An Investigation into the Influence of Hunger-State on the Mate-Choice Copying Phenomenon in Trinidadian Guppies *(Poecilia reticulata)*

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

This study investigates the effect of hunger state on the propensity for female Trinidadian guppies *(Poecilia reticulata)* to mate-choice copy. It is predicted that the hungrier the individual, the more likely they will use social information over asocial information when making effective decisions. This prediction is based on evidence suggesting that an individual should rely on other sources of information when asocial information is costly (Hoppitt and Laland, 2013; Bandura, 1977). An evolutionary trade-off between cheap yet inaccurate data, and expensive and accurate data (Boyd and Richerson, 1985). This study is a new and novel approach to the reversal experiment outlined by Dugatkin and Godin (1992) where females make a decision based on asocial information alone, and are then offered social information influencing the contrary decision. The female is then allowed to re-take that decision, pitting asocial information against the new social information. This experiment aimed to teach female guppies an association using a colour cue and the location of a high-quality male. The association would act as a female's asocial information, and the behaviour of a demonstrator female would provide social information. These females were starved to varying degrees and this was then statistically analysed alongside their decision making. This study produces evidence to suggest that the hungrier the female, the more likely she is to copy the demonstrator's decision. This study then debates these findings. This paper shows an original and exciting approach to state-dependent mate-choice copying phenomenon found by female guppies that may stimulate new research in this field.

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1. Introduction

1.1 What is asocial and social information?

Organisms sample their environment to improve their chances of making a correct decision, reduce the risk and damage of making uninformed decisions and to reduce vulnerability to uncertainty (Dall et al, 2005). Natural selection shapes each being producing a rich diversity of species ranging, for example, from the mantis shrimp with their 16 colour receptors to cartilaginous fish such as shark and ray's electroreception. Regardless of the novelty, each organism has evolved a way to take information from its environment that is used to influence decision making. The more reliable and accurate information, the greater the effectiveness of the decision. More evidence prior to making a decision, the smaller the risks associated with making such a decision.

Information is taken from the environment in many forms, from abiotic and biotic sources. Where an organism gets its information can directly correlate to how reliable or accurate that information is, and this can have fitness benefits and costs for the organism. The two competing sources of information that are being assessed in this experiment are "social information" and "asocial information"

Asocial information refers to data that an individual learns, usually from trial-and-error with its environment (Kendal et al, 2005). The repeated exposure to a cue and a biological stimulus builds associations. Where one is, the other may also be there. This link makes decisions easier and quicker to make. Usually associations are based around a biological reward or punishment (positive or negative reinforcement) which enforces the association. A cue that makes something good happen for an individual will be remembered, the same goes with a cue that has a negative impact.

Pollinator species, such as a honeybee, for example, identify which plant species are most beneficial to visit in terms of cost and reward by assessing the colour and shape of the flower (Srinivasan, 2010). To achieve this, photoreceptors in the pollinator pick up varying levels of stimuli, and receptors sensitive to certain frequencies of light fire signals to the honeybee's brain. The bee's own asocial information, acquired from its environment, allows it to combat the arms race that exists between plant-pollinators and maximise its own fitness. Each species will want to take advantage of the other, skewing the cost-reward ratio in their favour.

The case of the honeybee is particularly pertinent as it is a species that exhibits many forms of information acquisition, including asocial and social forms. Honeybees are incredibly strong associative learners, and exploit classical conditioning to ensure fitness benefits in a world of noisy, ineffective data.

The advantage to using asocial information is that it can be very informative. This means it reduces uncertainty to improve decision making. Personal preference is built over many previous experiences, and so provides a large amount of information content but does come at a high cost. Cost in decision making is measured not only in energy expenditure but also in the time it takes to sample, risk of injury or predation, or "missed opportunity costs" (Kendal et al, 2005).

One of the drawbacks of asocial information is that it takes a long time to perfect, as it is difficult to "block-out" inaccurate information when there is a high quantity of data available. Essentially, there is a huge amount of information that is not beneficial to the individual and should be ignored. It also has a high energetic cost, the bee, for example, spends a huge amount of energy testing flowers that may not be the most effective choice for its time and effort. Furthermore, large amounts of time spent

sampling increases risk of predation or wasting time and energy. Natural selection will favour the individual that's decision making improves its relative fitness in surviving, reproducing and ensuring offspring survival. Mistakes, using false information, following social information when it's incorrect, spending too much time on sampling for asocial information are all bad for an individual as they jeopardise that individual's relative fitness.

In contrast to asocial information, social information is information taken from the environment also but instead of sampling key ecological variables the individual instead observes another individual's interactions with those variables. It is important to note that, these individuals do not need to be related, and do not always need to be members of the same species. Intra-specific social information use is information that is transferred from one species to another, often inadvertently (Valone, 2007; Danchin et al, 2004). Inter-specific social information use is information that is given between members of the same species (Seppänen et al, 2007; Goodale et al, 2010) both on purpose (Danchin et al, 2004; Dall et al, 2005),

Initially, the honeybee is working with information provided by another species, the plant. However, this plant is the object, and not an organism interacting with an object, and so it remains asocial information. If the honeybee observes another individual interact with the plant, and makes new decisions based on these observations then these could have been influenced by social information. Recruitment tactics such as the waggle-dance (Von Frisch, 1967) are used to inform hive-mates to areas of high-quality resource (Riley et al, 2005). The dance does also provide odour and information on how good the quality of the resource is, with more waggling meaning greater worth (Gruter, Leadbitter and Ratnieks, 2010). This is a clear example of social information being used, information is given off by one individual to many others in the

hive to inform decision making. They then use this information to benefit themselves, and their relatives in the hive (Gruter, Leadbitter and Ratnieks, 2010). This information will also benefit the plant, making them more attractive to their pollinator.

To conclude the honeybee example, the species is open to taking information from a variety of sources. These sources include the direct environment or from social sources. An individual honeybee can maximise its fitness by picking the most cost-effective information source for the current scenario. During times of more stable resource availability the costs of asocial sampling could be considered worthy. High-cost/ high-reward scenarios can benefit an individual's fitness greatly, if the cost can be afforded. There are also times when the costs of asocial are not fully justifiable, and the copying of conspecifics is the most proficient of decisions.

There is a wealth of evidence for social information leading informed decision-making in many species to combat a series of day-to-day ecological problems. The most common uses of social information are in food discovery and in mate choice. These are both important choices that each individual must make to survive and attain reproductive success. The key difference between the two are the frequency at which the organism will have to make these decisions.

Foraging and resource acquisition is an area saturated with evidence for social information influencing decision making in a wide variety of species such as Norway Rats (Rattus norvegicus) (Galef and Bennett, 1990), European Starlings (Sturnus vulgaris) (Templeton and Valone, 1996), Red Crossbills (Loxia curvirostra) (Smith, Benkman and Coffey, 1999) and many more (Valone, 1989; Danchin et al, 2004; Valone and Templeton, 2002). Discovering and maintaining a constant supply of food to support growth, development and reproduction is vital to the success of the majority

of species. Therefore, it is understandable that finding alternatives to high-risk strategies, expensive foraging behaviours and exploiting asocial information is commonplace. To maintain a constant food supply, some species are known to copy foraging habits from other species to reduce the costs of foraging via asocial means (Krebs, 1973; Powell, 1985; Seppänen et al, 2007). This information flow is known to influence animal social structure and dynamics (Goodale et al, 2010) and can explain the appearance of novel interactions between species over a short amount of time, such as the mutualism between the honeyguide (Indicatoridae) of Africa and Asia and humans (Spottiswoode, Begg and Begg, 2016).

The second most popular field of social information use is found in mate-choice. There are many mechanisms in which the behaviours and decisions in choosing the correct mate exist such as Fisher's runaway model (Fisher, 1930). This model explains how the genetic trait for attraction to another, particular trait, join each other in one individual. (Pomiankowski and Iwasa, 1993; Stoner and Breden, 1988). This model can be used to debate the evolution of exaggerated male ornamentation (Andersson and Simmons, 2006).

Indicators of fitness and survivability are dominant and are found in all mate-choice copying decisions for example winning fighting bouts during lekking (male-dominated sexual selection contests) (Gibson and Bradbury, 1985; Gibson et al. 1991; Widemo and Owensi, 1995). In some cases, the ability to afford the weight of characteristics that reduce immediate survivability is an honest signal of quality. This is encapsulated in the handicap principle by Zahavi (Zahavi 1975; Johnstone, 1995; Zahavi 1999). In most species, there are numerous signals of mate-quality (Bro-Jørgensen, 2010), both positive and negative and the ratio of these will shift throughout time as an organism's

ecological conditions change (Blows et al, 2004). However, the mechanism of mate-choice that this study is based upon is the phenomenon of “mate-choice copying”.

1.2 What is mate-choice copying?

Mate choice copying is a phenomenon found in a variety of vertebrates such as birds (Galef and White, 1998), fish (Witte and Ryan, 2003) and humans (Waynforth, 2007). The majority of evidence is laboratory based, however, with examples of its frequency in the wild lacking (Galef and White, 1998). It is observed when an individual has its mate-choice decisions influenced by the choices of others (Dugatkin, 1992; Danchin, 2004). This is usually shown through a reversal experiment. This is broken down into 3 major steps: 1, an individual is left to make a decision between two prospective mates; 2, she then observes a demonstrator female go through the same choice, but pick differently; 3, she is then given the chance to make the decision again. If she changes her mind and chooses a mate that was favoured by the demonstrator, she most likely used social information to reverse her choice.

