Investigating shoal dynamics: Behavioural flexibility in the grouping of Trinidadian guppies

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Abstract

Flexibility in behaviour can be crucial to the survival and success of fish in fast-shifting variable environments, both natural and human-induced. Grouping or shoaling behaviours are affected by numerous factors acting in parallel, including evolutionary history, experience, and current conditions, and can be particularly consequential when fish are faced with novel threats. Here, I present studies investigating how previous experience moulds grouping behaviour in Trinidadian guppies (*Poecilia reticulata*). In a first set of experiments (Chapter 2), I investigate how reinforcement training with food reward can alter an individual's preference to shoal with a group of conspecifics. I find that female guppies show some evidence of learning an association between a social cue and reward, but this learning is not transferred to a subsequent test in the form of an increase in shoaling, pointing to the context-dependent nature of learning and flexible grouping. In a second set of experiments (Chapter 3), I examine how prior familiarisation to other individuals influences shoal choice. Previous studies have shown that shoals of familiar individuals are often preferred as grouping partners. Using a binary-choice paradigm, I investigate the preference of female guppies to group with familiar versus unfamiliar shoals. I find no evidence of a familiarity preference in my experiments, though subjects did show a significant preference to join a shoal. I consider potential explanations for this discrepancy with previous work, including population and methodological differences, the context-specificity of learning, and subjects' past experiences. Investigating the flexibility of social grouping and the factors that underlie it can provide valuable insights on more general ecological and evolutionary questions such as how sociality can be modified in the wild under varying selection pressures as well as how different species and populations adapt and learn in fast changing environmental conditions.

Résumé

La flexibilité du comportement peut être cruciale pour la survie et le succès des poissons dans des environnements hautement variables, à la fois naturels et induits par l'homme. Les comportements de regroupement ou de formation de bancs sont affectés par de nombreux facteurs agissant en parallèle, notamment l'histoire évolutive, l'expérience et les conditions écologiques, et peuvent avoir des conséquences particulièrement importantes lorsque les poissons sont confrontés à de nouvelles menaces. Ici, je présente des études examinant comment l'expérience antérieure façonne le comportement de regroupement chez les guppys Trinidadiens (*Poecilia reticulata*). Dans une première série d'expériences (Chapitre 2), j'étudie comment l'entraînement par renforcement avec récompense alimentaire peut modifier la préférence d'un individu à se regrouper avec un groupe de congénères. Je trouve que les guppys femelles montrent des signes d'apprentissage d'une association entre un signal social et une récompense, mais cet apprentissage n'est pas transféré à un test ultérieur sous la forme d'une augmentation du temps passé en groupe, ce qui souligne la nature dépendante du contexte de l'apprentissage et du regroupement flexible. Dans une deuxième série d'expériences (Chapitre 3), j'examine comment la familiarisation préalable avec d'autres individus influence le choix du banc. Des études antérieures ont montré que les groupes d'individus familiers sont souvent préférés comme partenaires de regroupement. En utilisant un paradigme de choix binaire, j'étudie la préférence des guppys femelles à se regrouper avec des bancs familiers ou inconnus. Je ne trouve aucune preuve d'une préférence de familiarité dans mes expériences, bien que les sujets aient montré une préférence significative à rejoindre un banc. J'examine les explications potentielles de cet écart avec des travaux antérieurs, notamment les différences de populations et de méthodologies, la spécificité du contexte de l'apprentissage et les expériences passées des sujets. L'étude de la flexibilité des groupements sociaux et des facteurs qui les sous-tendent peut éclairer des questions évolutives et écologiques plus générales telles que la manière dont la socialité peut être modifiée dans la nature sous diverses pressions de sélection, ainsi que la manière dont différentes espèces et populations s'adaptent et apprennent dans des conditions environnementales en évolution rapide.

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Contribution of authors

Chapter 1

General Introduction

Adithi S. Rao wrote the chapter with guidance and input from Simon M. Reader and Jon T. Sakata.

Chapter 2

Examining shoaling flexibility in Trinidadian guppies using reinforcement training

Adithi S. Rao, Simon M. Reader and Jon T. Sakata designed the study, Adithi S. Rao piloted, conducted the experiment and collected the data. Adithi S. Rao analysed the data and wrote the chapter with input from Simon M. Reader and Jon T. Sakata. All authors approve submission of this study as part of the present thesis.

Chapter 3

The role of familiarity in the grouping decisions of Trinidadian guppies

Adithi S. Rao and Simon M. Reader designed Test 1, Adithi S. Rao piloted, conducted the experiment and collected the data. Nina P. C. Alves and Simon M. Reader designed Test 2 based on Test 1. Adithi S. Rao analysed the data and wrote the chapter with input from Simon M. Reader, Jon T. Sakata and Nina P. C. Alves. Manuscript to be submitted to Ethology. All authors approve submission of this study as part of the present thesis.

Chapter 4

General Discussion and Conclusion

Adithi S. Rao wrote the chapter with guidance and input from Simon M. Reader and Jon T. Sakata.

Contribution to original knowledge

This thesis is manuscript-based, with chapters containing original scholarship and contributions to knowledge. In Chapter 2, I examine grouping flexibility in guppies without changes in external conditions such as competition or predation, providing evidence suggestive of the contextual nature of grouping and the context-specificity of learning. In Chapter 3, I show that our female guppies had no preference for familiar conspecifics, using two different testing approaches and cohabitation durations. As predicted, subjects did show a strong shoaling preference, but this preference did not seem to carry over to familiar conspecifics. This is, to the best of my knowledge, one of the two documented results of a lack of familiarity preference in female guppies towards female shoals of the same size, in the setting of a binary-choice experiment. My work thus provides the basis for exciting future research on the evolution of sociality under changing conditions, offers suggestions for future areas of interest and methodological improvements, and establishes an unconventional experimental set-up that can be utilized to measure learning and shoaling preferences.

Chapter 1

General Introduction

Grouping and social behaviours

Social living is ubiquitous in the animal kingdom, extending between taxa, regions and environmental conditions. However, while social living is widespread, there is incredible diversity in its extent and expression, with variation both across and within species. It extends from simple mutual attraction to more complex behaviours such as joint defence, joint brood care, dominance and submissive displays, and intraspecific communication (Haynes and Yeargan 1999; Korb et al. 2012; Manara et al. 2022). Even in species that engage in relatively fewer social interactions, group living can be pivotal to their fitness, with many species aggregating at least for part of their lives (e.g., Wagner et al. 2008, Lodé et al. 2021). Therefore, social attraction, which promotes proximity among individuals across space and time, is the underlying mechanism of group living (Ward and Webster 2016).

The prevalence of social grouping in nature can be explained by the fitness advantages it confers to individuals. Under the right conditions, individuals can gain fitness benefits such as enhanced anti-predator strategies, access to mating opportunities, and a reduction in the energetic costs of locomotion (Krause and Ruxton 2002; Markham and Gesquiere 2017). In terms of predator protection, associating with a larger group can dilute the likelihood of getting captured, as well as increase the chances of detecting approaching predators faster (Cresswell and Quinn 2011; Sorato et al. 2012). This is both because there are probably a greater number of individuals scanning for threat at any given moment, and because individuals can pool their information to maximize the accuracy of correctly identifying threats - the 'many-eyes effect' (Ward et al. 2011; Hammer et al. 2023). Of course, group living can also arise through other means, such as aggregation around shared resources, and the causes of association can have implications for competition, social recognition and other social interactions (Ebensperger and Hayes 2016).

However, in addition to constraints on grouping such as the inability to keep up with or being excluded from a group, grouping behaviours can carry significant costs for individuals. For example, grouping often creates the ideal set of conditions for increased transmission of parasites between individuals (Schmid-Hempel 2021). This can potentially be made even worse owing to the injury and stress associated with competing group members (Ward and Webster 2016). Other costs of grouping include competition for mates and resources, misdirected parental care, and increased rates of predator attacks (Lachlan et al. 1998; Krause and Ruxton 2002; Wilson et al. 2003).

Because of these costs and benefits to grouping, individuals can maximize net benefits by making choices about joining or leaving a group as well as considering whom to group with, making grouping typically non-random (Pitcher and Parish 1993; Whitehead 2003; Proops et al. 2021). Extensive research on grouping tendencies and preferences has examined the impact of factors such as individual morphology (e.g. body length, colour, size), group characteristics such as parasite load and group size, and characteristics such as relatedness or familiarity with individuals (Krause and Godin 1994; Griffiths and Magurran 1998; Lachlan et al. 1998; McRobert and Bradner 1998; Hoare and Krause 2003; Jones et al. 2010; Patterson and Ruckstuhl 2013).

Grouping is particularly well-studied in teleost fish. Their social structures can range from fully solitary, to simple aggregations of individuals or “shoals”, to polarized and synchronized “schooling” groups (Pitcher and Parish 1993; Miller and Gerlai 2012). In general, fish group preferentially with conspecifics over heterospecifics, individuals of similar body size over ones of varying sizes, and familiar over unfamiliar individuals (Barber et al. 2000; Krause and Ruxton 2002; Griffiths and Ward 2011). This wide spectrum of grouping behaviour is one of many reasons fish are a valuable system in which to study sociality. For example, one can investigate the impact of environmental factors such as food availability and predation pressure on the evolution of social behaviour (Brännäs et al. 2001) or even the co-evolution of multiple traits simultaneously (Endler 1995). In addition, flexibility and plasticity in grouping behaviours can be examined as a function of experience and environment.

There have been past investigations into the flexibility in grouping behaviours and decisions (e.g. Hoare et al. 2004), including in our research group (see e.g. Mühlhoff and Reader 2011, Lindeyer et al. 2015, Swaney et al. 2015, Vassileva 2019). Other studies that have investigated behavioural flexibility in shoaling have focussed on how contextual environmental factors such as predation or competition influence grouping (Krause and Ruxton 2002). For instance, it is known that local ecological factors such as high predation result in increased cohesion and lower variability in behaviour (Ioannou et al. 2017). However, few studies examine the effects of direct manipulation of the costs and benefits experienced with grouping. Vassileva (2019), in particular, found that female guppies’ baseline shoaling propensities can be changed through reinforcement training, which has implications on our understanding of the flexibility in shoaling and its adaptability in the wild. When thinking about whether we can modify individuals' tendency to group, an important consideration becomes that of the factors affecting their preference to shoal in the first place. Since it is known that individuals prefer familiar conspecifics over unfamiliar ones (Griffiths and Magurran 1997a), it raises the question of the presence of such a preference across populations and time periods.

Learning in groups and cue association

A benefit to grouping is the possibility of gaining information and learning from groupmates; this includes socially learning about danger, foraging locations, or even forming social associations that can provide useful information making future encounters more effective (Reader 2016; Ward and Webster 2016). For example, ungulate bighorn sheep (*Ovis canadensis*) are likely to learn about the seasonal distribution of high-quality forage in the form of culturally transmitted information (Jesmer et al. 2018). Similarly, wild meerkat (*Suricata suricatta*) pups learn their prey-handling skills from more experienced adults (Thornton and McAuliffe 2006). In addition to this, learning itself can be shaped through social interaction and grouping. The social intelligence hypothesis for instance proposes that social complexity is cognitively demanding and is therefore a major selective force shaping the evolution of intelligence and large brains in certain groups of extant animals (Shultz and Dunbar 2006; Johnson-Ulrich 2017). There is also some such work on fish species (Triki et al. 2024). Many animals including fish species have been shown to possess a large repertoire of social cognitive skills such as reciprocation, coordination and cooperation, as well as transitive inference (Bshary et al. 2014). Therefore, there is a significant role of learning in grouping itself, as well as learning in turn facilitated through grouping, both of which make learning an important factor to consider.

Social interactions play a crucial role in acquiring and developing learned behaviours (Lefebvre and Palameta 1988) and can certainly help in enabling animals to learn about their environments efficiently and quickly without having to engage in possibly dangerous or energetically costly learning trials (Reader 2016). There are numerous areas where learning may play a pivotal role in the development of fish behaviour, such as learning about predation, foraging, and group member characteristics (Brown and Laland 2001). For instance, juvenile, naïve fish might not initially recognize predators or respond correctly in their presence like experienced individuals would. But through repeated exposure to predators and experienced conspecifics, they are able to improve their anti-predator responses (Brown and Smith 1996; Mirza and Chivers 2000). Similarly, fish are able to learn the location of food patches and the most efficient route to get there directly from conspecifics, with this foraging information diffusing through the population (Laland and Williams 1997; Reader and Laland 2000). It is thus clear that fish are capable of learning not just through trial and error but also through social interaction and observation (Suboski and Templeton 1989; Brown and Laland 2003; Kendal et al. 2018). However, in addition to learning from conspecifics, animals can also learn about their surroundings individually.

An example of this individual learning is spatial learning. Edward Tolman marked the beginning of spatial cognition research on animals with work on maze learning in rats, demonstrating concepts such as latent learning (a type of learning that might not have immediate utility) and cognitive maps (a mental representation of one's physical environment) (Tolman 1948). Rats (*Rattus norvegicus*) can rapidly learn to solve complex problems such as mazes (reviewed in Thinus-Blanc 1996). In the classic set-up, a rat is released into a maze with no reward, where they tend to explore the maze with no set goal. When a reward is later included in the maze, the rats previously exposed to the maze were able to locate the reward much faster than their naïve counterparts; they had learnt the maze and created a cognitive map latently (Wang and Hayden 2021). Studies of maze performance have also been performed in fish species such as guppies (Warren 1957; Odling-Smee and Braithwaite 2003a; Lucon-Xiccato and Bisazza 2017a; Hong et al. 2019; Jones et al. 2023), though typically with an explicit reward. Lucon-Xiccato and Bisazza (2017a) found that guppies are able to solve complex mazes, indicating that this ability might be more common across taxa than previously thought. Similarly, goldfish (*Carassius auratus*) can remember the spatial locations of multiple food patches using landmarks and also use visual cues to orient themselves (Ingle and Sahagian 1973; Warburton 1990; Rodríguez et al. 2021). Indeed, in their natural environments, individuals are exposed to a huge diversity of stimuli from which they must not only extract information about their current spatial location, but also prioritize various cues; however, there is still work to be done to uncover the exact learning and memory mechanisms that underpin this cue prioritization (Healy 1998; Odling-Smee and Braithwaite 2003a).

Familiarity as a determinant of grouping

As discussed previously, there is great diversity in the extent of social organisation across populations and species. This organisation often requires individuals to adapt their behaviours and responses to different individuals that they interact with. For this reason, social organisation is built on the foundation of social recognition, at least to some extent (Mateo 2004). The ability to recognize others allows for differentiation between the numerous individuals in an animal's environment: conspecifics or otherwise. For various animal interactions, including some of the largest aggregations of fish or krill, individuals often only require a primitive level of discrimination and basic social attraction rather than sophisticated recognition systems (Ward and Webster 2016). However, there are other instances of significant complexity in social aggregations; while not necessary for all animal grouping behaviour, this complexity encourages and develops associations among individuals (Kappeler 2019).

Social recognition is thus something individuals of numerous species are capable of. However, the extent to which they are able to recognize and discriminate between conspecifics is variable between species (Wiley 2013; Tumulty and Sheehan 2020). 'True' individual recognition, the ability to discriminate between multiple individuals, involves learning the unique identifying traits of another individual and allocating these characteristics to that specific individual. This type of recognition is especially seen in species that form stable hierarchical societies in which individuals encounter each other repeatedly, making it beneficial to recognize one another (Tibbetts and Dale 2007; Tibbetts et al. 2022). For example, the dwarf mongoose (*Helogale parvula*) is a cooperative mammal that might associate unique characteristics of vocalizations to individuals, showing 'true' recognition (Sharpe et al. 2013). Similarly, ring-tailed lemurs (*Lemur catta*) can form a mental representation of specific individuals by their unique scents (Palagi and Dapporto 2006). The ability to identify others might ultimately depend upon individuals' society structure and species ecology; indeed, it is probably minimally advantageous to identify every individual in large fission-fusion groups where the chances of encountering the same conspecific multiple times is relatively low.

Grouping based on familiarity might act as a time-saving mechanism by releasing some of the constraints on a single individual (Griffiths et al. 2004). Early familiarity studies regarding territoriality mention the 'dear enemy' effect (Ydenberg et al. 1988) where familiar neighbouring territory holders are less aggressive toward one another to instead focus their attention on dealing with unfamiliar intruders. This way, costly aggression can be avoided with familiar existing neighbours who are less likely to represent a threat to territory holders, owing to their existing territory (Tumulty 2022). Familiarity with conspecifics can provide stability to dominance hierarchies through allowing individuals to remember the outcome of previous altercations and avoid further aggression and associated costs (reviewed in Griffiths and Ward 2011). In fact, this even stretches to third-party observers where individuals may settle conflicts faster and with less aggression with familiar opponents that they have observed in previous altercations with other opponents than with unfamiliar counterparts (Johnsson and Åkerman 1998; Arnott and Elwood 2009). There are also other benefits to associating with familiar conspecifics. For example, Swaney et al. (2001) found that familiarity among grouping conspecifics facilitated the social learning of foraging information in guppies. Broadly, stability in social conditions and the reduction in aggression in familiar groups allows individuals to allocate time and energy to other crucial activities such as foraging or anti-predator behaviours (Griffiths and Ward 2011).

Many shoaling teleost species often exert a high level of choice regarding preferred group members. Familiarity is a crucial factor that shapes their interactions (Griffiths and Magurran 1999). However, Ward and Webster (2016) point out that we are still not aware of whether the mechanism underpinning familiarity development in fish involves complex, 'true' individual recognition, or merely the distinction of individuals into the categories of 'familiar' and 'unfamiliar'. These two possibilities are difficult to disentangle by testing for in isolation; the focus of existing research has been largely on the implications of familiarity, rather than the mechanisms that underpin it. Ward et al. (2009) addressed this gap by investigating recognition of familiar conspecifics through a specific form of recognition as well as a broader, group-level label in sticklebacks (*Gasterosteus aculeatus*) and guppies (*Poecilia reticulata*). They found that guppies seem to utilize both general and more specific means of recognition (thereby individually recognizing conspecifics as well as differentiating between groups of conspecifics based on resource and habitat-use cues) using a series of binary-choice experiments. The sticklebacks on the other hand demonstrated no evidence of individual recognition abilities following prior social interactions, instead only using broader labels (Ward et al. 2009). Similarly, Webster and Laland (2020) also found no evidence for individual recognition in three-spine or nine-spine sticklebacks (*Gasterosteus aculeatus* and *Pungitius pungitius*). This could be, in part, attributed to differences in social structures; the sticklebacks live in gregarious fission-fusion social groups that might not favour individual recognition, whereas guppies can form more hierarchical structures under the right conditions (Gorlick 1976; Wilson et al. 2014; Webster and Laland 2020).

Therefore, the body of work examining conspecific grouping preferences with regard to familiarity in teleost species does not exclusively find that fish prefer familiar individuals. Early research indicated that shoals comprising familiar individuals are more cohesive than shoals of unfamiliar conspecifics (Chivers et al. 1995; Ward and Hart 2003), providing anti-predator advantages to all group members. However, Griffiths (1997) found no increase in the preference of European minnows (*Phoxinus phoxinus*) for familiar conspecifics in the presence of a model predator. In theory, since the tendency to associate with familiar conspecifics varies with predator densities (Magurran et al. 1994), the benefits of familiarity should increase with predation threat (Godin et al. 2003). Brown (2002) reported results with rainbowfish (*Melanotaenia* spp.) and posited that in habitats where individuals experience predation risk relatively frequently, it might be adaptive to maintain a consistent preference for familiar individuals regardless of prevailing predation conditions. Kydd and Brown (2009) found that rainbowfish caught in the wild did indeed show a shoaling preference for familiar conspecifics, while captive-reared fish did not. As pointed out by Griffiths and Ward (2011), this follows the idea that domestication and artificial selection may have large impacts on social behaviour and therefore fishes' responses to prevailing conditions such as predation (Solberg et al. 2020), which need to be factored in when considering the implications of this research.

Thesis overview and study species

In this thesis, I study the Trinidadian guppy, *Poecilia reticulata*, to investigate flexibility in shoaling tendencies (Chapter 2) and examine the role of familiarity, which typically refers to the recognition of unrelated conspecifics than an individual has had experience with (Griffiths 2003), in these grouping behaviours (Chapter 3). The Trinidadian guppy is an extremely well-studied tropical freshwater fish (Magurran 2005) and is regularly used to investigate questions on social interaction and learning (Reznik et al. 2001; Song et al. 2001; Swaney et al. 2001; Brown & Laland 2002; Reader et al. 2003; Fox et al. 2024). The guppy is native to Trinidad and Tobago, Venezuela, Surinam and Guyana, though they are also reported to invade freshwater habitats worldwide (Deacon et al. 2011). It is largely their ability to thrive in drastically different ecosystems and environments worldwide and particularly in Trinidad that makes them ideal to study key evolutionary theories (Magurran 2005; for overviews, see Houde 1997, Travis et al. 2014, Reznick and Travis 2019). In Trinidad, these fish are found in numerous rivers and streams, with relatively little mixing between populations. This segregation creates a number of distinct habitats with diverse selective pressures, resulting in the formation of different populations (Magurran 2005).

Guppies generally have a stark preference to shoal with larger, more familiar groups, and individuals known to be poor competitors (Krause and Godin 1994; Metcalfe and Thomson 1995; Griffiths and Magurran 1997a; Lachlan et al. 1998; Morrell et al. 2007). Typically, upstream guppy populations contain fewer predators, higher competition, low primary productivity and high biomass, whereas downstream regions have a higher predator density, lower competition, high productivity, and low biomass. This variation, specifically in aquatic predator abundance, results in rapid evolution of behavioural and morphological traits in the various populations (Reznick et al. 2001). For instance, while downstream guppies shoal more, take fewer risks and are less aggressive to conspecifics, their upstream counterparts are the opposite (Seghers 1974; Templeton and Shriner 2004; Song et al. 2011; Heathcote et al. 2017). These striking differences in sociality can have important implications on other factors such as social learning, grouping, recognition, and the spread of information (Griffiths and Magurran 1997b; Hasenjager and Dugatkin 2016, 2017; Chouinard-Thuly & Reader 2019).

Their tractability in the laboratory setting as well as in the wild is part of what makes them a great model system. Guppies have been extensively studied, giving us valuable knowledge regarding their behaviours, evolution and life history (Templeton and Shriner 2004; Magurran 2005; Reznick and Travis 2019). Literature on their sociality is widespread, including work on dominance, innovation, learning, and information use (Gorlick 1976; Magurran and Seghers 1991; Trompf and Brown 2014; Chouinard-Thuly and Reader 2019). Guppies are known to socially learn in numerous contexts including regarding foraging locations, escape routes, anti-predator defences and mate choice (Dugatkin and Godin 1992a; Brown and Laland 2003; Kelley et al. 2003; Reader et al. 2003; Fan et al. 2022; Earl et al. 2024). Further, their ability to learn and use information from conspecifics differs across populations (Chouinard-Thuly and Reader 2019), and importantly, their behavioural preferences in the wild are preserved in the laboratory (Magurran and Seghers 1994; Huizinga et al. 2009).

In Chapter 2, I utilize food reinforcement training to manipulate the direct benefits of grouping while keeping prevailing conditions such as predation and competition unchanged. With preexisting knowledge of the wide array of variation in their shoaling behaviours in the wild, I ask whether these tendencies can be changed in a directed way. In Chapter 3, I examine familiarity preferences in guppies. While past studies have examined the development of a familiarity preference over short timespans (Griffiths and Magurran 1997a), an open question is whether this can be extrapolated to multiple guppy populations. Using two different experimental set-ups and populations, I measure familiarity preference at two separate time points following cohabitation with conspecifics.

In this thesis, I study only female guppies. Guppies demonstrate sexual and behavioural dimorphism, with differences in size, shape, colour, as well as characteristics such as grouping and sexual behaviours (Magurran 2005). Females have larger and less colourful bodies than males and are known to shoal more often and for longer periods of time (Magurran 2005). They are also more innovative, exploratory, and tend to utilize social information to a greater extent (Reader and Laland 2000; Piyapong et al. 2010; Lucon-Xiccato et al. 2016), making them good candidates in which to study grouping. Additionally, using only females ensures that we might avoid any confounding results caused by sexual interaction.

The guppy's diverse behavioural repertoire including contextual grouping and social information use, as well as their ease of manipulation, makes them a very valuable system to study social behaviour. Here, I attempt to lay the groundwork to answer some of these broader questions by first examining flexibility in shoaling propensities in guppies in the absence of natural drivers such as predation and competition, and then investigating the role of familiarity as a factor affecting this shoaling tendency. Further investigation into these topics can have wide-reaching implications on teleost sociality, potentially giving us more information on the evolution of sociality in the wild and how this changes across populations and conditions.

