred individuals exhibit an unusual tactile interaction behaviour during coordinated activities (Chapter 3) and following a startle response in aquarium settings. Wild fish were observed utilizing this behaviour in several small streams in the Madre de Dios locality of the Peruvian Amazon in 2011 and 2013, particularly when fleeing the observer (Riley, personal observation).

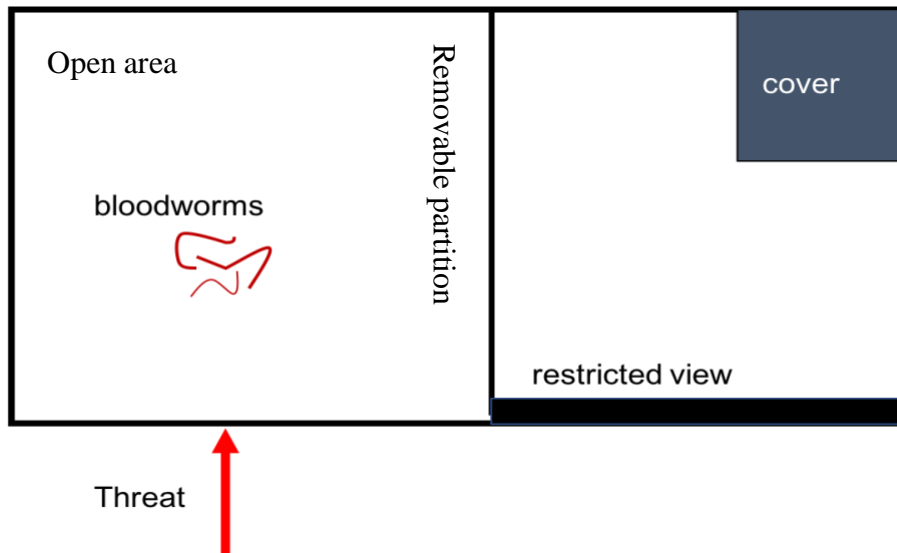
### **Husbandry**

I obtained Bronze Cory catfish from three local pet shops in Cambridgeshire: Maidenhead Aquatics Cambridge, Pet Paks LTD, and Ely Aquatics and Reptiles. All fish used in both experiments were at least 24 weeks of age and had been housed in the lab for at least six weeks prior to the start of experiments. I maintained the fish on reverse osmosis (RO) water purified to 15ppm or less total dissolved solids (TDS) and re-mineralized to 105-110ppm TDS using a commercially prepared RO re-mineralizing mix (Tropic Marin Re-mineral Tropic). The fish lived on a 12:12 light:dark cycle at a temperature of 23 +/- 1 degree Celsius. Prior to the start of the experiment, I housed the fish in mixed-sex social housing tanks (60cm x 30cm x 34cm) of 6-10 fish. The tanks were equipped with 4 Interpet Mini internal filters and an air stone. I fed the fish daily on a varied diet of alternating Hikari wafers (Hikari brand, USA), Tetra Prima granules (Tetra brand, Germany), and thawed frozen bloodworms (SuperFish, UK). The group

composition of social housing tanks was stable for at least six weeks prior to experiment, and unfamiliar fish (see below) had not been exposed to each other for at least six months prior to the experiment, if at all. At the conclusion of each experiment, all fish were returned to their respective social housing tanks.

### **Triplet study experiment procedure**

I investigated the behaviour of triplets over three weeks in May-June 2017, as in Chapter 3. I analysed 18 triplets for a total of 54 individuals. Each triplet consisted of two familiar individuals taken from the same social housing tank and an unfamiliar individual taken from a different tank. Triplets were composed of same-sex individuals to avoid courtship interactions, and fish were not fed prior to the trial to encourage exploratory movement in search of food. Each triplet was placed in a testing arena situated on a very low shelf 3cm off of the floor (figure 1; two identical setups were utilised in parallel). The arena had a thin layer of aquarium sand as substrate (mimicking the Bronze Cory catfish's natural habitat) and an opaque barrier so that each open (initially partitioned) arena was (47cm x 30 cm x 29 cm). I placed a piece of opaque acrylic outside the half of the tank where a sheltered 'cover' area was provided so that fish could not see any potentially threatening stimuli from outside the tank while in cover. Fish were left in the open part of the arena for an hour to acclimate to their group-mates and were filmed during this time so that nudging patterns at 'baseline' (in the absence of threat stimuli) could be analysed. The partition was then removed, and fish were allowed to explore the entire tank (47cm x 30cm x 91cm) for 30 minutes prior to the threat event period in order to explore the testing arena and become familiar with the location of cover. I used a GOPRO HERO 3 camera to film each triplet for the entire duration of the acclimation periods and threat events.



**Figure 1:** Schematic of test tank. Fish were initially allowed to acclimate in the open (initially partitioned) area without food. After acclimation, the partition was removed, and fish were allowed to explore the entire test tank. Bloodworms were then added to the open area to encourage fish to leave cover. The threat stimulus was only applied when fish were in the open area.

### Providing the threat stimulus

After fish were allowed to explore the entire test tank, i.e. 1.5 hours after introduction, the threat event period commenced. Threat events were delivered by Riva Riley or Elizabeth Gillie and were given through rapid approach of the test tank from a distance of 1.5 meters. Threat events were delivered in similar clothing every day (blue jeans and the same shoes during each event) and happened with an approach speed of roughly 2-2.5 m/s. Triplets responded to the vast majority of these threat events with a clear anti-predator response, and most responses to threat events fell into two categories: (i) fish responded to the threat by swimming rapidly to cover, often at speeds that required frame-by-frame video analysis for data extraction. (ii) fish responded to the threat with rapid movement, then froze in place outside of cover. I considered both reaching cover and freezing (following an initial burst of movement) as complete threat responses, thus considering a given threat event as ‘complete’ once all fish of a triplet had fled to cover or frozen. Following each threat event, I used an aquarium net to chase into cover any

individuals in the open area. If the aquarium net appeared before all individuals had frozen or reached cover, the threat was considered incomplete.

Threats were only delivered when all fish were out of cover in the open area. Thawed frozen bloodworms were delivered to the open area prior to the first threat event and after subsequent threat events to provide an incentive for fish to leave cover. Triplets were allowed to recover in between threat events, which occurred at least 4 minutes apart. Groups varied in how long they spent in the open area, as well as how long they needed to recover from the threat event and resume normal activities following a threat event; the threat event period was not to exceed 4 hours (the longest threat event period was 3.8 hours). After the threat event period, all individuals were returned to social housing tanks. Consequently, the total number of threats each triplet received differed.

## **Recorded behaviours**

For each triplet, I recorded (1) how many threat events took place, (2) the order that the group responded in (i.e. which individual responded first, second, or third), (3) how many times each group member nudged each of its groupmates, (4) whether any given nudge resulted in a previously stationary fish initiating a flight response, a case I defined as ‘recruitment’, and (5) the ‘flight time’ of each individual, defined as the time taken by each individual to either reach cover or freeze; ‘flight time’ refers to the duration of each individual’s flight response, starting at the onset of the threat event and ending when the individual reached cover or froze in place. For the analysis of mean flight times, I only used threat events that were considered complete. Finally, I assessed general measures of cohesion. These include whether or not all three group members were in proximity (within 7cm, or roughly two body lengths) to one another 30 seconds before the threat, three seconds before the threat, and three seconds after the threat event.

## **Analysis**

All statistical analyses were carried out in R version 3.2.2 (R core developer team), and generalized linear mixed effects models (GLMMs) were fitted using the lme4 package (Bates et

al 2013). All GLMMs were used to investigate count data and were thus fitted assuming a Poisson error distribution.

To test whether a triplet's baseline nudging tendency was correlated with their nudging tendency during flight responses, I used a Spearman's rank correlation test. I tested the correlation between a triplet's total number of nudges during the acclimation phase and that triplet's average number of nudges per threat event.

I used a GLMM to analyse whether familiarity influenced individual nudging preferences during threat events. The model included the number of nudges an individual initiated as response variable, the familiarity between initiator and receiver as explanatory variable (binary: receiver familiar or unfamiliar to the initiator), and two random effects (initiator ID and group ID).

Similarly, to analyse whether familiarity influenced recruitment rates during threat events, I used a GLMM with the number of successful recruitments by an individual as response variable. As above, the model included the familiarity between initiator and recruit as explanatory variables (binary: recruit familiar or unfamiliar to initiator), and two random effects (initiator ID and group ID).

To test whether nudging frequency during threat events was correlated with the probability of group cohesion following a threat event, I used a Spearman's rank correlation test. I tested the correlation between the average number of nudges a triplet performed during threat events and the proportion of all threat responses that ended with that triplet showing group cohesion (as defined above).

I analysed whether nudging rate during threat events influenced flight times and/or whether nudging rates changed throughout consecutive exposures to threat events using a further GLMM. The model included median flight time during a threat event (i.e. the median time it took a triplet's individuals to complete their threat response to a given threat event) as response variable, two explanatory variables (total number of nudges performed by the triplet during that threat event; count of threat events the triplet had experienced), and group ID as random effect.

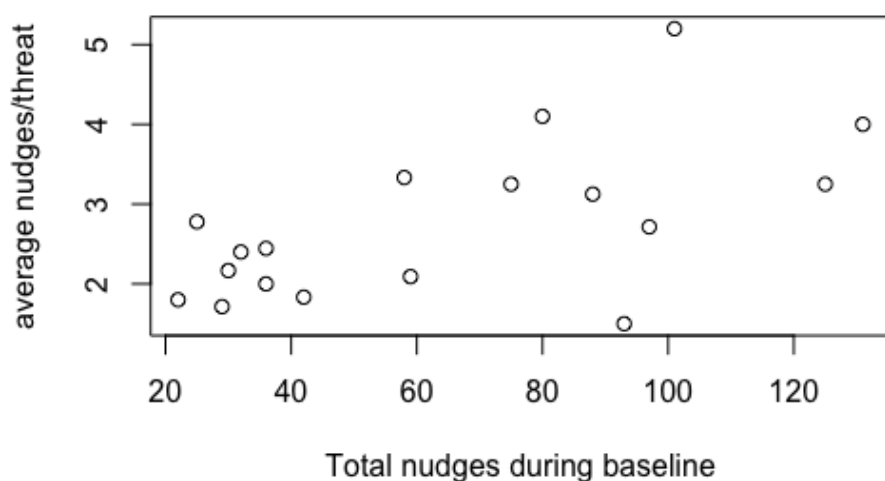
Finally, to analyse whether the order in which individuals of a triplet reacted to the threat influenced the number of nudges a given individual initiated and/or whether this changed throughout consecutive exposures to threat events, I used a GLMM. The model included the total number of nudges an individual initiated during a given threat event as response variable, two

explanatory variables (that individuals rank in the order of response: 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> responder; count of threat events the triplet had experienced), and group ID as random effect.

## RESULTS

### Comparison to baseline

Group nudging behaviour at baseline was significantly correlated with the average number of nudges per threat event (Spearman's rank correlation,  $S = 427.44$ ,  $p\text{-value} = 0.016$ , figure 2). Groups that nudged more during a set duration while exploring in the absence of simulated threats tended to nudge more during threat events.



**Figure 2:** the mean number of nudges per threat event vs the total nudges at baseline. There was a significant correlation between the number of nudges a triplet deployed during the observation period (i.e. at baseline) and the average number of nudges a triplet deployed per threat event.