Mate-choice copying in nature requires multiple conspecifics to be present at the same time (Galef and White, 1998). It also requires them to be of sexual maturity with an interest in mating i.e. not a non-receptive pregnant individual. The best place to start investigating this behaviour was therefore in lekking species (Hoglund et al. 1990, 1995; Gibson et al. 1991). However, it is difficult to distinguish what is a copying event, and what is a shared attraction to the best of that selection regardless of social information or competition (Clutton-Brock and McComb, 1993). Therefore, a slightly altered definition would suggest that the copying event exists if the choice made is actually for a lesser quality mate by all other quality assessments, with the only reason that mate being chosen is due to other conspecifics favouring it.

There is evidence of mate-choice copying existing in various species that also exhibit these social, lekking-esque mating trials such as the sailfin molly (Witte and Ryan, 1998; 2002), the black grouse (Höglund et al, 1995), the Japanese quail (Galef and White, 1998) and most recently in humans also (Waynforth, 2007). These papers show a remarkable increase in the attractiveness of some mates, usually males, after viewing the male being courted by a competitor.

As previously stated the quality of a mate is different between populations, and fluctuates with ecological condition (Feldman et al, 1996). Therefore, as pressures on each individual change the propensity to copy may also change. If mate-choice is directly correlated to energy use then as a constraint on energy increases, the chance of an individual taking a cheap alternative to make the mate-choice may increase. This will again change with species as the variety of mating systems, reproductive methods and skews in parental care differ between all species. For example, a female with a long gestation, in a monogamous relationship with high levels of female parental care will be less inclined to make quick, cheap mate choice. However, a female with a quicker generational turnover, in a male-competitive population with more room for error may make that riskier choice more often.

This, of course then raises the question, when should an individual copy, and when should it rely on its personally acquired asocial information? There are three leading theories to explain a copying event; 1, copy when asocial information is costly; 2, copy when asocial information is out-dated; 3, copy when uncertain.

An individual should rely on other sources of information when asocial information is costly (Hoppitt and Laland, 2013; Bandura, 1977). There is an evolutionary trade-off between cheap yet inaccurate data, and expensive and accurate data (Boyd and

Richerson, 1985). The costly information hypothesis (Bandura, 1977) states that the more hazardous the mistake, the heavier the reliance on observational learning from more competent examples (Bandura, 1977; Galef, 1995). It is also not just the attainment of this information that is costly; the use and processing of the data may also be costly (Galef, 1995). There is evidence of the "costly information hypothesis" in many species including in a European Starlings foraging study (Templeton and Giraldeau, 1996) a study on guppies (Laland and Williams 1998), and in humans (Bikhchandani et al, 1992).

There are, however, risks when copying if asocial information is too costly. It is possible that it may lead to what are known as informational cascades (Bikhchandani et al, 1992; 1998). This occurs when errors are carried forward in sequential copying events, with the individual at the end of the line suffering a massive drop in accuracy of information, like the phenomenon of "Chinese whispers". The greater the cost of engaging in erroneous cascades (Bikhchandani et al., 1992, 1998) the greater the selective pressure to ignore the decisions of others, forcing individuals to fall back upon asocial information (Giraldeau et al, 2002).

As outlined previously, social information may be used if the previously acquired asocial information is out-dated, and the chance of it being no longer relevant is high (Hoppitt and Laland, 2013). Social information use is thought to be favoured when the rates of change in an organism's environment is both minor and slow (Boyd and Richerson, 1985, 1988). With low rates of environmental change there is a reduced risk of information becoming out-dated and interactions with said environment will be easier to predict. Moscarini et al looked at the effect of the climate change on informational cascades (Bikhchandani et al, 1992; 1998) and predicted that copying and social learning may only occur for a limited amount of time, and in the more

unpredictable the organism's future the trade-off between social and asocial learning will favour self-taught information (Moscarini et al, 1998). A fluctuating environment that increases the costs of asocial learning will then push individuals to copy information culturally from conspecifics, most likely from a pool of out-dated data (Kameda and Nakanishi, 2002; Doligez et al, 2003). It is then predicted that natural selection will then act against reliance on social learning should a variety of environments become more unstable and unpredictable to the species within them.

When uncertain, an individual may be more likely to use immediate sources of social information to reduce uncertainty (Hoppitt and Laland, 2013; Boyd and Richerson, 1988). Uncertainty in any situation is very dangerous for any organism (Dall et al, 2005). Laland (2004) states that when prior information means that the individual is certain on what to do then they should ignore all social information (Laland, 2004). A repeat study by van Bergen et al (2004) shows that sticklebacks will ignore all social information when their own prior knowledge is adequate (originally carried out by Coolen et al in 2003). It is also the case that in some species it may be difficult for younger individuals to gain knowledge on important decisions. The harder it is to discriminate the right decisions from the wrong ones, the more likely it is an individual will copy (Nordell and Valone, 1998). A theoretical model by Stohr on this principle predicts the uncertainty of young individuals discriminating what the best quality mate is can lead to the evolution of mate-choice copying (Stohr, 1998).

It may also be the case that a copying event depends on all three of these considerations, with each carrying a varying degree of influence depending on an organism's ecological conditions at any given moment. It is also key to recognize that in nature temporal change leads to new pressures which affect the costs of information, therefore the out-datedness of prior information, and uncertainty brought

by change is expected to reduce reliance on copying behaviours (Stohr, 1998; Nordell and Valone, 1998)

1.3. The Trinidadian Guppy

The Trinidadian Guppy (Poecilia reticulata) is one of the most widely used species in evolutionary and behavioural ecology today due to its easy management, short generation times and quick adaptability and the wealth of background data (Magurran, 2005). Guppies are poeciliids which are a group of fish characterised by internal fertilization and viviparity (Wourms, 1981). Female guppies store sperm and this can be used to fertilize ova for up to 8 months (Winge, 1937). Mate choice in this species is dominated by female choice, with her making the decisions as to which male to accept fertilisation from. This has led to the evolution of two novel methods of acquiring copulations for the males. Males can either display their quality for consensual courtship, or they can inseminate uncooperative females through sneaky mating's (Baerends et al, 1955). Females are more responsive to mate with males as virgins or a few days after giving birth (Liley, 1966), reproduction also occurs all year round (Alkins-Koo, 2000).

Males differ morphologically to females, showing ornamental fins and a range of coloured spots all over the body, it is this colouration that acts as a basis for female choice in the guppy (Houde, 1987; 1997). The size (genetics) and brightness (dietary carotenoids) of the males coloured spots, including melanin dark spots, directly relates to the quality of the male (Kodric-Brown, 1989; Long and Houde, 1989; Houde, 1987). Orange colouration is the most obvious sign of quality and the more orange the male, the more attractive he is to the female (Long and Houde, 1989). "Orangeness" is not only attractive because of the ability to survive the cost of conspicuousness in high-

predator dense environments, but it has also been shown to correlate to male swimming strength and foraging efficiency (Kodric-Brown, 1989; Head, Wong and Brooks, 2010).

There is evidence that female guppies also value personality when selecting optimal mating partners, using boldness as a cue (Godin and Dugatkin, 1996). Boldness positively correlates to visual conspicuousness of the colour patterns on the male. By picking bolder males, the females are also picking, on average, more colourful males (Godin and Dugatkin, 1996). Inspective behaviours not leading to mortality produce more informed individuals, less likely to be harmed by uncertainty (Dugatkin, 1992). It is not just males that exhibit this personality, with bolder females excelling in associative learning tasks over their less bold compatriots (Dugatkin and Alfieri, 2003).

Female preference for males does fluctuate between populations, as with many species, this is partly due to the conflicting natural-selection and sexual-selection pressures acting on high colouration (Stoner and Breden, 1988). Achieving bright coloured spots in a low predation risk area with high density dietary carotenoids is not as attractive as achieving brightly coloured spots in high-predator, high-parasite loaded, low food quality environments (Kodric-Brown, 1989; Houde and Torio, 1992).

Based on the evidence, it can be established that guppies exhibit multi-component signalling systems when it comes to mate choice, with dynamic variation in the selective pressures on the many independent populations found in streams/rivers throughout tropical ecosystems (Bro-Jorgensen, 2010). The strongest indicator of quality is how colourful the male is, predominantly orange colouration (Houde, 1987; 1997; Long and Houde, 1989; Magurran, 2005). This cue will be used to determine quality in the following experiment.

In 1992, Dugatkin built an experiment to test the existence and frequency of mate choice copying behaviours in the Trinidadian guppy (Poecilia reticulata). He concluded that female preference can be influenced by social information, and imitation can predict mate choice in the species (Dugatkin, 1992). Then the reversal of previously made decisions was investigated, providing evidence that females can be made to change a priori estimations of quality following an influx of social information (Dugatkin and Godin, 1992). Briggs et al (1996) then explicitly tested if the mate-choice copying phenomenon was affected by the cost of predation. Contrary to primary hypotheses there was no evidence of increased copying frequency under high predation risk, but instead the opposite. Guppies disregarded their personal preference or asocial information and chose mates according to apparent preference of other females in the absence of the immediate threat of predation (Briggs et al, 1996). It was then found in 1998 that food-deprived guppies did not show an enhanced tendency to use social information despite the fact that they would be facing high energetic costs in acquiring asocial data. In fact, these guppies were less likely to use social information as deprivation increased (Dugatkin and Godin, 1998).

Despite the body of evidence supporting the existence of mate-choice copying in guppies, there is, likewise, evidence that it does not always occur. A study showed that the female's ease of discrimination based on ornamentation did not influence the use of social information in mate-choice events in guppies (Brooks, 1996). A re-evaluation of Dugatkin's (1992) experiment failed to provide the same results under the same conditions, observing no relationship between mate choice and copying (Lafleur and Sclafani, 1997). They concluded more work needed to be done. One of the prominent criticisms of these reversal experiments was their reliability. Critics hypothesised that focal females only reverse their decision to a new male because of

the disruption of seeing another female interaction (Applebaum and Cruz, 2000; Dugatkin, Druen and Godin, 2003). This predicts the focal female, the female doing the observation, would subsequently and consistently prefer the male she initially rejected. However, Dugatkin, Druen and Godin (2003) found no evidence for that, rather provided further support for mate-choice copying in the guppy. More recent evidence continues to support mate-choice copying in the guppy (Godin, Herdman and Dugatkin, 2005).