[See Bibliography, page 83, for Chapter 1 and 4 citation lists]

Chapter 2

Examining shoaling flexibility in Trinidadian guppies using reinforcement training

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Abstract

Grouping behaviours offer widespread benefits to individuals across numerous taxa, such as information transfer, foraging advantages, and predator protection. However, grouping can also come with associated costs such as increased competition and disease transmission, which makes it crucial that individuals "balance" these costs and benefits. Freshwater guppies (*Poecilia reticulata*) exhibit considerable variation in shoaling behaviours, making them an ideal model in which to study these trade-offs. While guppies generally prefer larger shoals, they can form smaller ones or are even solitary under certain predation conditions. We investigated shoaling flexibility in female guppies from two different populations through controlled food reinforcement training. Subjects were rewarded for associating with a shoal versus an asocial cue in two different experiments aiming to vary the direct benefits associated with grouping. Our first experiment was inconclusive, but our second experiment provided some evidence of learning, with subjects approaching the shoal faster than the asocial cue, but not significantly so. However, this effect was only observed during training, and did not translate into a stronger subsequent preference for shoals. Strikingly, despite reinforcement training to approach a shoal, guppies demonstrated a significant decrease in shoaling across preference tests. Thus, guppy shoaling tendencies may be flexible but the conditions under which this flexibility exists are more specific than previously thought.

Introduction

Many species benefit from living in groups for a variety of reasons, including efficient sharing of social information, increased foraging efficiency, and predator avoidance (Krause and Ruxton 2002; Hoare and Krause 2003). The complexity of animal groups varies from basic mutual temporary attraction to stable communities with complex division of labour (Wilson 1971; Ward and Webster 2016), and this has been studied in numerous animal taxa including insects, fish, birds and mammals (Meunier 2015; Couzin 2018). However, grouping is also associated with certain costs, such as increased competition for resources, food, and mates (Pitcher and Parish 1993), as well as heightened disease transmission (Wilson et al. 2003; Schmid-Hempel 2021). To optimize net benefits, individuals often have to be selective in when, with whom, and under what circumstances they group (Pitcher and Parish 1993; Proops et al. 2021). Examining this flexibility in grouping will give us valuable information regarding not only the effects of the environment on behaviour, but also aid in our understanding of the evolution of sociality in the wild.

Group living has been particularly well-studied in teleost fish. Fish generally prefer to group (or ‘shoal’) with larger over smaller groups of conspecifics (Lachlan et al. 1998; Sorato et al. 2012), likely due to the fitness advantages it confers. Freshwater Trinidadian guppies, *Poecilia reticulata*, typically demonstrate high propensities to shoal. They too tend to prefer larger shoals over smaller shoals (Magurran 2005; Cabrera-Alvarez et al. 2017). However, this preference varies across populations, with females observed even as singletons in the Paria river to a maximum group size of around 40 individuals (Croft et al. 2003; Magurran 2005). Geographical isolation and physical barriers between upstream and downstream populations and the consequent reduction in geneflow, differences in predation, biomass and productivity in the wild are proposed to underlie population variation in shoaling propensities (Reznick et al. 2001). Guppies in high predation downstream sites tend to form larger shoals, whereas individuals from low predation upstream sites form smaller, less cohesive groups. This variation in shoaling (partially genetic – see Magurran 2005; Huizinga et al. 2009; Hockley et al. 2014) makes Trinidadian guppies a useful system for revealing the factors that shape sociality.

Much like other species (see Webster and Hart 2006), guppies have been shown to utilize a variety of factors when making grouping decisions, including composition and familiarity of shoal mates and their morphology (body length, colour, size), shoal size, parasite load, and predation risk (Krause and Godin 1994; Griffiths and Magurran 1998; McRobert and Bradner 1998; Jones et al. 2010; Patterson and Ruckstuhl 2013). Recent research has indicated that one of these factors – group size preferences – can be varied by directly manipulating the benefits of grouping using food reinforcement. Vassileva (2019) rewarded subjects with food over 20 trials for joining either a 6-fish or 2-fish shoal and found an increase or decrease in relative preference for the large shoal, despite there being no changes in external factors such as competition and predation. This rapid learning is in line with previous research on fish including guppies, with regard to foraging, orientation, and threat (reviewed in Odling-Smee and Braithwaite 2003; Warburton 2003; Lucon-Xiccato and Bisazza 2017a; Fan et al. 2022).

To examine flexibility in shoaling propensities in female guppies as well as to see if the Vassileva (2019) finding extended to training to approach or avoid a shoal with a modified design, here we manipulated the benefits associated with shoaling using a binary-choice paradigm. Being aware of their broad range of shoaling preferences, we used two different populations of guppies – Paria-origin (from the Paria river in Trinidad) and a "domestic" population. Though there is value in studying both sexes of guppies and their interactions (Lucon-Xiccato et al. 2020), we used exclusively female guppies for our experiments, as females tend to be more exploratory and innovative, shoal more in general, and are more likely to use social information (Lindström and Ranta 1993; Reader and Laland 2000; Lucon-Xiccato et al. 2016). Additionally, using females alone could avoid any possible effects of sexual interaction between individuals, which may impact behaviour (Houde 1988).

We ran two experiments (hereafter 'Experiment 1' and 'Experiment 2'). In Experiment 1 (shoal/no shoal), we tested the shoaling preferences of adult female guppies before, during, and after a 15-day, 30-trial reinforcement training period. We trained guppies to selectively approach or to not approach a shoal of 4 fish, using food as a reward. We chose four fish as the midpoint between the large and small shoals used by Vassileva (2019). A third of the experimental fish were rewarded for approaching the shoal, another third were rewarded for approaching the side without fish or the "isolation side", and the final third served as a control that were rewarded in the middle of the tank. This experiment differs from Vassileva (2019) in that we are training for shoal/no shoal, as opposed to a larger/smaller shoal. In Experiment 2, we used the shoal as a social cue once more but contrasted it with an asocial cue. Based on the results of Experiment 1, Experiment 2 exclusively investigated whether we could increase shoaling preference through food reward. Therefore, all subjects were rewarded when exposed to the social cue and not rewarded in the presence of the asocial cue.

We expected individuals from the domestic population to have a high initial preference to shoal (Krause and Ruxton 2002; Cabrera-Alvarez et al. 2017), which the training would either increase or decrease based on treatment. Rewarding the fish to seek out isolation was predicted to decrease shoal preference while rewarding them to shoal should in turn increase shoal preference. While a similar experiment has been performed with a different strain of domestic guppies (Vassileva 2019), there is a lack of literature on the Paria population and how flexible their preferences might be. While Paria are asocial in the wild (with a median group size of 1) as well as in captivity (Magurran and Seghers, 1991; Seghers et al. 1995; Jacquin et al. 2017), the individuals we used have been housed in group tanks for many generations. For this reason, we expect that they might also prefer shoaling over isolating, pre-training, but to a lesser degree than the domestic strain.

Experiment 1

Materials and Methods

Overview

This experiment consists of two alternating phases – training and testing (Figure 1). In the training phase, subject fish were presented with a shoal tank on one side of their home tank and an empty tank on the other (Figure 2). Fish were rewarded with food for making a particular choice based on pre-assigned treatments. The three reinforcement conditions were reward for approaching the shoal ("shoal-reinforcing"), reward for isolating ("isolation-reinforcing" or moving away from the shoal), and a control treatment. This training took place for 15 consecutive days (2 trials per day for a total of 30 trials). Preference tests took place before (PT0 or initial preference test), during (PT1 and PT2), and after (PT3 or final preference test) training in order to determine initial and post-training shoaling propensity (Figure 1).

Figure 1: Experimental overview.

Test Subjects

We used 24 female guppies, *Poecilia reticulata* (12 Paria-origin and 12 domestic-origin, henceforth referred to as 'Paria' and 'domestics'). Paria-origin guppies were gifts of the Rodd Laboratory (University of Toronto, Canada); descendants of guppies brought from the "Houde" tributary of the Paria river in Trinidad in 2008 (and supplemented in 2016), considered a low-predation locality (Houde 1997; Magurran 2005; Li et al. 2022). Domestic-origin guppies, termed the "greenhouse" strain, were of mixed domestic origin. Given the variation in generation times in this species, it is difficult to calculate the exact number of generations that individuals have been housed in captivity. All subjects were outbred in laboratory conditions in mixed-sex tanks; they had not previously been used in any other studies. They were housed in 150L stock tanks with filters, gravel substrate, a heater, and artificial aquarium plants and a terracotta pot for enrichment. The tanks were maintained at 25^{O}C and a 12:12 light-dark cycle. Weekly 30% water changes with conditioned water and water testing (pH, hardness, nitrites, nitrates, and ammonia) were performed on stock tanks. The fish were fed daily with tropical fish flakes (TetraMin, Tetra, Germany), and supplemented with decapsulated brine shrimp eggs (*Artemia sp.*, Brine Shrimp Direct, USA) three times a week before the experiment.

The subjects in our experiment were housed in trios and placed in home tanks (*l* 40 x *w* 20 x *h* 25 cm; water depth 15 cm). Fish were grouped according to relative body size - with one large, one medium, and one small female in each tank, allowing individual recognition without subjecting fish to potentially stressful marking procedures. Social housing was done to avoid any isolation stress throughout the experiment. Over the course of the experiment, fish were fed TetraMin flake food every three days and on each day preceding a preference test, and a mixture of TetraMin flakes and bloodworms (*Chironomidae* spp., Nutrafin basix, Canada) as the food reward during daily reinforcement training.

Every housing tank contained sand to provide substrate and increase the visibility of the fish. An artificial plant and an inverted terracotta pot for enrichment, a thermometer, as well as a filter and heaters were included. Tanks were lined with opaque white plastic boards to avoid the fish being able to see their reflections or other tanks/conspecifics through the glass. The front of the tanks was divided and marked into 3 sections at different distances from the side of the tank with a shoal - shoaling (S) (within 10 cm of the shoal), a central neutral zone (N) (10-30 cm from the shoal), and isolation zone (I) (within 10 cm of the empty side, 30 cm from the shoal) (Figure 2). The 10 cm shoaling zone was defined as "shoaling" since fish being within 4 body lengths can be classified as shoaling following Pitcher and Parish (1993) and Morrell et al. (2008). The fish were video recorded with a Sony 4K Camcorder from the front for behavioural scoring, the markings were visible clearly from the outside of the tanks. Subjects were given 5-7 days to acclimatize to the new housing set-up before the start of the experiment.

The subject fish were ranked by shoaling tendency (see below) and pseudo-randomly allocated into three groups (shoal-reinforcing group, isolation-reinforcing group, and control) such that initial shoaling tendencies were not different across groups.

Stimulus Fish

28 female stimulus fish originally from the same population stock tanks (14 Paria and 14 domestics) were housed in two groups of 14, in two home tanks of dimensions *l* 30 x *w* 15 x *h* 20 cm, with the water depth at 15 cm. The Paria population stimulus fish were used for the Paria subjects, and the domestics as stimulus fish for the domestic subjects. Housing for stimulus fish was similar to that of the subjects' (including the artificial plant and terracotta pot). We ensured that each 4-fish shoal we used as social stimuli consisted of fish of similar body size range to the subject fish. For each trial, a subset of 4 fish was chosen from the respective population's stimulus fish home tank, and once they were used as stimuli for a particular day, they were mixed back into their home tank to avoid reuse of exactly the same shoal on the same day for the same subject. This set up allows for the same stimulus fish to be used more than once per day but reduces the likelihood of familiarity (or avoidance of a particular individual) from developing between the subjects and stimulus fish. Further, since we use medium-sized shoals, we are potentially less likely to encounter a ceiling effect (caused by guppies' predisposition to shoal with larger groups). An initial strong preference for large shoals could act as a ceiling, preventing detection of a further shoaling increase (see Vassileva 2019).

Preference Test

Preference tests were performed in the subjects' home tanks. A medium-sized shoal (4 fish) was revealed on one side of the home tank, and the tank on the other side remained empty (Figure 2a). In both stimulus tanks, we included airstones so that there was motion on each side. The individual subject was gently separated from its companions with an opaque plastic barrier and covered with a transparent glass cylinder in the centre of the home tank. The companions were constrained behind this barrier for the duration of the test, out of visual contact with the subject and test procedure. After a 5-minute acclimation period during which both stimuli were visible, the subject was released from the cylinder in a single smooth motion. When the fish was released, observation began for 5 minutes (and was video recorded). Latency (time to make a choice - i.e., move into either of the side zones) and overall choice (side on which the subject spent a greater amount of time) was measured. In the 2 days prior to the first preference test, subjects had been habituated to the testing procedure twice daily, for a total of four habituation sessions. This minimized the chance that the initial preference test was a novel and therefore highly stressful situation. Preference tests were performed before the commencement of training, as well as every 5 days of training, to examine changes in subjects' shoaling preferences over time.

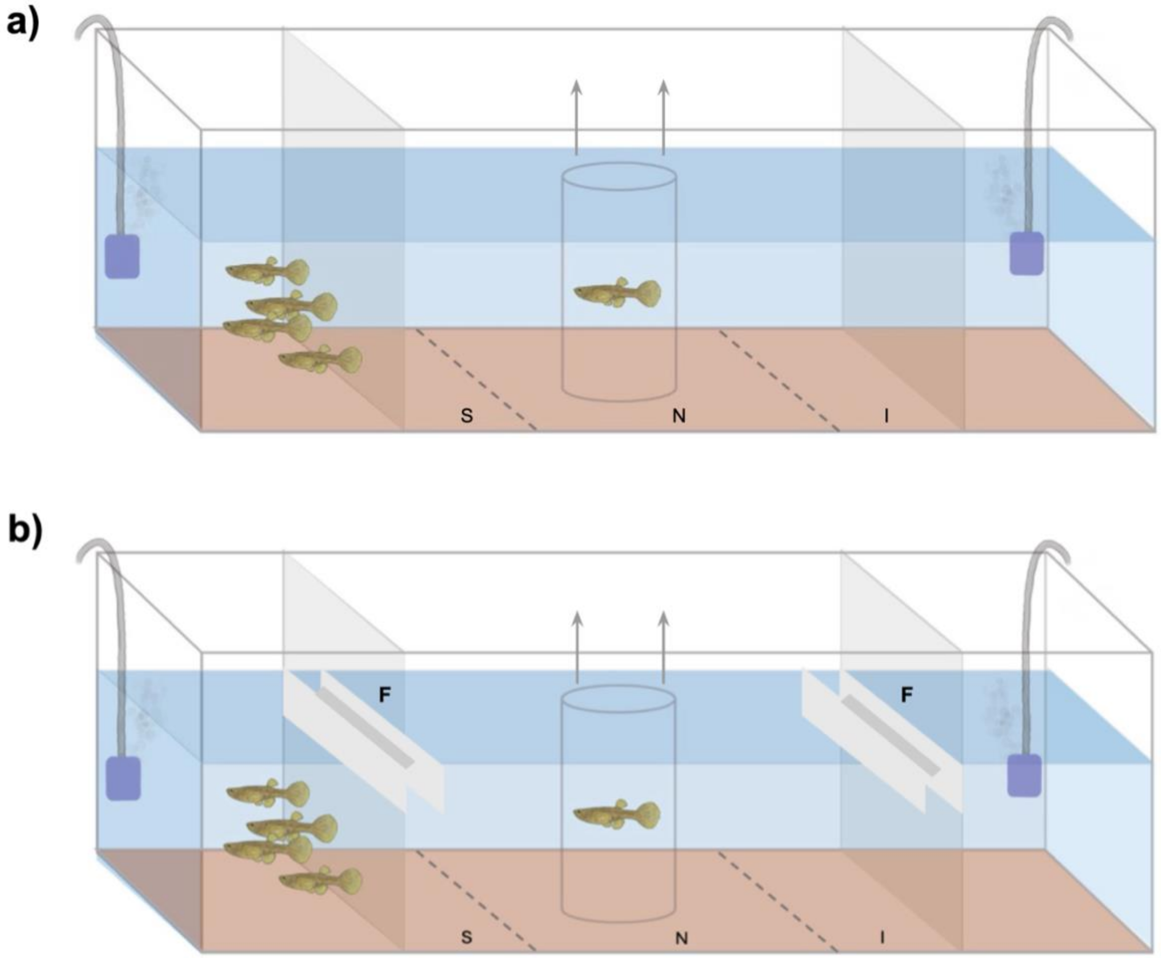


Figure 2: Experiment 1 set-up. *Subject in central cylinder habituating, then released.* ***a) Preference test apparatus*** *with shoal on one side and other side empty.* ***b) Training apparatus*** *identical to preference testing apparatus but including feeders (F) for reinforcement. Reward accessible on reinforcing side, based on pre-assigned treatments. S - shoaling zone; N - neutral zone; I - isolation zone. Airstones on both sides to reduce differences in visual motion.*

Training

For the training, the subjects' home tank was positioned between two stimulus tanks as in the preference tests (Figure 2b). Each subject was tested individually, with its companions (the two other subjects it was housed with) behind an opaque barrier. During training, a shoal of four guppies was revealed on one side of the home tank, and the stimulus tank on the other side remained empty (with airstones on both sides). Following a 5-minute habituation, the subject was rewarded with food for approaching either the shoal or the empty tank (n = 8 per treatment). The control group (n = 8) went through the same "training", but the food reward was administered by placing the feeder in the middle of the tank - the rationale for this was to examine consistency in shoaling propensities and examine the baseline level with identical feeding regimes. This training was done twice daily for 15 days, between 08:00 and 12:00 in the morning, and 13:00 and 17:00 in the afternoon. The location of the shoal was randomized daily, but with the constraint of not being on the same side for more than two days (four trials) in a row.

Instead of utilizing feeders wherein the food was visible to the subjects from the centre of the tank, we crafted a feeder that allowed food to either be accessible or inaccessible through a mesh depending on where in the feeder the food was dropped. On the reward side, the food was made accessible, and the non-reward side had the same amount of food, inaccessible to the subject (in the mesh pocket). This way, the subjects were unable to view the reward before they made a decision, and the set-up equalized any odour cues since the amount of food on both sides was kept constant.

All testing and training was recorded from behind a blind to avoid disturbing the subjects. Immediately following each trial, subjects were reintroduced to their companion fish.

Behavioural Measures and Statistical Analysis

For the preference test we noted the time spent by the subject in each zone. We calculated shoaling preference - a measure of the preference for the shoal, relative to no shoal - by subtracting the time spent on the empty side from time spent with the shoal. This measure takes into account the time spent on both sides, which provides a better overall picture of the subjects' behaviour. The difference between the shoaling preference from before training and after training gives us insight into the effects of the training treatment on the individual's shoaling preference. All statistical analyses were performed in R, with the R base package (R Core Team, 2022), using the lmerTest and lmer4 packages (Bates et al. 2015) to fit the linear and multi-level mixed models. Residual diagnostics were performed to assess model fit, including checks for normality (Shapiro-Wilk test) and homoscedasticity of residuals.

Initially, we compared baseline shoaling preferences within and between the two populations using two-sample and one-sample t-tests. Next, we quantified shoaling preference (time with shoal minus time with empty tank) across four preference tests throughout training and analysed how shoaling preference varied as a function of treatment (shoal-reinforcing, isolation-reinforcing, vs. control), population (Paria vs. domestics) and time or preference test number (PT0, PT1, PT2, PT3) using a mixed effects model (with fish ID as a random factor).

Results and Discussion

We did not observe the predicted shoaling difference between the Paria and domestic populations during the baseline test (two-sample t-test: t(18) = 0.278, p = 0.78) (Figure 3). While domestic population guppies had a significant baseline preference for the shoal (one-sample t-test: t(23) = 3.857, $p < 0.001$), the Paria population individuals did not (one-sample t-test: t(11) = 1.800, p = 0.09).

We next assessed how differential reinforcement for associating with a shoal or with an empty tank (away from shoal) affected shoaling propensities over time in the two populations of guppies. Overall, there were no significant effects of test number, population, treatment or their interactions on shoaling preference. Regardless of the experimental treatment or population, shoaling preferences seemed to decrease across the repeated tests, although this effect was not statistically significant (F(3,71) = 0.728; p = 0.54) (Figure 4).

A pertinent question when using more than one population in a study such as ours is whether there is a difference in their respective baseline behaviours. Given Paria guppies' asociality in the wild (Seghers et al. 1995), we predicted a lower shoaling baseline preference, but did not observe this. This might be attributed in part to the group rearing conditions of the Paria population. However, this population did not show a significant preference to shoal, potentially

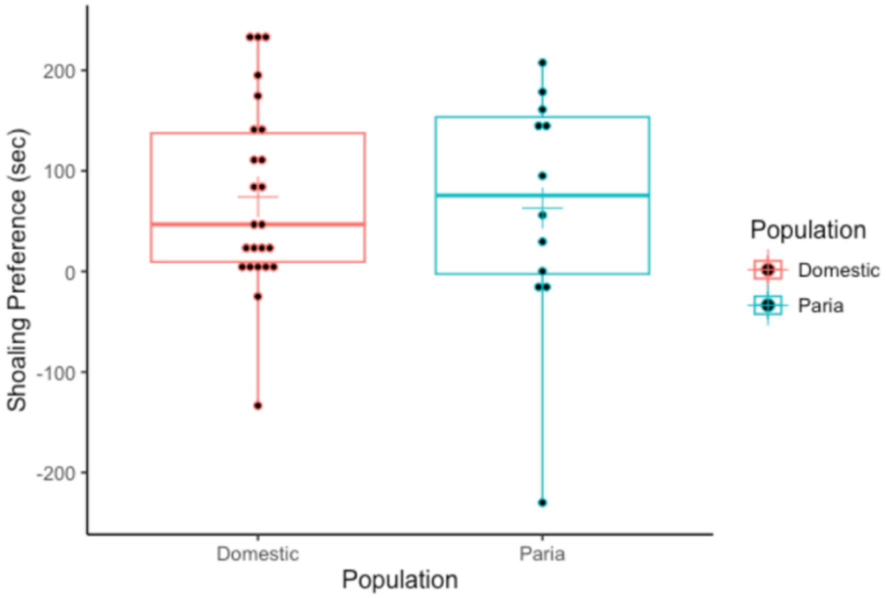


Figure 3: Boxplot of baseline shoaling preference of subjects in Experiment 1, colour-coded by guppy population *- Domestics (red) and Paria (blue). Each dot represents a single subject.*

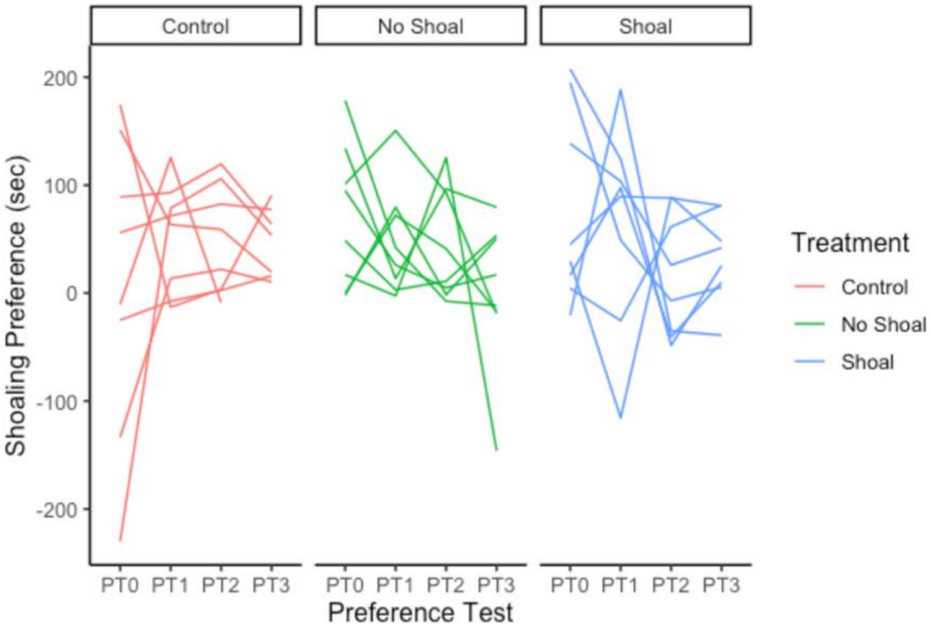


Figure 4: Faceted line graph of shoaling preference (time shoaling minus time on empty side) across 4 preference tests for both populations - domestics and Paria, Experiment 1. *PT0 - initial preference test prior to day 1 of training; PT1 - following 5 days (10 trials) of training; PT2 - following 10 days (20 trials) of training; PT3 - final preference test following 15 days (30 trials) of training. Each line represents a subject, colour-coded by treatment assigned where red - control (n = 8), green - isolation-reinforcing (n = 8) and blue - shoal-reinforcing (n = 8).*

the result of a small sample size. As there was no significant difference in baseline shoaling propensity across both populations, we used a domestic population for all following experiments. While Vassileva (2019) also used domestic guppies, note that it was a different strain.

Although we only tested 8 individuals for each condition in Experiment 1, there is no compelling evidence that the individuals have learned to associate cue (shoal/no shoal) with reward, despite a general reduction in variability in shoaling tendencies as training progressed. This could be due to numerous potential reasons, but we focussed on methodological changes for Experiment 2.