### General

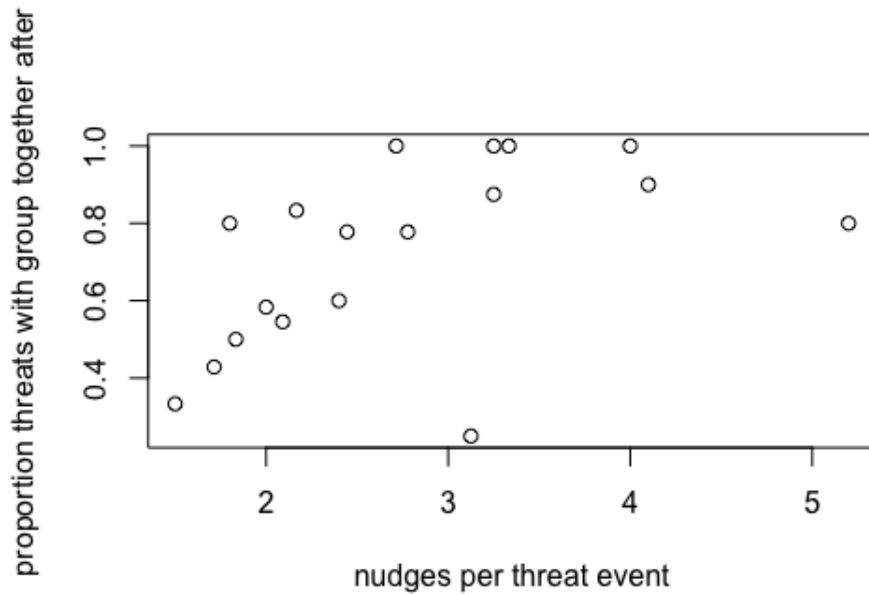


Overall, out of 135 threat events, 121 threat events were complete (all fish of a triplet fled to cover or froze in place). The 14 incomplete events involve fish either not responding to the stimulus at all, or still being in motion by the time the aquarium net chased them to cover. Out of the 121 complete threat events, 105 threat events involved one or more nudges. Groups varied in the number of threats they received: the range was 3-12 with a mean of 7.5 threats per group. The threat event period also differed between groups, with a range of 62-228 minutes and a mean of 105.9 minutes. The amount of time between threats varied as well: the range was 4-25 minutes with a mean of 14.11 minutes.

Individuals displayed no preference for delivering nudges with familiar partners as opposed to unfamiliar partners (Poisson generalized mixed effects model with individual and group ID as random effects,  $\chi^2_1=2.8$ ,  $p=0.093$ ). There was also no effect of familiarity on nudge frequencies during baseline (Chapter 3). The proportion of nudges that resulted in recruitment was 0.26. Familiar individuals did not tend to preferentially recruit their familiar group-mate over their unfamiliar group-mate (GLMM with individual and group ID as random effects,  $\chi^2_1=1.11$ ,  $p=0.292$ )

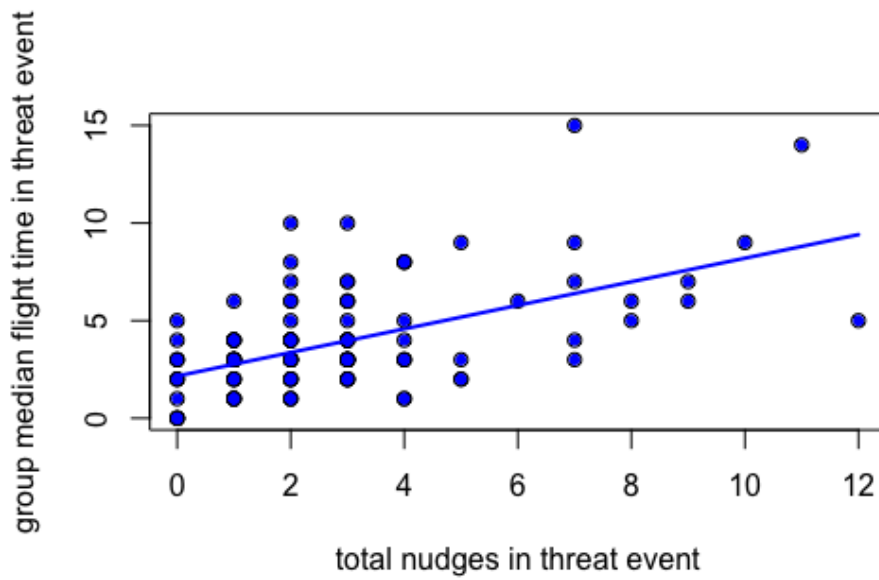
### **Nudging and group cohesion**

I found a significant correlation between a group's mean number of nudges per threat event and the proportion of threat events where all group-members were together three seconds following the event (Spearman's rank correlation,  $S = 335.24$ ,  $p\text{-value} = 0.003$ , figure 3). Groups that had a higher mean number of nudges per threat event exhibited higher cohesion following the threat event.



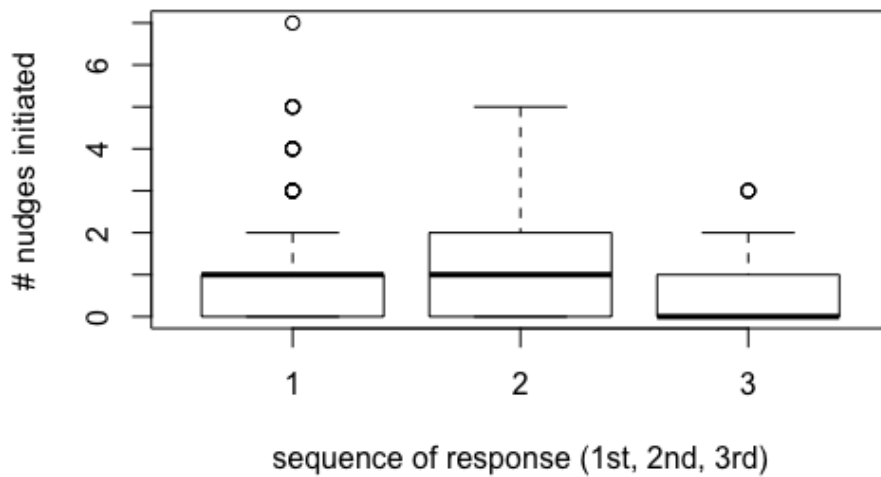
**Figure 3:** proportion of threats that ended with all group members together vs mean number of nudges per threat event. Triplets that tended to exhibit more nudges during threat events were more likely to end the threat event in close proximity to one another.

Threat events in which group members exhibited more nudges had longer flight times (generalized mixed-effects model with Poisson error structure; Group ID as a random effect,  $\chi^2_1 = 41.7$ ,  $p < 0.001$ , figure 4.) The threat event number did not have a significant effect on group median flight time ( $\chi^2_1 = 0.20$ ,  $p = 0.653$ ).



**Figure 4:** group median flight time (s) vs total nudges in threat event. The line indicates the line of best of fit for the relationship between a threat event’s total nudges and the group median flight time for the threat event. Threat events in which a triplet deployed more nudges tended to last for a longer duration of time.

The sequence in which individuals responded to the threat event had an effect on the number of nudge initiations: earlier responders initiated more nudges (generalized mixed-effects model with Poisson-distributed error structure; Group ID as a random effect  $\chi^2_1=4.6$ ,  $p=0.032$ , figure 5). The threat event number did not have a significant effect on the number of nudges an individual initiated ( $\chi^2_1=2.86$ ,  $p=0.09$ ).



**Figure 5:** nudges initiated by the first, second, and third fish to respond to a threat. There is a significant association between the order in which individuals perceive the threat event and the number of nudges the individual initiates during the threat event.

## DISCUSSION

My results show that, for triplets, nudging patterns are consistent across context, and groups that nudge more frequently during environmental exploration nudge more frequently during a flight response. Familiarity of group-mates did not impact nudging behaviour, and unfamiliar and familiar individuals were equally likely to initiate or receive nudges following a potential threat. This is somewhat surprising, as familiarity has clear effects on individual preference in other taxa, and individuals are more likely to assist in the defence of familiar conspecifics (Grabowska-Zhang et al., 2012a). However, given the high costs associated with a potential predator attack, and the fact that the Bronze Cory catfish have been shown to use nudging to compensate for lack of familiarity when foraging in the absence of a threat (Chapter 3), it seems likely that Bronze Cory catfish can use nudging to overcome familiarity in such high-stakes circumstances.

In Bronze Cory catfish, nudging was beneficial to all group members, and had a potentially selfish advantage for the initiator of the nudge. During a flight response, nudges were

positively associated with a higher likelihood of maintaining cohesion following threat events and longer flight times. Meanwhile, an individual's early detection of a threat relative to its group-mates is associated with initiating more nudges during the group's threat response. These results suggest that individuals deploy nudges in response to potential threats, and that it fundamentally alters a group's behaviour by increasing the probability of group cohesion. For this reason, familiarity may not affect an individual's decision of who to nudge, as the incentive to maintain cohesion is paramount. Furthermore, Bronze Cory catfish nudge group-mates extensively regardless of familiarity and can use increased nudging to coordinate effectively with unfamiliar partners (Chapter 3). Given the serious consequences of a potential predator attack and the ubiquity of nudging directed to both familiar and unfamiliar individuals, it is perhaps practical that familiarity does not affect the flight response. This mirrors findings in other systems, such as rats, in which reciprocity of cooperative behaviours is related solely to prior experience of cooperation, and not familiarity with the current beneficiary of cooperation (Rutte and Taborsky, 2007).

Furthermore, social animals with an incentive to maintain proximity with conspecifics have often been shown to use behaviours to coordinate with others. In green woodhoopoes, vocalizations are used to maintain group cohesion while moving to new territory (Radford, 2004), and white-tailed deer exhibit a low-cost flagging alarm signal to recruit other individuals to join it in a flight response to a potential predator (LaGory, 1987). The Bronze Cory catfish also appears to utilise an interactive behaviour in order to influence the dynamics of its group following a flight response to a potential threat. The nature of this behaviour, a tactile nudge, perhaps lends itself to maintaining cohesion, as the initiator must be in such close physical proximity as to touch the receiver of its nudge.

Bronze Cory catfish may have evolved this behaviour for a variety of reasons. They tend to live in small streams with low visibility (Nijssen in Lambourne, 1995), which I observed at my field site, and were observed in other experiments to have poor vision (Kohda et al., 2002), a characteristic I have also noted in wild and laboratory populations. Under these conditions, living in shallow water with poor vision, a tactile mode of interacting with one another might be the most effective way for individuals to transfer information and maintain contact. This might encourage groups to maintain higher levels of cohesion: if an individual loses contact with its group-mates, it might be difficult to find them again. Tactile communication is present in another

interspecific association, the well-documented shrimp-goby association, under similar circumstances, as the shrimp in that association has relatively poor vision (Kramer et al., 2009). In this system, shrimps convey their location outside the burrow by touching the goby with their antennae, and gobies, who have superior vision and serve as lookouts for predators, convey information about predators to their shrimp via a flick of the tail, a tactile signal that the shrimp can perceive, and after which both the shrimp and goby take shelter inside the burrow dug by the shrimp (Preston, 1978).

In this way, the Bronze Cory catfish's poor eyesight may have led to the evolution of an intraspecific tactile interaction method that can be deployed to spread information about predators and maintain cohesion following an attack. Individuals that reacted to a threat earlier initiated more nudges, which implies that this behaviour may be used more frequently by individuals who have already perceived the threat and are altering their behaviour in a way that transfers information to a group-mate. The fact that some of these nudges resulted in 'recruitment' in the sense that the receiver had been stationary prior to the nudge and then initiated a flight response following the nudge implies that nudges can alter the behaviour of receivers and potentially alert them to the presence of a threat. Finally, the association between increased nudging rates and mean flight time has implications for the potential costs and benefits of this behaviour. In the context of this experiment, the higher nudging rates may be a cost in the form of a lengthened flight response that could make individuals conspicuous to a potential predator for a longer duration of time. In some social species, an individual's response to early detection of a predator can be a potentially costly alarm call (Bergstrom and Lachmann, 2001), so it is possible that nudges are a costly behaviour. Furthermore, Bronze Cory catfish are very robust fish, armoured with bony plates that protect them from mechanical stress, and possessing locking, venomous spines on their dorsal, pectoral, and anal fins that increase their effective size and make them difficult prey (Sands, 1986). This may allow Bronze Cory catfish to prioritize proximity with its group-mates. In addition, the Bronze Cory catfish's poor eyesight (Kohda et al., 2002) may mean that an individual who was separated from its group following a flight response would struggle to find its group-mates after, and therefore nudging to maintain group-cohesion is worth the potential cost of an extended flight response.