1.4 The Experiment

1.4.1 Aims and Predictions

The aim of this study is to experimentally test the potential influence of the hunger state, as manipulated by food deprivation, of female guppies on their tendency to mate-choice copy. The prediction of this experiment is that females will be more likely to mate-choice copy when they are hungrier. It predicts that females will show preference for social information with increasing hunger levels, the hungrier they are the more likely they will copy. It's also predicted that fully satiated females on the other hand would be able to sustain these costs of sampling, and the risks of missed opportunities (Kendal et al, 2005).

This study will take on a novel approach that integrates associative learning to produce a source of asocial information. This takes the form of a colour cue that indicates the presence of a high-quality male. The female's association will then be pitted against the social information given to the her via a demonstrator (Dugatkin and Godin, 1992). This study is therefore broken down into two sections; a conditioning phase and a testing phase. In the conditioning phase, a female is given her asocial information through associative learning of a colour cue being located

near a high-quality male. This is then followed immediately by the testing phase where her loyalty to this information will be tested against social information.

1.4.2. The Conditioning Phase

The aim of the conditioning phase is to produce the asocial information in the form of a colour cue. The colour cue will be taught to the female through repetitive exposure to the stimuli, in this case a male, and a non-biological cue, in this case a colour cue. This is known as sexual conditioning (Holloway and Domjan, 1993) and is a novel approach for associate learning in Trinidadian guppies. The phase is based around two central questions; 1, has an association been formed? ; 2, how does this association differ when comparing the start and the end of the trial?

Predictions in this phase consist of females building an association between coloured cues and the location of high-quality mates based on the extensive literature on mate choice in guppies (Kodric-Brown, 1989; Long and Houde, 1989; Houde, 1987, Head, Wong and Brooks, 2010). The formation of the association will be measured in two ways: the time spent near the male and which male the female approaches first. The prediction is, therefore, that the female will not only approach the higher-quality male first (more-often than random) but also spend more time near that male, proportionally to the other male. These measurements have been shown previously to be strong indicators of mating preference (Dugatkin and Godin, 1992). The conditioning phase will continue for 8 days, with females being exposed to the stimuli and non-biological cue every day.

It is also predicted that the strength of this association will grow over time due to repeated exposure to this stimuli and cue. Therefore, it's expected that females will first approach the stimuli, with the corresponding cue to her learning, at a greater

frequency later in the week. Day 1 measurements versus day 8 measurements will be compared. Evidence of successful associative learning would be a significant difference from random first choice of which stimuli approached first.

After the conditioning phase, the females will be subjected to varying levels of starvation. The population of females will be split into three groups, one of these will not experience any limitations on their food supply. The other two groups will go without any food, one group for 24 hours, the other for 48 hours. After the females have been fasted for the correct time they will be introduced into the reversal experiment of the testing phase.

1.4.3. The Testing Phase

The testing phase will then test the individual females' propensity to use social information. The female will first make her own, asocial choice. She will then act as an observer, watching a demonstrator make the opposite choice to the one she made just minutes before. To finish the reversal experiment, observer will then be given the opportunity to choose again, re-entering the test and can either stick to her previous, asocial choice, or change her preference to that matching the demonstrator female.

The actions of each female will be recorded and then tested statistically to look for evidence for any significant difference between treatment groups in their use of different forms of information. There are 3 central questions to this phase; 1, is there any single effect that impacts how a female chooses a mate; 2, is there any joint interaction that impacts how a female picks a mate; 3, what do these interactions look like?

The prediction of this phase is a significant interaction between demonstrator's actions and treatment imposed, showing evidence for state-dependent social information use in mate-choice. It is predicted that hungrier females will be more likely to base their decisions on social information, rather than stick with the costlier asocial information. This prediction is based on the "copy when asocial costly" hypothesis (Bandura, 1977; Hoppitt and Laland, 2013). This was not seen by Dugatkin and Godin in 1998.

The fully satiated, non-starved individuals are expected to prefer the use of asocial information of social information, as they can afford the costs (Bandura, 1977) and the asocial conditioning will reduce their uncertainty (Hoppitt and Laland, 2013; Boyd and Richerson, 1988). Females that have been starved for 24-hours are predicted to use social information over asocial, as they use the behaviours of the demonstrator female to guide their decision making. This is because the cost of using asocial is high in comparison to social. The 48-hour treatment group are predicted to only use social information, showing the greatest propensity to mate-choice copy.

This paper aims to broaden the debate on conflict between asocial and social information sources, opening doors for new research into why, and how organisms copy. Using guppies as a model this approach also aims to encourage more general research on the presence and frequency of copying behaviours in multi-signalling mating systems. If copying is found to be energy-dependent, then questions can be asked on how much value an organism puts on pieces of information and how costly uncertainty is on mate-choice.

2. Methods

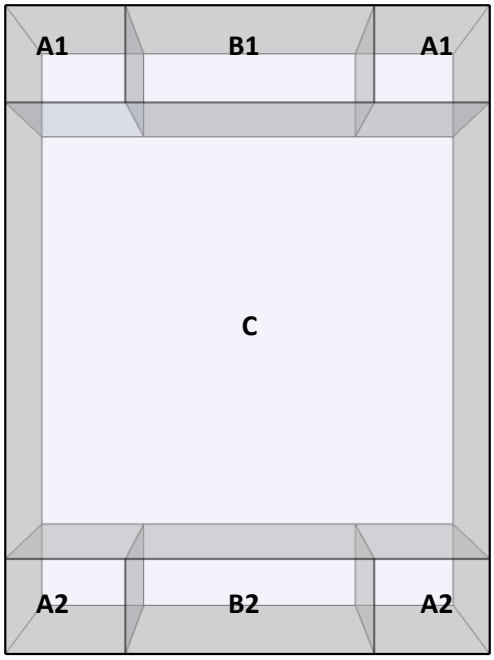
2.1 Introduction

The conditioning phase aims to teach the females an association between a colour cue and the quality of the male nearby. This will act as their asocial information. Over the course of 8 days the females will be consistently exposed to this colour cue and a male of certain quality. This phase is immediately followed by the testing phase, where the asocial information is offered alongside social information. In between these two phases some females will experience food-deprivation. The propensity of each female to use asocial or social information will then be analysed as a factor of their food-deprivation state. The following methodology explains how the females were given the opportunity to form associations which will then act as their asocial information.

The conditioning phase was conducted in a tank sized 42.5cm length by 25cm width by 25cm height (figure 1). The tank is split into 7 sections. Four 7.5cm by 7.5cm by 25cm boxes on each corner (marked by A1 and A2), with two 7.5cm by 10cm by 25cm joining cells running cross-width between these boxes that will house the males (B1 and B2). Corner boxes show certain coloured sheets of coloured plastic (A1 shows one colour, A2 shows a different colour). The central zone (C), measuring 27.5cm by 25cm by 25cm which is where the female will be left to explore and be monitored. Water fills up 4cm deep. The quality of the male held in the chamber (B1 or B2) between the coloured boxes (A1 or A2) will depend on the focal female in the central zone (C). For example, if this female is forming an association with high-quality males and the colour blue, then a high-quality male will be found in the chamber (B1/B2) in-between two corner boxes (A1/A2) containing blue coloured

plastic. At the other end, there would be a low-quality male in a chamber in-between another colour, for example pink.

Figure 1: The Conditioning Tank: Top Down View



Top down view of the experimental apparatus for the conditioning phase of this experiment. IT consists of a rectangular aquarium (42.5cm length by 25cm width by 25cm height). All walls of this aquarium are transparent. White walls placed against outside of tank. Fish behaviour was viewed using a video camera mounted 50cm above the centre of the apparatus. A1, corner box containing plastic sheet of colour 1; B1 clear chamber for male of corresponding quality to colour 1; C, central chamber that female is placed in to roam freely and is monitored by camera; A2, corner box containing plastic sheet of colour 2; B2, clear chamber for male of corresponding quality of colour 2.

The rationale for the sizes as listed above was to ensure a healthy amount of free-roaming space for all live-subjects used in this experiment. The central section (C), where the female will explore, is small enough for the female to investigate within the time limit and make her choice. It is also large enough so that there is some cost to this sampling. The corner sections (A1/A2) are large enough for the colours to be obvious to the females in the central chamber, but not so big that they impact the male's welfare. The end chambers (B1/B2), that house the males, are large enough for the male to inhabit without any damaging effects to their welfare, but small enough to ensure that when the female is nearby she will always see the male.

During any experimentation, a camera above records footage from inside the tank and relayed it through “Viewer” software. This software records the movement of the fish inside the tank, where it goes and what it does and for how long. To more objectively measure the activity within the tank the inner area (C, figure 1) was split into 3 zones. The size of these zones was determined to be just greater than the size of the largest female guppies (no larger than 3cm). This was because if the female faces the male area (A1/2 and B1/2, figure 1) head on, it was important to not lose any information should a reference point, e.g. the tail, leave the area and spoil results. The zone had to be big enough to contain the largest, whole female but not so large that a female would enter it and not see the male.