Of the modifications we made in Experiment 2, the most pertinent one was changing the stimuli. While the subjects may have been able to begin associating reward with the shoal in Experiment 1, the no-shoal side might not have been recognizable as a second stimulus. Instead of using shoal/no shoal stimuli as in Experiment 1, we used shoal/Lego blocks. In using blue Lego blocks (Toure and Reader 2022), we hoped to make the contrast between the two stimuli clearer to the subjects. Another key change was training the subjects in the presence of a single cue at a time. Individuals in Experiment 1 seemed to have developed an alternative strategy to access the reward as efficiently as possible. Instead of associating the intended cue with the reward, they appeared to pick a side at random and dart back and forth across the tank until they located the reward in one of the two feeders during training. Instead of exposing the subject to both stimuli and two feeders simultaneously during training, we ran the training trials with each stimulus independently. Since there was now only a single feeder in the tank per training trial, we hoped to strengthen the stimulus-reward association and ensure that the subjects would no longer attempt to swim back and forth between two stimuli as they did in Experiment 1. Finally, to attempt to increase the salience of the food reward and motivation to learn through hunger, we fed the subjects solely during training (and on the evening before the preference tests, since they do not receive food on testing days). Importantly, individuals in Experiment 2 also went through double the number of training trials (4 sub-trials a day per individual) in an attempt to further enhance learning.

Experiment 2

Materials and Methods

Overview

This experiment also consisted of a training and testing phase (Figure 1). There were initially the following conditions planned: “shoal-reinforcing” and “asocial-reinforcing”, with each individual being trained in the presence of both stimuli. In the training phase, subject fish in a cylinder were exposed to a 4-fish shoal, and a 4-Lego block stimulus in an adjacent tank successively, and then released post acclimation. Since we were yet to observe any increase in shoaling propensity (Figure 4), we decided to only run a single condition, and trained all individuals to associate reward with the shoal side. Fish were rewarded with food in the feeder after entering the shoal zone (described below) in the presence of the social stimulus and received no reward in the presence of the asocial stimulus. This training took place twice daily for 15 days (30 trials, 60 sub-trials). Preference tests took place before (PT0 or initial preference test), during (PT1 and PT2), and after (PT3 or final preference test) training to determine initial and post-training shoaling propensity.

Set-up and Housing

Set-up and housing was identical to Experiment 1 of this study, including tank sizes and contents, as well as pre-experiment feeding and cleaning regime.

Test Subjects

This iteration utilized 12 “greenhouse” domestic female guppies (mean body length: X + SE = 20.55 + 0.904 mm). Unlike Experiment 1, the subjects were not fed outside of training. They were fed a mixture of freeze-dried bloodworm and TetraMin flakes during reinforcement training. This was done to increase the salience (not measured) of food, thereby potentially increasing the impact of training. The front of the subject home tanks were marked into 5 sections at different distances - two outermost ‘tight’ shoaling/Lego zones measuring 5 cm from the shoal, two ‘loose’ shoaling/Lego zones a further 5 cm from the shoal (Pitcher and Parish 1993; Morrell et al. 2008), and the central ‘neutral’ zone measuring 20 cm. We recorded both tight and loose shoaling measures (two and four body lengths respectively) to verify the robustness of the measures. Once again, subjects were housed in trios according to body length. Housing and acclimatization was identical to Experiment 1, but the sociality ranking was not required since this experiment only involved a single condition.

Stimulus Fish

The same domestic stimulus fish used in Experiment 1 were also used as shoal fish in this experiment (with 4 replaced before the start of Experiment 2 due to natural deaths over the course of the study). Housing for stimulus fish was similar to that of the subjects' (including the artificial plant and terracotta pot for enrichment).

Preference Test

Preference test procedure for this iteration was identical to Experiment 1, except that the two stimuli were the social (shoal) and asocial (Lego blocks) stimuli, as opposed to shoal and no shoal as in Experiment 1 (Figure 5a). Once again, in the 2 days prior to the first preference test, subjects were habituated to the testing procedure twice daily for a total of four habituation sessions.

Training

Subjects were trained in their home tanks (Figure 5b), after being gently separated from their companion fish using a net and an opaque plastic barrier as separation. Training involved two sub-trials: in one sub-trial the subject was exposed to a 4-fish stimulus shoal in an adjacent container on one side and in the following sub-trial, the subject was exposed to 4 blue one-by-three studded Lego blocks in the adjacent container. For both stimuli, a ring feeder was placed adjacent to the stimulus compartment in the subject's tank.

The subject was initially placed in a glass cylinder (with the feeder and stimulus visible next to it). Once the 5-minute acclimation period had passed, the subject was released. If the subject entered the shoal zone in the shoal stimulus trial, a mixture of bloodworm and TetraFlakes was added to the feeder manually. To feed, the subject needed to swim into the ring feeder. After a 5-minute period, the trial ended. In the non-rewarded asocial stimulus (Lego blocks), the feeder remained empty throughout the sub-trial. For both stimuli, we included airstones to provide motion. The feeder was not removed in the Lego block trials to avoid fish potentially developing an aversion to it. The side as well as order of the stimuli was randomized daily with the constraint that it could not be on the same side for more than two sub-trials in a row. Note that during training, the subject was always only exposed to a single stimulus tank/feeder at a time, and not both simultaneously (as in Experiment 1). Each subject was exposed to the shoal and Lego blocks in pseudorandom order, comprising two sub-trials per trial.

This was repeated twice (four sub-trials) for every subject for 15 days, between 07:00 and 12:00 in the morning, and 13:00 and 18:00 in the afternoon. As before, all testing and training was recorded from behind a blind to avoid disturbing the subjects. Immediately following each trial, subjects were reintroduced to their companion fish.

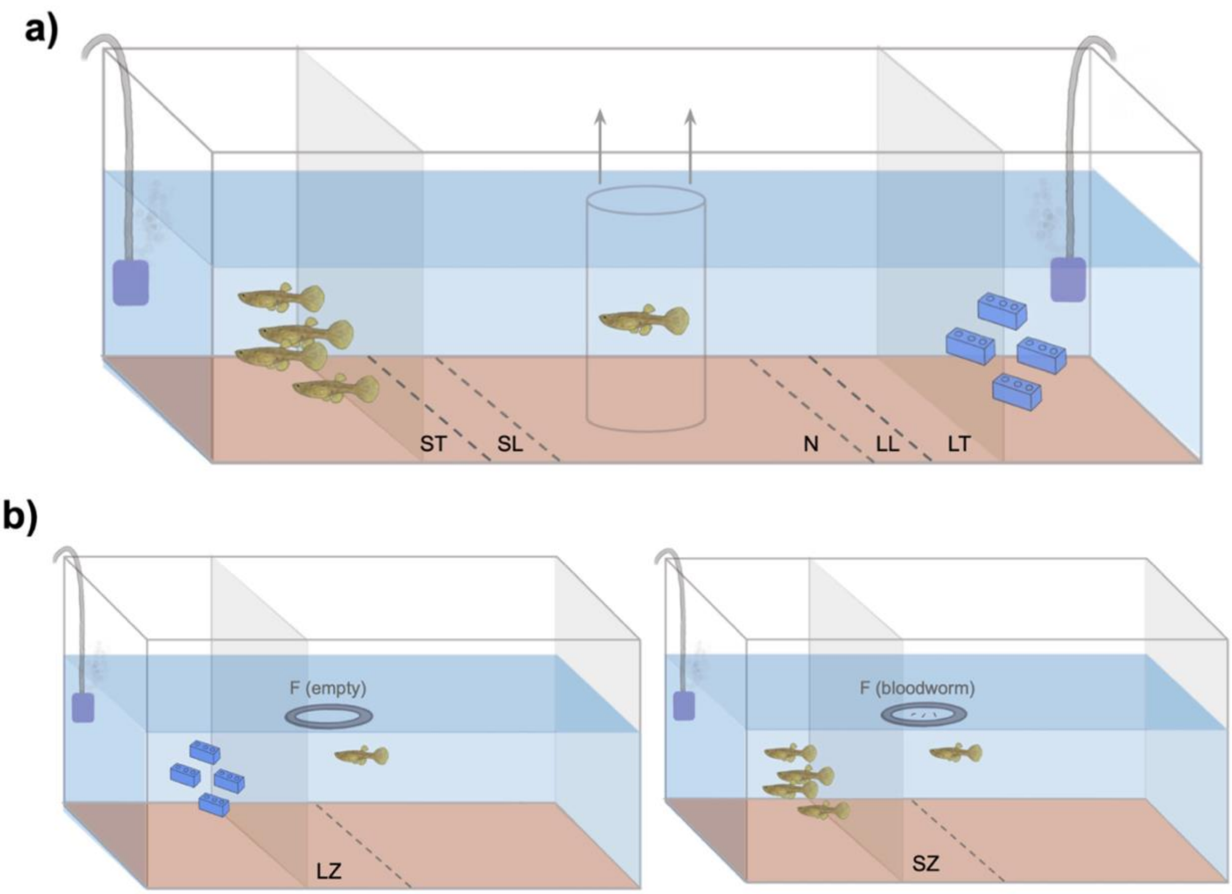


Figure 5: Experiment 2 set-up. *Subject in central cylinder habituating, then released.* ***a) Preference test apparatus*** *with shoal on one side and asocial cue (Lego blocks) on the other. ST - tight shoaling; SL - loose shoaling; N - neutral; LL - Lego loose shoaling, LT - Lego tight shoaling.* ***b) Training***

***apparatus** including feeders (F) for reinforcement. Same subjects trained with each cue individually, successively. Reward dropped into shoal feeder; Lego feeder remains empty. SZ - shoal zone; LZ - Lego zone. Airstones included to control for motion.*

Behavioural Measures and Statistical Analysis

Again, the test subjects were recorded from the front of the tank. Following initial data analysis, the tight and loose shoaling zones were combined; therefore, as in Experiment 1, any instance where the individual was in the 10 cm shoaling zone was defined as shoaling. Once more, we noted time spent in each zone and measured shoaling preference as the preference for the social stimulus, relative to the asocial stimulus. We predicted that the reinforcement to shoal would increase their overall shoaling tendency and therefore the amount of time they spent with the social stimulus in the preference tests.

All statistical analyses were performed in R, with the R base package (R Core Team, 2022), using the lmerTest and lmer4 packages (Bates et al. 2015) to fit the linear and multi-level mixed models, and post hoc Tukey pair-wise comparisons (when applicable) were conducted with the help of the emmeans package (Lenth et al. 2018). Residual diagnostics were performed to assess model fit, including checks for normality (Shapiro-Wilk test) and homoscedasticity of residuals. Where applicable, non-normal distributions were modelled using generalized linear mixed models with an appropriate link function.

We measured shoal preference (time with social stimulus minus time with asocial stimulus) in the four preference tests throughout training and investigated the extent to which shoal preference changed across tests using a mixed effects model (with fish ID as a random factor). The random intercept variance for fish ID was estimated at 75.33 (SD = 8.68). We then ran post-hoc analyses using Tukey's method with a Kenward-Roger degrees-of-freedom adjustment to compare the four different tests (PT0, PT1, PT2, PT3). The estimated marginal means were computed for each level of the 'test' variable in the linear mixed model.

We next explored the effect of the day of training and stimulus (shoal or Lego blocks) on the latency to enter the feeder, with fish ID as a random factor using a linear mixed-effects model followed by pairwise comparisons. The random intercept variance for fish ID was estimated at 0.21 (SD = 0.46).

Finally, to quantify whether for the final preference test behaviour during testing can be affected by trials immediately preceding the testing day, we performed a chi-squared analysis. We classified the response of the subjects in PT3 – i.e., whether they preferred the side that was previously associated with food reward (scored as "1") or the side that was not associated with reward (scored as "0") in the last training trial ("side choice").

Results

In Experiment 2, we exposed subjects to only a single cue (stimulus) and single feeder during each training sub-trial. Specifically, we rewarded subjects with food for approaching a shoal of 4 fish and did not reward them for approaching a group of 4 Lego blocks, the asocial stimulus (30 sub-trials for each stimulus). We observed a significant difference in shoaling preference across tests (Figure 6; mixed effects model: F(3, 33) = 3.705; p = 0.02). Post-hoc comparison results revealed a significant decrease in shoaling propensity between PT0 and PT3 (p = 0.01). No other pairwise comparisons revealed significant differences.

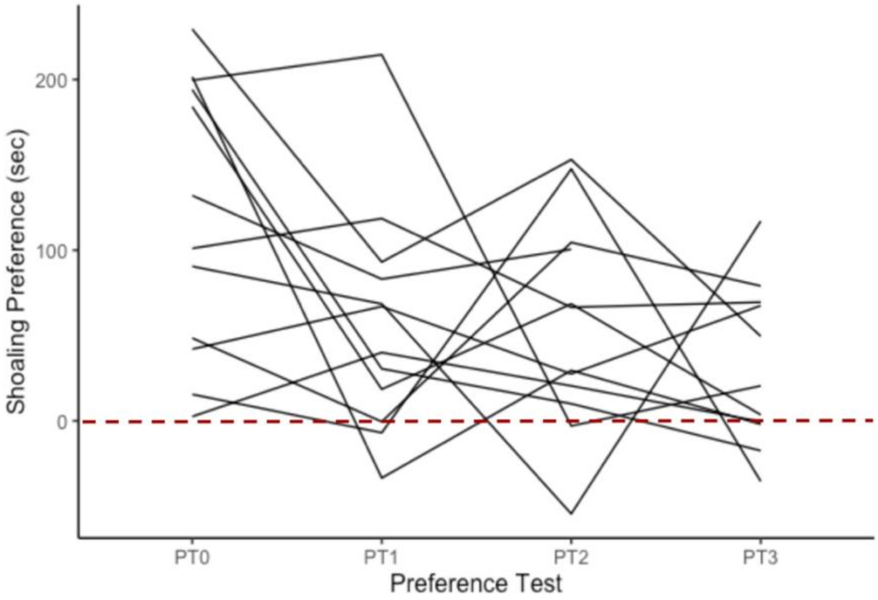


Figure 6: Line graph of shoaling preference (time shoaling minus time on empty side) across 4 preference tests, Experiment 2. *PT0 - initial preference test prior to day 1 of training; PT1 - following 5 days (20 trials) of training; PT2 - following 10 days (40 trials) of training; PT3 - final preference test following 15 days (60 trials) of training. Each line represents a single subject, Domestic population.*

Another measure of learning is a change in the latency to make a choice during training. Therefore, we measured the latency of the subjects to enter the feeder associated with a shoal or the feeder associated with Lego blocks during training days 2, 5, 10, and 15 (the last training day). Video data were not available for day 1. There was a significant effect of day of training on mean latency (F(3,83) = 22.183, $p < 0.001$) with coefficients of -1.487 (day 2 vs. day 5), -1.812 (day 2 vs. day 10), and -1.276 (day 2 vs. day 15), all $p < 0.001$. Latencies were shorter to enter the feeder associated with the shoal than to enter the feeder associated with the Lego blocks, but not significantly so (F(1,83) = 3.649, p = 0.06). There was no significant interaction between day of training and stimulus on the latency to enter the feeder (F(3,83) = 1.032, p = 0.38).

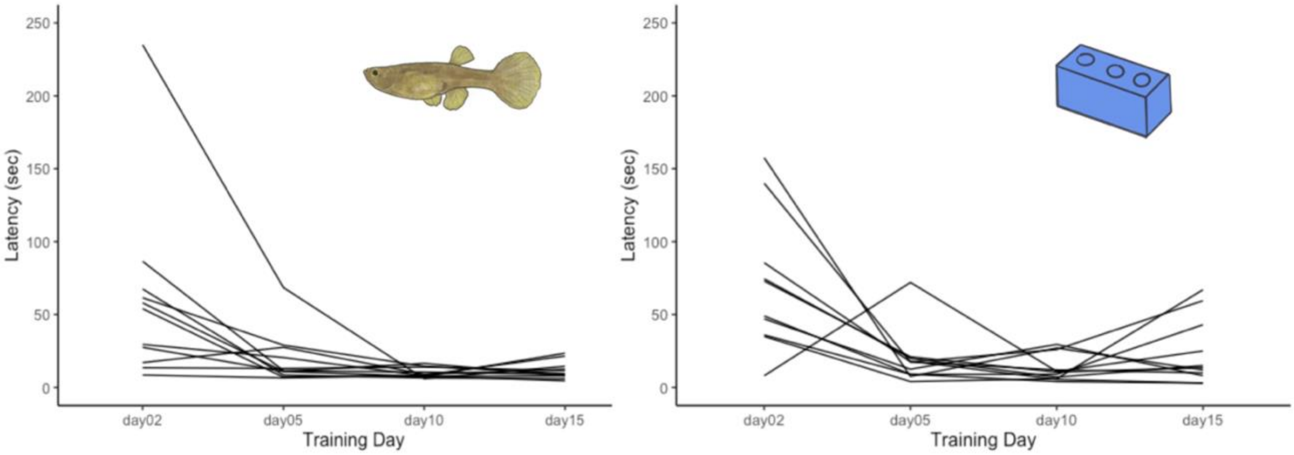


***Figure 7: Line graph of shoaling latency to enter the feeder during training** across the 15 training days, for each stimulus **(left) shoal; (right) Lego blocks (asocial cue) in Experiment 2.** Each line represents a single subject, Domestic population.*

We then investigated for the final preference test whether behaviour during testing can be affected by trials immediately preceding the testing day. We found that in PT3, 11 out of 12 subjects had an overall preference for the side on which they last received food reward, a significant difference from the chance expectation (X2(1, N = 12) = 8.333, p = 0.004).

The full dataset is available at: https://doi.org/10.5683/SP3/LVQKZ2.

General Discussion

In this study, we used reinforcement training to vary the benefits associated with grouping, which was predicted to change female guppies' tendency to shoal. In both our experiments, we found little evidence that female guppies can be trained to selectively approach or not approach a 4-fish shoal. When trained with a stimulus-reward pairing for 15 days, they were unable to learn to associate said stimulus with reward; or perhaps if this association had indeed formed in training, it did not translate to the testing set-up. Some evidence for learning is seen in Experiment 2; although subjects in exposed to both cues got faster to enter the feeder over time, subjects' latency to enter the shoal feeder tended to be lower than the latency to enter the Lego block feeder, though not significantly so. However, when later tested in a preference testing paradigm, this learning does not seem to translate to an increased preference for the shoal.

Vassileva (2019) was able to increase or decrease subjects' preferences for a larger shoal compared to a smaller one using food reward. While the current study and Vassileva (2019) addressed similar questions, some differences exist that could have led to this discrepancy in results. First, Vassileva (2019) contrasted a small and large shoal, rather than here a shoal and no shoal (Experiment 1) or a shoal and plastic blocks (Experiment 2). Further, Vassileva 2019 utilized two cylinders containing the stimulus fish that were simultaneously pulled away from the subject, for the preference test. In my set-up, however, the stimulus fish were placed in a stationary external tank. We know that fish utilize chemical cues and olfaction in conspecific detection (Ward et al. 2002; Gherardi and Tiedemann 2003; Campos et al. 2017). Potentially, the odour of companion fish held behind an opaque partition in the home tank could have also influenced behaviour in the current study. A huge variety of experimental factors can impact data acquisition and replicability of behavioural measures across studies (Jones et al. 2023). There has been work demonstrating that even factors such as tank size (Stewart et al. 2012) and acclimation time (O'Neill et al. 2018) can have effects on behavioural traits like activity and its repeatability. For this reason, it is important to disentangle the effects and biases that various methodological set-ups introduce into a study such as this one.

Strikingly, in Experiment 2, we see a significant decrease in shoaling propensity following training that was intended to increase it. Past work has shown that rapid learning through reinforcement training affects shoaling propensities even in preference tests (Vassileva 2019), but we did not see direct evidence for this in our subjects and populations. While the shoaling preference decrease we saw was surprising, there have been previously reported instances of similar gradual decreases in shoaling over time (Houslay et al. 2019; Schons et al. 2021). We know that guppies quickly habituate to experimental set-ups (Schons et al. 2021), especially following repeated exposure (Houslay et al. 2019). As habituation to the set-up occurs, a reduction in stress levels may well lead to a reduction in shoaling motivation, which is ultimately an anti-predator stress response (Dugatkin and Godin 1992; Botham et al. 2006; Brown and Irving 2013). This decrease in social motivation corresponding to exposure to an unfamiliar tank has also been seen in male guppies in the absence of mating opportunity (Lucon-Xiccato and Bisazza 2017a) as well as other teleost species such as sticklebacks, *Gasterosteus aculeatus* (Thünken et al. 2014; Lucon-Xiccato et al. 2016). It is likely that in our experiment too, repeated trials caused the set-up to become more familiar to the subjects, thereby reducing anti-predator behaviour and motivation to shoal (see Lucon-Xiccato and Bisazza 2017a). A possible future direction for this study would be to perform it with negative training stimulus rather than a preferred one. Using alarm cue, for instance, might increase learning and reduce the likelihood of habituation. Since alarm cue is caused by mechanical damage to the prey epithelial tissue, it is a direct indication of real-time danger and is therefore a highly salient cue (Brown 2003; Chivers et al. 2007; Ferrari et al. 2010; Fan et al. 2022).

Despite the lack of a shoaling increase in our study, there was a behavioural response difference between stimuli, wherein individuals seemed to potentially withhold approaches to the feeder in the Lego condition (in addition to getting faster to reach the shoal feeder, perhaps). In other words, as the latencies begin to decrease as training progresses, we see an increase in approach latencies to the Lego stimulus (but not the shoal) towards the end of training, though this was not significant. This might imply that the subjects are learning that they would only be rewarded in the presence of the social stimulus and are therefore withholding response to the Lego blocks. Note that since rewards were only provided after fish entered a feeder, subjects cannot be following visual or odour food cues.

If this is the case, why, then, does the learning not translate to the preference tests? One potential reason is the presence of a disconnect between training and testing. It is indeed possible that subjects are able to learn to associate the intended cues with reward as seen in training latency differences but are unable to translate that learned behaviour to the preference tests. There could be numerous reasons for this, including this type of learning potentially being more context-dependent than previously thought. Perhaps learning is easier in the context of foraging but does not translate as smoothly to the preference tests, where there is no food reward present. This is in line with research that shows that if an individual learns a stimulus-response-reinforcer relationship in a certain context that is then tested in another context, that same stimulus that modified the behaviour in the first context will have a lower effect in the new one (Gazit et al. 2005).

Finally, in addition to the changing training latencies, we saw some preservation of memory of the last location at which reward was received. In the final preference test of Experiment 2, 11 out of the 12 subjects spent a significantly larger proportion of time on the same side (right/left) they last received a reward on the previous day of training. This memory for spatial cues potentially overrides any learned association with the shoal in training. It seems that while in the training set-up, subjects are able to utilize the association from reinforcement training but when placed in the testing apparatus, they use location instead. There are numerous studies showing evidence of spatial memory retention in guppies (see e.g. De Waele et al. 2022). In changing environments, it is known that animals rapidly incorporate learned spatial and environmental cues to navigate their surroundings. For example, honeybees can locate their hives with the use of spatial features they learnt on a prior flight (Degen et al. 2016), and some bird species can memorize the locations of multiple food caches (Male and Smulders 2007). Spatial learning has also been documented in numerous fish species, examples of which include goldfish (*Carassius auratus*), fifteen-spined stickleback (*Spinachia spinachia*), and butterflyfishes (family Chaetodontidae) (Reese 1989; Hughes and Blight 2000; Rodríguez et al. 2021), with a significant amount of work on mazes (Lucon-Xiccato and Bisazza 2017b). For instance, goldfish can not only locate food reward through using landmarks as indirect reference points, but also remember multiple food patches and use cues to find an endpoint, even when approaching it from a novel direction (Ingle and Sahagian 1973; Warburton 1990; Rodríguez et al. 1994). This indicates that in addition to learning locations, there is a certain degree of prioritization of spatial cues, which could partially explain our results.

Despite the potential context-specificity of learning, this experimental methodology proves beneficial in that it allows one to examine the costs and benefits of grouping through direct manipulation (Vassileva 2019). Our results seem to suggest that learning is taking place in the context of foraging, but a set-up change may be impeding the translation of this learning to the choice test. This study fills a valuable gap in knowledge since though it is safe to say that flexibility in grouping behaviours is likely to exist, the specific circumstances under which it appears are not fully known. Further integration of research investigating grouping behaviour at the individual and population level is necessary to address questions of the extent of learning and in what specific contexts this learning is beneficial, as well as how context plays a role in flexibility and adaptation.

References

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67,* 1-48.

Botham, M. S., Kerfoot, C. J., Louca, V. & Krause, J. (2006). The effects of different predator species on antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*. *Naturwissenschaften*, *93*, 431–439.

Brown, C., & Irving, E. (2013). Individual personality traits influence group exploration in a feral guppy population. *Behavioral Ecology*, *25*(1), 95-101.

Brown, G. E. (2003). Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries*, *4*(3), 227-234.

Cabrera-Álvarez, M., Swaney, W. T., & Reader, S. M. (2017). Forebrain activation during social exposure in wild-type guppies. *Physiology & Behavior*, *182*, 107-113.

Campos, S. M., Strauss, C., & Martins, E. P. (2017). In space and time: territorial animals are attracted to conspecific chemical cues. *Ethology*, *123*(2), 136-144.

Couzin, I. D. (2018). Synchronization: The key to effective communication in animal collectives. *Trends in Cognitive Sciences*, *22*, 844-846.

Croft, D. P., Arrowsmith, B. J., Bielby, J., Skinner, K., White, E., Couzin, I. D., Magurran, A. E., Ramnarine, I., & Krause, J. (2003). Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. *Oikos*, *100*(3), 429–438.

De Waele, H. et al. (2022). Jumping out of trouble: evidence for a cognitive map in guppies (*Poecilia reticulata*). *Behavioral Ecology*, *33*, 1161–1169.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., ... & Menzel, R. (2016). Honeybees learn landscape features during exploratory orientation flights. *Current Biology*, *26*(20), 2800-2804.