Alternatively, my observations of wild fish suggest that the extended flight time may represent a more thorough flight. Instead of freezing in close proximity to the potential threat,

wild fish tended to flee to deeper pools with ample leaf litter, logs, and other hiding places within the stream. In fact, exploiting acquired knowledge of these hiding pools was how I captured wild fish- they were too difficult to catch in more open areas of the stream (Riley, personal observation). In any event, it seems very likely that nudges modify the behaviour of groups following a potential threat and provide the benefit of increasing the likelihood of maintaining group cohesion while also encouraging a group to flee from a potentially dangerous area. Studying the behaviour of larger groups of Bronze Cory catfish in a larger arena (or in a natural habitat) that provides more area and refuges for the fish to flee into would add more insight into how groups use nudges to coordinate, and whether these nudges may lead to a more thorough (i.e. fleeing further from the threat stimulus) flight response.

This study suggests that the Bronze Cory catfish's nudging behaviour can provide benefits when groups are responding to a potential attack. Nudges were also associated with coordinated movements in pairs of fish (Chapter 3), and the consistency in nudging in triplets during exploration/foraging and while responding to potential threats suggests that nudging is a behaviour that is useful for coordination under different conditions, and that nudging more frequently during exploration/foraging may contribute to a group's coordination during responses to a potential threat.





## **Chapter 5: Development of tactile interactions in *Corydoras aeneus* larvae**

*This chapter will be submitted as a first author manuscript with contributions from the following co-authors. Tom Roe assisted with experimental design, execution, video coding, data analysis, manuscript input, and manuscript editing; Beth Gillie assisted with experimental design and execution; Neeltje Boogert assisted with experimental design and analysis; Andrea Manica assisted with experimental design, data analysis, and manuscript editing.*

### **ABSTRACT**

Very young animals develop life skills as they mature, and in social animals this includes the acquisition of social abilities such as communication. Many animals exhibit changeable patterns of social behaviour based on development, and social experience during the juvenile period can be vital for the development of necessary social behaviours in adulthood. I investigated the development of a distinctive tactile interaction behaviour in *Corydoras aeneus*, the Bronze Cory catfish. Adults use this behaviour to coordinate group activities during foraging and flight responses from predators, and the development of this behaviour in larvae is of interest in investigating how communication and social behaviours develop as an individual matures, and which factors affect their development. I found that larvae respond to applied tactile stimulation with a flight response far less often as larvae matured, implying that larvae become less sensitive to tactile stimulation with age. Given that adults frequently interact with one another tactilely, this development is consistent with developing appropriate social behaviour in adulthood. I also found that social exposure affects the development of the larval response to tactile interactions with conspecifics, and that tactile isolation in the earliest larval stage leads to a greater likelihood of responding to a tactile interaction with a conspecific with a flight response. This suggests that

social exposure is important for developing an appropriate response to tactile stimulation in social settings and underscores the particular importance of early life experiences in the development of sociality.

## INTRODUCTION

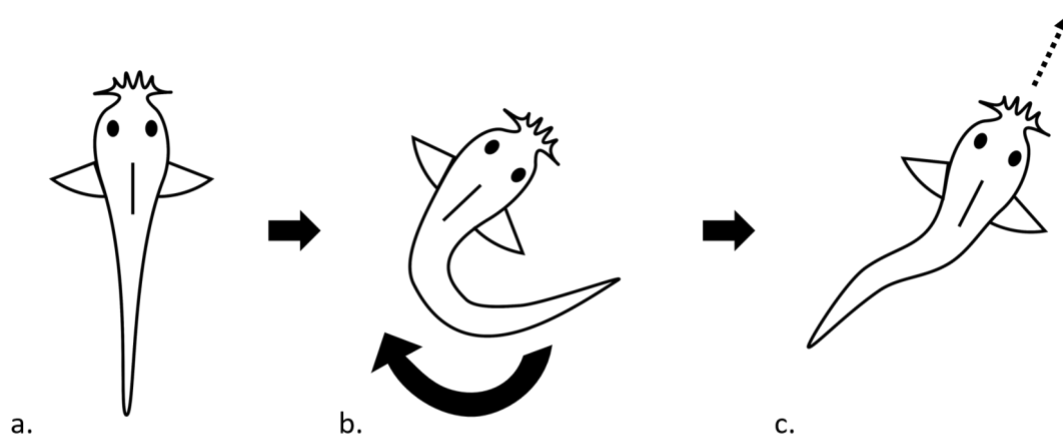
Group living confers a number of advantages to social animals, including a reduced risk of predation (Magurran, 1990) and increased foraging efficiency (Pitcher et al., 1982). In order for social animals to reap the benefits of group living, individuals must coordinate their behaviour with their group-mates'. Many social animals, however, begin their lives as undeveloped juveniles whose behaviour gradually adapts to social interactions over time. For example, the labyrinth fish develops the ability to communicate vocally later in development than the ability to perceive conspecific vocalizations (Wysocki and Ladich, 2001), and passerine birds develop the ability to sing first in a generalised learning stage and later in development in a more specific learning stage (Irwin, 1988). The progression of social skills as individuals grow and mature has important consequences for group coordination.

Many types of group coordination, including cohesion, develop in groups alongside physical development in individuals. In the catfish *Corydoras paleatus*, the development of group cohesion and aggregative behaviours is highly correlated to anatomical developmental stage (Rodríguez-Ithurrealde et al., 2014). The development of cohesive group behaviour with age has also been documented in zebra fish, which begin to form cohesive shoals as individuals age (Buske and Gerlai, 2011). In Florida scrub jays, mobbing behaviour, a group behaviour used to deter predators, first appears in the fledgling stage and gradually develops into adult mobbing behaviour over about three months (Francis et al., 1989).

The development of behaviour over a young animal's maturation to adulthood is vital for individuals and groups, and the social environment an animal experiences during development can have a profound impact on the social behaviours it exhibits as an adult (Slagsvold et al., 2002). This is apparent in species that experience imprinting, as when very early experiences determine mate preferences in great tits (Slagsvold et al., 2002). In zebrafish, a preference to shoal with conspecifics with particular coloration patterns is highly influenced by the patterns of the group-mates that an individual was raised with (Spence and Smith, 2007). The way social

behaviour can be determined by early experiences highlights how the mechanisms of development can have cascade effects on an animal's behaviour through its life. In rats, for example, individuals who experience early social isolation subsequently develop a behavioural condition which shares core features with schizophrenia (Fone and Porkess, 2008). Furthermore, many species have 'critical periods' in which developing individuals must experience specific stimuli in order to develop adult behaviours. Examples include language development in humans, in which individuals must be exposed to language within a window of development to fully develop language abilities (Kuhl et al., 2005) and song tuition in zebra finches, which must occur within a specific period of development for a young bird to properly learn to sing (George et al., 1995).

Due to the importance of social development in group-living animals, the development of communication between individuals is of particular interest, as communication and interactions between individuals is a driving factor of group coordination in many species (Conradt and Roper, 2005). Adults of my study species, the Bronze Cory catfish *Corydoras aeneus*, utilise tactile interactions that seem to facilitate group coordination (Chapter 3) and mediate flight responses from a potential threat (Chapter 4). The usage of tactile interactions in the Bronze Cory catfish is unusual, as tactile stimulation often triggers a stereotyped threat response in fish called a 'c-start' that is deployed involuntarily when an individual perceives an urgent potential threat (Kimmel et al., 1980). The c-start threat response was first reported in detail by (Weihs, 1973) in trout and pike and is characterised by a two stage motor pattern (figure 1). Stage 1 lasts 15–40 msec and is characterised by the ipsilateral contraction of axial muscle on one side of the body (Eaton and Emberley, 1991). The fish orientates away from the threat and resembles a 'C shape' from above at the end of stage one. The head and caudal fin flap lie in the same direction. Stage 2 is characterised by the straightening of the axial skeleton and acceleration caused by the lateral side of the fish displacing water (Eaton et al., 1988). This allows the fish to be propelled forwards in an escape trajectory. The underlying neural command appears to be ballistic, and the reflex is unaffected by sensory information once it is initiated (Eaton and Emberley, 1991). The fact that the Bronze Cory catfish interact tactilely with conspecifics without a c-start response suggests that Bronze Cory catfish must either have an innate trait that stops them from interpreting tactile stimulation as a potential threat, or that they must develop a tolerance to tactile stimulation during development in order to interact with conspecifics.



**Figure 1 | C-Start Schematic Diagram – Dorsal View**

a.) Larva pre C start

b.) Stage 1 – larva adopts 'C shape' during the fast phase.

c.) Stage 2 – larva straightens body and is propelled forwards in an escape trajectory

Furthermore, the Bronze Cory catfish provides a unique study system for investigating the development of social interaction, as patterns of tactile interactions can be observed and responses to tactile interactions can be clearly recorded. This allows us to ask questions about the way individual larvae respond to tactile interactions during development, and about the role of social exposure in developing a tolerance to tactile stimulation. How do individuals develop the ability to interact and coordinate with others, and how do their experiences with conspecifics during early development affect their responses to tactile interactions later on?

I conducted two experiments to investigate the development of communication and sociality in larval Bronze Cory catfish. First, I investigated the ontogeny of the response to tactile stimuli by stimulating individuals within stable groups over regular intervals in the

developmental period. I predicted that older larvae would respond to the tactile stimulation with a c-start less often than younger larvae. I also predicted that individuals that did respond to the tactile stimuli would be more likely to initiate tactile interactions later in development. Second, I investigated the effect of tactile isolation on sociality and communication in larvae that ranged from 14 to 21 days post hatching (dph) in age. I predicted that isolated larvae would spend less time together, participate in fewer tactile communication interactions and be more likely to respond to tactile interactions with conspecifics with a c-start.

## **METHODS**

### **Study subjects**

Adult *C. aeneus* were maintained as detailed in Chapter 3; the adult breeding stock used in this experiment were the same individuals that were used in Chapter 3. The conditions under which fish were kept were ideal for this species (Lambourne, 1995) and the adult fish readily bred under these conditions. Although the exact relatedness of my breeding stock is unknown, they were obtained at different times from different retailers, and there is no reason to believe that the adults were related to one another. Eggs were collected in the morning from stock tanks that had been previously designated for egg collection due to high egg production. This allowed me to maximise the number of full siblings, as all eggs from the same clutch are full siblings due to the unique sperm drinking behaviour of females (Kohda et al., 1995).

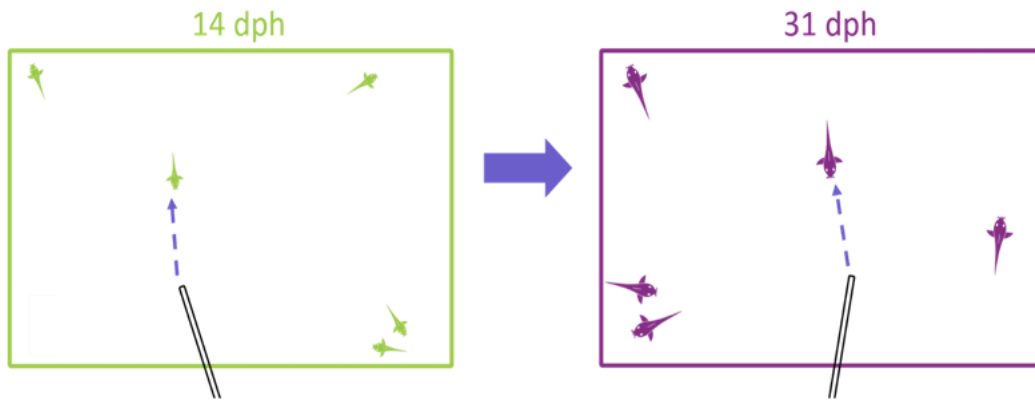
### **Development of Response to Tactile Stimulation**

This experiment was done in two batches, with two weeks in between hatching. Eggs from three clutches per batch were hatched, and larvae were placed with full siblings in groups of five into a transparent, 21L plastic fish tank at 12 days post hatching (dph). Each tank had a small air driven aquarium filter for biological filtration. Larvae were fed “Interpet Liquifry Number 1” and “Interpet liquifry Number 3” daily. Aufwuchs from adult Bronze Cory tanks was introduced for larvae to graze on. In addition, larvae were fed twice a week with live *Panagrellus redivivus*. Larvae were always fed after experimentation on days when they underwent tactile

stimulation. Larvae were given two days to acclimate to their enclosures and were stimulated tactilely on 14, 24, and 31 dph. I chose those time points for developmental reasons. I started filming at 14dph because at that age, larvae of a closely related *Corydoras* species, *Corydoras paleatus*, have definitively entered the pterygiolarval phase and have begun interacting with other larvae; they are also reasonably physically robust (Rodríguez-Ithurrealde et al., 2014) and able to withstand tactile stimulation without risk of injury. Filming ended at 31dph because, in line with the same study, at this age larvae were still in the pterygiolarval phase and had not developed their bony armour yet. For this reason, if larvae exhibited desensitisation to tactile stimulation, it could not be due to lack of sensation or greater defence. I choose 24dph as an intermediate day to assess how the larval response to tactile stimulation develops through this crucial developmental window.