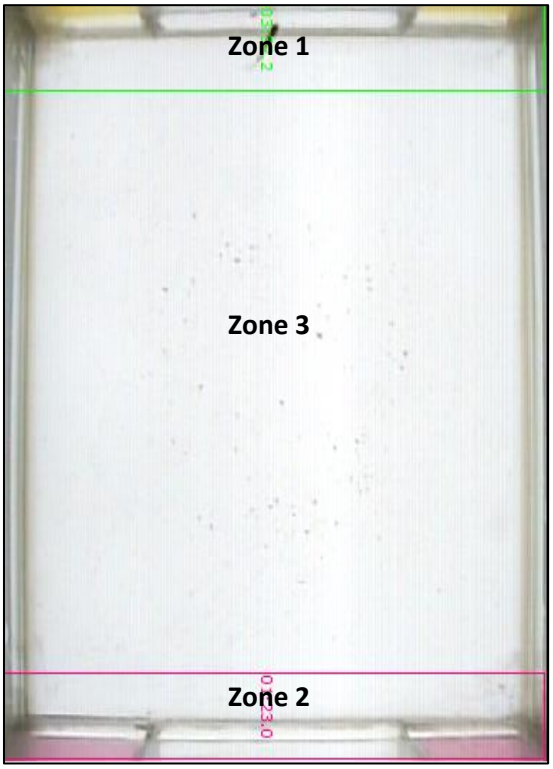


Figure 2: The Zones of the Conditioning Tank

Top down view of the experimental apparatus as seen by the camera mounted 50cm above centre. Image taken directly from software. Zone 1, 4cm length by 25cm width zone directly beside areas A1 and B1 shown in figure 1; Zone 2, 4cm length by 25cm width zone directly beside areas A2 and B2 shown in figure 1; Zone 3, the remaining 34.5cm length by 25cm width zone of the central chamber (C, figure 1).

These zones were placed directly next to male areas (chambers A1/A2 and B1/B2, figure 1; zones 1 and 2, figure 2). Time in these zones, near the males, was recorded and used in this study as a proxy for interest in that male. The rationale for using this as a proxy is that it has been used to show interest in other guppy mate-choice experiments (Dugatkin, 1992). Which zone (1 or 2, not 3) the female enters first is also recorded.

One of the first steps in associative learning is picking a cue that retains no previous association with the individual. It is well established that female guppies primarily base mate choice decisions on the colours shown by a male's phenotype (Houde, 1997). Therefore, the guppy retina contains cones that are differentially sensitive to wavelengths (Endler et al, 2001). There is evidence to suggest that guppies also have some pre-existing colour biases (Rodd et al, 2002; Grether et al, 2005). Because of the ability to form associations with colours previously shown in the guppy (Endler et al, 2001; Rodd et al, 2002; Grether et al, 2005), colour cues were used to form new associations in this experiment. However, because of pre-existing colour biases, it was important to run preliminary testing to determine of these biases exist before teaching new associations.

2.2 Subjects

All guppies used in the following experiment were taken from a Trinidadian wild population in a high predation risk environment. These were kept for approximately 10 generations in the lab. All fish were fed twice a day on flake food and frozen daphnia and live brine shrimp. Fish were kept under identical abiotic conditions in tanks with a through-flow system. Temperatures remained at 24°C with a neutral ph.

2.2.1 Females

A sample of females was taken from a stock tank of thousands of mixed gender guppies. These females were isolated for one month away from males. Any pregnant females had offspring removed upon birth. The gestation time for Trinidadian guppies is 21 to 30 days. Isolation for one month ensured no new copulations would occur and no pregnant females would be used in the study. Female size was measured but only to ensure a rough equal age of female being used in the study. Females with a body length between 1cm and 3cm were used. These size measurements take into consideration sexual maturity as a factor of living in a high-predation risk population, like their ancestral relatives.

The remaining females were separated into 4 groups each with their own tank, all maintained to identical conditions (table 1). These groups were assigned colours at random. Once given a colour, which would eventually become their non-biological cue, this colour would not change for them for the entirety of the trial. For example, the group assigned the colour green would only ever see a high-quality male next to a green cue throughout the conditioning phase. After segregation into smaller shoal sizes mortality increased for some of the groups (table 1).

A full run through of the conditioning phase and the testing phase is a single trial. After the completion of a conditioning phase and following testing phase, the female group would be rested, with unlimited food, for two weeks. This was determined to be enough time for them to return to fresh state that they were in before any conditioning began. This allowed for new isolated female guppies to be introduced into these resting populations, to maintain a reliable sample size. This does mean that some females were exposed to multiple trials and some were not.

During the conditioning phase, the females will not experience any food deprivation. Unfortunately, the female groups showed a high mortality rate. Table 1 shows the female population numbers for each group. During the resting time between trials, new females (isolated and size/age restricted) were introduced to make up for this. This will be accounted for in the discussion section as a potential confounding element to this study.

Trial	Color	Number of females at the start	Number of females at the end	Females lost	Previous females	Number of new females added
1	blue	8	8	0	0	8
1	pink	8	7	1	0	8
2	green	8	6	2	0	0
2	pink	8	8	0	7	1
3	pink	10	8	2	8	2
3	yellow	10	8	2	0	10
4	blue	8	7	1	8	0
4	green	8	7	1	6	2
5	pink	8	6	2	8	0
5	yellow	6	4	2	6	0
6a	blue	8	5	3	0	8
6a	green	7	5	2	0	7
6b	pink	7	7	0	0	7
6b	yellow	8	8	0	0	8

7a	pink	6	5	1	7	0
7a	yellow	7	5	2	8	0
7b	blue	7	4	3	5	2
7b	green	5	4	1	5	0
					Total females:	63

Table 1: Table to show female population numbers throughout trials.

Table shows the sample sizes of all females used throughout experiment. Trial, this refers to a single 9-day slot of 8 days of conditioning and testing on the 9th day; Colour, these are which colours were used for that particular trial; Number of females at the start, this refers to how many females started this experiment, taking into consideration that new females may have been added; Females at the end refers to how many females survived the trial and how many were left. If this number was too low to place into a new study, new females would be introduced. Females lost, refers to how many females died during that trial; new females added refers to how many isolated females that had not been tested before were added into the stock before trial began.

2.2.2 Males

Males, like the females, were taken from the same stock tank and held in isolation for a minimum of one month. Like the females this was to ensure no sexual activity, from courtship to copulation. 30 males were taken into isolation. Each male was sedated and photographed (figure 3). These photographs were used to analyse the quality of the male using “ICY” software (de Chaumont et al, 2012). According to female guppies, orange colouration is a strong indicator of male quality; the more orange the male, be it the number or the size of orange coloured spots, the more attractive the male (Kodric-Brown, 1989; Long and Houde, 1989; Houde, 1987). All

spots were measured, along with basic measurements such as body length and total body area for each male. The males were given a rating with the larger males with lots of orange colouration being regarded as the high quality, and smaller males with not much orange colouration being considered low quality (figure 3).

Males were then separated into pairs of equally differing quality (figure 3). Firstly, the two males, if they were to be paired, the high-quality male had to have a positive difference in overall colour, as measured as a portion of their total body area, of at least 10mm^2. This difference could be no more than 13mm^2. Secondly, the higher quality male also needed to show more orange colouration (see figure 3). The higher-quality male had to show at least 1mm^2 more orange than the lower-quality male in the pair. The last determining characteristic of the pair was that the higher-quality male needed to be at least 0.5mm larger in length in comparison to the lower-quality male of the pair. Because of the exactness of these measurements (table 2), many males did not fit into a category and were released back into stock. A total of 10 males, in 5 pairs, were then selected to be used in this study.

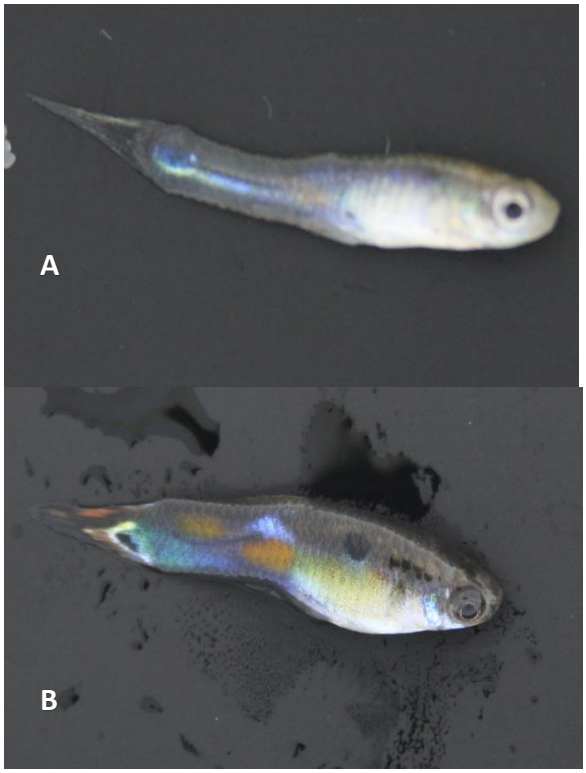


Figure 3: Pair of males

The image is taken from photos before measurements were taken from them. This image represents a typical one of the 5 pairs of males used. A, low quality male showing little/no orange colouration; B, higher quality male showing lots of orange colouration and a larger size than A.

The 5 pairs are shown in table 2, where their measurements are also listed. There was no male death in this experiment. A male pair would be selected at random for their use in that day's experiment by rolling a dice. The rationale behind these size measurements was that the high-quality male must always be larger than the low-quality male. However, as colouration is deemed more important the measurement for length was relatively small, at 0.5mm. Orange is the most effective indicator, so a difference is required. A difference of 1 mm^2 ensured that one male was considerably more orange than his counterpart. Total body colouration was used also. 10 mm^2 is a very large portion of a male Trinidadian guppy. This makes it obvious to the female which is the higher quality male.

Pair Number	High-quality male ID	Low-quality male ID	Difference in Colour (mm^2)	Difference in Orange (mm^2)	Difference in length (mm)
1	mh28	mh20	10.89	2.03	0.76
2	mh27	mh4	10.92	1.54	1.11
3	mh25	mh1	10.55	1.85	0.93
4	mh23	mh18	12.71	1.08	1.47
5	mh26	mh22	12.50	1.88	2.14

Table 2: The pairings of male Trinidadian guppies

Table to show the 5 pairs of males that were used in all experimentation and how they differed statistically. Difference in colour is measured as the proportion of the total body area (mm^2) that shows any colour compared with the pair. Difference in orange is measured as the proportion of the total body area (mm^2) of the male that shows the colour orange.