Dugatkin, L. A., & Godin, J. G. J. (1992). Predator inspection, shoaling and foraging under predation hazard in the Trinidadian guppy, *Poecilia reticulata*. *Environmental Biology of Fishes*, *34*, 265-276.

Fan, R., Reader, S. M., & Sakata, J. T. (2022). Alarm cues and alarmed conspecifics: Neural activity during social learning from different cues in Trinidadian guppies. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1981).

Gazit, I., Goldblatt, A., & Terkel, J. (2005). The role of context specificity in learning: the effects of training context on explosives detection in dogs. *Animal Cognition*, *8*, 143-150.

Gherardi, F., & Tiedemann, J. (2004). Chemical cues and binary individual recognition in the hermit crab *Pagurus longicarpus*. *Journal of Zoology*, *263*(1), 23-29.

Griffiths, S., & Magurran, A. (1998). Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour*, *56*(3), 689-693.

Hoare, D. J., & Krause, J. (2003). Social organization, shoal structure and information transfer. *Fish and Fisheries*, *4*(3), 269-279.

Hockley, F. A., Wilson, C. A., Graham, N., & Cable, J. (2014). Combined effects of flow condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, *68*(9), 1513–1520.

Houde A. E. (1997) Sex, color, and mate choice in guppies. Princeton, NJ: Princeton University Press.

Houde, A. E. (1988). The effects of female choice and male-male competition on the mating success of male guppies. *Animal Behaviour*, *36*, 888–896.

Houslay, T. M., Earley, R. L., Young, A. J. & Wilson, A. J. (2019). Habituation and individual variation in the endocrine stress response in the Trinidadian guppy (*Poecilia reticulata*). *General and Comparative Endocrinology*, *270*, 113–122.

Hughes, R. N., Blight, C. M. (2000). Two intertidal fish species use visual association learning to track the status of food patches in a radial maze. *Animal Behaviour*. *59*(3), 613–621.

Huizinga, M., Ghalambor, C. K., & Reznick, D. N. (2009). The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, *Poecilia reticulata*. *Journal of Evolutionary Biology*, *22*(9), 1860-1866.

Ingle, D., & Sahagian, D. (1973). Solution of a spatial constancy problem by goldfish. *Physiological Psychology*, *1*(1), 83-84.

Jacquin, L., Dybwad, C., Rolshausen, G., Hendry, A. P., & Reader, S. M. (2017). Evolutionary and immediate effects of crude-oil pollution: depression of exploratory behaviour across populations of Trinidadian guppies. *Animal Cognition*, *20*, 97-108.

Jones, K. A., Croft, D. P., Ramnarine, I. W., & Godin, J.-G. J. (2010). Size-assortative shoaling in the guppy (*Poecilia reticulata*): The role of active choice. *Ethology*, *116*(2), 147-154.

Jones, N. A. R., Cortese, D., Munson, A., Spence-Jones, H. C., Storm, Z., Killen, S. S., Bethel, R., Deacon, A. E., Webster, M. M., & Závorka, L. (2023). Maze design: Size and number of choices impact fish performance in cognitive assays. *Journal of Fish Biology*, *103*(5), 974–984.

Krause, J. D., & Ruxton, G. D. (2002). Living in Groups. Oxford University Press, USA.

Krause, J., & Godin, J.-G. J. (1994). Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): Effects of predation risk, fish size, species composition and size of shoals. *Ethology*, *98*(2), 128-136.

Lachlan, R. F., Crooks, L., & Laland, K. N. (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour*, *56*(1), 181-190.

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). Emmeans: Estimated marginal means, aka least-squares means. *R Package*. *1*(7).

Li, A., Richardson, J. M., & Helen Rodd, F. (2022). Shoaling in the Trinidadian guppy: costs, benefits, and plasticity in response to an ambush predator. *Behavioral Ecology*, *33*(4), 758-766.

Lindstron, K., Ranta, E. (1993). Social preferences by male guppies, *Poecilia reticulata*, based on shoal size and sex. *Animal Behaviour*, *46*, 1029-1031.

Lucon-Xiccato, T., & Bisazza, A. (2017a). Complex maze learning by fish. *Animal Behaviour*, *125*, 69-75.

Lucon-Xiccato, T. & Bisazza, A. (2017b). Sex differences in spatial abilities and cognitive flexibility in the guppy. *Animal Behaviour*, *123*, 53–60.

Lucon-Xiccato, T., Dadda, M., & Bisazza, A. (2016). Sex differences in discrimination of shoal size in the guppy (*Poecilia reticulata*). *Ethology*, *122*, 481-491.

Lucon-Xiccato, T., Gatto, E. & Bisazza, A. (2020). Male and female guppies differ in problem-solving abilities. *Current Zoology*, *66*, 83–90.

Magurran, A. E. (2005). *Evolutionary ecology: the Trinidadian guppy*. Oxford University Press, USA.

Magurran, A. E., & Seghers, B. H. (1991). Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour*, *118*(3-4), 214-234.

Male, L. H. & Smulders, T. V. (2007). Memory for food caches: not just for retrieval. *Behavioral Ecology*, *18*, 456–459.

McRobert, S. P. & Bradner, J. (1998) The influence of body coloration on shoaling preferences in fish. *Animal Behaviour*, *56*, 611–615.

Meunier, J. (2015). Social Immunity and the evolution of group living in insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1669), 20140102.

Morrell, L. J., Croft, D. P., Dyer, J. R., Chapman, B. B., Kelley, J. L., Laland, K. N., & Krause, J. (2008). Association patterns and foraging behaviour in natural and artificial guppy shoals. *Animal Behaviour*, *76*(3), 855-864.

O'Neill, S. J., Williamson, J. E., Tosetto, L., & Brown, C. (2018). Effects of acclimatisation on behavioural repeatability in two behaviour assays of the guppy *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, *72*, 1-11.

Odling-Smee, L., & Braithwaite, V. A. (2003). The role of learning in fish orientation. *Fish and Fisheries*, *4*, 235-246.

Patterson, J. E., & Ruckstuhl, K. E. (2013). Parasite infection and host group size: a meta-analytical review. *Parasitology*, *140*(7), 803-813.

Pitcher, T. J. Parrish, J. K. (1993). Functions of Shoaling Behaviour in Teleosts. In T. J. Pitcher (Ed.), The Behaviour of Teleost Fishes (pp. 294-337). Boston, MA: Springer US.

Proops, L., Troisi, C. A., Kleinhappel, T. K., & Romero, T. (2021). Non-random associations in group housed rats (*Rattus norvegicus*). *Scientific Reports*, *11*(1), 15349.

R Core Team (2022). *R: A Language and Environment for Statistical Computing, 2022.* R Foundation for Statistical Computing.

Reader, S. M., & Laland, K. N. (2000). Diffusion of foraging innovations in the guppy. *Animal Behaviour*, *60*, 175-180.

Reese, E. S. (1989). Orientation behavior of butterflyfishes (family Chaetodontidae) on coral reefs: spatial learning of route specific land- marks and cognitive maps. *Environmental Biology of Fishes*. *25*(1), 79–86.

Reznick, D., Butler Iv, M. J., Rodd, H., & Dolph, S. (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *The American Naturalist*, *157*, 126-140.

Rodríguez, F., Durán, E., Vargas, J. P., Torres, B. & Salas, C. (1994). Performance of goldfish trained in allocentric and egocentric maze procedures suggest the presence of a cognitive mapping system in fishes. *Animal Learning and Behaviour*, *22*, 409-420.

Rodríguez, F., Quintero, B., Amores, L., Madrid, D., Salas-Peña, C., & Salas, C. (2021). Spatial cognition in teleost fish: strategies and mechanisms. *Animals*, *11*(8), 2271.

Schmid-Hempel, P. (2021). Sociality and parasite transmission. *Behavioral Ecology and Sociobiology*, *75*(11), 156.

Schons, R. F., Vitt, S. & Thünken, T. (2021). Environmental habituation and sexual composition affect juveniles' shoaling activity in a cichlid fish (*Pelvicachromis taeniatus*). *Journal of Fish Biology*, *99*, 1307–1317.

Seghers, B. H., Shaw, P. W., & Carvalho, G. R. (1995). The behavioral diversity and evolution of guppy, *Poecilia reticulata*, populations in Trinidad. *Advances in the Study of Behavior*, *24*, 155-202.

Sorato, E., Gullett, P. R., Griffith, S. C., & Russell, A. F. (2012). Effects of predation risk on foraging behaviour and group size: adaptations in a social cooperative species. *Animal Behaviour*, *84*(4), 823-834.

Stewart, A. M., Gaikwad, S., Kyzar, E., & Kalueff, A. V. (2012). Understanding spatio-temporal strategies of adult zebrafish exploration in the open field test. *Brain research*, *1451*, 44-52.

Thünken, T., Eigster, M., & Frommen, J. G. (2014). Context-dependent group size preferences in large shoals of three-spined sticklebacks. *Animal Behaviour*, *90*, 205e210.

Toure, M. W. & Reader, S. M. (2022). Colour biases in learned foraging preferences in Trinidadian guppies. *Ethology*, *128*, 49–60.

Vassileva, I. (2019). *Assessing Flexibility in Shoaling and Learning in the Trinidadian Guppy*. MSc Thesis, McGill University, Montreal.

Warburton, K. (1990). The use of local landmarks by foraging goldfish. *Animal Behaviour*, *40*, 500-505.

Warburton, K. (2003). Learning of foraging skills by fish. *Fish and Fisheries*, *4*(3), 203-215.

Ward, A. J. W., Axford, S., Krause, J. (2002). Mixed-species shoaling in fish: the sensory mechanisms and costs of shoal choice. *Behavioral Ecology and Sociobiology*, *52*(3):182–187.

Ward, A., & Webster, M. (2016). Sociality: the behaviour of group-living animals. Berlin, Germany: Springer.

Webster, M. M., & Hart, P. J. (2006). Subhabitat selection by foraging threespine stickleback (*Gasterosteus aculeatus*): previous experience and social conformity. *Behavioral Ecology and Sociobiology*, *60*(1), 77-86.

Wilson, E. O. (1971). The insect societies. Cambridge, Massachusetts, USA, Harvard University Press [Distributed by Oxford University Press, USA].

Wilson, K., Knell, R., Boots, M., & Koch-Osborne, J. (2003). Group living and investment in Immune Defence: An interspecific analysis. *Journal of Animal Ecology*, *72*(1), 133–143.

Linking Statement to Chapter 3

In Chapter 2, I found that female guppies (*Poecilia reticulata*) did not change their shoaling propensity as predicted by 15 days of reinforcement training. Despite some evidence that individuals were indeed learning the association between cue and reward, this did not translate to a shoaling preference test, where shoaling tendency was instead significantly reduced. Flexibility in behaviours can confer numerous fitness benefits to individuals in fast changing environments; however, it is likely that the circumstances under which this flexibility appears are more specific than previously thought. The two experiments I performed in Chapter 2, in addition to existing work on shoaling, then raise the question of the factors that influence this flexibility and shoaling tendency, and how this changes across populations of the same species. In Chapter 3, I go on to investigate preference for familiar shoals in two different guppy populations, using two different experimental set-ups.

Chapter 3

The role of familiarity in the grouping decisions of Trinidadian guppies

Rao, A. S., Alves, N. P. C., Sakata, J. T., Reader, S. M.

Abstract

Social grouping is widely observed in numerous taxa, providing grouping individuals with various benefits including efficient information exchange and predator protection. However, grouping also comes with costs such as an increase in competition for both resources and mates, which means that individuals often have to make decisions regarding joining or leaving a group. Past work has shown that fish have the ability to not only distinguish between familiar and unfamiliar conspecifics, but also use this distinction when making grouping choices. Trinidadian guppies (*Poecilia reticulata*), well known for their variation in shoaling preferences across populations, are an excellent system in which to study the role of familiarity in grouping decisions. In the current study, we examine preferences for familiar individuals following both a 15-day and 54-day cohabitation period in two different guppy populations. Utilizing two binary-choice experimental designs, we find no evidence for a familiarity preference in either population or following either cohabitation duration, despite observing a high preference to group with conspecifics. These results suggest that various context and population-level differences may impact familiarity preferences, contributing to the growing body of work on the evolution of social organization and grouping decisions in teleost species.

Introduction

Social grouping is a widespread phenomenon and has been researched extensively in behavioural and evolutionary biology (Krause and Ruxton 2002). Despite being associated with costs such as increased competition and disease transmission (Wilson et al. 2003; Schmid-Hempel 2021), grouping can confer numerous benefits to the individual. Access to social information, the ability to tackle large prey, diluted risk of capture, and more coordinated movements are just a few examples of these benefits, some of which also make grouping an effective anti-predator strategy (Neill and Cullen 1974; Mooring and Hart 1992; Barber and Wright 2001; Ward and Webster 2016).

Social recognition, at least at a primitive level, underlies all social organization (Wiley 2013; Ward and Webster 2016). However, many species possess the ability to not only recognize conspecifics, but also discriminate between them. For example, Gheusi et al. (1997) found that rats (*Rattus norvegicus*) are not only able to individually recognize conspecifics using odour cues, but also retain this memory for at least 24 hours (also see de la Zerda et al. 2020). Experiments indicate that wild bottlenose dolphins (*Tursiops truncatus*) utilize signature whistles to recognize conspecifics (Sayigh et al. 1999; Loth 2017) and that only a few, brief exposures are required to form a memory of individuals in Bengalese finches (Toccalino et al. 2016). Similarly, American lobsters (*Homarus americanus*) can individually recognize opponents, which aids in social organization (Karavanich and Atema 1998).

A number of teleost species are also known to be able to recognize and discriminate between individuals. By decreasing the risk of predation, increasing information exchange and foraging efficiency (Krause and Ruxton 2002), shoaling can be extremely beneficial to individuals. Depending on the associated costs and benefits, individuals can make decisions regarding whether or not to partake in grouping behaviours, as well as with whom (Krause and Godin 1994; Griffiths and Magurran 1998; Hoare and Krause 2003). Indeed, grouping advantages can be particularly pronounced when individuals associate with familiar individuals, i.e., unrelated conspecifics that an individual has had prior experience with (House et al. 1988; Seeman and McEwen 1996; Silk 2007). In stable social groups, individuals may develop knowledge regarding conspecifics' behaviours across contexts and perform individualized roles within their groups (Krause and Ruxton 2002). Shoaling specifically with familiar conspecifics further enhances anti-predator responses such as earlier detection, increased cohesion and coordination (Chivers et al. 1995; Barber and Wright 2001; Nadler et al. 2021; Lucon-Xiccato et al. 2022). Therefore, one crucial influence on grouping decisions is the potential shoaling partners (Dugatkin and Sih 1995).

The freshwater guppy, *Poecilia reticulata*, is a species that is known to be social, regularly demonstrating shoaling behaviours (Magurran 2005; Cabrera-Alvarez et al. 2017). It is known that guppies can recognize conspecifics; Dugatkin and Alfieri (1991) found that individuals remember the outcome of past encounters when choosing partners for predator inspection behaviours (but see Veiros et al. 2024). Lachlan et al. (1998), following from an experiment performed by Griffiths and Magurran (1997a), demonstrated that female guppies use familiarity as one of the cues to assess potential shoaling partners. That is, female guppies prefer to follow familiar individuals. Further, Swaney et al. (2001) reported that guppies are also more likely to learn from familiar than unfamiliar conspecifics. There are other benefits to familiarity; for instance, grouping with familiar conspecifics might result in the formation of dominance hierarchies, which are crucial in avoiding escalation of conflict in social species, thereby reducing aggression and its associated costs (Drews 1993; Reddon et al. 2021). However, while potentially widespread, familiarity generally takes some time to develop.

Griffiths and Magurran (1997a) found that female guppies associated preferentially with familiar conspecifics (their own tank-mates) after a period of 12 days. This preference remained even following a 5-week period of separation from each other, indicating that persistence and the non-transitory nature of recognition might be one potential mechanism in structuring groups in the wild (Bhat and Magurran 2006). Further, shoaling preferences for familiar individuals were found to diminish as group size increased (Griffiths and Magurran 1997b). This familiarity can override even kin recognition in shoaling decisions, perhaps indicating that naturally occurring kin groups form as a result of familiarity, as opposed to as an immediate consequence of kin recognition (Griffiths and Magurran 1999). While the findings in other species are consistent with these familiarity studies done in guppies, less is known about familiarity preferences in different populations of the same species.

Here, we use previous methodologies (Magurran et al. 1994) to examine familiarity preferences in two populations of guppies following both a 15-day and 54-day cohabitation period using two different experimental apparatuses. We predicted that as in Griffiths and Magurran (1997a), subjects would demonstrate a preference for the familiar individuals following 15 days of familiarisation. This study consists of two experiments (hereafter 'Test 1' and 'Test 2'). In Test 1, we examined preferences for familiar conspecifics in two different guppy populations. Subjects were housed in groups of six that were classified as either 'familiar' (conspecifics from the same housing tank) or 'unfamiliar' (conspecifics from any other housing tank). Following 15 days of cohabitation with conspecifics, we gave subjects a binary-choice preference test wherein the subject could pick between a familiar and unfamiliar shoal (Test 1). Based on our results from this experiment, we repeated it with the same subjects in a modified set-up (Test 2). This was done following approximately 54 days of familiarization in total. In Test 2, we also performed a preference test for a shoal versus an empty tank to verify that our procedure and apparatus could detect any shoaling preferences, should they exist.

Test 1

Materials and Methods

The fish used in this study consisted of individuals from two different populations. The first, the ‘Aripo/Quare cross’ population are descendants resulting from a cross between Aripo and Quare high-predation populations collected in Trinidad in 2009 and 2010 (Gotanda 2015). The ‘mixed wild origin’ population was a gift of the Rodd Laboratory (University of Toronto, Canada) in 2018. Both populations have since been outbred in laboratory conditions. These guppies were not previously used in other studies. Before the experiment, they were housed in 150L mixed-sex population stock tanks of about 50-70 individuals each. In previous work, similar group sizes were large enough to prevent familiarity development among individuals (Griffiths and Magurran 1997b). The tanks included filters, gravel substrate, a heater, and artificial aquarium plants and a terracotta pot for enrichment, and were maintained at 25^{O}C and a 12:12 light-dark cycle. Weekly 30% water changes and water testing (pH, hardness, nitrites, nitrates, and ammonia) were performed on stock tanks. Fish were fed daily with tropical fish flakes (TetraMin, Tetra, Germany), and supplemented with decapsulated brine shrimp eggs (*Artemia sp.*, Brine Shrimp Direct, USA) three times a week before the experiment.

We took 60 fish from their stock home tanks described above and grouped them in tanks (l 40 x w 20 x h 25 cm) of 6 fish from the same population, visually and olfactorily isolated from other tanks. In each tank, 3 fish were randomly chosen to be subjects and the remaining 3 as stimulus fish on the morning of the test (body length for subjects: X + SE = 19.84 + 0.394 mm; stimulus fish: X + SE = 20.58 + 0.903 mm). The sizes of the subjects and the stimulus fish were not significantly different from each other (two-sided t-test: t(20) = 0.755, p = 0.46). Individuals were first tested after being housed in these tanks for a period of 15 days with identical feeding and housing conditions as the stock tanks, including enrichment. Fish that shared a single tank were considered ‘familiar’, while fish in different tanks, ‘unfamiliar’.

Preference Test

We conducted a preference test using a similar procedure to that adopted by Griffiths and Magurran (1997a) (Figure 1). The subject was moved by net into the centre of the testing tank (*l* 90 x *w* 45 x *h* 30 cm; 70 cm neutral zone; water depth 20 cm), into a glass cylinder to habituate. Stimulus shoal fish were placed 6 cm from either end of the testing tank in two perforated and clear 1 litre bottles (diameter: 8 cm) with the tops cut off and covered with plastic cups once the stimulus fish were in. The perforations (about 4 mm in diameter, 5 cm apart, around the circumference of the bottle) allowed olfactory cues to pass through without allowing physical contact between subjects and stimulus fish. In each bottle on either side in the tank was placed a shoal of 3 fish, with familiar fish on one side (the 3 non-subjects from the same tank as the subject), and unfamiliar fish on the other (3 non-subject fish chosen randomly from any other one tank, ensuring each tank's stimulus fish were picked an equal number of times overall). The subject could thus shoal with the familiar or unfamiliar fish (or remain outside the shoaling zones). After 15 minutes of habituation, the subject was released and video recorded from above using a Sony 4K Camcorder.

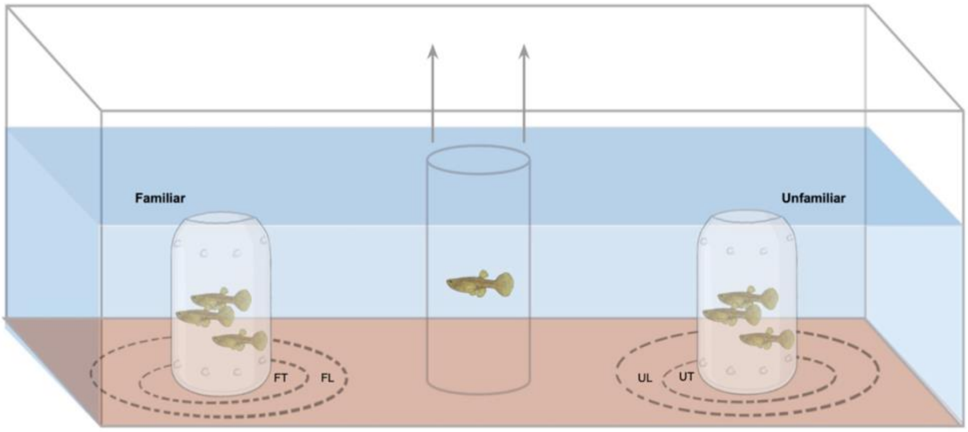


Figure 1: Test 1 familiarity preference test set-up. *Subject in central cylinder habituating, then released. Familiar 3-fish shoal on one side and unfamiliar 3-fish shoal on the other in perforated plastic bottles, covered with plastic cup (not pictured). Radial shoaling zones associated with each stimulus; FT - familiar tight shoaling zone; FL - familiar loose shoaling zone; UT - unfamiliar tight shoaling zone; UL - unfamiliar loose shoaling zone. Tight zones are 0-5 cm from the shoal and loose zones are 5-10 cm from the shoal.*

Shoaling was measured as the duration of the 15 minute trial that the subject spent within 10 cm of either shoal (0-5 cm: tight shoaling zone, 5-10 cm: loose shoaling zone), as fish being within 2 or 4 body lengths can be classified as shoaling following Pitcher and Parish (1993) and Morrell et al. (2008). We therefore recorded both tight and loose shoaling measures (two and four body lengths respectively) to verify the robustness of the measures. Following a trial, subjects were immediately netted back into their home tanks, split by an opaque white barrier from stimulus fish of the same tank. No fish was tested more than once. We also randomized the order of subjects and the position of the bottles (as in Griffiths & Magurran 1997a) to control for any order or side bias. The water in the testing tank was mixed using a net between every trial to minimize the presence of any localized odour cues.

Behavioural Measures and Statistical Analysis

All statistical analyses were performed in R, with the R base package (R Core Team, 2022), using the lmerTest and lmer4 packages (Bates et al. 2015) to fit the linear and multi-level mixed models. Residual diagnostics were performed to assess model fit, including checks for normality (Shapiro-Wilk test) and homoscedasticity of residuals.

We analysed the total time individual guppies from different populations (mixed wild origin vs. Aripo/Quare cross) spent with familiar vs. unfamiliar shoals using a linear regression model (with fish ID and tank ID as fixed effects). Since there was little variation in the results when the tight and loose shoaling zones were analysed individually, we combined them to better account for shoaling as defined in literature (Pitcher and Parish 1993; Morrell et al. 2008).

Results and Discussion

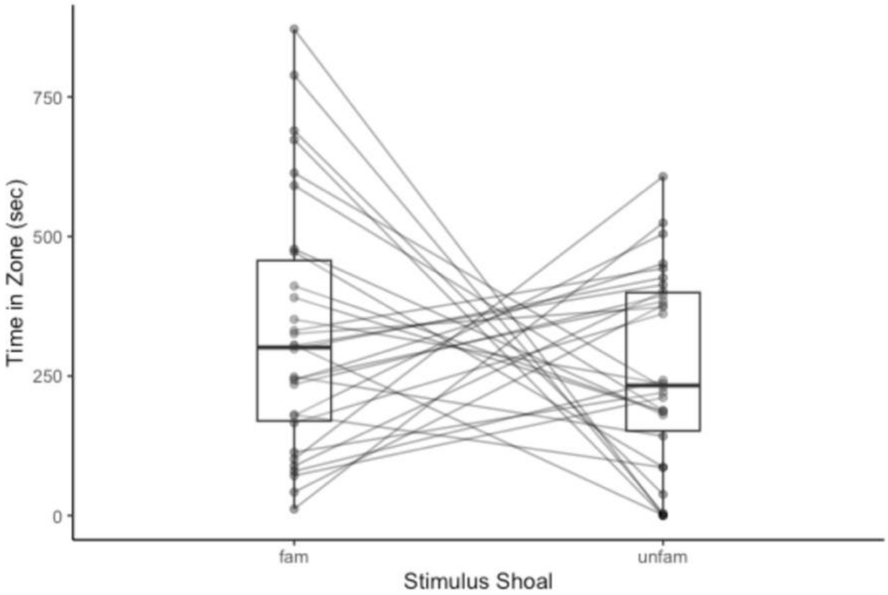


Figure 2: Boxplot of Test 1, total time spent shoaling on familiar ('fam') and unfamiliar ('unfam') sides*. Each line represents a single subject and the time it spent on either side, in seconds.*

All subjects approached at least one of the shoals and spent most of the test shoaling. On average, subjects spent 68.2% of the 15-minute test grouping with either shoal in what represents 14.0% of the total tank volume. However, there was no significant difference in the amount of time guppies spent with familiar versus unfamiliar fish (Figure 2; F(1,28) = 0.954, p = 0.34), and no significant effect of or interaction with population (Population: F(1,28) = 0.006, p = 0.94; Population*Condition: F(1,28) = 0.292, p = 0.59).