I filmed the groups' response to tactile stimulation from a dorsal perspective (i.e. from above). The order in which the groups were filmed being stimulated was randomly determined. To optimise filming, before tactile stimulation commenced, the water level in the tanks was reduced to 11cm, and the air driven filter removed as was any detritus on the base of the aquarium. The larvae were left for 15 minutes to acclimatise prior to tactile stimulation.

Tactile stimulation was performed with a 25cm long glass rod which tapered to a 1mm blunt end, which was used to tactilely stimulate the larvae. Tactile stimulation was applied by Riva Riley and Thomas Roe, and stimulation was performed in rounds. Each of the five fish in a group was stimulated in turn during a round before the next round commenced. Rounds were separated by a period of at least 60s to avoid stressing the larvae excessively. Stimulation was standardised (figure 2). A test cohort was used to develop the tactile stimulation protocol, from which no data were collected, before the experiment began. During this test cohort period, we practised applying tactile stimulation to the fry and developed a technique that closely matched the level of tactile stimulation delivered by nudges from larval conspecifics. No fry were harmed during the test cohort period, and we gained practise with the tactile stimulation rods so that we could reliably apply tactile stimulation without undue disturbance to the fish.



**Figure 2:** Dorsal view of tactile stimulation protocol dorsal view. The glass rod began at least two body lengths away from the larva being stimulated. The 1mm diameter blunt end was swept toward the larvae along the bottom of the aquarium until it clearly but gently contacted the larva such that the larva was not physically moved from the tactile contact. The rod was then withdrawn and used to tactilely stimulate the next larvae in that round.

This ensured that the tactile stimulations were consistent and reproducible when data were collected. Each group of larvae underwent 10 or 11 rounds of tactile stimulation with each individual receiving tactile stimulation at maximum once per round. Stock tanks containing full siblings for all the experimental groups were maintained under identical conditions so that any deceased larvae could be replaced with full siblings. Over the course of the experiment, 11 larvae died (of either apparent deformities, or no obvious cause) in seven different groups, and were replaced with full siblings from the same clutch at least 24 hours before tactile stimulation was applied.

I scored the immediate reaction of a fish to tactile stimulation with a glass rod and recorded whether a tactile stimulation event led to that fish initiating a tactile interaction with a group-mate(s). I defined the immediate reaction as occurring within the first second after stimulation. Fish either “ignored”, c-started or performed a non-c-start flight response. A c-start was scored if a clear c-shaped body morphology was observed in conjunction with rapid movement. A response was scored as “ignored” if the larva being stimulated remained static. A non-c-start flight response was scored if the larva did not remain static in response to tactile stimulation but also did not undergo a c-start. If a larva responded with a flight response of any

sort (c-start or non-c-start), I also recorded if it subsequently initiated a social interaction with another larva within 5 seconds of the initial tactile stimulation.

An ethogram was developed to score the tactile stimulation of larvae filmed dorsally. Videos were scored by two researchers. Both researchers observed one full session of tactile stimulation (10 rounds of stimulation) together to develop scoring protocol for an ethogram. Four training videos were then scored independently by both researchers. Scores were compared, and inconsistencies resolved by referring to the scoring protocol and reaching a consensus about each interaction. A further 2 videos were scored independently and the scores of each measure were compared. All measures (immediate reaction, initiates an interaction, incidental or non-incidental) were within 90% agreement between both scorers and so the scoring was assessed as being consistent. The remaining videos were randomly divided up between the two researchers and were scored independently.

### **Effect of Isolation on Sociality and Communication**

To investigate the effect of tactile isolation on sociality and communication, larvae were reared in groups of 3-5 or in isolation until testing. All larvae were raised in identical mesh enclosures of roughly 9.5cm diameter suspended in 20L aquariums equipped with an Interpet MINI filters. Each 20L aquarium held 2-6 mesh enclosures. Larvae were tested when they reached the age range of 14-21 dph; as with the previous experiment, we chose an age range at which fish would be reasonably robust and in the early stages of social development (Rodríguez-Ithurralde et al., 2014). After a 30 minute acclimatization period, larval behaviour was filmed from dorsally for a 1-hour period. The camera was fixed 30cm above the water surface. There was no pseudo replication between videos.

I defined a tactile interaction as an event when one fish physically touches another. Interactions were only scored if both fish were on the bottom of the enclosure. Larvae were scored as together if they were within 2 body lengths of each other using the body length of the smallest fish in that group. Time was recorded as soon as two fish were within this distance and were on the bottom. Number of fish in the group was also recorded. Togetherness (i.e. cohesion) was terminated if one fish began side fixating having been on the bottom of the enclosure prior to that. If a fish briefly moved more than two body lengths away from its partner(s) but returned



to within 2 body lengths in less than three seconds, then this was scored as one continuous period of togetherness without a break for the brief separation period. Periods of togetherness were only scored if individuals were together for at least 3 seconds.

Each tactile interaction had an initiator and a receiver. An initiator is the individual whose movement resulted in the interaction. A receiver is the individual who was touched by the movement of the initiator. I recorded the response of initiators and receivers to tactile interactions. A c-start was scored if a clear c-shaped body morphology was observed in conjunction with rapid movement, as is characteristic of the fast phase of the c-start response.

To assess the consistency of scoring protocol, I developed an ethogram to score the interactions between group-members. Videos were scored by Riva Riley and Thomas Roe. Scoring always began 30 minutes after the start of filming. Both researchers observed an hour of randomly selected footage together to develop scoring protocol for an ethogram. Three training videos were then scored independently by both researchers. Scores were compared, and inconsistencies resolved by referring to the scoring protocol and reaching a consensus about each interaction. A further three videos were scored independently and the scores of each measure were compared. All measures (total time together, total number of interactions, total initiator c-starts, total receiver c-starts, total spontaneous c-starts) were within 90% agreement between both scorers and so the scoring was assessed as being consistent. The remaining videos were randomly divided up between the two researchers and were scored independently. One researcher assigned codes for the videos so that the other could score them blind.

## **Data analyses**

I used a General Linear Model (GLM) with quasibinomial error structure to assess the differences between larvae ages 14, 24, and 31 dph in the proportion of tactile stimulation events that resulted in a c-start and that resulted in fish not responding (or ‘ignoring’) the stimulus. For this analysis, the proportion of events that resulted in a c-start is the number of responses resulting in a c-start divided by the total number of tactile stimulation events (i.e. the sum of the responses that resulted in an ignoring response, a non-c-start flight response, or a c-start). Similarly, the proportion of events that resulting in an ignoring response is the number of

ignoring responses divided by the total number of tactile stimulation events (i.e. the sum of responses that resulted in an ignoring responses, a non-c-start flight responses, or a c-start).

Similarly, I used a GLM with quasibinomial error structure to assess the proportion of tactile stimulation events where an individual responded to the stimulus in which the response involved a tactile interaction with another individual. For all analyses, I used group ID as a blocking factor.

I used a GLM with a Poisson error structure to assess the differences between socially-housed and isolation-housed groups in the total interactions each group underwent. I used a linear model (LM) to assess whether socially-housed and isolation-housed groups differed in the amount of time individuals were in proximity to one another. For all analyses, I used group ID as a blocking factor.

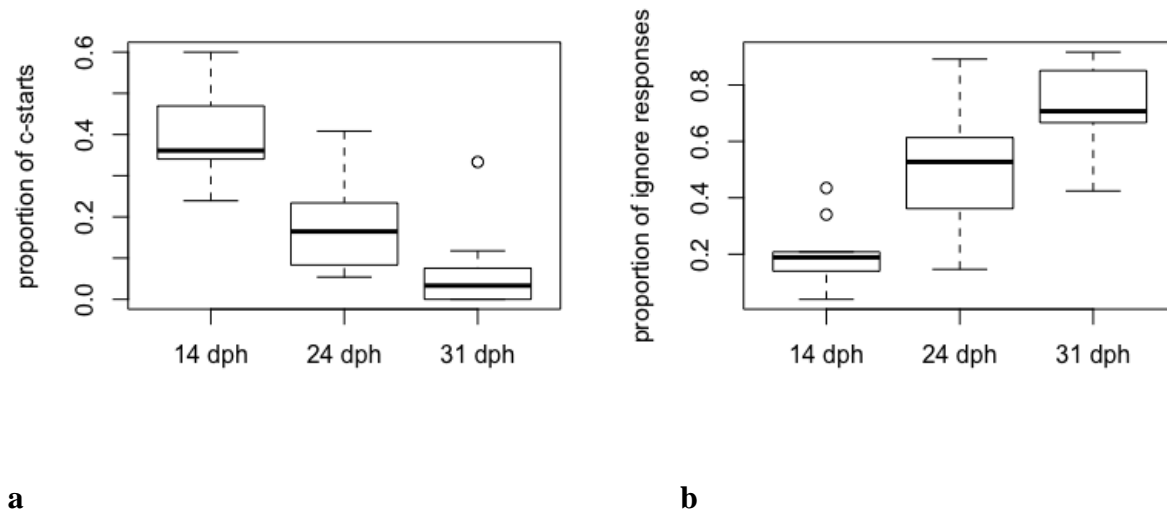
Statistical analysis was performed in R version 3.4.2.

## **RESULTS**

### **Development of response to tactile stimulation**

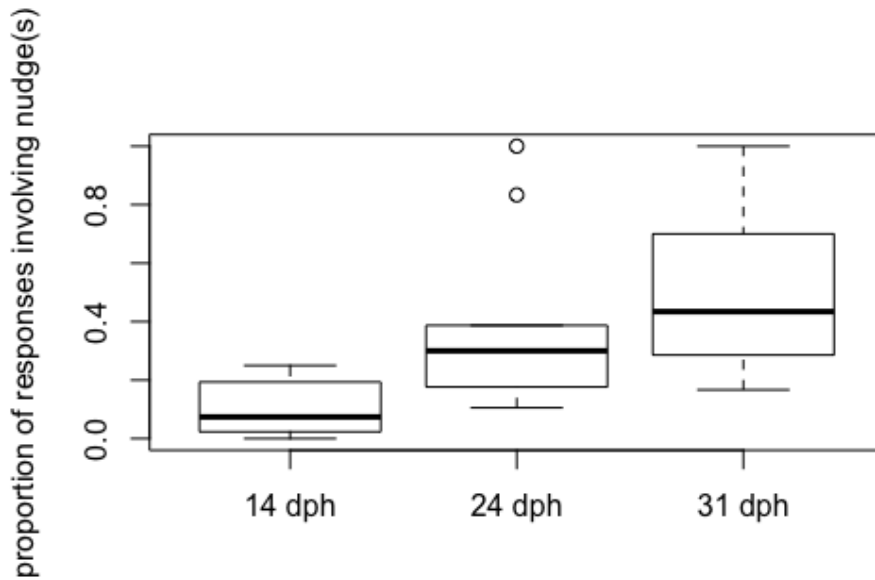
Bronze Cory catfish larvae underwent a behavioural transformation in response to applied tactile stimulation starting from hatching and continuing until about 31 dph. Larvae responded to tactile stimulation with a c-start response at lower rates as they mature (quasibinomial GLM:  $F_{1,19} = 61.8$ ,  $p < 0.001$ , Figure 3a). Furthermore, as larvae matured, they were significantly more likely to respond to tactile stimulation by remaining stationary and

‘ignoring’ the stimulus (quasibinomial GLM:  $F_{1,19}= 44.5$ ,  $p<0.001$ , figure 3b)



**Figure 3:** a) the proportion of tactile stimulation events that resulted in a c-start response in fry days 14, 24, and 31 dph; b) the proportion of tactile stimulation events that results in an ignoring response on days 14, 24, and 31 dph. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5$ IQR, and points beyond the whiskers are indicated as outliers. As fry develop, they become less likely to c-start following tactile stimulation and more likely to ignore the stimulation.