2.3 Preliminary testing

For the preliminary testing, the tank was set up almost identically to how it would be used in the experiment. The only difference was that no males were used in this. The zoning (figure 2) was the same and recorded identically to how it would in conditioning phase. This was to determine whether the females had any specific bias for the colours being used. A control was ran using no colour, to determine if females had any bias for any end or aspect of the tank. For preliminary testing methodology see below procedure without using males. The results for this preliminary bias testing is shown below in table 2. There is no significant bias for any of the 4 colours used (table 3). The “no-colour” test was conducted separately from the others. There is no significant bias for any zone in the absence of colour (table 3). With these results, the experiment can continue.

Colour	Average time spent (s)	Average first entry	Number of observations	Probability of observation
Pink	142.58	0.61	22	0.055
Yellow	169.84	0.49	18	0.132
Blue	155.81	0.47	17	0.125
Green	142.03	0.43	15	0.081
No Colour	153.18	0.48	17	0.125

Table 3: Table to show results from preliminary colour-bias testing

Table shows the results from preliminary tests on females entering conditioning tank (figure 1) and recording behaviour. Probability of observation was taken from a two-tailed binomial test.

2.4 The Procedure

After preliminary testing was complete the conditioning trials could begin. Some added components were used. Figure 5 shows these components and how they will be used alongside the procedure.

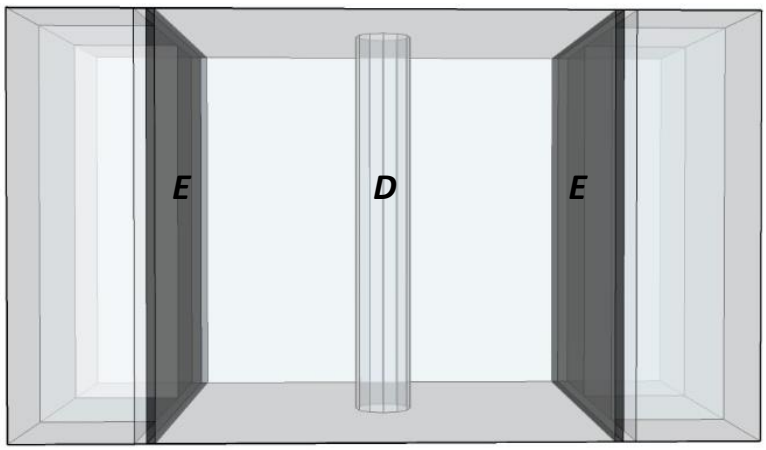


Figure 4: Sideview of the Conditioning Tank

This shows the same tank shown in figure 1 but from the side view. This shows extra components not shown in previous figures. D, moveable transparent plastic cylinder 4cm in diameter and 25cm in height; E, moveable opaque plastic partitions that can be used to block female view of colours (A1/2, figure 1) and males (B1/2, figure 1).

To help acclimatise the female after she has been placed into this new environment it was important to reduce the number of stimuli to reduce stress. Figure 4 shows the sideview of the conditioning tank. The moveable transparent cylinder is placed directly into the centre of the tank (D, figure 4). The cylinder is large enough to comfortably house the female for a short amount of time. The cylinder is punctured with holes, allowing for water to flow through. In this cylinder, the female will be able to see the entire tank. To stop the female being able to see the colours (A1/2, figure 1) or the males (B1/2, figure 1) two partitions are placed at the ends of the tank (E, figure 4). The female can now be entered into the water of the tank and left to acclimatise to her surroundings. The partitions can be moved and the female can then see the colours and the males whilst still within the cylinder. This means she can have time to make

decisions before sampling. She will sample the males (figure 3) by entering the zones (figure 2) and seeing the males in their chambers (B1/2, figure 1). The rationale to this placement of this cylinder is also that it ensures the female enters the exact centre of the chamber, allowing for no bias for fish entering a particular side/zone more. The cylinder is also far enough away from each side so that sampling of the males is not possible without closer inspection. This should attract the females to approach and sample males.

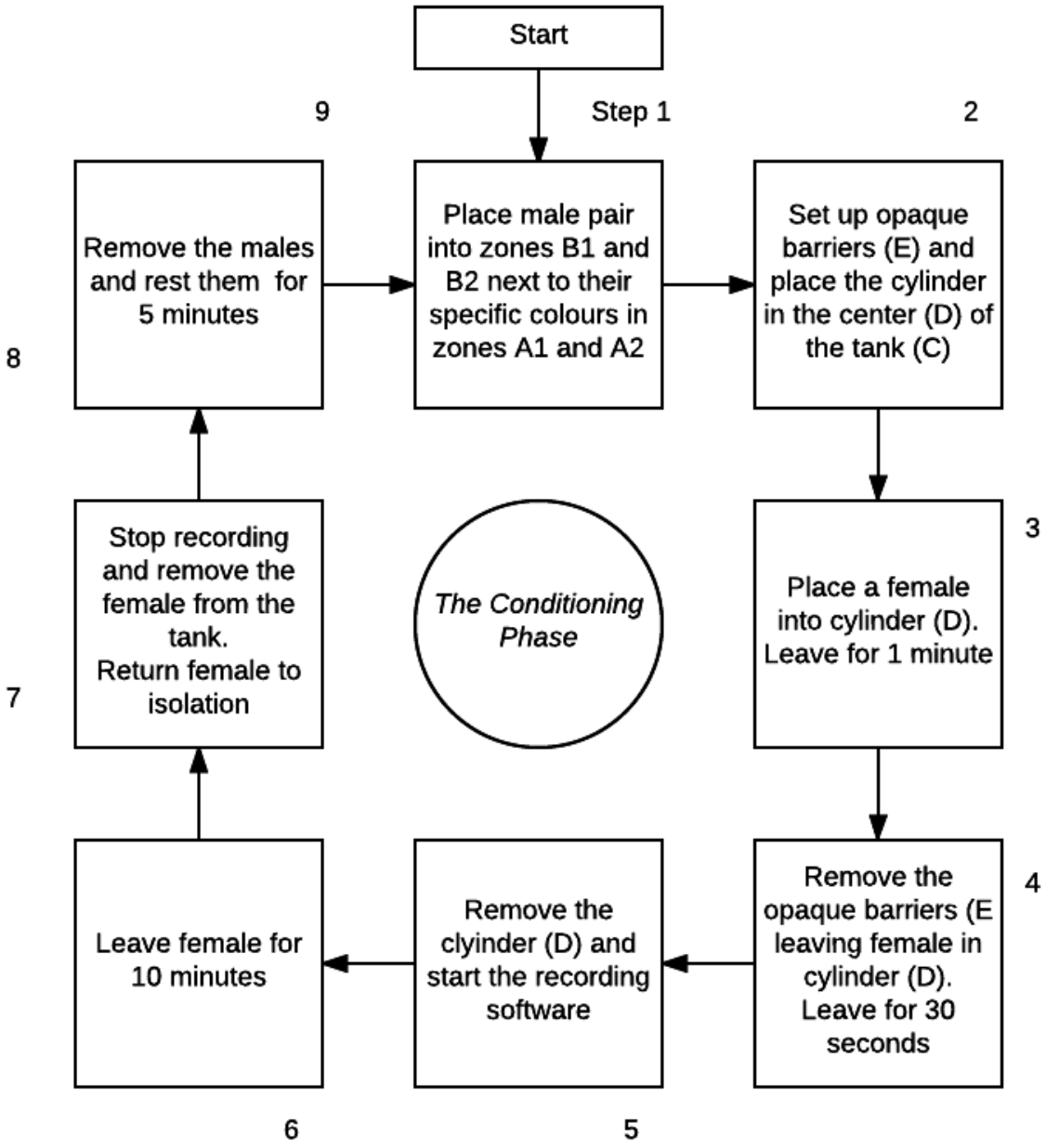


Figure 5: The procedure for the Conditioning Phase

Flow chart to show the 9 steps of each run through of the conditioning phase. Each of the boxes is marked with a number and these are all discussed blow. Arrows indicate movement from one step, forward onto the next.

With the tank (figure 1) ready, the zoning marked out and prepared to record (figure 2), the extra components added (figure 4), the females isolated, grouped and prepared (figure 3; table 1) and the males assessed and paired up (figure 3; table 2), we can begin the conditioning phase. Figure 5 below shows the procedure of this phase

Before starting, all subjects must be prepared for this particular experiment. The conditioned colour must match to the high-quality male. The end in which the high-quality male of the pair is placed is done via random choice (dice roll). Although preliminary experiment showed no bias to a particular end of the tank, it is still worth taking into consideration. Temperature of the water in the tank was checked to make sure it was the same as that of the tanks in which the females are housed (24c). This is to reduce the shock the female will experience in entering a new environment.

Step 1 (figure 5), a pair of males is chosen at random and placed into the male chambers (B1/B2, figure 1). Depending on the female that is about to be tested, the colours in boxes (A1/A2, figure 1) is changed so that the high-quality male is next to the conditioned cue. Step 2 is to place the opaque barriers shown in figure 5 in their place, blocking the view of the males (B1/B2) and colours (A1/A2) from the inner chamber (C, figure 1). Also place the plastic cylinder (D, figure 4) into the centre of the inner chamber (C). Making sure the tank is ready for the female to be entered into it.

Step 3 is to place the female, carefully, into the cylindrical holding cell (D). From here she will be in the centre of the inner chamber, she will not be able to explore the chamber yet, however. She cannot yet see the colours (A1/A2) nor the males (B1/B2). She is left for 1 minute to acclimatise to her new surroundings and to calm down after the human contact she received whilst moving between tanks.