We thus saw no indication of a familiarity preference after 15 days for cohabitation. Although our methods and duration of familiarization closely matched previous work that had detected a familiarity preference (Griffiths and Magurran 1997a), we reasoned that perhaps the duration of familiarization or the precise methodology used could have impacted our findings. We thus retested the same subjects after around 54 days of familiarization (i.e., after an additional ~40 days), using a different choice test set-up where the two stimulus shoals were alongside each other as opposed to on opposite sides of the tank.

Test 2

Materials and Methods

Set up and housing conditions were generally identical to Test 1, and the same subjects were reused. Two subjects were replaced at the beginning of Test 2 (one wild origin and one Aripo/Quare cross) due to natural deaths over the course of the study. Note that the two replaced subjects had an overall fewer number of familiarization days than all other subjects (~39 days as opposed to ~54 days). A key difference was that the Aripo/Quare cross females were housed in pairs to increase the subject sample size and potentially also the opportunity to develop a familiarity preference while the other population was not manipulated and housed in larger groups as in Test 1. During the preference test, the subjects of the Aripo/Quare population could choose between an individual familiar fish (their housing companion), and an unfamiliar fish (chosen randomly from one of the other Aripo/Quare tanks). The mixed wild origin females were housed in five tanks of six fish each (in the same tanks as in Test 1). In the preference test, they were once again able to choose between a familiar and unfamiliar 3-fish shoal.

Unlike in Test 1, fish used as part of the stimulus shoal on day 1 were reused as subjects the following day to increase the sample size. Following day 1 of preference testing, an opaque white barrier was placed between subjects and stimulus fish overnight, to be able to identify them for this reason the next day.

Preference Tests

The preference tests consisted of two stimuli being presented - a familiar stimulus, which was either a 3-fish shoal (for wild origin subjects: Figure 3a) or an individual fish (for Aripo/Quare cross subjects: Figure 3b) from the subject's home tank, or an unfamiliar stimulus which was either a shoal (mixed wild origin) or single fish (Aripo/Quare cross) from a randomly chosen different tank. Stimuli were presented in front of the subject testing tank (*l* 54 x *w* 40 x *h* 40 cm; water at 20 cm), separated from each other by an opaque black partition. Each rectangular shoal zone was 27 cm long. During a 3-minute acclimation period where the subject was placed into a glass cylinder, it was able to observe both stimuli simultaneously before making a choice. When released following acclimation, behaviour was video recorded for a period of 5 minutes.

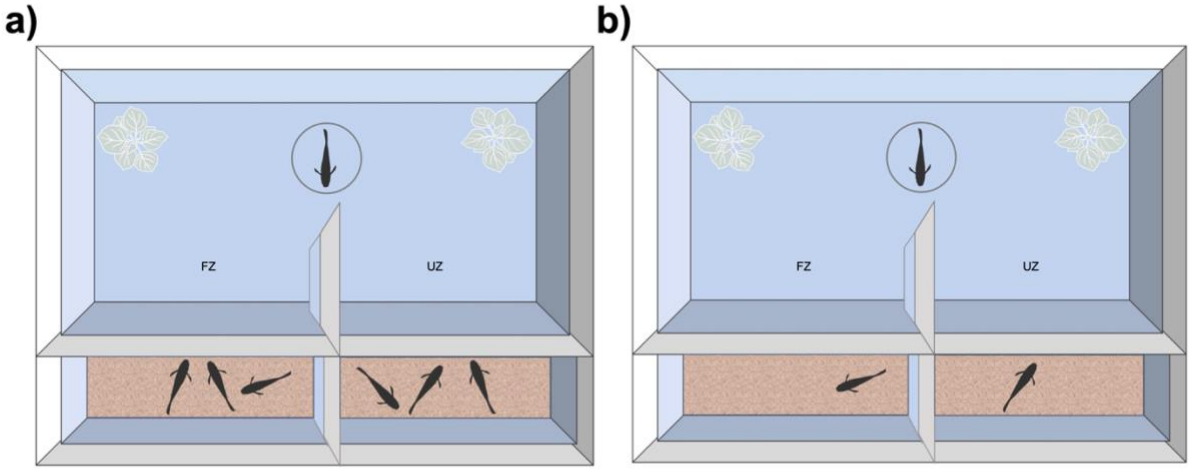


Figure 3: Test 2 familiarity preference test set-up. *Subject in central cylinder habituating, then released.* ***a) mixed wild origin population*** *- Familiar 3-fish shoal on one side and unfamiliar 3-fish shoal on the other in external tank and* ***b) Aripo/Quare cross population*** *– Familiar individual fish on one side and an unfamiliar fish on the other in external tank. Opaque black barriers between the two stimulus tanks, artificial plants on either end of testing tank. FZ - familiar zone; UZ - unfamiliar zone.*

Time shoaling with the familiar and unfamiliar stimuli (0-5 cm: tight shoaling zone, 5-10 cm: loose shoaling zone) was measured following Pitcher and Parish (1993) and Morrell et al. (2008). Each test consisted of two trials between which the side of the familiar stimulus was switched to account for any side bias, and the subject was given an additional minute to acclimate. The familiarity preference testing took two days, each day in a random subject order with a random unfamiliar stimulus to avoid any possible bias.

Shoaling Test

After the familiarity preference test was completed for all subjects, we performed a shoaling test with the same individuals and methodology. This test occurred on the 2 days following the familiarity preference test, between which subjects were put back into their familiarity housing tanks. The apparatus was identical to the preference testing apparatus described above (Figure 4).

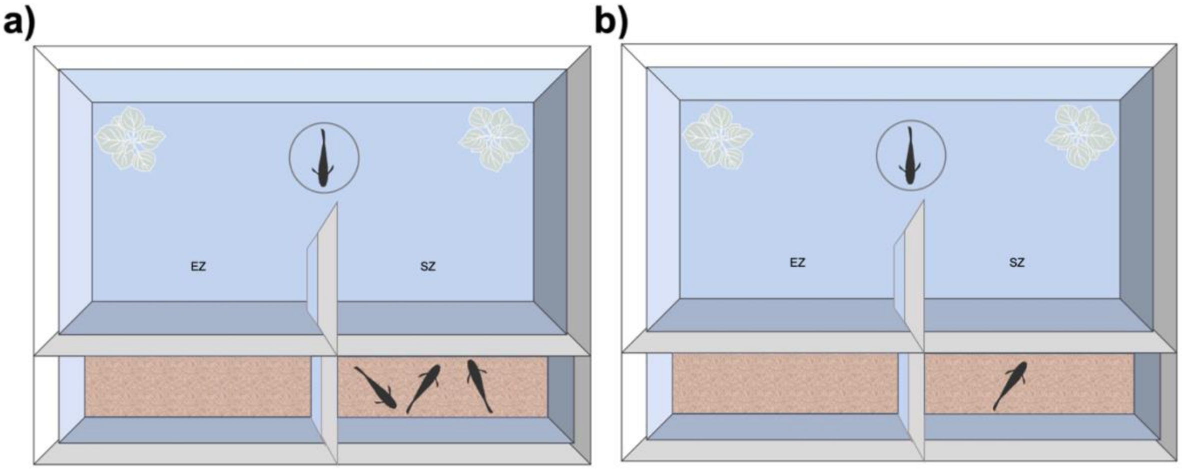


*Figure 4: **Test 2 shoaling preference test set-up.** Subject in central cylinder habituating, then released. **a) mixed wild origin population** - 3-fish shoal on one side and the other side empty, both tanks external and b) **Aripo/Quare cross population** - single fish on one side and the other side empty, both tanks external. Opaque black barriers between the two stimulus tanks, artificial plants on either end of testing tank. EZ - empty zone; SZ - shoaling zone.*

We had two stimuli - a tank with a social stimulus (a shoal of familiar fish for the mixed wild origin: Figure 4a and a single familiar fish for the Aripo/Quare cross population: Figure 4b) and an empty tank. Using the same methodology as in the previous test, the social stimulus and the empty tank were both revealed in front of the testing tank, separated from each other with an opaque black barrier. The subject was netted from its home tank into a transparent glass cylinder in the centre of the testing tank from where both stimuli were simultaneously visible.

After a 3-minute acclimation period, the subject was released from the cylinder. When the fish was released, video recording began for 5 minutes. Time shoaling with the social stimulus and time spent on the empty side was measured once more.

Behavioural Measures and Statistical Analysis

After initial analysis, the tight and loose shoaling zones were combined for each stimulus. Due to the difference in housing and testing conditions between the two populations in Test 2, they were analysed separately. All statistical analyses were performed in R, with the R base package (R Core Team, 2022), using the lmerTest and lmer4 packages (Bates et al. 2015) to fit the linear and multi-level mixed models.

We first analysed the total time individual guppies (mixed wild origin and Aripo/Quare cross) spent with familiar vs. unfamiliar individuals using a paired t-test, since we did not need a linear model to account for population as was required in Test 1. We then similarly analysed the total time individual guppies (mixed wild origin and Aripo/Quare cross) spent with the shoal vs. the empty side using a second paired t-test.

Results

Familiarity test

We found that there was no significant difference in the time spent with the familiar versus unfamiliar stimulus in both mixed wild origin (Figure 5a; paired t-test: t(24) = 0.229, p = 0.82) and Aripo/Quare cross populations (Figure 5b; paired t-test: t(27) = -0.927, p = 0.36).

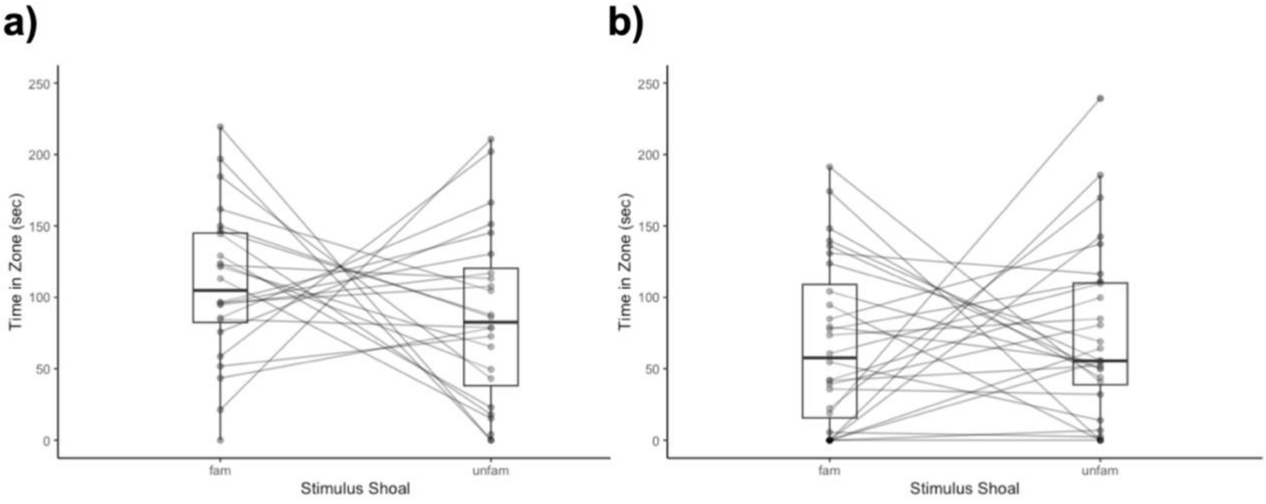


Figure 5: Boxplot of Test 2, total time spent shoaling on familiar ('fam') and unfamiliar ('unfam') side *for* ***a) mixed wild origin*** *and* ***b) Aripo/Quare cross populations****. Each line represents a single subject and the time it spent on either side, in seconds.*

Shoaling test

Subjects had a significant preference to spend time on the shoal side (versus the empty side) in both wild origin (Figure 6a; paired t-test: t(24) = 6.472, p < 0.0001) and Aripo/Quare cross populations (Figure 6b; paired t-test: t(24) = 3.612, p = 0.001).

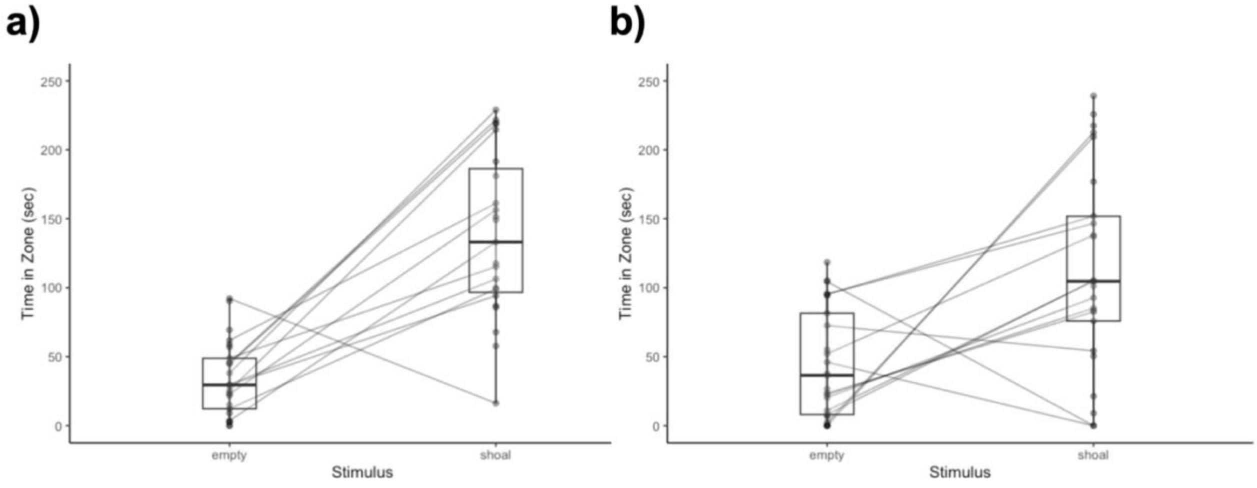


Figure 6: Boxplot of Test 2, total time spent on shoal side and empty side *for* ***a) mixed wild origin*** *and* ***b) Aripo/Quare cross populations****. Each line represents a single subject and the time it spent on either side, in seconds.*

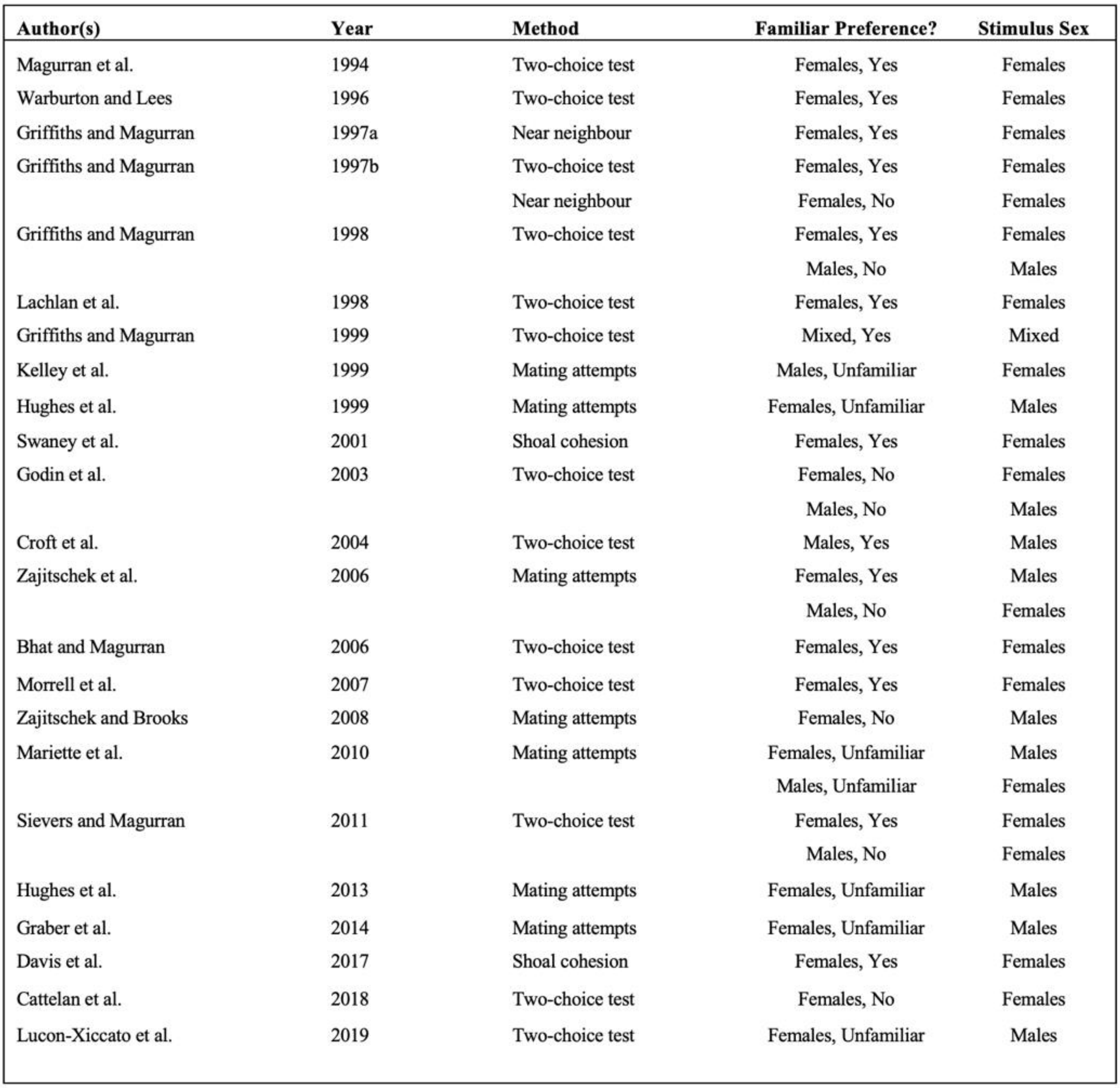
The full dataset is available at: https://doi.org/10.5683/SP3/LVQKZ2.

General Discussion

We know that associating with familiar conspecifics increases many of the benefits of grouping, leading to more cohesive shoals, more effective information transfer, and even faster learning (Chivers et al. 1995; Barber and Wright 2001; Swaney et al. 2001; Lucon-Xiccato et al. 2022). In Test 1, female guppies were housed in groups of six for a period of 15 days before being given a binary-choice preference test. Previous research has indicated that individuals begin to school preferentially with a 4-fish shoal comprising tank-mates after a period of about 12 days (Griffiths and Magurran 1997a; Bhat and Magurran 2006).

However, in our current experiments, we did not detect any such preference for familiar tank-mates following 15 days of cohabitation. This was also the case in Test 2, where the same females had been cohabiting for around 54 days and were tested using a different apparatus. However, the results of Test 2 did demonstrate a strong preference for a shoal over an empty compartment, suggesting that both populations were able to demonstrate grouping preferences in the experimental apparatus.

In addition to the study that we attempted to replicate (Griffiths and Magurran 1997a), there are several other studies that indicate that female guppies prefer familiar individuals (summarized in Table 1). For example, Sievers and Magurran (2011) show that female high-predation descendant guppies develop a familiarity preference in 12 days, though this was not seen in male subjects, who did not seem to differentiate between familiar and unfamiliar females. Cattelan et al. (2019) demonstrated that while guppies that were previously familiarized in 2-fish or 6-fish groups had no preference for familiar conspecifics overall, there was significant consistency in their preference (or lack thereof) for familiar individuals. Other than guppies (Magurran et al. 1994), this familiarity is also seen in numerous other species of freshwater fish such as cory catfish (*Corydoras aeneus*: Riley et al. 2019), paradise fish (*Macropodus opercularus*: Miklósi et al. 1992), mangrove rivulus (*Kryptolebias marmoratus*: Martin et al. 2022) and bluegill sunfish (*Lepomis macrochirus*: Dugatkin and Wilson 1992).



Author(s)	Year	Method	Familiar Preference?	Stimulus Sex
Magurran et al.	1994	Two-choice test	Females, Yes	Females
Warburton and Lees	1996	Two-choice test	Females, Yes	Females
Griffiths and Magurran	1997a	Near neighbour	Females, Yes	Females
Griffiths and Magurran	1997b	Two-choice test	Females, Yes	Females
		Near neighbour	Females, No	Females
Griffiths and Magurran	1998	Two-choice test	Females, Yes	Females
			Males, No	Males
Lachlan et al.	1998	Two-choice test	Females, Yes	Females
Griffiths and Magurran	1999	Two-choice test	Mixed, Yes	Mixed
Kelley et al.	1999	Mating attempts	Males, Unfamiliar	Females
Hughes et al.	1999	Mating attempts	Females, Unfamiliar	Males
Swaney et al.	2001	Shoal cohesion	Females, Yes	Females
Godin et al.	2003	Two-choice test	Females, No	Females
			Males, No	Males
Croft et al.	2004	Two-choice test	Males, Yes	Males
Zajitschek et al.	2006	Mating attempts	Females, Yes	Males
			Males, No	Females
Bhat and Magurran	2006	Two-choice test	Females, Yes	Females
Morrell et al.	2007	Two-choice test	Females, Yes	Females
Zajitschek and Brooks	2008	Mating attempts	Females, No	Males
Mariette et al.	2010	Mating attempts	Females, Unfamiliar	Males
			Males, Unfamiliar	Females
Sievers and Magurran	2011	Two-choice test	Females, Yes	Females
			Males, No	Females
Hughes et al.	2013	Mating attempts	Females, Unfamiliar	Males
Graber et al.	2014	Mating attempts	Females, Unfamiliar	Males
Davis et al.	2017	Shoal cohesion	Females, Yes	Females
Cattelan et al.	2018	Two-choice test	Females, No	Females
Lucon-Xiccato et al.	2019	Two-choice test	Females, Unfamiliar	Males

Table 1: Summary of studies on the effects of familiarity on guppy (Poecilia reticulata) shoaling decisions*. Discriminatory ability of individual fish was tested in two-choice tests or by looking at association with near neighbours. Familiarity has also been tested in the context of shoal cohesion, composition and aggression in the laboratory. Preference for familiars in the wild is inferred from shoal fidelity. (Adapted from Griffiths and Ward 2011).*

However, our results do link with other work indicating that the presence of a familiarity preference might not extend to all freshwater teleost species or even populations unequivocally. For example, Webster and Laland (2020) found no evidence of individual recognition in three-spine or nine-spine sticklebacks (*Gasterosteus aculeatus* and *Pungitius pungitius*). Male zebrafish (*Danio rerio*) similarly do not preferentially associate with familiar over unfamiliar male conspecifics (Blonder and Tarvin 2022). In guppies too, numerous studies have shown that males do not possess a familiarity preference (Griffiths and Magurran 1998; Godin et al. 2003; Zajitschek and Brooks 2008; Sievers and Magurran 2011). While familiarity preferences in female guppies towards other female shoals have been repeatedly reported, this finding is not universal (Table 1). Godin et al. (2003) found no evidence in wild female guppies of a preference to associate with familiar conspecifics, which they attributed in part to population differences. This points towards the potentially contextual nature of recognition and the conditions under which familiarity might evolve.

Our Test 1 set-up was nearly identical to that in Griffiths and Magurran (1997a). Differences included the size of the stimulus shoal (we used 3 fish instead of 4, matching Bhat and Magurran (2006)), number of days of cohabitation (15 days instead of 12 in the original study), and guppy populations used. While Griffiths and Magurran began seeing an increased familiarity preference at day 12, it appeared to peak around day 15, which is why we chose the latter in Test 1. Test 2 attempted to investigate the same question in the same subjects, but with a different experimental set-up and following around 54 days of familiarization time. However here too, we observed no familiarity preference in the subjects. Familiar and unfamiliar shoals were placed in perforated transparent containers on either side of the same testing tank. This was done specifically to allow for exchange of olfactory and chemical cues between subject and stimulus fish. We therefore believe that the lack of a familiarity preference in either of the tests in Chapter 3 was not due to the subjects' inability to perceive the odour of the stimulus fish. Regardless of this, there is no doubt that further work needs to be done to pick apart the effects that chemical cues have on recognition and conspecific shoal preference.