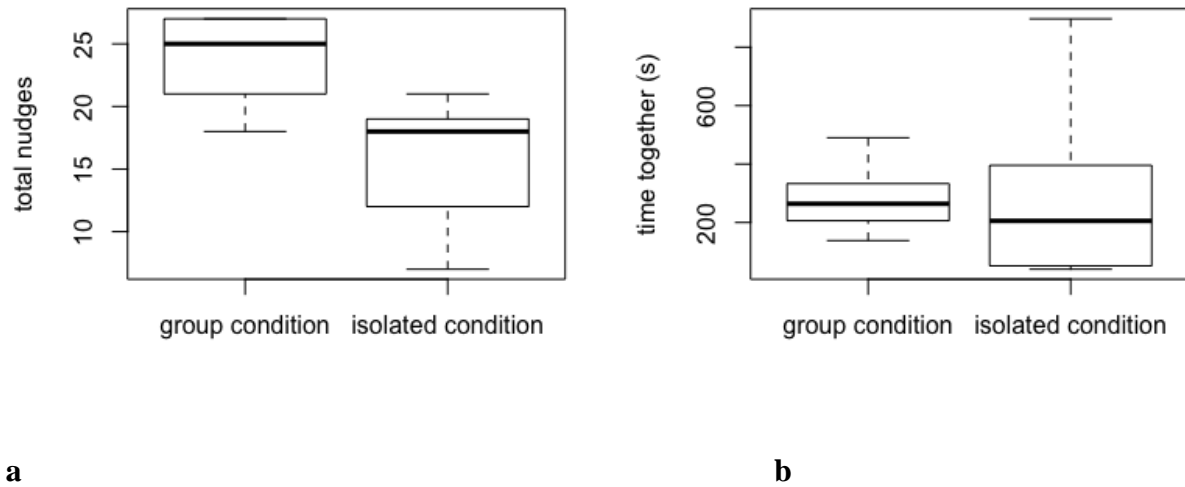
In addition, fry that do respond to a tactile stimulation event were much more likely to nudge their group-mates during their response. As fry mature, the proportion of tactile stimulation events that elicit a response increases significantly (binomial GLM:  $F_{1,19}=16.2$ ,  $p<0.001$ , figure 4). This shows that, if a fry does respond to a tactile stimulation event, it is more likely to initiate nudges with one or more group-mates with increasing age.



**Figure 4:** The proportion of responses involving one or more nudges (number of tactile stimulation events in which fry nudged a group-mate divided by the number of tactile stimulation events) in larvae 14, 24, and 31 days post hatching. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers. As fry develop, they are more likely to nudge group-mates during a flight response.

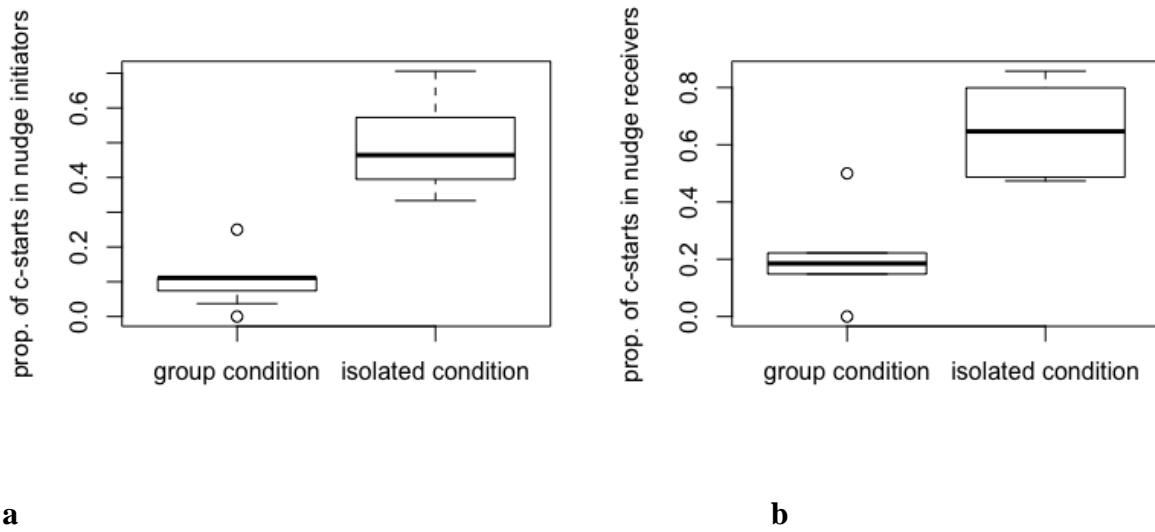
### **The role of social exposure in responding to tactile interactions with group-mates**

Groups consisting of fry raised in isolation exhibited significantly fewer nudges as compared to groups consisting of socially-reared fry (GLM:  $\chi^2_1 = 12.9$ ,  $p < 0.001$ , figure 5a). However, there was not a significant difference in the time groups spent together based on social vs isolated housing (LM,  $F_{1,12} = 0.88$ ,  $p = 0.367$ , figure 5b)



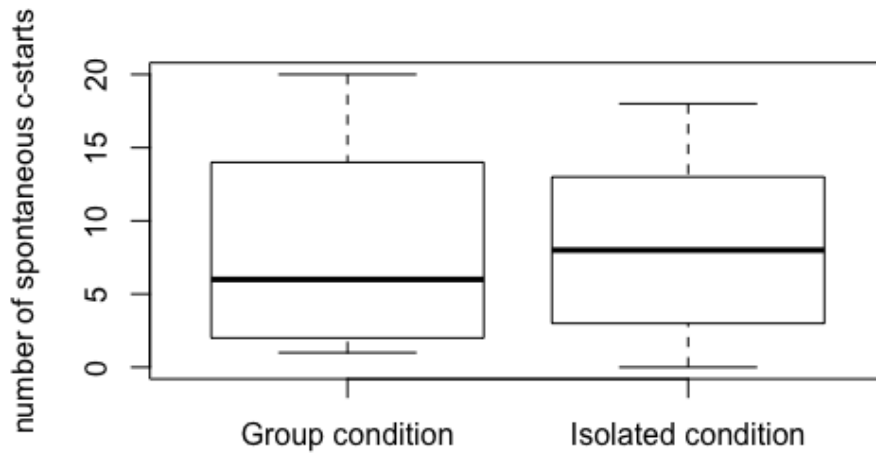
**Figure 5:** a) total nudges in groups of three fry reared socially and fry reared in tactile isolation; b) time together in fry reared social and fry reared in tactile isolation. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers. While total nudges were significantly higher in fry reared in groups, time together did not vary between group- and isolation-housed fry. Therefore, the difference in nudging frequency was not due to lower cohesion in isolation-housed fry.

Fry raised in isolation are also significantly more likely to respond to a nudge with a c-start both when initiating (quasibinomial GLM:  $F_{1,12}=34.0$ ,  $p<0.001$ , figure 6a) and receiving (quasibinomial GLM:  $F_{1,12}=21.3$ ,  $p<0.001$ , figure 6b) a nudge.



**Figure 6:** a) proportion of total interactions in which the initiator c-started following a nudge; b) proportion of total interactions in which the receiver c-started following a nudge. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers. Tactile isolation leads to a higher probability of an individual c-starting following contact with a conspecific, whether the individual initiated or received the nudge.

However, it does not seem that individuals in the isolation condition were merely more reactive generally: the social- and isolation-reared individuals performed a similar number of spontaneous c-starts (Poisson GLM:  $\chi^2_1 = 0.95$ ,  $p = 0.329$ , figure 7).



**Figure 7:** the number of spontaneous c-starts in socially- and isolation-reared groups. Lines inside boxplots indicate medians, boxplot boundaries indicate interquartile range, whiskers indicate  $\pm 1.5\text{IQR}$ , and points beyond the whiskers are indicated as outliers. Individuals reared in tactile isolation were not more susceptible to spontaneous c-starts.

## DISCUSSION

My results show that Bronze Cory catfish larvae are more likely to c-start in response to tactile stimulation when in early developmental stages, and increasingly tolerate tactile stimulation as they develop. My results also suggest that more developed larvae tend to initiate tactile interactions with conspecifics when disturbed by a tactile stimulation event. Increased toleration of tactile stimulation imply that the larvae may have perceived the stimulation as a threat less often with age, may have already developed the ‘freeze’ threat response common in adult threat responses (Chapter 4), or their morphological development could have prevented them forming the c-shape necessary for a c-start to be scored.

Morphological constraints seem unlikely. Previous work on *C. plateaus* found that *Corydoras* larvae are in the late pterygiolarval phase at 33 days post fertilisation (dpf) which is roughly 29dph allowing for a four-day incubation (Rodríguez-Ithurralde et al., 2014). Their cranial bones are still cartilaginous at 33dpf and their morphology is not very robust. It is

reasonable to assume that the 31dph Bronze Cory catfish larvae in this study were at the same stage in development because *C. plateaus* has a very similar adult size to the Bronze Cory catfish and the larvae are a similar size for a given age (*C. plateaus* 10.7mm Standard length (SL) 33dpf, *C. aeneus* 10.0mm SL 31dpf). In *Corydoras arcuatus*, another related, similarly sized species, the cartilaginous precursors to scutes do not develop until larvae are at least 15mm SL (Sire, 1993). The literature therefore suggests that morphological development at 31dph does not restrict the lateral flexion of larvae. It also seems unlikely that larvae would adopt a freeze response to a threat before the development of prominent anterior pectoral fin rays or armoured scutes because they have very little protection from predation (Rodríguez-Ithurrealde et al., 2014) and would rely on camouflage alone. Therefore, it seems that the most likely explanation for the change in response to tactile stimulation is that older larvae were less likely to perceive tactile stimulation as a threat. The ability to control and modulate c-start responses has been observed in archer fish in the context of feeding (Wöhl and Schuster, 2007) and it may be that the modulation of the c-start reflex occurs in Bronze Cory catfish so that tactile interaction is tolerated.

The fact that older larvae tend to initiate more tactile interactions with group-mates when they do respond to tactile stimuli is likely to be due, at least in part, to having greater social exposure with more advanced development. This is consistent with the results of the tactile isolation experiment, in which larvae housed in groups (and which therefore have ample opportunities to interact socially with group-mates) were far less likely to respond to a tactile interaction with a group-mate with a c-start response. This underscores the importance of tactile exposure for these fish, as they were not entirely socially isolated- several isolated fish were kept in mesh enclosures in the same tank and were exposed to chemical cues from other fish kept separately but in the same water. Despite the presence of olfactory social exposure, the lack of tactile exposure led to markedly different behaviours in response to tactile interactions with larval conspecifics. Given the prevalence of nudging behaviours in adult Bronze Cory catfish (as in Chapters 3 and 4), it seems very likely that tactile interactions are vital to Bronze Cory catfish sociality and that tactile exposure to conspecifics is a crucial aspect of social exposure during development. Consequently, it seems that tactile isolation in Bronze Cory catfish leads to similar effects of social isolation in many other species.