Step 4 is to remove the opaque barriers (E, figure 4). Now the female in the cylinder (D) will be able to see the colours (A1/A2) and the males (B1/B2) but she still cannot move towards them as she is still contained within the cylinder. She is left for 30 seconds in the cylinder. Step 5 is the removal of the cylinder (D), setting the female free to explore the inner chamber (C). At the same time of the removal of the cylinder, the recording starts and all movement in the 3 zones (figure 2) is recorded. Step 6 is to leave the female for 10 minutes.

After 10 minutes, step 7 requires the stopping of the recording and the removal of the female from the inner chamber. The female can then be taken back to her home tank to rest. She will be left with an abundance of food for 24 hours when she will then be conditioned again. Each female is conditioned a total of 8 times over 8 days. Step 8 is to remove the males from their chambers. They are now left to rest for 5 minutes with food and shelter from human interference.

Step 9 is the repeat of this entire procedure: the males are placed back into their corresponding chambers depending on the conditioning colour cues to their sides and a new female is prepared to enter. The Conditioning Phase will end for the day once all females have experienced full experimentation. The male pair stays the same throughout that day of testing. The following day a new male pair will be used,

again chosen at random with the condition that they cannot have 2 days of testing in a row.

After 8 days of repeated exposure to the stimulus and cue, the females will move on to the testing phase. During this shift into the testing phase there are some requirements. The 0-hour treatment group, the group that will experience no starvation, are ready to immediately enter into the testing phase 24 hours after their last conditioning phase on day 8. The 24-hour treatment group are also able to start the testing phase the following day (day 9), however when they are returned to their tank on day 8, step 7, there will be no food in that tank for them. The tank will have been cleaned of all food debris and no new food will be given to them until full completion of the testing phase over 24hours later.

The 48-hour treatment group require 48 hours of starvation. It would not be fair for them to be starved whilst still in the conditioning phase. This may affect how they make associations. Therefore, the food deprivation starts immediately after day 8, just like it does for the 24-hour treatment group, but they are not tested the next day, and instead they are moved on to the testing phase on day 10. They are rested on day 9 without any experimentation or food.

The testing phase continues on day 9 for the 0-hour and 24-hour treatment groups, and on day 10 for the 48-hour treatment groups. Because of the methodology for the testing phase, the 48-hour treatment group were tested a day earlier than all other

groups. This was to make the day of testing the same for all groups. All females from all treatments were tested on the same day under the same test conditions.

2.5 Statistical Analysis

The results from the conditioning phase will be analysed to determine whether an association has been formed between the high-quality male and the colour cue. This will be done in two ways. The first method of statistically analysing this data is to look at the probability of a female entering a zone on day 1 compared with day 8. The probability will be analysed using a binomial test. The second method of statistical analysis will be to look at the time spent in each zone by the female. This will compare how much time is spent in the zone marked with the correct cues and stimuli versus the zone with an opposing colour and lower-quality male. The results from day 1 will then be compared against the results of day 8 and analysed via a t-test. There are two central questions for this phase and they are as follows:

1. Do fish show a preference for their zone of conditioning? This will just look at how often a female first enters her zone of training, and how long she spends in the zone of training. This will look at both days, 1 and 8.

2. Is there any change in preference between day 1 and day 8 of the trial? This question looks at the difference between measurements taken on day 1 to day 8. It will look to determine if an association is forming.

2.6 Predictions

The prediction of this phase is that females will enter the zone of her training more frequently on day 8 when compared to day 1. The prediction is also that this will be significantly different from random choice of a zone. Another prediction is that the

female will spend significantly more time in zones of her training. This is also predicted to increase, become more significant, on day 8 when compared with day 1.

2.7. The Testing Phase

2.7.1 Introduction

Note: For the entirety of this experiment, the term demonstrator is the individual that is providing social information and the term observer is the individual who is watching the demonstrator, taking in their social information and making the decision.

This experiment sets out to determine whether or not females have a greater propensity to copy a demonstrator female when they are hungrier, instead of relying on their own asocial information. The females have just come out of the conditioning phase where they were given the opportunity to form associations with coloured cues and potential mates. If the conditioning phase was successful the female will be in possession of our asocial information, it is now time to test whether or not they will stick with that information when presented opposing social information from the demonstrator. 24-hour and 48-hour treatment groups enter this stage with 24 hours and 48 hours of food deprivation respectively. The 0-hour treatment group enters this stage with no food deprivation.

The following testing phase will take place in a new tank, built specifically for this purpose. The tank is shaped as a “Y” maze, named as such as the shape resembles the letter “Y”. Figure 6 shows the Testing Phase tank. The tank is designed out of an identical rectangular tank used in the conditioning phase to the dimensions of 42.5cm length by 25cm width by 25cm height. Partitions have been added to create the maze shape, offering the demonstrator (I2, figure 6) a choice of two paths, left or

right. Each path is marked with a colour (G1/G2, figure 6). At the end of each path lies a small chamber to the dimensions of 6.25 cm length by 5cm width by 25cm height (F1/F2, figure 6). These chambers will house the males. These chambers are right in the corners of the rectangular tank. These chambers cannot be seen until the demonstrator (I2) has fully investigated that arm of the maze. The tank is filled to 5cm deep of water, deeper than in the conditioning stage but this is because the maze is smaller in total area, so more water gives the females more space, reducing stress.

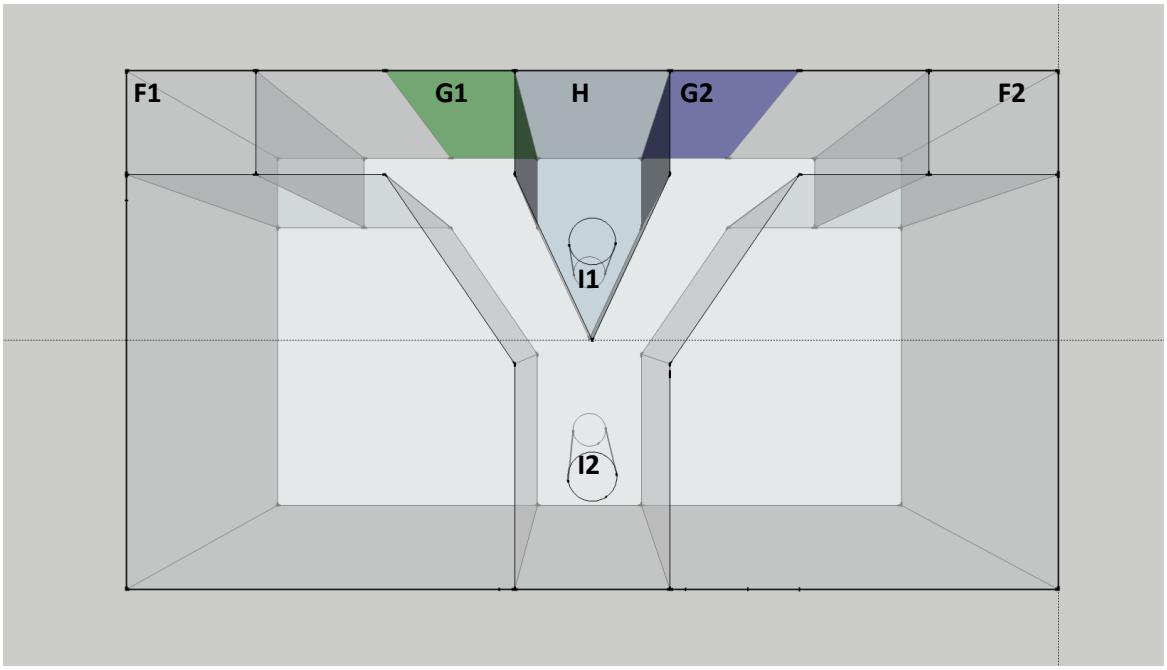


Figure 6: The Testing Tank

Figure 6 represents the "Y" shaped maze used to test the propensity of mate-choice copying in the guppy. The rectangular aquarium (42.5cm length by 25cm width by 25cm height) has been split up into various sections. F1/F2, these are the end chambers (6.25cm length by 5cm width by 25cm height) that will house the male pair; G1, here a colour is shown (in this case green); G2, here another colour is shown (in this case blue); H, the observer chamber (irregular pentagonal prism, 67cm^2 area); I1, the observer; I2, the demonstrator.

The rationale for these sizes is based on what is visible for the demonstrator female whilst she explores the maze. The maze requires full exploration of one arm for the female to see a male. Therefore, the size of the male's enclosure takes this into consideration and cannot be too big. It is also not so small that it raises concerns for the male's quality of life. The size of the observer chamber (H) is large enough for the female to view all the maze, whilst splitting the arms into a large enough size for the demonstrator to explore. The arms are long enough to make sampling them fully have some cost, if it was cheap to explore then even the hungriest of females could afford to do it. 5 minutes was determined to be long enough to record all necessary behaviours. Any longer than this would make the entire ordeal of multiple testing too long for the female, risking her health.

At the top of the "Y" shape is a pentagonal shaped chamber (H, figure 6). This is where the observer (I1, figure 6) female will be housed. From this vantage point the observer (I1) can see almost the entire maze. The observer female cannot see the end of the arms of the maze, and therefore she cannot see the males. The colours (G1 and G2) used in this testing phase are presented identically to how they were presented to the females in the conditioning phase; they are coloured sheets of plastic that are placed against the transparent glass.

The aim of this phase is to allow a demonstrator female (I2) decide as to which arm of the maze she investigates first and most based on the colours (G1/G2) and the males (F1/F2). The demonstrator will be watched by an observer (I1) who will already have gone through this maze, and made her choice. After being presented with the demonstrator's information, the observer runs the experiment for a second time. This second time gives her the opportunity to change her decision based on

the new, social information she has been given. She may, however decide to not change her mind, basing her decision on her own asocial information.