Webster and Rutz (2020) recently proposed the STRANGE framework, which aims to help researchers minimize sampling biases. Numerous species show large amounts of variation in behaviour, and only specific subsets of this variation might be detected and accounted for in test samples. It is of utmost importance that we consider the numerous factors that go into shaping an organism's behaviours both in the laboratory and in the wild. This includes Social background (S), Trappability and self-selection (T), Rearing history (R), Acclimation and habituation (A), Natural changes in responsiveness (N), Genetic make-up (G) and Experience (E) (Webster and Rutz 2020). These factors can influence a range of things from which animals are sampled for a test and how readily those individuals partake in the study, to the repertoire of behaviours they perform over the trials, which is of particular importance in studies like ours.

Given the well-studied population differences in guppies, it is valuable to consider their impact on familiarity and sociality. Stark differences in morphology, life history and behaviours have been documented across populations (Shaw et al. 1991), giving us useful evolutionary insights (Magurran and Seghers 1990a). These differences often have to do with predation, with guppies from high-predation populations being more cohesive shoalers (Seghers 1974; Magurran and Seghers 1990b). Regarding familiarity specifically, Kelley et al. (1999) found that male guppies from Tunapuna and Tacarigua that were from confined pools preferentially attempted to mate with unfamiliar females from different pools, indicating that they did possess a familiarity preference. However, males from open rivers demonstrated no such preference.

Griffiths and Magurran (1997a) utilized female guppies that were Lower Tacarigua descendants, from high predation conditions. Our fish on the other hand were both mixed populations, comprising either high-predation Aripo/Quare cross individuals or low-predation mixed wild origin guppies. The fish were also reared in the laboratory for many generations before being tested. Domesticated guppies not only shoal less than their feral counterparts (Swaney et al. 2015) but are also exposed to lower predation and an increase in social structure uniformity, which can have pivotal implications on social behaviour (Wright et al. 2006a, 2006b). Thus, it is indeed plausible that the lack of familiarity preferences in our experimental fish are at least in part due to these factors, making it valuable to consider population differences, experiences, and rearing conditions as characteristics affecting sociality – all of which can hamper generalizability and reproducibility of results (Webster and Rutz 2020).

Upon analysing the results from Test 1, we modified the experimental set-up and re-tested the subjects following prolonged cohabitation. However, we still saw no evidence of a familiarity preference. One plausible reason could be differences in the physical characteristics of the apparatus. While we replicated the methodology and set-up from Griffiths and Magurran (1997a) and our tank size was identical to theirs, there could have been issues with factors such as the dimensions of the habituation cylinder, or even the distance between the subject and stimulus shoal, for instance. Past studies have shown that guppies are able to distinguish between stimuli up to 120 cm away (Mühlhoff and Reader 2011), so it is unlikely that the lack of preference was due to subjects’ inability to see and distinguish between the stimuli, which in Test 1 were only ~39 cm away from the centre of the tank and about 78 cm away from each other. We also know that the preference test set-up in Test 2 is indeed able to detect shoaling preferences. The significant shoaling preference we observed in our Test 2 shoaling test is well in line with existing literature (Magurran 2005; Cabrera-Alvarez et al. 2017), as guppies typically tend to form shoals. Therefore, this lack of familiarity preference is likely not a lack of preference detection, unless the behaviour was more subtle than the expected shoaling preference. However, it is now well appreciated that methodological details can have large impacts on the ability to measure behaviours and performance and importantly, to compare them between studies and assays (Stewart et al. 2012; O'Neill et al. 2018; Jones et al. 2023). In light of this, a nuanced investigation of how specific physical design discrepancies affect the detection and measurement of subject participation and behaviour is a pivotal next step in sociality studies like this one (Salena et al. 2021; Jones et al. 2023). A crucial question that then needs to be addressed is whether familiarity-based shoal preferences are observed in the wild, and if so, under what conditions.

It is established that possessing a familiarity preference can be very beneficial for a social species such as guppies for numerous reasons (Chivers et al. 1995; Barber and Wright 2001; Swaney et al. 2001). Even though several past studies have seen this preference form in guppies (see Griffiths and Magurran 1997a; Lachlan et al. 1998; Bhat and Magurran 2006), we did not find this in our current study. This suggests that familiarity might be contextual in nature, only developing under specific conditions or in certain populations. There is a strong case for further research to investigate how context, population, and set-up can play a role in familiarity preference and its development over time. Specifically, formal population comparisons would provide much needed background on behaviours and the specific conditions under which they evolve both in the laboratory and in the wild. Studies like this one contribute to the growing knowledge base of the conditions under which grouping preferences are most beneficial.

References

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67,* 1-48.

Barber, I. & Wright, H. A. (2001). How strong are familiarity preferences in shoaling fish? *Animal Behaviour*, *61*, 975–979.

Bhat, A., & Magurran, A. E. (2006). Benefits of familiarity persist after prolonged isolation in guppies. *Journal of Fish Biology*, *68*(3), 759–766.

Blonder, A. F., & Tarvin, K. A. (2022). Male zebrafish (*Danio rerio*) do not preferentially associate with familiar over unfamiliar conspecifics. *Journal of Fish Biology*, *100*(4), 1025-1032.

Cabrera-Álvarez, M., Swaney, W. T., & Reader, S. M. (2017). Forebrain activation during social exposure in wild-type guppies. *Physiology & Behavior*, *182*, 107-113.

Cattelan, S., Lucon-Xiccato, T., Pilastro, A., & Griggio, M. (2019). Familiarity mediates equitable social associations in guppies. *Behavioral Ecology*, *30*(1), 249-255.

Chivers, D. P., Brown, G. E., & Smith, R. J. F. (1995). Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Canadian Journal of Zoology*, *73*(5), 955-960.

Croft, D. P., Arrowsmith, B. J., Webster, M., & Krause, J. (2004). Intra-sexual preferences for familiar fish in male guppies. *Journal of Fish Biology*, *64*(1), 279-283.

Davis, S., Lukeman, R., Schaerf, T. M., & Ward, A. J. (2017). Familiarity affects collective motion in shoals of guppies (*Poecilia reticulata*). *Royal Society Open Science*, *4*(9), 170312.

de la Zerda, S. H., Netser, S., Magalnik, H., Briller, M., Marzan, D., Glatt, S., & Wagner, S. (2020). Social recognition in rats and mice requires integration of olfactory, somatosensory and auditory cues. *BioRxiv*, 2020-05.

Drews, C. (1993). The concept and definition of dominance in animal behaviour. *Behaviour*, *125*, 283-313.

Dugatkin, L. A. & Alfieri, M. (1991). Guppies and the Tit for Tat strategy: preference based on past interaction. *Behavioral Ecology and Sociobiol*ogy, *28*, 243–246.

Dugatkin, L. A. & Sih, A. (1995). Behavioral ecology and the study of partner choice. *Ethology*, *99*, 265–277.

Dugatkin, L. A., Wilson, D. S. (1992). The prerequisites for strategic behaviour in bluegill sunfish, *Lepomis macrochirus*. *Animal Behaviour*, *44*, 223-230.

Gheusi, G., Goodall, G. & Dantzer, R. (1997). Individually distinctive odours represent individual conspecifics in rats. *Animal Behaviour*, *53*, 935–944.

Godin, J.-G. J., Alfieri, M. S., Hoare, D. J., & Sadowski, J. A. (2003). Conspecific familiarity and shoaling preferences in a wild guppy population. *Canadian Journal of Zoology*, *81*(11), 1899-1904.

Gotanda, K. M. (2015). *Adaptation and the spatiotemporal mosaic of selection*. Doctoral dissertation, McGill University, Montreal.

Graber, R. E., Senagolage, M., Ross, E., Houde, A. E., & Hughes, K. A. (2015). Mate preference for novel phenotypes: a fresh face matters. *Ethology*, *121*(1), 17-25.

Griffiths, S. W., & Magurran, A. E. (1997a). Familiarity in schooling fish: how long does it take to acquire? *Animal Behaviour*, *53*(5), 945-949.

Griffiths, S. W., & Magurran, A. E. (1997b). Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proceedings of the Royal Society B: Biological Sciences*, *264*(1379), 547-551.

Griffiths, S. W., & Magurran, A. E. (1999). Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology*, *45*(6), 437-443.

Griffiths, S. W., & Ward, A. (2011). Social recognition of conspecifics. *Fish Cognition and Behavior*, *2*, 186-216.

Griffiths, S., & Magurran, A. (1998). Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour*, *56*(3), 689-693.

Hoare, D. J., & Krause, J. (2003). Social organization, shoal structure and information transfer. *Fish and Fisheries*, *4*(3), 269-279.

House, J. S., Umberson, D., & Landis, K., R. (1988). Structure and processes of social support. *Annual Review of Sociology*, *14*, 293–318.

Hughes, K. A., Du, L., Rodd, F. H., & Reznick, D. N. (1999). Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Animal Behaviour*, *58*(4), 907-916.

Hughes, K. A., Houde, A. E., Price, A. C., & Rodd, F. H. (2013). Mating advantage for rare males in wild guppy populations. *Nature*, *503*(7474), 108-110.

Jones, N. A. R., Cortese, D., Munson, A., Spence-Jones, H. C., Storm, Z., Killen, S. S., Bethel, R., Deacon, A. E., Webster, M. M., & Závorka, L. (2023). Maze design: Size and number of choices impact fish performance in cognitive assays. *Journal of Fish Biology*, *103*(5), 974–984.

Karavanich, C. & Atema, J. (1998). Individual recognition and memory in lobster dominance. *Animal Behaviour*, *56*, 1553–1560.

Kelley, J. L., Graves, J. A., & Magurran, A. E. (1999). Familiarity breeds contempt in guppies. *Nature*, *401*(6754), 661-662.

Krause, J. D., & Ruxton, G. D. (2002). Living in Groups. Oxford University Press, USA.

Krause, J., & Godin, J.-G. J. (1994). Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): Effects of predation risk, fish size, species composition and size of shoals. *Ethology*, *98*(2), 128-136.

Lachlan, R. F., Crooks, L., & Laland, K. N. (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour*, *56*(1), 181-190.

Loth, A. (2017). Individual recognition in bottlenose dolphins (*Tursiops truncatus*): from acoustic distinctiveness in signature whistles to visual self-recognition.

Lucon-Xiccato, T., Bisazza, A., & Pilastro, A. (2019). Exploratory behaviour covaries with preference for unfamiliar males in female guppies. *Animal behaviour*, *155*, 217-224.

Lucon-Xiccato, T., Cattelan, S., & Griggio, M. (2022). Familiarity effects on fish behaviour are disrupted in shoals that contain also unfamiliar individuals. *Behavioral Ecology and Sociobiology*, *76*(7), 100.

Magurran, A. E. (2005). *Evolutionary ecology: the Trinidadian guppy*. Oxford University Press, USA.

Magurran, A. E., & Seghers, B. H. (1990a). Population differences in the schooling behaviour of newborn guppies, *Poecilia reticulata*. *Ethology*, *84*(4), 334-342.

Magurran, A. E., & Seghers, B. H. (1990b). Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour*, *112*(3-4), 194-201.

Magurran, A. E., Seghers, B. H., Shaw, P. W., Carvalho, G. R. (1994). Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. *Journal of Fish Biology*, *45*, 401-406.

Mariette, M. M., Zajitschek, S. R. K., Garcia, C. M., & Brooks, R. C. (2010). The effects of familiarity and group size on mating preferences in the guppy, *Poecilia reticulata*. *Journal of evolutionary biology*, *23*(8), 1772-1782.

Martin, K. E., Blewett, T. A., Burnett, M., Rubinger, K., Standen, E. M., Taylor, D. S., Trueman, J., Turko, A. J., Weir, L., West, C. M., Wright, P. A., & Currie, S. (2022). The importance of familiarity, relatedness, and vision in social recognition in wild and laboratory populations of a selfing, hermaphroditic mangrove fish. *Behavioral Ecology and Sociobiology*, *76*(3), 34.

Miklosi, A., Haller, J., Csanyi, V. (1992). Different duration of memory for conspecific and heterospecific fish in the paradise fish (*Macropodus opercularus* L.). *Ethology*, *90*, 29-36.

Mooring, M. S. & Hart, B. L. (1992). Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. *Behavior*, *123*, 173–193.

Morrell, L. J., Croft, D. P., Dyer, J. R., Chapman, B. B., Kelley, J. L., Laland, K. N., & Krause, J. (2008). Association patterns and foraging behaviour in natural and artificial guppy shoals. *Animal Behaviour*, *76*(3), 855-864.

Morrell, L. J., Hunt, K. L., Croft, D. P., & Krause, J. (2007). Diet, familiarity and shoaling decisions in guppies. *Animal Behaviour*, *74*(2), 311-319.

Mühlhoff, N., Stevens, J. R. & Reader, S. M. (2011). Spatial discounting of food and social rewards in guppies (*Poecilia reticulata*). *Frontiers in Psychology*, *2*, 10129.

Nadler, L. E., McCormick, M. I., Johansen, J. L., & Domenici, P. (2021). Social familiarity improves fast-start escape performance in schooling fish. *Communications Biology*, *4*(1), 897.

Neill, S. R. St. S. & Cullen, J. M. (1974). Experiments on whether schooling by their prey affects the hunting behaviour of cephalopods and fish predators. *J. Zool., Lond.*, *172*, 549–569.

Pitcher, T. J. Parrish, J. K. (1993). Functions of Shoaling Behaviour in Teleosts. In T. J. Pitcher (Ed.), The Behaviour of Teleost Fishes (pp. 294-337). Boston, MA: Springer US.

R Core Team (2022). *R: A Language and Environment for Statistical Computing, 2022.* R Foundation for Statistical Computing.

Reddon, A. R., Ruberto, T. & Reader, S. M. (2021). Submission signals in animal groups. *Behaviour*, *159*, 1–20.

Riley, R. J., Gillie, E. R., Horswill, C., Johnstone, R. A., Boogert, N. J., & Manica, A. (2019). Coping with strangers: how familiarity and active interactions shape group coordination in *Corydoras aeneus*. *Royal Society Open Science*, *6*(9), 190587.

Salena, M. G., Turko, A. J., Singh, A., Pathak, A., Hughes, E., Brown, C., & Balshine, S. (2021). Understanding fish cognition: A review and appraisal of current practices. *Animal Cognition*, *24*, 395–406.

Sayigh, L. S., Tyack, P. L., Wells, R. S., Solow, A. R., Scott, M. D., Irvine, A. B. Individual recognition in wild bottlenose dolphins: a field test using playback experiments. *Animal Behaviour*, *57*, 41–50 (1999).

Schmid-Hempel, P. (2021). Sociality and parasite transmission. *Behavioral Ecology and Sociobiology*, *75*(11), 156.

Seeman, T.E., & McEwen, B. (1996). Impact of social environment characteristics on neuroendocrine regulation. *Psychosomatic Medicine*, *58*, 459–471.

Seghers, B.H. (1974). Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. *Evolution*, *28*, 486-9.

Shaw, P. W., Carvalho, G. R., Magurran, A. E., & Seghers, B. H. (1991). Population differentiation in Trinidadian guppies (*Poecilia reticulata*): patterns and problems. *Journal of Fish Biology*, *39*, 203-209.

Sievers, C., & Magurran, A. E. (2011). Context dependent acquisition of familiarity recognition in Trinidadian guppies. *Behaviour*, 843-855.

Silk, J. B. (2007). The adaptive value of sociality in mammalian groups. *Phil. Trans. R. Soc. B*, *362*, 539–559.

Swaney, W. T., Cabrera-Álvarez, M. J., & Reader, S. M. (2015). Behavioural responses of feral and domestic guppies (*Poecilia reticulata*) to predators and their cues. *Behavioural Processes*, *118*, 42-46.

Swaney, W., Kendal, J., Capon, H., Brown, C., & Laland, K. N. (2001). Familiarity facilitates social learning of foraging behaviour in the guppy. *Animal Behaviour*, *62*(3), 591–598.

Toccalino, D. C., Sun, H., & Sakata, J. T. (2016). Social memory formation rapidly and differentially affects the motivation and performance of vocal communication signals in the Bengalese Finch (*Lonchura striata* var. domestica). *Frontiers in Behavioral Neuroscience*, *10*, 113.

Veiros, A. L., Schweinfurth, M. K. & Webster, M. M. (2024). On closer inspection: Reviewing the debate on whether fish cooperate to inspect predators. *Ethology*, *130*, e13427.

Warburton, K., & Lees, N. (1996). Species discrimination in guppies: learned responses to visual cues. *Animal Behaviour*, *52*(2), 371-378.

Ward, A., & Webster, M. (2016). Sociality: the behaviour of group-living animals. Berlin, Germany: Springer.

Webster, M. M., & Laland, K. N. (2020). No evidence for individual recognition in threespine or ninespine sticklebacks (*Gasterosteus aculeatus* or *Pungitius pungitius*). *Royal Society open science*, *7*, 191703.

Webster, M. M., & Rutz, C. (2020). How STRANGE are your study animals? *Nature*, *582*(7812), 337–340.

Wiley, R. H. (2013). Specificity and multiplicity in the recognition of individuals: implications for the evolution of social behaviour. *Biological Reviews*, *88*(1), 179-195.

Wilson, K., Knell, R., Boots, M., & Koch-Osborne, J. (2003). Group living and investment in Immune Defence: An interspecific analysis. *Journal of Animal Ecology*, *72*(1), 133–143.

Wright, D., Nakamichi, R., Krause, J., Butlin, R. K. (2006a). QTL analysis of behavioral and morphological differentiation between wild and laboratory zebrafish (*Danio rerio*). *Behavior Genetics*, *36*(2), 271–284.

Wright, D., Ward, A. J. W., Croft, D. P., & Krause, J. (2006b). Social organization, grouping, and domestication in fish. *Zebrafish*, *3*, 141-155.

Zajitschek, S. R., & Brooks, R. C. (2008). Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *The American Naturalist*, *172*(6), 843-854.

Zajitschek, S. R., Evans, J. P., & Brooks, R. (2006). Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. *Behavioral Ecology*, *17*(6), 911-916.

Chapter 4

General Discussion and Conclusion

Summary of main findings

My thesis investigated flexibility in shoaling and the role of familiarity in grouping behaviours in the Trinidadian guppy, *Poecilia reticulata*. In Chapter 2, I asked whether female guppies could associate a social or asocial cue with reward and in turn change their tendency to group with conspecifics. My results show that the populations of guppies I studied show some indication of cue-reward association in training, but this does not translate to the subsequent preference test, where they instead significantly decreased their shoaling propensity over the course of the experiment. In Chapter 3, I investigated whether guppies were able to form a familiarity preference following 15 days and 54 days of cohabitation with conspecifics and found no such evidence for a familiarity preference. However, these same fish demonstrated the expected preference for a shoal. In other words, though fish preferred shoaling with conspecifics over remaining isolated, they demonstrated no detectable preference towards familiar conspecifics. Our results are in line with the idea that there are numerous factors that underpin shoaling decisions. Shoaling might be flexible, as to allow for rapid adaptive responses to changing environmental conditions (Hoare et al. 2004), but this flexibility is likely contextual, only appearing under a certain set of circumstances and differing across populations and external conditions.

Thesis overview

Chapter 2

Group living and sociality in general offers individuals a wide array of benefits (Krause and Ruxton 2002). Studies examining grouping behaviours have shown that grouping can increase foraging efficiency, predator protection and exchange of social information regarding mates and resources (Hoare and Krause 2003). In my thesis, I explore these group dynamics, furthering our understanding of the complex nature of social behaviours. In past work, shoaling has been utilized as a reward in several learning experiments (e.g., Burns 2008, Lucon-Xiccato and Bisazza 2017b, Kurvers et al. 2018). In Chapter 2 of my thesis, I take a different approach and investigate specifically whether shoaling tendencies can be shaped experimentally in a directed way. I used food reinforcement to ask whether female guppies can be trained to change their shoaling tendencies. In Experiment 1, I rewarded individuals for either grouping or isolating (and also included a control condition) and in Experiment 2, I investigated whether we could simply increase shoaling tendencies by rewarding guppies for shoaling with conspecifics. I found that female guppies did not readily increase their shoaling propensities following reinforcement. This stands in contrast to previous results indicating that reinforcement could favour shoaling with a small versus large shoal (Vassileva 2019). Strikingly, the individuals in our study instead significantly decreased their shoaling preference even when reinforced for approaching shoals. It is possible that they did indeed learn the association between cue and reward (as subjects tended to approach the rewarded-cue feeder faster than the asocial-cue feeder that was not rewarded). However, this effect did not reach statistical significance and did not carry over to the preference tests.

There are numerous factors that might account for why shoaling propensity did not change following reinforcement training in our experiment, which I discuss below. It could have to do with the focus on a choice between a shoal and an asocial cue; perhaps this is less open to experiential effects than the choice between two shoals. However, the results could also be due to the methodology; for example, our stimulus fish were placed outside of the subject tank, which might reduce the subjects' ability to recognize them. Similarly, the control condition involved rewarding subjects in the middle of the tank, perhaps reinforcing a preference for this region. Alternatively, it is possible that the particular way in which we ran our preference test did not elicit a response, despite learning having occurred in training. Finally, the lack of a shoaling tendency change could also have to do with the background of our subjects, including population, past experiences or personality (Webster and Rutz 2020).

Chapter 3

In the wild, animals need to allocate sufficient time and energy for tasks such as foraging and predator vigilance, which puts constraints on foraging efficiency (Griffiths et al. 2004). Shoaling is an effective anti-predator strategy that can also provide foraging advantages (Pitcher et al. 1982; Morgan and Colgan 1987; Magurran 1990; Griffiths 2003) that are often strengthened through familiarity (Bhat and Magurran 2006). For these reasons, in Chapter 3, I first asked whether female guppies show a familiarity preference for conspecifics following 15 days of cohabitation in Test 1 (partially a replication of Griffiths and Magurran 1997a). I found no evidence of such a preference in my study. To follow-up this experiment, we retested the same subjects after about 54 days of cohabitation using a different experimental set-up, but still did not observe a familiarity preference (Test 2). However, we did see a strong preference for shoals (vs. an empty tank) in our control shoaling test as expected (Lachlan et al. 1998; Cabrera-Alvarez et al. 2017). Thus, this implies that the testing apparatus was able to detect and measure shoaling preferences, suggesting that the negative finding might indicate a lack of a familiarity preference as opposed to merely the inability to detect it.

As in Chapter 2, it is pertinent to consider a variety of reasons for not detecting a familiarity preference in our subjects. Methodologically, our set-up in Test 1 was nearly identical to Griffiths and Magurran (1997a). However, we do know that even apparently minute apparatus changes can have large effects on the replicability of behavioural studies (Jones et al. 2023). Further, it is crucial to draw the distinction between preference and performance; it is possible that familiarity did indeed develop, but a preference is either not being displayed or not being detected, which will need to be investigated further. Finally, it is once more important to consider subjects' background, including their population, experiences, and rearing history, which are all factors that might affect experimental outcomes (Webster and Rutz 2020).

General Discussion

Learning of cues and training-testing disconnect

It has been extensively documented since the work of Pavlov and Thorndike that learning shapes adaptive behaviour (reviewed in Castro and Wasserman 2010). However, there are different approaches that can be taken to study these questions, and there has been a major expansion in the paradigms utilized in studying learning (Marler and Terrace 1983; Staddon 2016). These contrasting and complementary methods greatly shape animal learning theories and experiments. While Thorndike demanded replicability and control in experimental studies through operant conditioning, Pavlov gave behavioural biologists the very first comprehensive explanation of association formation through demonstrating conditioned responses (Bitterman et al. 2012). The acquisition of a conditioned response through the use of a conditioned stimulus is still one of the most common methodologies for studying animal learning. Similarly, Thorndike explained that the consequences of an act determine the probability of this act reoccurring in the future. That is, the 'Law of Effect', wherein if a particular behaviour is followed by a satisfying consequence, that behaviour becomes more likely to happen in the future. Conversely, if a behaviour is followed by a negative consequence, it becomes less likely to reoccur. Building on this, contemporary animal learning theories posit that previously paired stimuli are encoded in associations (Pearce and Bouton 2001).

Learning has been observed in a variety of contexts in fish including escape routes, foraging location, and mate choice (Brown and Laland 2003; Magurran 2005; Reader and Biro 2010). For example, female Siamese fighting fish (*Betta splendens*, Osphronemidae) observe aggressive interactions between two male conspecifics and utilize this knowledge of fighting ability in their subsequent mate-choice decisions (Doutrelant and McGregor 2000). Instead of relying on the newest information received in the context of foraging, nine-spined sticklebacks (*Pungitius pungitius*) can shape decisions based on the reliability of the external cues and the duration between contradictory asocial information (van Bergen et al. 2004). In guppies too, Kendal et al. (2004) shows that in circumstances where social and asocial information are contradictory, individuals are able to adjust their reliance on specific information according to the cost. These are only few of the numerous examples indicating that fish in general and guppies specifically have been known to learn complex behaviours and responses across multiple contexts.

In Chapter 2, I used food reinforcement to train female guppies to join (or not join, in Experiment 1) shoals of conspecifics. I attempted to control for a variety of factors that are known to affect learning, such as population differences, personality and information access (Carlier and Lefebvre 1997; Dawson et al. 2013; Mesoudi et al. 2016). I did so by modifying the protocol to include only a single population (in Experiment 2, domestic-strain guppies), exposing subjects to the same cues in pre-training and training, keeping housing conditions constant, and testing subjects for differences in baseline sociality. While there was a non-significant change in latency to enter the feeder depending on stimulus in training that potentially provides some evidence for learning, our sample size was relatively low and further experiments would be required to determine the robustness of any effect. Even with the extended training, I was unable to find significant evidence for learning in the preference tests.