Social exposure has been found to influence and ultimately weaken responses to potential threats in paradise fish larvae (Miklósi et al., 1997), as I saw with the developing larvae in my



tactile stimulation experiment. It seems likely that paradise fish larvae became habituated to the continuous presence of larval conspecifics and generalised the experience of conspecifics with exposure to potential predators, leading to a weakened response to predators with increasing social exposure (Miklósi et al., 1997) I observed a similar decrease in response to a potential threat with age in Bronze Cory catfish larvae, which was also likely a result of increased social exposure. It may be that desensitization to tactile stimulation during early development in Bronze Cories may only occur in the presence of conspecifics to ensure that individuals do not erroneously ignore a potential threat. Nonetheless, the fact that older Bronze Cory catfish larvae were more actively social (i.e. initiated more nudges with group-mates) in response to tactile stimulation implies that the effect of social exposure in Bronze Cory catfish is not just limited to the downregulation of a threat response, as it appears to be in paradise fish larvae.

Although my experiment cannot elucidate the long-term effects of tactile isolation, the isolated larvae's increased likelihood of misinterpreting an interaction with a conspecific has parallels in other systems. The behavioural and neurological effects of tactile isolation early in development are well documented in rodent models (Einon and Morgan, 1977; Fone and Porkess, 2008; Makinodan et al., 2012). Impaired sensorimotor gaiting was a symptom of early social isolation in rats and this is one possible explanation for the higher c-start frequency I observed in isolated larvae. In many animals, including rodents (Fone and Porkess, 2008) social isolation early in development permanently alters an individual's social behaviour, rendering individuals incapable of normal social behaviour or cognition. In mice, social isolation in early development has a lasting effect on brain development, but only if mice are isolated during their critical period in development (Makinodan et al., 2012). Because critical periods are so fundamental to the development of social behaviour in many group-living animals, the study of social isolation and its effects in more diverse taxa may have the potential to improve our understanding of some psychological and behavioural disorders, and the role social exposure plays in their aetiology. Indeed, in humans, social isolation is as significant a risk factor as smoking or obesity for morbidity and mortality, effects that have also been observed in animal models (Cacioppo et al., 2011) .

The role of social exposure in the development of adult-like behaviour in Bronze Cory catfish larvae implies that there may be a developmentally vital period during which individual social behaviour develops through social exposure. In the tactile stimulation experiment, the

decrease in c-start frequency seemed to occur gradually over time, with 14 dph larvae c-starting frequently, 24 dph larvae c-starting significantly less frequently, and 31 dph larvae c-starting very seldomly at all. This progression seems gradual over this time period, without a developmental ‘switch’ that very rapidly modifies behaviour, but this window of time may represent a critical period for social skill acquisition. Although this gradual decrease could be the result of acclimation to the tactile stimulus, the result that isolated larvae in the isolation experiment exhibited significantly more c-starts in response to nudges from conspecifics strongly suggests that sustained social exposure leads to the development of toleration for tactile stimulation.

Although my tactile isolation experiment provides evidence that nudging requires tactile exposure to properly develop, it is unclear whether or not there is a critical period of tactile exposure for the development of nudging. In my isolation experiment, the isolation of larvae was confounded when they formed groups of three for filming and the development of isolated individuals was not followed post filming due to the difficulty of tagging larvae. Future work may aim to determine if the effect of tactile isolation I observed in isolated larvae can be overcome by social exposure later in life or if Bronze Cory catfish larvae show a critical period for social exposure. Social behaviour may be plastic throughout a fish’s life, or it could be determined by early developmental environment. If sociality in the Bronze Cory catfish does display a critical period for development, then it is ecologically very important for an individual to have social exposure and interact with other larvae during that critical period. If a larva does not, then it may become unable to interact effectively with the rest of the shoal later in life, potentially misinterpreting the social interactions of conspecifics as a threat. Their readily observable tactile interaction behaviour and response paradigm make the Bronze Cory catfish a compelling model system for investigating the development of social behaviour and its consequences in later life.



## **General Discussion**

Coordination is a fundamental characteristic of sociality, and different groups across the animal kingdom employ diverse strategies to accomplish their goals, which may include predator evasion and foraging, among many other aims (Frank, 1998). The role of individuals varies substantially in different groups, and the mechanisms by which individuals coordinate with one another are often poorly understood, even as characteristics of group behaviour are increasingly well studied. The variety of complexity within animal aggregations is apparent in the example of dolphins hunting a shoal of fish, a scenario in which two very different taxa have formed groups for opposing purposes. The dolphins form groups in order to hunt, in those groups individuals adopt differentiated roles, and the spatial arrangement of individuals is relatively simple and based primarily on the dolphins' reaction to the behaviour of the fish shoal (Gazda et al., 2005). On the other hand, individual fish in the shoal aggregate to avoid predation, each following the same rules for spatial proximity to group-mates, and the group forms sophisticated three dimensional arrangements in space (Katz et al., 2011; Tien et al., 2004). These contrasting examples of social coordination highlight the disconnect between social complexity, group coordination, and individual behaviour: simple individual behaviours can lead to simple social complexity and complex group coordination (Lukeman et al., 2010), and complex individual behaviours can lead to high social complexity and simple group coordination (Gazda et al., 2005). Although group coordination and social complexity can be dramatic and readily observable, individual organisms are the fundamental units in social biology, and individual behaviour is the basis of group behaviour. The role of individual behaviours in determining the features of groups is fundamental to sociality.

## **RESULTS OVERVIEW**

The research laid out in this thesis explores how individual behaviours and characteristics contribute to group coordination in two distinctive systems, three spined-sticklebacks and Bronze Cory catfish. Three-spined sticklebacks are an established model organism with many advantages for investigating group behaviours, including a well-documented boldness assay that returns consistent boldness scores for sticklebacks housed individually (Jolles et al., 2016) as well as an established assay for assessing coordination in pairs (Harcourt et al., 2010; Jolles et al., 2015). My first chapter explores how individual personality and familiarity impact leadership dynamics in pairs of sticklebacks of differing personality. I found that individual boldness scores predict trip initiation likelihoods only in unfamiliar pairs; familiar pairs seem to operate based on leadership dynamics that supersede the effects of individual personalities.

To further investigate the role of individual interactions on other aspects of group coordination, I performed a second experiment with sticklebacks in which triplets of fish were placed in a novel task together. This experiment demonstrates that individual success and position, as well as group cohesion, in the maze is associated with the individual's relative boldness to its group-mates, the average boldness of the group, and the familiarity of the group. It is apparent that familiarity and individual personalities can play a role in determining group behaviours, and the effect of individual boldness on group cohesion is only apparent in familiar groups. This has implications for how familiarity affects groups, and it is possible that familiarity modifies group behaviour in part because individuals in familiar groups know one another's personalities (i.e. behavioural tendencies) and can coordinate accordingly.

I then turned to Bronze Cory catfish, a new system that has been rarely utilised in behavioural research. I observed a unique tactile interaction behaviour in wild Bronze Cory catfish while on fieldwork in the summers of 2011 and 2013. I described this behaviour, termed 'nudging', in chapter three, and used it to investigate how individual interactions shape group coordination. I first established that nudging rates in pairs of fish were significantly higher during coordinated movements than when fish were close to one another but not engaged in a coordinated movement. I then investigated the effects of familiarity on nudging, cohesion, and coordination in pairs and triplets of captive-bred Bronze Cory catfish. Triplets of Cory catfish consisting of one unfamiliar and two familiar individuals exhibit patterns of cohesion based on familiarity, with unfamiliar individuals tending to spend more time alone compared to their

familiar group-mates; nudging rates were the same between familiar and unfamiliar partners, and familiar individuals nudged their unfamiliar partner at a similar rate to their familiar partner. In pairs, however, individuals did not have a choice of partner, and a different pattern emerged. Familiar and unfamiliar pairs displayed similar levels of cohesion and coordination (a measure which could be defined in pairs but not triplets). However, unfamiliar pairs underwent a significantly higher nudging rate than familiar pairs in order to achieve the same level of coordination and cohesion.

The use of nudges has many ramifications for the ecology of wild Bronze Cory catfish, and I next investigated how nudging affected group coordination during a flight response from a potential threat. Increased nudging was associated with a greater probability of group cohesion, which is obviously preferable for a species that forms groups to avoid predation: remaining cohesive following one predator attack would put the group at its best advantage in case of another attack. The increased flight time associated with nudging, which may represent a cost of nudging, certainly resulted in a modification of the group's flight response. It may be that the cost of nudging, increased flight time, is worth the benefit of increased cohesion. Furthermore, the potential spread of social information is of interest. The spread of social information about the presence of a predator occurs in many species, most obviously through alarm calls (Bergstrom and Lachmann, 2001; Schel et al., 2010), and nudging represents a unique case of possible social transmission of information about a potential predator. Unlike an alarm call, an auditory signal that broadcasts information to many individuals (Clutton-Brock et al., 2002; Hollén et al., 2011), a tactile sensory modality can only transmit information from one individual to another for each nudge. This incentivizes individuals to undergo multiple interactions with different group-mates and shows how individual interactions can shape important aspects of group coordination.

Finally, given the relevance of nudging for Bronze Cory catfish coordination, I investigated how nudging develops in larval fish. I found that nudging requires social exposure to properly develop, with larvae raised in isolation responding to tactile contact from a conspecific with an increased likelihood of a c-start response, a reflex that the vast majority of fish use to rapidly escape from predators (Kimmel et al., 1980). Larvae housed socially develop a tolerance to tactile stimulation as they develop; they also develop the tendency to initiate nudges.

## **FAMILIARITY AND PERSONALITY**

Individual personality can play a pivotal role in group coordination. Studies have shown that personality affects individual social behaviour by affecting which individuals tend to lead (Kurvers et al., 2009; Leblond and Reebs, 2006) and how responsive individuals are to the behaviour of their group-mates (Nakayama et al., 2012). Shy three-spined sticklebacks are more sensitive to the behaviour of others, as shy fish are deterred by previous failure to recruit a conspecific and subsequently tend to lead less often, while bold fish do not alter their behaviour based on the behaviour of their partner (Nakayama et al., 2012). Although many species exhibit social behaviour that varies with individual personality, the connection between personality and sociality in sticklebacks has been more difficult to deconstruct. Individual sticklebacks have consistent boldness scores when housed in isolation but fail to perform consistently when housed socially (Jolles et al., 2015). This presents a challenge in investigating how individual personalities influence group coordination and poses interesting questions about how group characteristics affect the individuals within the group.

It is also well-known that familiarity impacts individual behaviour and group coordination in important ways. Familiar groups outperform similar but unfamiliar groups and tend to coordinate more effectively during foraging and predator evasion (Chivers et al., 1995). Moreover, individuals tend to prefer to join groups of familiar rather than unfamiliar individuals (Frommen et al., 2007; Griffiths and Magurran, 1997; Kohn et al., 2015), and they tend to preferentially associate with familiar group-mates while in groups with both familiar and unfamiliar conspecifics (Kohn et al., 2015). My first three chapters all reflect the importance of familiarity on group coordination. In pairs of sticklebacks, an individual that was alone out of cover was far more likely to choose to return to cover as compared to individuals in unfamiliar pairs, which were generally less likely to respond to their partner's absence by returning to cover. This is consistent with what I would expect based on the tendency for individuals to prefer familiar conspecifics, and the tendency of familiar groups to be more cohesive (Chivers et al., 1995). In triplets of sticklebacks, familiarity was necessary for personality to have expected effects on cohesion- groups comprised of shyer individuals, which are generally more sociable than bold individuals, were only more cohesive than bold groups if group-mates were familiar with one another. In triplets of Bronze Cory catfish, I also found that familiar group-mates

exhibited higher levels of cohesion with one another, and the unfamiliar group-mate was more often left alone.

In both of my systems, the effects of familiarity on individuals and groups were apparent. I used three-spined sticklebacks to investigate the interaction between familiarity and personality because there does not exist an assay to test Bronze Cory catfish boldness. In sticklebacks, as in most other systems, the most reliable personality assays require the testing of isolated individuals, and the Bronze Cory catfish does not tolerate isolation, even in the short term. Our attempts to establish a boldness assay in the Bronze Cory catfish led to erratic behaviour and ‘freezing’ in the exposed area of the boldness test, an anti-predator tactic for the cryptically coloured Bronze Cory catfish that confounded the results of the boldness assay commonly used for sticklebacks. It may be possible to establish personality based on behaviours that can be measured in small groups, such as nudging, but this is problematic as I have shown that group members affect one another’s behaviour. Consequently, sticklebacks were the ideal system for investigating how personality and familiarity interact.