Just like in the conditioning phase the colours will correspond to the males as per the association. For example, females who have spent 8 days prior making the association of the colour blue and the presence of a high-quality male, will find the high-quality male down the arm of the maze marked with the colour blue. Again, just like in the conditioning phase, time and zone of first entry are used as measurements for females interests and decisions.

Figure 7: The Zones of the Testing Tank

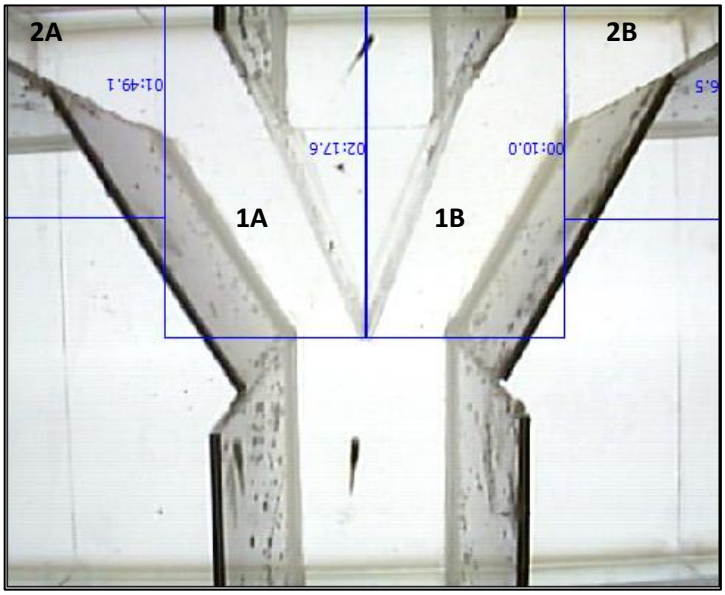


Image taken from camera above testing tank showing the zones drawn. 1A, the first zone representing the right arm (left on image); 2A, the end zone of the right arm, where the male is visible; 1B, first zone representing the left arm (right on image); 2B, the end zone of the left arm, where the male is visible.

Just like the conditioning stage the testing tank is recorded by a camera suspended 50cm above. This camera feeds into a computer where “Viewer” software records the movements of the fish. The tank is split into zones by the software (figure 7). There are 4 zones, two zones per arm of the maze. The first part of the arm is covered by Zones 1a and 1b (figure 7). These represent the zones of female first interest. The next two zones, Zones 2a and 2b (figure 7), represent the end of each

arm, where the male is visible. Entry and time spent in this zone is used as a proxy for male interest (Dugatkin, 1992). The rationale behind these zones is that a female could enter one arm but not see the male, so the zones are not equal in showing preference for a mate. The female can, however, see the colour in front of her (G1 or G2, figure 6).

2.8 The Procedure

The procedure is split into 3 stages. In the first stage, a female will be given the opportunity to explore the maze, see the colours and sample the males. She will then make a decision by entering a particular zone first, and showing preference for a zone by spending time in it (figure 7). The second stage this same female becomes an observer, watching a demonstrator make an opposing decision. In stage 3 the female is given the chance to change her decision as she runs the experiment a second time.

In the following procedure, the term “group” relates to a cohort of females that have all been taught the same colour association. The group number is just an arbitrary number to differentiate between the two colours. 1 could refer to the colour pink, 2 could refer to the colour green, for example.

The procedure starts by preparing 3 females. One of the females will be from group 1, and will spent the last 8 days being taught that colour 1 represents the presence of a high-quality male. The second female is from group 2 and will have spent the same amount of time forming associations as the first, but instead has been taught that colour 2 represents the presence of a high-quality male. All females from group 1 believe colour 1 to be the indicator of high-quality male presence whereas all of group 2 believe colour 2 to be the indicator. The third female is a dummy female, a female that has not been used in this experiment and has no information. The dummy is

required because the first stage of each experiment requires a fish to be in that chamber, to make it balanced for each test.

In preparation for the coming testing phase a pair of males will be selected at random (roll of a dice) and used for that days testing. All females running the experiment will be referred to as demonstrators. All females watching another female run the experiment will be referred to as observers. The following procedure is split into 3 stages. In the first stage, the female explores the maze and picks a male of preference by spending time in their zone. They are only in possession of their asocial information at this stage. In stage 2 they observe a demonstrator female run the same maze and make an opposing decision. This is manipulated by using a demonstrator with an opposing association. In stage 3 the observing female gets to run the maze a second time, now in possession of both social and asocial information.

2.8.1 Stage 1

Step 1 (figure 8), the males (F1/F2) and colours (G1/G2) are organised into their places in the maze (figure 6). Step 2 is to place the dummy female into the observer chamber (H). Here she acts as the observer, but she will never be tested upon. The first female is now to be placed into the experiment, acting as a demonstrator for the dummy female. In this step (3) she is placed into a plastic cylinder, identical to the one used in the conditioning stage (D, figure 4). The cylinder is placed at point I2 (figure 6). She is left in the cylinder for 1 minute, which helps her acclimatise to the new environment and calm her down after the stress of human handling. Step 4, the cylinder is removed and the recording begins. The female will explore the maze, directly ahead of her she will see the two colours she has seen every day for the last 8 days. One of these colours will be associated with the presence of a high-quality

male, the other will mark the presence of a lower quality male. The zones she enters (figure 7) will record how long she spends in each arm, along with which arm she entered first

After 5 minutes, the recording is stopped and the dummy observer is removed from the maze and rested, that's step 5. Step 6 is where the demonstrator female is moved into H, the observer chamber. Stage one has now ended (figure 8), the demonstrator is now the observer and will witness a new female enter the maze in stage 2 (figure 9).

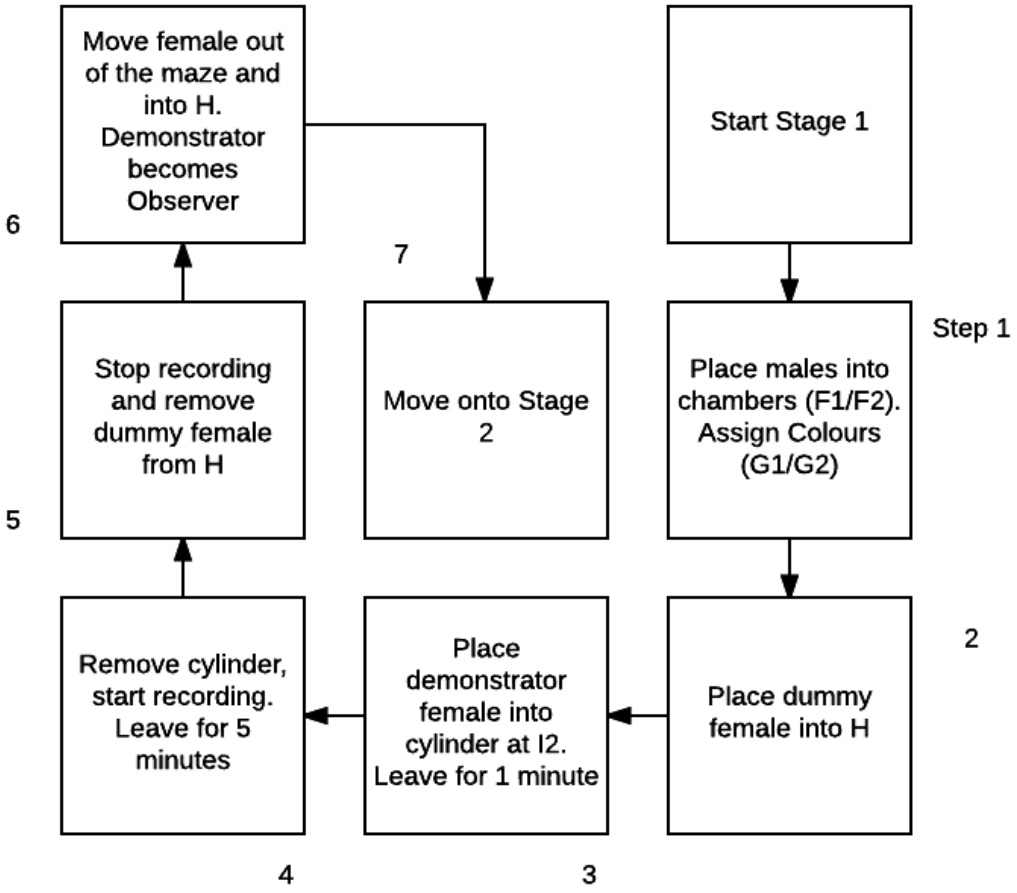


Figure 8: Stage one of the Testing Phase

Figure 8 shows the steps from 1 to 7. In this stage, the female explores the maze for the first time whilst being watched by a dummy female. She is only in possession of asocial information at this point.

2.8.2 Stage 2

The now observer female is resting in the observer chamber (H). The following steps are illustrated in figure 9. Before the new female can be introduced, the males have to be swapped around (F1/F2, figure 6). This is because the new female will have an opposing association to the female now acting as an observer. This second female will be from a different group, where the other colour is the one representing the high-quality male. The observer cannot see this change of males. Step 8 is to swap the males, step 9 is to place the cylinder back at point I2 (figure 6). The new female is entered into the cylinder at step 10, where she can acclimatise to the new environment. For step 11 the cylinder is removed and the new female, the demonstrator, explores the maze and is recorded for 5 minutes. It is predicted that her association will draw her to the opposing arm of the observer, as that is now where the high-quality male is, alongside the demonstrators preferred colour.