Early learning theorist Tolman (1932) made an important distinction between learning – the process whereby an association between events is formed – and performance – the expression of learning in overt behaviours. It is possible that learning did indeed occur in our Chapter 2 study, but learning can remain latent until something triggers overt action, such as prevailing conditions or foraging motivation (Castro and Wasserman 2010). It is still however surprising that we did not see the predicted changes in shoaling tendencies. This experiment was designed based in part on a previous study performed by Vassileva (2019). Vassileva (2019) found that female guppies could be trained to join social groups of certain sizes, showing that their shoaling tendencies are flexible. This effect was found after 15 days of training, two times per day. In Experiment 2 of Chapter 2, I saw no effect of training during testing even after doubling training to four times a day for 15 days, per subject. Therefore, it is unlikely that the lack of cue-reward association can be attributed to inadequate training.

Shoaling motivation and habituation

Repeatedly presenting a single stimulus to experimental subjects often results in changed behaviour, such as long-term habituation (Blumstein 2016). Numerous experiments in recent years have documented the effects of habituation on shoaling motivation and stress responses. Habituation is a form of learning occurring when repeated or prolonged application of a stimulus results in progressively weaker responses (Thompson and Spencer 1966; Houslay et al. 2019). For example, Schons et al. (2021) found a decline in both mean speed and total distance travelled by cichlids (*Pelvicachromis taeniatua*) over the course of the experimental fish being involved in the study. They also found declining reactions to shock stimuli (a mechanical disturbance cue) over time. They posit that because individuals repeatedly encountered the shock stimulus which was not followed by any true threat, stress responses declined over time. Similarly in guppies, Houslay et al. (2019) found that individuals were able to habituate quickly to repeated exposure to a stressor.

While shoaling has many benefits, it is foremost an anti-predator behaviour and stress response (Botham et al. 2006; Ward et al. 2011; Swaney et al. 2015). However, we know that social motivation derived from the exposure to an unfamiliar tank decreases with time (Thünken et al. 2014; Lucon-Xiccato et al. 2016). For instance, Lucon-Xiccato and Bisazza (2017b) found a reduction in shoaling in guppies over the course of an experiment, which the authors attributed in part to habituation to the test tank. Kurvers et al. (2018) similarly found that guppies shoaled less with companion fish over the course of the study. In Chapter 2, the observed decrease in shoaling over time could thus reflect habituation to the test tank. It is even possible that this habituation extends to the stimulus shoal fish, whose behaviour may have changed over time, affecting subjects' responses. Overall, habituation to the experimental conditions and apparatus can strongly affect variables as well as responses to stimuli, which needs to be considered in future behavioural experiments in fish and potentially other taxa as well (Schons et al. 2021).

Cues for conspecific recognition

A key factor that can shape social interactions is familiarity, i.e. the recognition of unrelated individuals. While there is no concrete consensus on the mechanisms that underlie familiarity across taxa, it is generally agreed upon that there exists a recognition template, which is a representation of certain characteristics that an individual may use to identify others (Ward and Webster 2016).

While recognition involves phenomena such as phenotype matching and learned individual characteristics (Ward and Webster 2016), chemical cues play a pivotal role. Even in systems where vision plays a large role, olfactory cues elicit a strong response (Brown 2003). For instance, hermit crabs (*Pagurus longicarpus*) rely heavily on olfaction as the dominant sensory channel. Providing subjects with a combination of two signal components from visual and olfactory channels resulted in an enhanced response by the receiver (Gherardi and Tiedemann 2003). Similarly, eastern fence lizards (*Sceloporus undulatus*) are attracted to territorial scent marks of conspecifics, which in turn affects space use (Campos et al. 2017). In fish too, it has been shown that individuals weigh visual and chemical cues in conspecific detection. In one study, subject fish were exposed to a binary-choice test and presented with a choice of conspecific visual cues together with heterospecific chemical cues versus heterospecific visual cues with conspecific chemical cues. Indeed, the fish chose to group with the shoal expressing conspecific chemical cues despite the visual cues being that of heterospecifics, which demonstrates the crucial role of chemical cues in social attraction (Ward et al. 2002).

Following from this, one other limitation of our Chapter 2 study is the potential confounding effect of the companion fish. In order to reduce the effects of isolation stress on the subjects, I housed them with two companion fish (also subjects) that were placed behind an opaque plastic barrier when training and testing took place in both experiments in Chapter 2. Given the high salience of chemical cues, it is possible that the presence of these companion fish in the testing tank could be detected by the subject, thereby reducing stress levels and therefore the motivation for the subjects to shoal. Despite keeping the subject visually isolated from the companion fish, it is unlikely that the plastic barrier and filters completely stop the transmission of odour cues. If this study were to be repeated, I would suggest that the subjects be tested in a separate testing tank instead. There is no doubt that further work needs to be done to pick apart the effects that chemical cues have on recognition and conspecific shoal preference.

Accounting for variability and bias

It is also possible that there is a publication bias skewing the results of familiarity studies in fish, and thus results are more mixed than the literature reports. There are other ecological examples indicating that published literature is likely a biased subsample. For instance, Sánchez-Tójar et al. (2018) investigated the status signalling hypothesis which is an explanation of intra-species variation in ornamentation in nature. Despite this being an established textbook example in evolutionary and behavioural biology, the authors found that the mean effect size of unpublished studies was essentially zero, while the mean effect size on published studies was medium. Additionally, a large percentage of the model's variance could be linked to whether the work was published or unpublished. Studies with statistically significant results are published more often and faster than those with non-significant findings ('the file-drawer problem', Rosenthal 1979), leading to a decline in reported effects over time (Jennions and Møller 2002). It is indeed possible that there were other studies investigating familiarity in teleost species that found a lack of such a preference but were not published. Replicable findings are the foundation of cumulative scientific research, making it paramount to minimize bias (Yang et al. 2023).

Above I discuss several specific factors that might be responsible for the experimental outcomes in Chapters 2 and 3. However, there are also potentially broader explanations that might apply to all the experiments performed and behaviours recorded, leading to bias in results. Webster and Rutz (2020) recently put forth the STRANGE framework, proposing careful consideration of genetic make-up, experience and social background when observing and attempting to explain animal behaviour, aiming to minimize sampling bias. STRANGE stands for Social background, Trappability and self-selection, Rearing history, Acclimation and habituation, Natural changes in responsiveness, Genetic make-up and Experience. These factors can influence numerous aspects of both laboratory and field experiments, from which individuals are sampled for testing, to how they behave during trials. Since comparison and replicability across studies is vital to scientific progress, it becomes crucial to account for this variation.

Population and generational effects

We know of widely documented differences in morphological traits, life histories, and social behaviour between populations of the same species, including in teleost species (Shaw et al. 1991). This type of intraspecific variation provides insight into the role of natural selection in shaping behaviours and is particularly valuable in situations where selection pressures differ between populations (Magurran and Seghers 1990a; Song et al. 2011). It is well known that guppies from highly predated populations are more cohesive in their shoaling behaviours (Seghers 1974; Magurran and Seghers 1991; Li et al. 2022) and also differ in predator assessment and risk sensitivity of courtship behaviour (Magurran and Seghers 1990b). Kelley and Magurran (2003a) found that wild-caught high-predation descendants react more strongly to predators than their low-predation counterparts and strikingly, show a reduced response when reared in the laboratory. We also see population and individual differences in social attraction and innovation (Wright et al. 2006a, 2006b; Kappeler et al. 2013; Sims and Reader 2021). Taken together, these studies might suggest that populations vary drastically in an array of factors ranging from cue reliance to behavioural responses (see Webster et al. 2019; Keagy et al. 2023).

In both Chapter 2 and 3, I used two different guppy populations each. While the original study in Chapter 2 was done using a "domestic" guppy population (Vassileva 2019), I used a different domestic strain to Vassileva (2019), as well as the Paria population in Experiment 1. Similarly in Chapter 3, Griffiths and Magurran (1997a) used guppies that were descendants of Lower Tacarigua guppies, which is a high predation population, while I used two different populations. The first, the 'Aripo/Quare cross' population are descendants resulting from a cross between Aripo and Quare high-predation populations collected in Trinidad in 2009 and 2010 (Alexander et al. 2006; Fraser et al. 2015; Gotanda 2015), while the 'mixed wild origin' population came from a mixture of wild Trinidadian populations. While several studies show familiarity preferences in guppies (see Table 1, Chapter 3), it is possible that this is more context and population dependent than previously thought. Different guppy populations may learn associations (Chapter 2) or develop and display familiarity preferences differently (Chapter 3), as has been shown for shoaling propensities (Shaw et al. 1991; Song et al. 2011).

Another related potential reason for the unexpected results in Chapter 2 might be the use of domesticated fish, despite there being evidence of learning in similar conditions in different strains of domestic guppies in our laboratory and others (Laland and Williams 1997, 1998; Lachlan et al. 1998; Swaney et al. 2001; Brown and Laland 2002; Kelley and Magurran 2003b; Franks and Marshall 2013; Leris and Reader 2016; Lucon-Xiccato and Bisazza 2017a; Vassileva 2019). For every population, the fish were reared in the laboratory for many generations before being tested. This type of domestication can result in a reduction in the need to forage extensively, reduction in predation, and homogeneity in social structures, all of which can have an effect on an individual's behaviour (Wright et al. 2006a, 2006b). For instance, Swaney et al. (2015) demonstrated that domesticated guppies shoal less than feral guppies, even in the presence of a novel predator. It is therefore crucial to make note of population differences, rearing conditions, and transgenerational traits when thinking about factors affecting learning and social behaviours.

Impacts of methodological factors

It is challenging to control for variation between and within individuals: a crucial consideration when attempting to increase comparability and replicability between studies (Manteca and Deag 1993; Stamps et al. 2012). Differences in experimental design and methodology only exacerbate this existing variation, extending to further differences in factors such as data acquisition and replicability of behavioural measures across studies (reviewed in Jones et al. 2023). For instance, Stewart et al. (2012) found that fish scale their locomotory activity corresponding to the size of the tank and O'Neill et al. (2018) demonstrated that acclimation time can affect behavioural repeatability (also see Biro et al. 2012). Enclosure size itself is also known to have an effect on numerous behaviours such as activity and aggression in a range of animals including white rabbits (*Oryctolagus cuniculus*: Valuska and Mench 2013) and chimpanzees (*Pan troglodytes*: Jensvold et al. 2001). Similarly, rearing and housing conditions such as environmental enrichment or predator threat can have an effect on the cognitive performance of fish (Odling-Smee and Braithwaite 2003b; Salvanes et al. 2013; Ferrari 2014;

Zhang et al. 2019). In other words, the methodological set-up and rearing environment can introduce biases into the experiment and its results.

There is a middle-ground to be reached in making a test extreme (i.e., either too easy or too difficult) to detect meaningful variation within a population. An extreme test would potentially lead to all individuals performing similarly, making it challenging to capture this variation even if present (Jones et al. 2023). In Experiment 1 of Chapter 2, I ensured fish could not choose the rewarded feeder by its odour, created feeders that did not allow the reward to be seen until a choice was made, and tried to avoid any opportunity for pattern formation with regard to side or stimulus in training. We considered that it was possible the training and testing was just too challenging for the subjects to form the intended association. Therefore, in Experiment 2, the training was more straightforward, with the subjects being exposed only to a single stimulus at a time and being fed only after the correct choice was made. I suggest repeating this experiment with a “challenging middle” set-up, which might reveal correlations with other aspects of phenotype that would be hidden in set-ups where individuals show similar high or low performance as we saw in our experiment (Jones et al. 2023). Individual variation in performance also needs to be further considered and controlled for since this variation could offer insight not just into differences in the primary trait being tested, but also differences in stress, motivation and temperament, including boldness, exploration, or sociality (Sih et al. 2004; Réale et al. 2007; Castanheira et al. 2016).

Further, I suggest aiming to make the training and testing paradigms as similar to each other as possible. In the experiments in Chapter 2, there were key differences in training and testing set-up, including the presence of food and feeders in the tank. The reliance on external cues and information can be highly context dependent (Kendal et al. 2004), and it is possible that social exposure with food (Chapter 2 training) and social exposure without food (Chapter 2 preference testing) might be incompatible motivational systems wherein subjects can learn associations linked to one context but not the other (Fernö et al. 2020). It is indeed possible that subjects are able to learn the necessary associations during training but are unable to apply them in a novel context – say, one that is no longer related to foraging – in the preference test. Further, it is possible that feeding subjects in the centre of the tank in the control condition reinforced a preference for that area, so I would recommend repeating the experiment with a no-reward treatment. It is safe to acknowledge that the binary-choice paradigm is valuable in that it can be used to make minute manipulations to aspects of cues available to a subject, identifying exactly which component of the overall cue is preferred (Ward and Webster 2016). However, a more nuanced understanding of how design discrepancies affect the detection and measurement of subject participation and behaviour is a necessary next step in fish sociality studies like ours.

Further questions and implications

Investigating the direct fitness consequences of grouping interactions and sociality can aid in our understanding of the selective pressures that allow for the development of numerous group dynamics and behaviours (Kutsukake 2009). A crucial component of learning and cue-reward association in the context of grouping is the salience of the stimulus provided. I suggest further experiments attempting to manipulate shoaling behaviours using different training stimuli. While we used bloodworms as the reward in our experiments in Chapter 2, the subjects seemed to habituate to the experimental set-up following repeated exposure. I suggest using negative stimuli such as alarm cue or temperature (see Kemp 1969) that subjects are unlikely to habituate to rapidly. Another useful extension of this experiment would be to replicate the methodology in Vassileva (2019) using different guppy populations. This will aid in better understanding the exact circumstances that promote shoaling flexibility across contexts and environments.

Many aforementioned grouping benefits are often known to be increased by associating with familiar conspecifics (Bhat and Magurran 2006). In Chapter 3, despite the existence of some other studies that have indicated the presence of a familiarity preference (Griffiths and Magurran 1997a), we found no evidence for the same. I suggest a follow-up study to attempt to narrow the possible reasons for the lack of a familiarity preference. Just as some sex differences in familiarity preferences are already documented (e.g., Griffiths and Magurran 1998, Godin et al. 2003), a next step would be to compare familiarity preferences across populations, which will also uncover valuable information on the ways in which different selective pressures affect behaviours and how these behaviours change across contexts. Similarly, investigating methodological aspects including shoal size, distance of the stimulus shoals from the subject, and habituation time are all valuable next steps.

General conclusion

The overarching aim of my thesis was to investigate shoaling flexibility and the factors that underlie it through reinforcement training and familiarity preference assays. My hypothesis in Chapter 2 that grouping tendencies in female Trinidadian guppies are flexible and can be manipulated through learned associations with stimuli was only partially supported. While there was some evidence of the subjects learning the cue-reward association, we saw no change in the shoaling tendencies corresponding with this reinforcement training. Past work suggests numerous factors including population differences and methodological aspects could have impacted these outcomes. In Chapter 3, I was surprisingly unable to observe a familiarity preference in female guppies but provide more supporting evidence for a significant grouping preference, the latter of which is in line with the existing body of work on grouping in guppies (Magurran 2005). Both studies in my thesis throw light on important questions of ecology and evolution; namely, the evolution of social behaviours and how specific species and populations adapt to rapidly changing conditions. This becomes especially important in the wake of novel threats caused by climate change, where group behaviours and sociality can have crucial impacts on animal survival and conservation challenges. Finally, I want to conclude by reiterating the importance of the Trinidadian guppy as a valuable system in which to study these questions, given the natural variation in their social behaviours and learning tendencies. Future work investigating the factors affecting grouping will certainly enhance our understanding of adaptations in the wild and the evolution of sociality across and within populations in the presence of varying social and environmental pressures.

Bibliography [Chapters 1 and 4]

Alexander, H. J., Taylor, J. S., Wu, S. S. T., & Breden, F. (2006). Parallel evolution and vicariance in the guppy (*Poecilia reticulata*) over multiple spatial and temporal scales. *Evolution*, *60*(11), 2352-2369.

Arnott, G., & Elwood, R. W. (2009). Assessment of fighting ability in animal contests. *Animal Behaviour*, *77*(5), 991-1004.

Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, *10*(2), 131-165.

Bhat, A., & Magurran, A. E. (2006). Benefits of familiarity persist after prolonged isolation in guppies. *Journal of Fish Biology*, *68*(3), 759–766.

Biro, P. A. (2012). Do rapid assays predict repeatability in labile (behavioural) traits? *Animal Behaviour*, *83*(5), 1295-1300.

Bitterman, M. E., LoLordo, V. M., Overmier, J. B., & Rashotte, M. E. (2012). *Animal learning: Survey and analysis* (Vol. 19). Springer Science & Business Media.

Blumstein, D. T. (2016). Habituation and sensitization: new thoughts about old ideas. *Animal Behaviour*, *120*, 255-262.

Botham, M. S., Kerfoot, C. J., Louca, V. & Krause, J. (2006). The effects of different predator species on antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*. *Naturwissenschaften*, *93*, 431–439.

Brännäs, E., Alanärä, A., & Magnhagen, C. (2001). The social behaviour of fish. *Social behaviour in farm animals*, *10*, 275-304.

Brown, C. (2002). Do female rainbowfish (*Melanotaenia* spp.) prefer to shoal with familiar individuals under predation pressure? *Journal of Ethology*, *20*(1), 89-94.

Brown, C., & Laland, K. (2001). Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology*, *59*(3), 471-493.

Brown, C., & Laland, K. N. (2002). Social learning of a novel avoidance task in the guppy: conformity and social release. *Animal Behaviour*, *64*(1), 41-47.

Brown, C., & Laland, K. N. (2003). Social learning in fishes: a review. *Fish and Fisheries*, *4*(3), 280-288.

Brown, G. E. (2003). Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries*, *4*, 227-234.

Brown, G. E. & Smith, R. J. F. (1996). Foraging trade-offs in fathead minnows (*Pimephales promelas*, Osteichthyes, Cyprinidae): Acquired predator recognition in the absence of an alarm response. *Ethology*, *102*, 776–785.

Bshary, R., Gingins, S., & Vail, A. L. (2014). Social cognition in fishes. *Trends in Cognitive Sciences*, *18*(9), 465–471.

Burns, J. G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology*, *122*(4), 344.

Cabrera-Álvarez, M., Swaney, W. T., & Reader, S. M. (2017). Forebrain activation during social exposure in wild-type guppies. *Physiology & Behavior*, *182*, 107-113.

Campos, S. M., Strauss, C., & Martins, E. P. (2017). In space and time: territorial animals are attracted to conspecific chemical cues. *Ethology*, *123*(2), 136-144.

Carlier, P., & Lefebvre, L. (1997). Ecological differences in social learning between adjacent, mixing, populations of Zenaida doves. *Ethology*, *103*, 772-784.

Castanheira, M. F., Cerqueira, M., Millot, S., Gonçalves, R. A., Oliveira, C. C., Conceição, L. E., & Martins, C. I. (2016). Are personality traits consistent in fish?—The influence of social context. *Applied Animal Behaviour Science*, *178*, 96-101.

Castro, L., & Wasserman, E. A. (2010). Animal learning. *WIRES Cognitive Science*, *1*(1), 89-98.

Chivers D. P., Wisenden B. D., Hindman C. J., Michalak T. A., Kusch R. C., Kaminskyj S. G. W., Jack K. L., Ferrari M. C. O., Pollock R. J., Halbgewachs C. F., Pollock M. S., Alemadi S., James C. T., Savaloja R. K., Goater C. P., Corwin A., Mirza R. S., Kiesecker J. M., Brown G. E., Adrian J. C., Krone P. H., Blaustein A. R. & Mathis A. (2007). Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 2611-9.

Chouinard-Thuly, L., & Reader, S. M. (2019). Population differences in how wild Trinidadian guppies use social information and socially learn. *bioRxiv*, 786772.

Cresswell, W., & Quinn, J. L. (2011). Predicting the optimal prey group size from predator hunting behaviour. *Journal of Animal Ecology*, *80*(2), 310-319.

Dawson, Erika H., Avarguès-Weber, A., Chittka, L., & Leadbeater, E. (2013). Learning by observation emerges from simple associations in an insect model. *Current Biology*, *23*, 727-730.

Deacon, A. E., Ramnarine, I. W., & Magurran, A. E. (2011). How reproductive ecology contributes to the spread of a globally invasive fish. *PloS one*, *6*(9), e24416.

Doutrelant, C., & McGregor, P. (2000). Eavesdropping and mate choice in female fighting fish. *Behaviour*, *137*(12), 1655-1668.

Dugatkin, L. A., & Godin, J. G. J. (1992a). Reversal of female mate choice copying in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society B: Biological Sciences*, *249*(1324), 179-184.

Earl, S. R., Johnson, L. E., Grant, E., Kasubhai, A., López-Sepulcre, A., Yang, Y., & Gordon, S. (2024). Disentangling genetic, plastic and social learning drivers of sex-specific foraging behaviour in Trinidadian guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B*, *291*(2018), 20232950.

Ebensperger, L. A., & Hayes, L. D. (2016). Sociobiology of caviomorph rodents: an integrative approach. John Wiley and Sons.

Endler, J. A. (1995). Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecology & Evolution*, *10*, 22–29.

Fan, R., Reader, S. M., & Sakata, J. T. (2022). Alarm cues and alarmed conspecifics: Neural activity during social learning from different cues in Trinidadian guppies. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1981).

Fernö, A., Folkedal, O., Nilsson, J., & Kristiansen, T. S. (2020). Inside the fish brain: cognition, learning and consciousness. *The Welfare of Fish*, *20*, 149-183.

Ferrari, M. C. (2014). Short-term environmental variation in predation risk leads to differential performance in predation-related cognitive function. *Animal Behaviour*, *95*, 9-14.

Ferrari, M. C. O., Wisenden, B. D. & Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, *88*, 698-724.

Fox, J. A., Toure, M. W., Heckley, A., Fan, R., Reader, S. M., & Barrett, R. D. H. (2024). Insights into adaptive behavioural plasticity from the guppy model system. *Proceedings of the Royal Society B: Biological Sciences*, *291*(2018), 20232625.

Franks, V. R., & Marshall, R. C. (2013). Mechanisms and extent of information transfer in socially foraging guppies, *Poecilia reticulata*. *Animal Behaviour*, *85*(1), 103-108.

Fraser, B. A., Künstner, A., Reznick, D. N., Dreyer, C., & Weigel, D. (2015). Population genomics of natural and experimental populations of guppies (*Poecilia reticulata*). *Molecular Ecology*, *24*(2), 389-408.

Gherardi, F., & Tiedemann, J. (2004). Chemical cues and binary individual recognition in the hermit crab *Pagurus longicarpus*. *Journal of Zoology*, *263*(1), 23-29.

Godin, J.-G. J., Alfieri, M. S., Hoare, D. J., & Sadowski, J. A. (2003). Conspecific familiarity and shoaling preferences in a wild guppy population. *Canadian Journal of Zoology*, *81*(11), 1899-1904.

Gorlick, D. L. (1976). Dominance hierarchies and factors influencing dominance in the guppy *Poecilia reticulata* (Peters). *Animal Behaviour*, *24*(2), 336-346.

Gotanda, K. M. (2015). *Adaptation and the spatiotemporal mosaic of selection*. Doctoral dissertation, McGill University, Montreal.

Griffiths, S. W. (1997). Preferences for familiar fish do not vary with predation risk in the European minnow. *Journal of Fish Biology*, *51*(3), 489-495.

Griffiths, S. W. (2003). Learned recognition of conspecifics by fishes. *Fish and Fisheries*, *4*, 256–268.

Griffiths, S. W., & Magurran, A. E. (1997a). Familiarity in schooling fish: how long does it take to acquire? *Animal Behaviour*, *53*(5), 945-949.

Griffiths, S. W., & Magurran, A. E. (1997b). Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proceedings of the Royal Society B: Biological Sciences*, *264*(1379), 547-551.

Griffiths, S. W., & Magurran, A. E. (1999). Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology*, *45*(6), 437-443.

Griffiths, S. W., & Ward, A. (2011). Social recognition of conspecifics. *Fish Cognition and Behavior*, *2*, 186-216.

Griffiths, S. W., Brockmark, S., Ho¨jesjo¨, J. & Johnsson, J. I. (2004). Coping with divided attention: the advantage of familiarity. *Proceedings of the Royal Society of London B*, *271*, 695–699.

Griffiths, S., & Magurran, A. (1998). Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour*, *56*(3), 689-693.

Hammer, T. L., Bize, P., Gineste, B., Robin, J. P., Groscolas, R., & Viblanc, V. A. (2023). Disentangling the “many-eyes”, “dilution effect”, “selfish herd”, and “distracted prey” hypotheses in shaping alert and flight initiation distance in a colonial seabird. *Behavioural Processes*, *210*, 104919.

Hasenjager, M. J., & Dugatkin, L. A. (2016). Familiarity affects network structure and information flow in guppy (*Poecilia reticulata*) shoals. *Behavioral Ecology*, *28*(1), 233-242.

Hasenjager, M. J., & Dugatkin, L. A. (2017). Fear of predation shapes social network structure and the acquisition of foraging information in guppy shoals. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1852).