I first tested how personality and familiarity affected coordination in pairs of sticklebacks. Individuals seemed to express their personality in different ways depending on whether or not they were familiar with their partner. Unfamiliar individuals seemed to behave in similar ways in the boldness test and the coordination test. However, individuals who were familiar with their partner were not ‘bold’ or ‘shy’ in the same way that they were while alone in the boldness test, but nevertheless personality predicted their response to being alone out of cover in the coordination test. It seems that an individual’s social environment can interfere with the way it expresses aspects of its personality. This has the potential to allow for behavioural plasticity for an individual under different social conditions, which could be highly adaptive for individuals in changeable social environments.

However, while this study showed how individual personality and familiarity affect coordination, it cannot give definitive insight into how individual behaviours affected pair coordination. This was largely due to the experimental set-up, in which individuals were physically separated by a transparent barrier. Fish could see one another and visually react to their partner’s movements but were held in separate spaces. This allowed for more thorough analysis of individual movements but prevented fish from directly interacting. While fish may have been able to communicate or interact through visually accessible means, it was impossible



to discern if fish were actively interacting, and leaders were behaving in ways specifically to recruit their partner to follow them. Fish did exhibit some degree of synchrony, with fish aligning their movements to their partner's and descriptively 'following' one another, but such coordination could have been achieved by one fish observing and following the other without any direct interaction between the two. Therefore, while individual personality and familiarity did impact group coordination, I was not able to assess whether or not individuals actively attempted to affect each other's behaviour.

To further investigate the role of individual interactions in group coordination, I performed a second experiment with sticklebacks in which triplets of fish had direct access to one another as they navigated a novel task as a group. Familiarity also seems to modify the expression of personality in this context. As in the pair experiment, shy fish do not seem to behave as shyly when they are familiar with their group-mates. Shy fish in familiar groups were increasingly successful foragers in groups of increasing boldness, which shows how familiarity might affect individuals of differing personalities. This is also consistent with previous work that shows that shy fish are more sensitive to previous social experience (Jolles et al., 2014), and this may be especially true for familiarity. The fact that personality expression is so different in an individual context as compared to a social one is intriguing for its social implications, and future work is needed to further understand how group dynamics affect individuals with different behavioural tendencies and preferences.

The results of Chapters 1 and 2 add insight to the complex challenges of studying personality across different contexts and demonstrate the multiple axes of potential inconsistency. First, measures of personality are difficult to define, and boldness is no exception. Many different assays purport to assess 'boldness', which presents a clear problem of inconsistency across assays, but even in cases where boldness is defined using the same assay and applied to the same individuals, different contexts lead to different individual and group results with respect to personality (as in Jolles et al, 2017). My stickleback chapters reflect the difficulty of interpreting personality across context: in Chapter 1, under the context of the coordination assay, familiarity did not have any effect on pair synchrony, but in Chapter 2, in a different testing paradigm with groups of three, familiarity interacted with personality to affect group cohesion. There were subtle but important differences in context between these chapters, including the fact the Chapter 2 utilises a problem-solving assay that requires exploration, which,

while related to risk taking, differs subtly from the type of risk taking that was directly assessed by the boldness assay I used.

The subtle differences in my first two chapters emphasize the challenge of coming to conclusions based on an established literature that present different associations between personality and coordination depending on apparently minute differences in context. It is even difficult to be certain of the extent of consistency in personality across social context. Some studies have found boldness to be consistent across social context, as Ward et al, who used a boldness assay related to recovery from a simulated predator attack on socially housed fish and found that the effect of boldness on shoaling tendencies and competition (among other measures) was consistent (Ward et al., 2004b). Other studies, however, have found that housing fish socially eliminates the expression of boldness measured when boldness is tested on fish housed in isolation (Jolles et al., 2016). This marked difference in consistency depending on the boldness assay and specific social context underscore the difficulty in making predictions about the effect of personality on social behaviour based on essential principles of personality. In a similar vein, my stickleback chapters reflect the challenge of untangling the effect of personality on coordination if the effect of personality depends on the experimental design and social circumstances. Further work is necessary to more fully characterize personality and its effect on individual and group coordination, and my work demonstrates the importance of precisely defining social and physical context while investigating personality.

## **THE ROLE OF ACTIVE INDIVIDUAL INTERACTIONS**

### **Three-spined sticklebacks**

The characteristics of an individual can clearly affect the behaviour of the group the individual belongs to, an effect that is particularly apparent in chapter 2, which shows how group mean personality affects cohesion and leadership tendencies. However, for sticklebacks, which I used in my first two chapters, I was not able to identify behaviours that could unambiguously be interpreted as an individual actively trying to interact with another individual. It is of interest to understand how individual interactions shape group behaviour because all groups involve some kind of interaction between individuals, but in sticklebacks we could not observe any type of

directed communication. Similarly, the triplet stickleback problem-solving experiment still had fundamental limitations for understanding how individual interactions affect group coordination, as it was still impossible to discern if leaders were actively trying to influence and recruit followers. This is to say, it is still unclear how and even whether individuals directly interacted in a way that affected coordination. To analyse group coordination, I had to use an individual's relative position with respect to the foraging patch as a proxy for leadership, where individuals that were closest to the foraging patch were defined as the leader because groups moved toward the foraging patch, and whichever individual was closest to the foraging patch was on the forefront of the group's movement. However, this analysis does not shed any light on how individuals interacted or related to one another, and why shy fish were more likely to be on the forefront of group movements in familiar groups of high mean boldness. For example, while shy fish in familiar groups showed increasing success with increasing group average boldness, shy fish in unfamiliar groups showed decreasing success with increasing group average boldness, but the underlying interactions between individuals that led to those results are unclear.

Similarly, the result that group cohesion decreased (i.e. the average distance between pairs of fish increased) with increasing group mean boldness in familiar groups, but not unfamiliar groups, is interesting because it complicates the potential effects of familiarity. Higher boldness is associated with decreased sociability in three-spined sticklebacks (Jolles et al., 2015), so it may just be that groups of higher mean boldness are simply less sociable. However, it is also possible that, in certain circumstances, familiarity does not benefit all of the individuals within the group. In some species, boldness and aggression are correlated, including a livebearing poeciliid (Bourne and Sammons, 2008) and, interestingly, three-spined sticklebacks: bold individuals tend to display more aggression (Bell and Stamps, 2004). It may be that the shyest fish in groups with high mean boldness experienced higher levels of aggression from their familiar group-mates, which leads to a preference to remain further from group-members if given the choice, and particularly when foraging and competing with group-mates for food. Sustained negative prior experience with another individual can lead to aversion instead of preference in many species, including parrotfish, a client of cleaner wrasses which avoid wrasses that ignored them or bit them in the past (Bshary and Schaffer, 2002). This makes intuitive sense in that, if familiarity allows individuals to gather information about their group-mates, and the information they gather is that their group-mates are aggressive, they might prefer to avoid close proximity

those group-mates. Familiarity may then induce aversion instead of preference in cases where an individual has negative experiences while becoming familiar with another individual.

However, I cannot say for certain that negative experiences led to aversion in this experiment. The effects of individual personality on group cohesion were clear, and the effect of group characteristics on individual behaviour and success were clear, but the role of individual behaviours in bringing about these effects was impossible to discern in this system. In sticklebacks, it is possible to track how individuals react to the behaviour of their group-mates by analysing spatial following during collective movements, but the inability to assess (or the absence) of a mechanism by which individuals actively interact with one another puts limits on the questions that can be asked about the individual's role in group coordination. This is similar to other common behavioural systems, including guppies and zebrafish, where so far it has been impossible to identify a specific behaviour that individuals use to interact with one another. In this way, these systems cannot be used to elucidate how individual interactions mediate group coordination.

### **Bronze Cory catfish**

In many bird and mammal species, individuals exhibit clear social role differentiation that makes the effects of individual behaviour on group coordination clear. In birds and meerkats, for example, sentinel behaviour by individuals contributes to the predator evasion function of the group (Bednekoff, 1997; Clutton-Brock et al., 2002), and an obvious individual behaviour (i.e. the alarm call) affects the behaviour of the entire group, which takes cover. In cooperatively hunting animals that exhibit task specialization, individuals within the group behave in differentiated ways that enhance the predatory function of the group (Gazda et al., 2005; Stander, 1992). Individuals in groups can clearly have a specific, profound impact on their group's behaviour, but it is difficult to identify the role of individual interactions with group-mates on group coordination and decision-making because so many animals do not actively interact with one another in apparent ways. The Bronze Cory catfish's discrete, observable tactile interaction style (i.e. the nudge) allowed me to investigate how individuals can behave in ways that influence others, and how that influence contributes to group coordination.

Familiarity has well documented effects in fish shoals, and the Cory familiarity experiment laid out in this thesis presents possibilities for how individual interactions could allow unfamiliar groups to gain the coordination advantages of familiarity. The ability to cope with unfamiliarity seems intuitive for species that can actively interact (or in particular, communicate) with one another: individuals may simply need to communicate more frequently with unfamiliar individuals in order to coordinate effectively, given the lack of acquired social information. Indeed, this is the effect I observed in pairs of Bronze Cory catfish, and unfamiliar pairs underwent more nudges than familiar pairs but exhibited similar levels of coordination. Given how common it is for unfamiliar groups to exhibit poorer coordination than familiar ones, this is a notable effect. This shows that, in some species, individuals can actively interact with one another in ways that affect group coordination. The potential ecological ramifications of this active coordination are important for a species that occupies a changeable environment. Some populations of Bronze Cory catfish, including one population I observed in the Madre de Dios region of the Peruvian Amazon, live in flood plains that seasonally flood (Nijssen in Sands, 1986). During the wet season, previously disjointed waterways become continuous and individuals that had been isolated from one another may come into contact. The ability to coordinate effectively with unfamiliar individuals may therefore be of particular importance to the Bronze Cory catfish, and *Corydoras* catfish in general. Active interaction-mediated group behaviour may also play an important part in mediating group coordination across the many taxa in which individuals actively coordinate with one another.

Considering that the Bronze Cory catfish inhabits a murky, changeable environment (Nijssen in Sands, 1986), it makes sense that their active tactile nudges also seems to confer advantages in the extremely ecologically relevant case of predator evasion. It also seems possible that, in such an environment, leadership (as described in other systems, with one individual at the forefront of a group's movement) is less relevant than broad participation. Individuals likely cannot rely on vision to coordinate (Kohda et al., 2002), and a nudge can only influence one recipient at a time. As a result, all individuals can meaningfully participate in mobilizing a group flight response. This is not the only egalitarian aspect of Bronze Cory catfish biology. One study found that males never compete with one another for courtship, and females simply choose to mate with the male that displayed the most courtship interactions toward her (Kohda et al., 2002). The lack of direct male/male competition is likely reflective of the Bronze Cory catfish's

generally peaceful demeanour: I have never encountered any evidence or even anecdotes of aggression in this species (a noted contrast to sticklebacks, whose reproduction is fraught with male/male competition). Perhaps this general tendency toward pacifism allows the Bronze Cory catfish to use a tactile nudge, which puts the initiator in close range of the recipient and could be risky for more aggressive species, including three-spined sticklebacks. I observed some individuals behaving aggressively in Chapter 2 (where individuals in groups had direct access to one another) and moreover, for sticklebacks aggression is correlated with boldness (Utne-Palm and Hart, 2000). This may help explain the reduced group cohesion in familiar groups of high mean boldness, a situation in which individuals had had the chance to interact over a duration of time and might avoid proximity to group-mates as the result of prior aggression. In this way, it seems logical that general features of an animal's social behaviour would impact the way familiarity affects group behaviour, and the Bronze Cory catfish's peaceful demeanour may also contribute to the ways its interaction patterns alter the effect of familiarity. In triplets, for example, familiarity did not impact pairwise nudging rates while exploring a new area, an individual's likelihood of maintaining cohesion with its group-mates following a threat, or the propensity of individuals to nudge group-mates following a threat. Although additional work involving more direct comparisons are necessary to explore this further, it appears that a lack of aggression and utilisation of a tactile interaction modality may have vital consequences for the manner in which the Bronze Cory catfish is affected by its social environment.

## **DEVELOPMENT OF NUDGING**

The unique behavioural features of the Bronze Cory catfish are fascinating from a natural and life history perspective, and the way that a tactile interaction modality develops is of interest in understanding how this behaviour might have evolved. It is not wholly innate and required social experience to properly develop. This may be a mechanism for specificity- wild Bronze Cory catfish larvae would likely only be touched in a non-threatening way by other Bronze Cory catfish larvae (and potentially the larvae of other *Corydoras* species, as it is likely that Bronze Cory catfish form mixed species schools with other *Corydoras* species as described in Prang, 2007, Paxton, 1997, Sands, 1986, and personal observation) and so should be exposed to multiple nudges before they accept nudging as an interaction and not as a potential threat.

The development of nudging also has ecological and evolutionary ramifications for Bronze Cory catfish, and most likely other *Corydoras* species as well. The Bronze Cory catfish lives in a variety of habitats (small streams to navigable rivers) and, accordingly, a range of group sizes and population densities (Lambourne, 1995). It seems possible that populations of different sizes in different habitats may experience markedly different social environments in early life that could impact their social behaviour. Furthermore, nudging almost certainly occurs in some if not most other *Corydoras* species- I have observed wild-caught *Corydoras elegans* utilizing this behaviour at my field site and groups of *Corydoras panda*, *Corydoras paleatus*, and *Corydoras trilleantus*, among others, using this behaviour in captive settings (personal observation). *Corydoras* species inhabit a wide geographical range in a variety of habitats and water conditions, and differ in various morphological and ecology measures, including their size, the extent to which they are armoured, the volume and turbidity of their habitats, and the amount of predation pressure they experience. It would be interesting to see how nudging varies across and within *Corydoras* species according to ecology, which affects the early social environment, and morphology. In particular, it would be interesting to see if populations of Bronze Cory catfish and other *Corydoras* species that live in more turbid water nudge earlier in development and rely more on nudging due to decreased visibility, and if populations and species living at higher population densities nudge earlier and more (or less) frequently in adulthood.

Finally, the possibility that nudging may have a critical period has implications for Bronze Cory catfish neurodevelopment. The mechanisms by which repeated exposure to nudging leads to tolerance of tactile stimulation could shed light on the neurological and developmental processes that underlie social behaviour. Initiating nudges is a discrete, observable behaviour with a clear response, and consequently the Bronze Cory catfish may be a useful system for investigating how developmental social experience leads to adult social behaviour. Sociality is a fundamental aspect of Bronze Cory catfish biology and understanding how it develops may contribute to our understanding of developmental processes and the evolution of sociality.

## **FUTURE WORK: SOCIAL LEARNING IN THE BRONZE CORY CATFISH**

Clearly identifying when social, rather than asocial, learning has occurred is difficult in many systems. The Bronze Cory catfish provides a system that contains an unambiguous,

discrete social process of active interactions that are initiated by an individual and receive a clear response. These interactions are clearly observable through a physical process, the nudge, that requires social experience to appropriately develop. Nudges indicate how individuals interact with one another and could potentially be used to show how the social behaviours of individuals facilitate the spread of information amongst group-mates. This makes for a potentially fruitful system for the study of different kinds of learning. Conceptual interpretations of social learning have challenged the role of sociality in social learning and contend that the effects on learning introduced through specific social processes, particularly ontogenetical effects, are necessary to truly distinguish social from asocial learning (Heyes, 2012). Assessing the role of active interactions between individuals on learning/demonstrator dynamics, as well as how the social environment during development modifies nudging and social learning later in life, could shed light on the social processes that underlie social learning.

This thesis shows that the Bronze Cory catfish is a promising system for investigating the role of individuals in group coordination. Furthermore, aspects of nudging, including what sorts of information it can communicate, a fuller accounting of when and how it is used, and how nudges might be differentiated to convey different types of information, remain open questions that showcase the Bronze Cory catfish as an ideal vehicle for behavioural ecology research.

## **FURTHER IMPLICATIONS: SOCIAL NICHE CONSTRUCTION**

All social animals are affected by the other individuals in their social group, which comprises an added environmental dimension that individuals must navigate alongside the other aspects of their surroundings. For social animals, the social environment can have a profound effect on an individual's life history and fitness, and the effects of the social environment are determined by the behaviour and characteristics of an individual's group-mates. Familiarity, a major theme of this thesis, is an obvious example of a factor that affects an individual's social environment. An individual's prior exposure and knowledge of its group-mates fundamentally affects how it behaves toward conspecifics, and familiar group-mates elicit less aggression and greater cohesion than unfamiliar ones. Individuals also learn more effectively from familiar conspecifics (Kavaliers et al., 2005; Swaney et al., 2001). Social learning is a vital component of the social lives of animals ranging from sticklebacks (Ostlund-Nilsson et al., 2006) to birds (Slagsvold and Wiebe, 2011) to higher order primates (Whiten, 2000), and variation in an



individual's social environment can have a substantial effect on this vital process. Similarly, the personality of an individual's group-mates can affect individual foraging success and group cohesion, as discussed earlier in three-spined sticklebacks. Social environments can have long-lasting effects on individuals, and the social environment experienced during development can affect dominance later in life (Boag and Alway, 1980). Dominance is also affected by the social environment experienced during adulthood, and in gorillas, the sex composition of a group affects males' cohesion to one another, affiliation with one another, aggression, wounding frequency, and social stability (Robbins, 1996). Sociality has immense benefits for social animals, but it also adds complexity to an individual's life, and different social environments can prove advantageous or disadvantageous to different individuals. In some cases, an individual's social environment can largely, or even entirely, determine its fitness. Species in which an individual is denied access to mates, as in gorillas (Robbins, 1996), or where its reproduction is actively impeded by conspecifics, as in meerkats (Young et al., 2006) and marmosets (Abbott, 1984), are clear examples of individuals suffering from low fitness due to their social environments. On the other hand, individuals can enjoy increased fitness from their social environments, as is the case of baboons, where females with stronger social bonds experience higher offspring survival (Silk et al., 2009).

Discussions of how an animal's environment influences its fitness are often centred on the individual's physical environment, that is, the biotic and abiotic factors that affect its life history, including predation and resource availability: it has been well documented that many animals have the capacity to alter their physical environments in ways that maximize their fitness. Niche construction, a concept embedded in Darwin's writings, is the process by which organisms can alter their environments in ways that increase their fitness. Darwin's classic example was earthworms, which effect immense change on their physical environments (e.g. soil) that positively feeds back on their own biological processes. Modern work on niche construction focuses on how niche construction can drive evolution: when organisms modify their environments, they can determine novel selection pressures (Laland et al., 2016, 1999). Particularly in humans, a special case of niche construction has been described, cultural niche construction. Cultural niche construction describes the process by which cultural transmission can accelerate the spread of behavioural traits through a population in ways that cause populations to modify their environments, and thus selection pressure acting on populations

(Laland et al., 2001). A common example in humans is dairy farming, in which the cultural practice of raising cattle and ingesting dairy selected for lactase persistence in dairy-consuming cultures (Simoons, 1969). Humans undergo a variety of specialized social processes, and perhaps it is unsurprising that specific variations of cultural transmission and cultural niche construction would occur in our species.

However, although the specific case of cultural niche construction may be unique to humans (or very rare in nature), individuals of many species are capable of altering their social environments without necessarily doing so via cultural factors. This is perhaps most obvious in cases where individuals can influence group membership, a phenomenon often mediated by social dominance. When relatively dominant individuals can choose to exile group members, they shape their social environments to their preferences, often to avoid competition. Male lions can evict young males that do not voluntarily disperse (Pusey and Packer, 1987), and banded mongooses of both sexes will drive off subordinates when it is favourable for them to do so (Cant et al., 2001). Regulation of group membership also occurs to maintain social structures, as in elephants, in which female elephants sustain complex matriarchal social structures that require the exile of male calves beyond a certain age (Sukumar, 2006). Modifying group membership is also carried out to improve subordinate individuals' reproductive success, and when subordinate individuals are prevented from interacting with others by a dominant group member, they can form alliances to depose a dominant and alter their social dynamics in advantageous ways, as in the case of chimpanzees (de Waal, 1986). In this way, altering group composition can have a huge effect on individual social environment and fitness.

Changing group membership, often via eviction or by killing undesired group members, is an obvious way to alter the social environment, and can have ramifications for all members of the group, not just the individual or individuals who affected the change in group composition. For example, if a subset of subordinates remove the dominant alpha that drove group decisions, as in the case of chimpanzees, the entire group will be affected by the alpha's absence, for the better if the alpha led the group in ways that were detrimental to most individuals, or for the worse if the alpha led the group in ways that largely benefitted most individuals. Most likely, different individuals performed differently in the original social environment, and the removal of the alpha will have accordingly variable effects on individuals. In this case, removal of the alpha was very risky to subordinates, who could have been injured or killed in the confrontation, and

the risk was only taken by individuals who were extremely constrained in the original social environment and therefore strongly incentivized to modify it. In situations where a dominant individual evicts one or more subordinates, as when a dominant gorilla evicts younger males who might pose a challenge to the alpha's dominance (and ability to mate) later in life, the modification of the social environment is much less costly and is likely to be undertaken more frequently.

It is clear that individuals experience social environments differently, that some individuals can modify their social environments by manipulating group membership, and that changing the social environment can impact an individual's fitness. However, it is little considered that the modification of the social environment can occur through means other than changing group composition. Individuals can also modify their social environments by modifying the behaviour of their group-mates, which can have important consequences for both the individuals affecting social change and the group-mates whose behaviour is being modified. To illustrate this, consider a hypothetical social group with very high aggression between group-members. The high aggression resulted in widespread injuries and reduced vigilance and foraging as a result. If an individual could reduce the aggressive behaviour of its group-mates, it would modify the social environment in a way that benefited not only the individual, but all of its group-mates, who would be in better condition. In this way, it is important to consider that individual behaviours can lead to a sort of 'social niche construction' in which individuals influence the behaviour of their group-mates and thus essentially modify their own social environment. A concrete example of social niche construction comes from vervet monkeys, in which unrelated individuals that participate in grooming with one another are more likely to respond to or receive aid from a request later (Seyfarth and Cheney, 1984). A similar scenario occurs in same-sex sexual behaviour in bonobos, and female bonobos seem to modify their social environment by reducing tension and mollifying potential conflicts by engaging sexually with conspecifics (Parish, 1996).

The ability of individuals to modify the behaviour of their group-mates is present in many species and may essentially modify the social environment an individual experiences. Like niche construction on an animal's physical environment, social niche construction can modify the selection pressures that act on a species' behavioural patterns. The effects of social niche construction have likely shaped the evolution of human behaviour, as well as the behaviour of

our closest relatives. The complexity of human and higher primates (as well as other complex social systems such as certain birds, elephants, and dolphins) sociality, including dominance hierarchies and cooperative structures, among other complicating factors, are fascinating to study, but pose challenges in deciphering the mechanisms of social niche construction and the ways individual behaviours and interactions with conspecifics can shape the social environment.

The Bronze Cory catfish is an excellent system for investigating questions related to the effects of social niche construction due to the presence of a clear behaviour individuals deploy that has important effects on group behaviour (for example, increased cohesion and coordination), but within a social structure that is relatively simple and straightforward to manipulate in experimental settings. Furthermore, as I described earlier, the Bronze Cory catfish is an extremely peaceful species, which allows for broad experimental procedures that cannot lead to unsuitable social groups that could result in unethical conditions for certain individuals. The particular sociality of the Bronze Cory catfish, including its unique nudging behaviour and peaceful nature, position the Bronze Cory catfish as a strong potential system for investigating social niche construction.

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