Haynes, K. F., & Yeargan, K. V. (1999). Exploitation of intraspecific communication systems: illicit signalers and receivers. *Annals of the Entomological Society of America*, *92*(6), 960-970.

Healy, S. E. (1998). *Spatial representation in animals*. Oxford University Press, USA.

Heathcote, R. J., Darden, S. K., Franks, D. W., Ramnarine, I. W., & Croft, D. P. (2017). Fear of predation drives stable and differentiated social relationships in guppies. *Scientific Reports*, *7*(1), 41679.

Hoare, D. J., & Krause, J. (2003). Social organization, shoal structure and information transfer. *Fish and Fisheries*, *4*(3), 269-279.

Hoare, D. J., Couzin, I. D., Godin, J. G. J., & Krause, J. (2004). Context-dependent group size choice in fish. *Animal Behaviour*, *67*, 155-164.

Hoare, D. J., Krause, J., Peuhkuri, N., & Godin, J. G. J. (2000a). Body size and shoaling in fish. *Journal of Fish Biology*, *57*(6), 1351-1366.

Hong, X., & Zha, J. (2019). Fish behavior: A promising model for aquatic toxicology research. *Science of the total environment*, *686*, 311-321.

Houde A. E. (1997) Sex, color, and mate choice in guppies. Princeton, NJ: Princeton University Press.

Houslay, T. M., Earley, R. L., Young, A. J. & Wilson, A. J. (2019). Habituation and individual variation in the endocrine stress response in the Trinidadian guppy (*Poecilia reticulata*). *General and Comparative Endocrinology*, *270*, 113–122.

Huizinga, M., Ghalambor, C. K., & Reznick, D. N. (2009). The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, *Poecilia reticulata*. *Journal of Evolutionary Biology*, *22*(9), 1860-1866.

Ingle, D., & Sahagian, D. (1973). Solution of a spatial constancy problem by goldfish. *Physiological Psychology*, *1*(1), 83-84.

Ioannou, C. C., Ramnarine, I. W., & Torney, C. J. (2017). High-predation habitats affect the social dynamics of collective exploration in a shoaling fish. *Science advances*, *3*(5), e1602682.

Jennions, M. D., & Møller, A. P. (2002). Relationships fade with time: a meta-analysis of temporal trends in publication in ecology and evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1486), 43-48.

Jensvold, M. L. A., Sanz, C. M., Fouts, R. S., & Fouts, D. H. (2001). Effect of enclosure size and complexity on the behaviors of captive chimpanzees (*Pan troglodytes*). *Journal of Applied Animal Welfare Science*, *4*(1), 53-69.

Jesmer, B. R., Merkle, J. A., Goheen, J. R., Aikens, E. O., Beck, J. L., Courtemanch, A. B., Hurley, M. A., McWhirter, D. E., Miyasaki, H. M., Monteith, K. L. & Kauffman, M. J. (2018). Is ungulate migration culturally transmitted? Evidence of social learning from translocated animals. *Science*, *361*(6406), 1023-1025.

Johnson-Ulrich, L. (2017). The Social Intelligence Hypothesis. In T. K. Shackelford & V. A. Weekes-Shackelford (Eds.), *Encyclopedia of Evolutionary Psychological Science* (pp. 1–7). Springer International Publishing.

Johnsson, J. I., & Åkerman, A. (1998). Watch and learn: preview of the fighting ability of opponents alters contest behaviour in rainbow trout. *Animal Behaviour*, *56*(4), 771-776.

Jones, K. A., Croft, D. P., Ramnarine, I. W., & Godin, J.-G. J. (2010). Size-assortative shoaling in the guppy (*Poecilia reticulata*): The role of active choice. *Ethology*, *116*(2), 147-154.

Jones, N. A. R., Cortese, D., Munson, A., Spence-Jones, H. C., Storm, Z., Killen, S. S., Bethel, R., Deacon, A. E., Webster, M. M., & Závorka, L. (2023). Maze design: Size and number of choices impact fish performance in cognitive assays. *Journal of Fish Biology*, *103*(5), 974–984.

Kappeler, P. M. (2019). A framework for studying social complexity. *Behavioral ecology and sociobiology*, *73*, 1-14.

Kappeler, P. M., Barrett, L., Blumstein, D. T., & Clutton-Brock, T. H. (2013). Constraints and flexibility in mammalian social behaviour: introduction and synthesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1618), 20120337.

Keagy, J., Lehto, W., Minter, R., Machniak, S., Baird, O., & Boughman, J. W. (2023). Repeated parallel differentiation of social learning differences in benthic and limnetic threespine stickleback fish. *Biology Letters*, *19*(7), 20230208.

Kelley, J. L., & Magurran, A. E. (2003a). Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behavioral Ecology and Sociobiology*, *54*, 225-232.

Kelley, J. L., & Magurran, A. E. (2003b). Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries*, *4*(3), 216-226.

Kelley, J. L., Evans, J. P., Ramnarine, I. W., & Magurran, A. E. (2003). Back to school: can antipredator behaviour in guppies be enhanced through social learning? *Animal Behaviour*, *65*(4), 655-662.

Kemp, F. D. (1969). Thermal reinforcement and thermoregulatory behaviour in the lizard *Dipsosaurus dorsalis*: an operant technique. *Animal Behaviour*, *17*, 446-451.

Kendal, R. L., Boogert, N. J., Rendell, L., Laland, K. N., Webster, M., & Jones, P. L. (2018). Social learning strategies: Bridge-building between fields. *Trends in cognitive sciences*, *22*(7), 651-665.

Kendal, R. L., Coolen, I., & Laland, K. N. (2004). The role of conformity in foraging when personal and social information conflict. *Behavioral Ecology*, *15*, 269-277.

Korb, J., Buschmann, M., Schafberg, S., Liebig, J., & Bagnères, A. G. (2012). Brood care and social evolution in termites. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1738), 2662-2671.

Krause, J. D., & Ruxton, G. D. (2002). Living in Groups. Oxford University Press, USA.

Krause, J., & Godin, J.-G. J. (1994). Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): Effects of predation risk, fish size, species composition and size of shoals. *Ethology*, *98*(2), 128-136.

Kurvers, R. H. J. M., Drägestein, J., Hölker, F., Jechow, A., Krause, J., & Bierbach, D. (2018). Artificial light at night affects emergence from a refuge and space use in guppies. *Scientific Reports*, *8*(1), 14131.

Kutsukake, N. (2009). Complexity, dynamics and diversity of sociality in group-living mammals. *Ecological Research*, *24*, 521-531.

Kydd, E., & Brown, C. (2009). Loss of shoaling preference for familiar individuals in captive-reared crimson-spotted rainbowfish *Melanotaenia duboulayi*. *Journal of Fish Biology*, *74*(10), 2187-2195.

Lachlan, R. F., Crooks, L., & Laland, K. N. (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour*, *56*(1), 181-190.

Laland, K. N. & Williams, K. (1997). Shoaling generates social learning of foraging information in guppies. *Animal Behaviour*, *53*, 1161–1169.

Laland, K. N., & Williams, K. (1998). Social transmission of maladaptive information in the guppy. *Behavioral Ecology*, *9*, 493-499.

Lefebvre, L., & Palameta, B. (1988). Mechanisms, ecology, and population diffusion of socially learned, food-finding behavior in feral pigeons. *Social learning: Psychological and biological perspectives*, 141-164.

Leris, I., & Reader, S. M. (2016). Age and early social environment influence guppy social learning propensities. *Animal Behaviour*, *120*, 11-19.

Li, A., Richardson, J. M., & Helen Rodd, F. (2022). Shoaling in the Trinidadian guppy: costs, benefits, and plasticity in response to an ambush predator. *Behavioral Ecology*, *33*(4), 758-766.

Lindeyer, C. M., Langen, E. M., Swaney, W. T., & Reader, S. M. (2015). Nonapeptide influences on social behaviour: effects of vasotocin and isotocin on shoaling and interaction in zebrafish. *Behaviour*, *152*(7-8), 897-915.

Lodé, T., Lélias, M. L., Lemasson, A., & Blois-Heulin, C. (2021). Solitary versus group living lifestyles, social group composition and cooperation in otters. *Mammal Research*, *66*, 13-31.

Lucon-Xiccato, T., & Bisazza, A. (2017a). Complex maze learning by fish. *Animal Behaviour*, *125*, 69-75.

Lucon-Xiccato, T. & Bisazza, A. (2017b). Sex differences in spatial abilities and cognitive flexibility in the guppy. *Animal Behaviour*, *123*, 53–60.

Lucon-Xiccato, T., Dadda, M., & Bisazza, A. (2016). Sex differences in discrimination of shoal size in the guppy (*Poecilia reticulata*). *Ethology*, *122*, 481-491.

Magurran, A. E. (1990). The adaptive significance of schooling as an antipredator defence in fish. *Annales Zoologi Fennici*, *27*, 51–66.

Magurran, A. E. (2005). *Evolutionary ecology: the Trinidadian guppy*. Oxford University Press, USA.

Magurran, A. E., & Seghers, B. H. (1990a). Population differences in the schooling behaviour of newborn guppies, *Poecilia reticulata*. *Ethology*, *84*(4), 334-342.

Magurran, A. E., & Seghers, B. H. (1990b). Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour*, *112*(3-4), 194-201.

Magurran, A. E., & Seghers, B. H. (1991). Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour*, *118*(3-4), 214-234.

Magurran, A. E., & Seghers, B. H. (1994). Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behaviour*, *128*, 121-34.

Magurran, A. E., Seghers, B. H., Shaw, P. W., Carvalho, G. R. (1994). Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. *Journal of Fish Biology*, *45*, 401-406.

Manara, V., Ruberto, T., Swaney, W. T., & Reddon, A. R. (2022). Subordinate submissive responses are predicted by dominant behaviour in a cooperatively breeding fish. *Behaviour*, *160*(2), 127-144.

Manteca, X., & Deag, J. M. (1993). Individual differences in temperament of domestic animals: a review of methodology. *Animal Welfare*, *2*(3), 247-268.

Markham, A. C., & Gesquiere, L. R. (2017). Costs and benefits of group living in primates: an energetic perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727), 20160239.

Marler, P., & Terrace, H. S. (1983). The Biology of Learning [electronic resource]: Report of the Dahlem Workshop on the Biology of Learning Berlin, October 23–28.

Mateo, J. M. (2004). Recognition systems and biological organization: the perception component of social recognition. In *Annales Zoologici Fennici* (pp. 729-745). Finnish Zoological and Botanical Publishing Board.

McRobert, S. P. & Bradner, J. (1998) The influence of body coloration on shoaling preferences in fish. *Animal Behaviour*, *56*, 611–615.

Mesoudi, A., Chang, L., Dall, S. R. X., & Thornton, A. (2016). The evolution of individual and cultural variation in social learning. *Trends in Ecology & Evolution*, *31*, 215-225.

Metcalfe, N. B., & Thomson, B. C. (1995). Fish recognize and prefer to shoal with poor competitors. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *259*(1355), 207-210.

Miller, N., & Gerlai, R. (2012). From schooling to shoaling: patterns of collective motion in zebrafish (*Danio rerio*). *PloS one*, *7*(11), e48865.

Mirza, R. S. & Chivers, D. P. (2000). Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Canadian Journal of Zoology*, *78*, 2198–2208.

Morgan, M. J. & Colgan, P. W. (1987). The effects of predator presence and shoal size on foraging in bluntnose minnows. *Environmental Biology of Fishes*, *20*, 105–111.

Morrell, L. J., Hunt, K. L., Croft, D. P., & Krause, J. (2007). Diet, familiarity and shoaling decisions in guppies. *Animal Behaviour*, *74*(2), 311-319.

Mühlhoff, N., Stevens, J. R. & Reader, S. M. (2011). Spatial discounting of food and social rewards in guppies (*Poecilia reticulata*). *Frontiers in Psychology*, *2*, 10129.

O'Neill, S. J., Williamson, J. E., Tosetto, L., & Brown, C. (2018). Effects of acclimatisation on behavioural repeatability in two behaviour assays of the guppy *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, *72*, 1-11.

Odling-Smee, L., & Braithwaite, V. A. (2003a). The role of learning in fish orientation. *Fish and Fisheries*, *4*, 235-246.

Odling-Smee, L., & Braithwaite, V. A. (2003b). The influence of habitat stability on landmark use during spatial learning in the three-spined stickleback. *Animal Behaviour*, *65*, 701-707.

Palagi, E., & Dapporto, L. (2006). Beyond odor discrimination: demonstrating individual recognition by scent in *Lemur catta*. *Chemical Senses*, *31*(5), 437-443.

Patterson, J. E., & Ruckstuhl, K. E. (2013). Parasite infection and host group size: a meta-analytical review. *Parasitology*, *140*(7), 803-813.

Pearce, J. M., & Bouton, M. E. (2001). Theories of associative learning in animals. *Annual Review of Psychology*, *52*(1), 111-139.

Pitcher, T. J. Parrish, J. K. (1993). Functions of Shoaling Behaviour in Teleosts. In T. J. Pitcher (Ed.), The Behaviour of Teleost Fishes (pp. 294-337). Boston, MA: Springer US.

Pitcher, T. J., Magurran, A. E., & Winfield, I. J. (1982). Fish in larger shoals find food faster. *Behavioral Ecology and Sociobiology*, *10*, 149-151.

Piyapong, C., Krause, J., Chapman, B. B., Ramnarine, I. W., Louca, V., & Croft, D. P. (2010). Sex matters: a social context to boldness in guppies (*Poecilia reticulata*). *Behavioral Ecology*, *21*(1), 3-8.

Proops, L., Troisi, C. A., Kleinhappel, T. K., & Romero, T. (2021). Non-random associations in group housed rats (*Rattus norvegicus*). *Scientific Reports*, *11*(1), 15349.

Reader, S. M. (2016). Animal social learning: associations and adaptations. *F1000Research*, *5*.

Reader, S. M., & Biro, D. (2010). Experimental identification of social learning in wild animals. *Learning & Behavior*, *38*, 265-283.

Reader, S. M., & Laland, K. N. (2000). Diffusion of foraging innovations in the guppy. *Animal Behaviour*, *60*, 175-180.

Reader, S. M., Kendal, J. R. & Laland, K. N. (2003) Social learning of foraging sites and escape routes in wild Trinidadian guppies. *Animal Behaviour*, *66*, 729-39.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291-318.

Reznick D., Travis J. (2019). Experimental studies of evolution and eco-evo dynamics in guppies (*Poecilia reticulata*). *Annual Review of Ecology, Evolution, and Systematics*, *50*, 335–354.

Reznick, D., Butler Iv, M. J., Rodd, H., & Dolph, S. (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *The American Naturalist*, *157*, 126-140.

Rodríguez, F., Quintero, B., Amores, L., Madrid, D., Salas-Peña, C., & Salas, C. (2021). Spatial cognition in teleost fish: strategies and mechanisms. *Animals*, *11*(8), 2271.

Rosenthal, R. (1979). The file drawer problem and tolerance for null results. *Psychological Bulletin*, *86*(3), 638.

Salvanes, A. G. V., Moberg, O., Ebbesson, L. O., Nilsen, T. O., Jensen, K. H., & Braithwaite, V. A. (2013). Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1767), 20131331.

Sánchez-Tójar, A., Nakagawa, S., Sanchez-Fortun, M., Martin, D. A., Ramani, S., Girndt, A., Bókony, V., Kempenaers, A. L., Westneat, D. F., Burke, T., & Schroeder, J. (2018). Meta-analysis challenges a textbook example of status signalling and demonstrates publication bias. *Elife*, *7*, e37385.

Schmid-Hempel, P. (2021). Sociality and parasite transmission. *Behavioral Ecology and Sociobiology*, *75*(11), 156.

Schons, R. F., Vitt, S. & Thünken, T. (2021). Environmental habituation and sexual composition affect juveniles' shoaling activity in a cichlid fish (*Pelvicachromis taeniatus*). *Journal of Fish Biology*, *99*, 1307–1317.

Seghers, B.H. (1974). Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. *Evolution*, *28*, 486-9.

Sharpe, L. L., Hill, A., & Cherry, M. I. (2013). Individual recognition in a wild cooperative mammal using contact calls. *Animal Behaviour*, *86*(5), 893-900.

Shaw, P. W., Carvalho, G. R., Magurran, A. E., & Seghers, B. H. (1991). Population differentiation in Trinidadian guppies (*Poecilia reticulata*): patterns and problems. *Journal of Fish Biology*, *39*, 203-209.

Shultz, S., & Dunbar, R. I. M. (2006). Both social and ecological factors predict ungulate brain size. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 207–215.Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*(7), 372-378.

Sims, P. Q., & Reader, S. M. (2021). An investigation of population variation in maze exploration and its predictors in wild Trinidadian guppies (*Poecilia reticulata*). *Behavioural Processes*, *192*, 104475.

Solberg, M. F., Robertsen, G., Sundt-Hansen, L. E., Hindar, K., & Glover, K. A. (2020). Domestication leads to increased predation susceptibility. *Scientific Reports*, *10*(1), 1929.

Song, Z., Boenke, M. C. & Rodd, F. H. (2011). Interpopulation differences in shoaling behaviour in guppies (*Poecilia reticulata*): Roles of social environment and population origin. *Ethology*, *117*, 1009-18.

Sorato, E., Gullett, P. R., Griffith, S. C., & Russell, A. F. (2012). Effects of predation risk on foraging behaviour and group size: adaptations in a social cooperative species. *Animal Behaviour*, *84*(4), 823-834.

Staddon, J. E. R. (2016). *Adaptive behavior and learning*. Cambridge University Press.

Stamps, J. A., Briffa, M., & Biro, P. A. (2012). Unpredictable animals: individual differences in intraindividual variability (IIV). *Animal Behaviour*, *83*(6), 1325-1334.

Stewart, A. M., Gaikwad, S., Kyzar, E., & Kalueff, A. V. (2012). Understanding spatio-temporal strategies of adult zebrafish exploration in the open field test. *Brain research*, *1451*, 44-52.

Suboski, M. D. & Templeton, J. J. (1989). Life skills training for hatchery fish: social learning and survival. *Fisheries Research (Amsterdam)*, *7*, 343–352.

Swaney, W. T., Cabrera-Álvarez, M. J., & Reader, S. M. (2015). Behavioural responses of feral and domestic guppies (*Poecilia reticulata*) to predators and their cues. *Behavioural Processes*, *118*, 42-46.

Swaney, W., Kendal, J., Capon, H., Brown, C., & Laland, K. N. (2001). Familiarity facilitates social learning of foraging behaviour in the guppy. *Animal Behaviour*, *62*(3), 591–598.

Templeton, C., & Shriner, W. (2004). Multiple selection pressures influence Trinidadian guppy (*Poecilia reticulata*) antipredator behavior. *Behavioral Ecology*, *15*(4), 673–678.

Thinus-Blanc, C. (1996). *Animal spatial cognition: Behavioural and brain approach*. World Scientific Publishing Company.

Thompson, R. F., Spencer, W. A., (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychology Review*, *73*, 16–43.

Thornton, A., & McAuliffe, K. (2006). Teaching in wild meerkats. *Science*, *313*(5784), 227-229.

Thünken, T., Eigster, M., & Frommen, J. G. (2014). Context-dependent group size preferences in large shoals of three-spined sticklebacks. *Animal Behaviour*, *90*, 205e210.

Tibbetts, E. A., Dale, J. (2007). Individual recognition: it is good to be different. *Trends in Ecology & Evolution*, *22*(10), 529–537.

Tibbetts, E. A., Pardo-Sanchez, J., & Weise, C. (2022). The establishment and maintenance of dominance hierarchies. *Philosophical Transactions of the Royal Society B*, *377*(1845), 20200450.

Tolman, E. C. (1932). Purposive Behavior in Animals and Men. New York: Appleton-Century-Crofts.

Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychology Review*, *55*, 189-208.

Travis, J., Reznick, D., Bassar, R. D., López-Sepulcre, A., Ferriere, R., & Coulson, T. (2014). Do eco-evo feedbacks help us understand nature? Answers from studies of the Trinidadian guppy. In *Advances in ecological research* (Vol. 50, pp. 1-40). Academic Press.

Triki, Z., Zhou, T., Argyriou, E., Sousa De Novais, E., Servant, O., & Kolm, N. (2024). Social complexity affects cognitive abilities but not brain structure in a Poeciliid fish. *Behavioral Ecology*, *35*(3), arae026.

Trompf, L., & Brown, C. (2014). Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. *Animal Behaviour*, *88*, 99-106.

Tumulty, J. P. (2022). Dear enemy effect. *Encyclopaedia of Animal Cognition and Behaviour* (pp. 1937-1940). Springer International Publishing.

Tumulty, J. P., & Sheehan, M. J. (2020). What drives diversity in social recognition mechanisms? *Frontiers in Ecology and Evolution*, *7*, 517.

Valuska, A. J., & Mench, J. A. (2013). Size does matter: The effect of enclosure size on aggression and affiliation between female New Zealand White rabbits during mixing. *Applied Animal Behaviour Science*, *149*(1-4), 72-76.

van Bergen, Y., Coolen, I., & Laland, K. N. (2004). Nine-spined sticklebacks exploit the most reliable source when public and private information conflict. *Proceedings of the Royal Society B: Biological Sciences*, *271*, 957-962.

Vassileva, I. (2019). *Assessing Flexibility in Shoaling and Learning in the Trinidadian Guppy*. MSc Thesis, McGill University, Montreal.

Wagner, A. P., Frank, L. G., & Creel, S. (2008). Spatial grouping in behaviourally solitary striped hyaenas, *Hyaena hyaena*. *Animal Behaviour*, *75*(3), 1131-1142.

Wang, M. Z., & Hayden, B. Y. (2021). Latent learning, cognitive maps, and curiosity. *Current Opinion in Behavioural Sciences*, *38*, 1-7.

Warburton, K. (1990). The use of local landmarks by foraging goldfish. *Animal Behaviour*, *40*, 500-505.

Ward, A. J. W., Axford, S., Krause, J. (2002). Mixed-species shoaling in fish: the sensory mechanisms and costs of shoal choice. *Behavioral Ecology and Sociobiology*, *52*(3):182–187.

Ward, A. J. W., Herbert-Read, J. E., Sumpter, D. J. T., & Krause, J. (2011). Fast and accurate decisions through collective vigilance in fish shoals. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 6.

Ward, A. J. W., Webster, M. M., Magurran, A. E., Currie, S. & Krause, J. (2009). Species and population differences in social recognition between fishes: a role for ecology? *Behavioral Ecology*, *20*, 511–516.

Ward, A. J., & Hart, P. J. (2003). The effects of kin and familiarity on interactions between fish. *Fish and Fisheries*, *4*(4), 348-358.

Ward, A., & Webster, M. (2016). Sociality: the behaviour of group-living animals. Berlin, Germany: Springer.

Warren, J. M. (1957). The phylogeny of maze learning. (1) Theoretical orientation. *The British Journal of Animal Behaviour*, *5*(3), 90-93.

Webster, M. M., & Laland, K. N. (2020). No evidence for individual recognition in threespine or ninespine sticklebacks (*Gasterosteus aculeatus* or *Pungitius pungitius*). *Royal Society open science*, *7*, 191703.

Webster, M. M., & Rutz, C. (2020). How STRANGE are your study animals? *Nature*, *582*(7812), 337–340.

Webster, M. M., Chouinard-Thuly, L., Herczeg, G., Kitano, J., Riley, R., Rogers, S., Shapiro, M. D., Shikano, T., & Laland, K. N. (2019). A four-questions perspective on public information use in sticklebacks (Gasterosteidae). *Royal Society Open Science*, *6*(2), 181735.

Whitehead, H. (2003). Sperm whales: social evolution in the ocean. University of Chicago Press, Chicago

Wiley, R. H. (2013). Specificity and multiplicity in the recognition of individuals: implications for the evolution of social behaviour. *Biological Reviews*, *88*(1), 179-195.

Wilson, A. D., Krause, S., James, R., Croft, D. P., Ramnarine, I. W., Borner, K. K., Clement, R. G., & Krause, J. (2014). Dynamic social networks in guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, *68*(6), 915-925.

Wright, D., Nakamichi, R., Krause, J., Butlin, R. K. (2006a). QTL analysis of behavioral and morphological differentiation between wild and laboratory zebrafish (*Danio rerio*). *Behavior Genetics*, *36*(2), 271–284.

Wright, D., Ward, A. J. W., Croft, D. P., & Krause, J. (2006b). Social organization, grouping, and domestication in fish. *Zebrafish*, *3*, 141-155.

Yang, Y., Sánchez-Tójar, A., O'Dea, R. E., Noble, D. W., Koricheva, J., Jennions, M. D., Parker, T. H., Lagisz, M., Nakagawa, S. (2023). Publication bias impacts on effect size, statistical power, and magnitude (Type M) and sign (Type S) errors in ecology and evolutionary biology. *BMC Biology*, *21*(1), 71.

Ydenberg, R. C., Giraldeau, L.-A., & Falls, J. B. (1988). Neighbours, strangers, and the asymmetric war of attrition. *Animal Behaviour*, *36*, 343–347.

Zhang, Z., Zhang, X., Li, Z., & Zhang, X. (2019). Effects of different levels of environmental enrichment on the sheltering behaviors, brain development and cortisol levels of black rockfish *Sebastes schlegelii*. *Applied Animal Behaviour Science*, *218*, 104825.