The next step (figure 9) is to stop the recording, remove the observer female from her chamber (H) and place her in an intermediary tank. The demonstrator female is then taken from the maze and entered into the observer chamber (H). Step 13 is to place a cylinder at point I2 (figure 6). The final step of this stage is to place the observer female into the cylinder, beginning her re-acclimatisation of the maze as the next step is to move onto stage 3.

The aim of stage 2 (figure 9) is to present the observer female with social information. She has explored the maze in stage one, acquiring her own asocial information and she has now been presented with social information, potentially opposing her previous

decision. The demonstrator from this stage has now experienced her first run through of the maze, and stage 3 (figure 10) will be her chance to acquire social information.

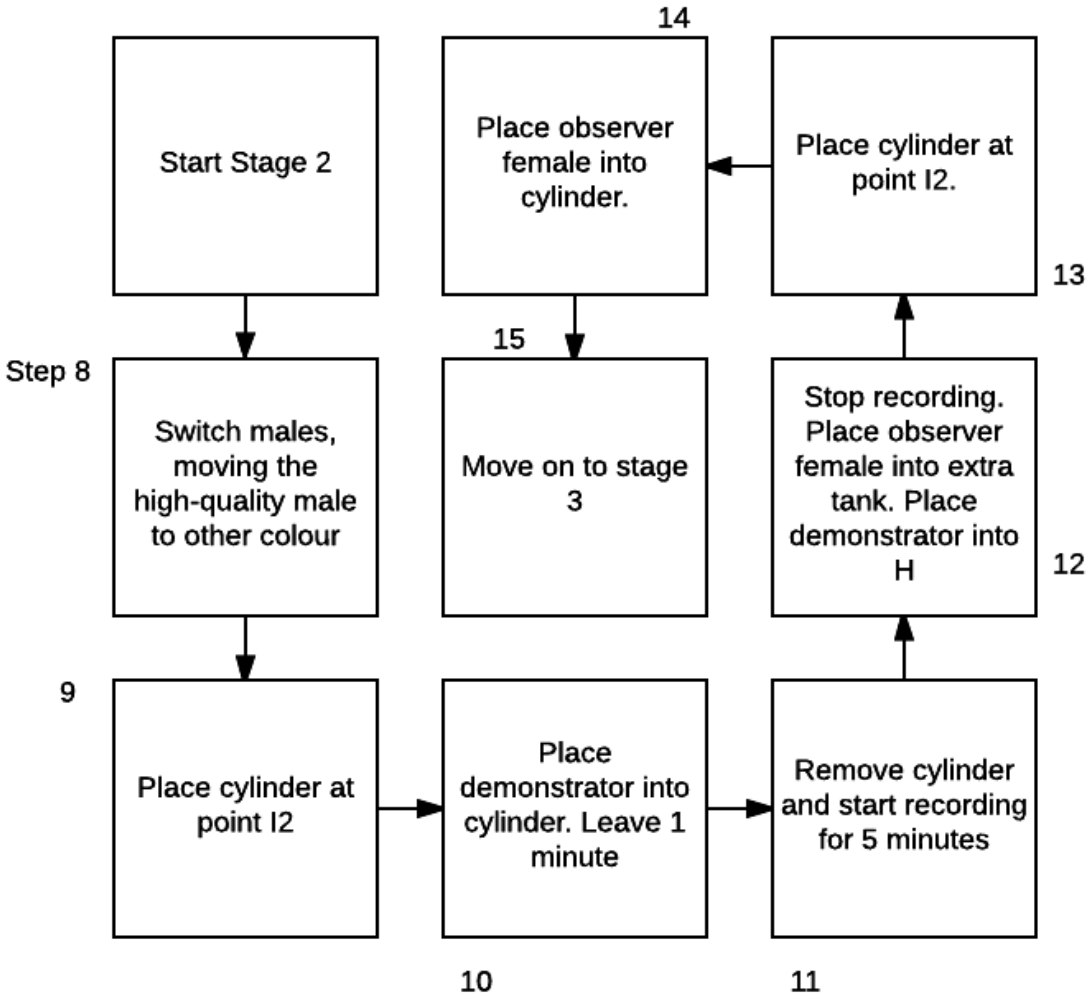


Figure 9: Stage 2 of the Testing Phase

Steps 8-15 are shown in this flowchart, explaining how to provide the observer female with social information as she becomes an observer, watching a demonstrator run the maze she just came out of.

2.8.3 Stage 3.

The final stage of this procedure. The aim of this stage is to test whether the social information given to the female (currently waiting in the cylinder from stage 2, step 14, figure 9) has changed her mind away from her decision funded by asocial information

in stage 1. This female is now the demonstrator, and we follow on from stage 2 with step 16 (figure 10).

Step 16 is to remove the cylinder after 1 minute of rest time. The demonstrator is then free to explore the maze and all of her actions are recorded. Step 17 is to stop recording after 5 minutes. Now the colours need to be swapped around. This ensures that the demonstrator goes the opposing way in the eyes of the observer.

Step 18 is to remove the demonstrator female and place in an empty intermediary tank. Place the cylinder back at point I2. Step 19 is to place the observer into the cylinder, and the demonstrator from the intermediary tank into H, the observer chamber. Step 20 is to remove the cylinder after 1 minute of acclimatisation and begin recording. Step 21 is to stop recording after 5 minutes. The testing phase is now complete. The final steps are to take the females back to their home tanks where they can eat and rest. The males are also taken out of the experiment to rest before the next test starts. The final step, (23, figure 10) is to return to stage 1 with 2 new females and a dummy female.

The two females firstly explored the maze with only asocial information to guide them. They then observed each other demonstrate an opposing behaviour. They were then

both offered the chance to change their minds, to copy the demonstrator that went before them. If they changed their minds on which mate was attractive then they have shown mate-choice copying. If they did not change their mind, they then are preferring to use their own information to guide their mate-choice.

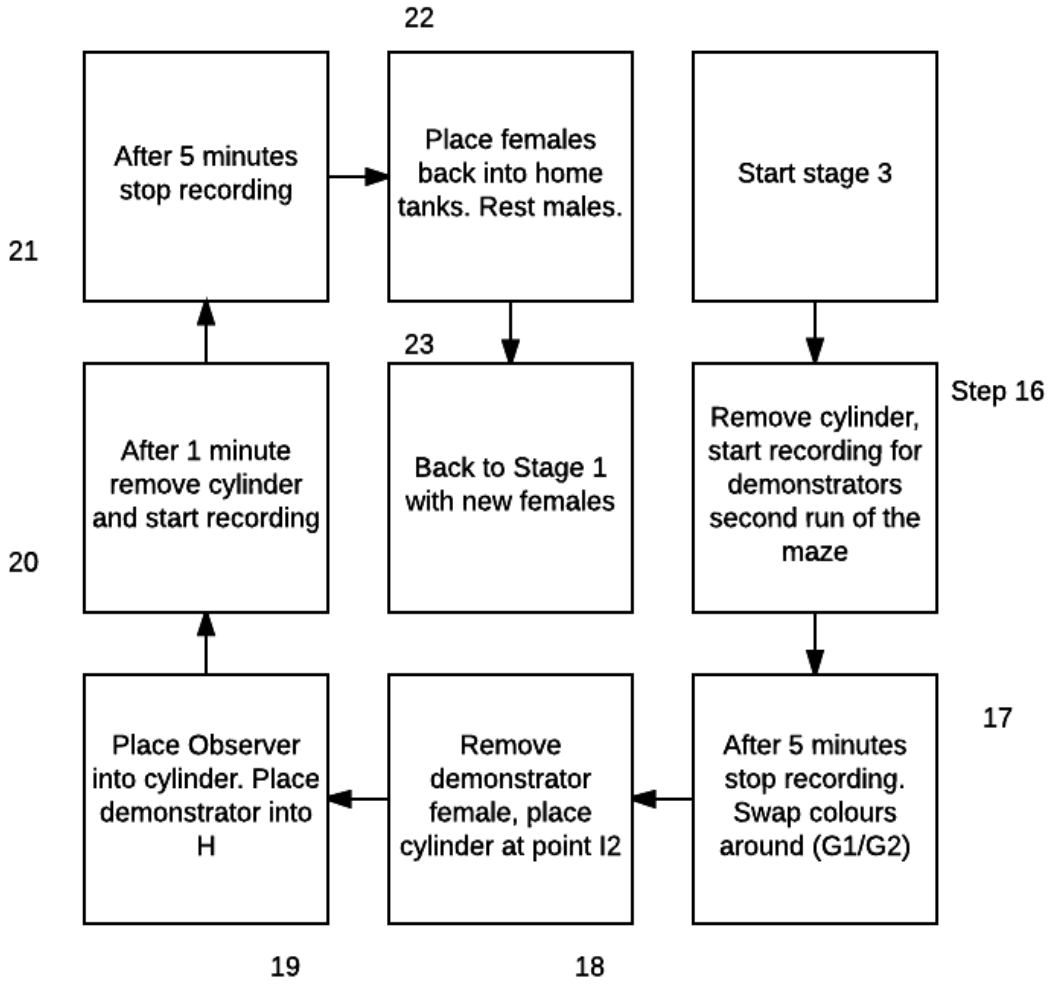


Figure 10: Stage 3 of the Testing Phase

This flowchart shows steps 16 to 23. This explains how the third stage of the testing phase is used to provide the observer with a chance to change her choice (step 17). This stage also acts as the second stage for the second female, in this stage she is given her social information and then at step 20 offered a chance for her to change her decision.

These 3 stages (figures 8;9;10) will then be repeated for all treatments of females. Each run through of the maze lasts for 5 minutes (see stages 1-3; figures 8-10). The rationale behind this was to ensure that no female was being used for too long on a day of testing. Not including movement time and acclimatisation time each individual female would spend 10 minutes as a demonstrator and 5 minutes as an observer (minimum, sometimes 10 minutes as observer if then being used as a dummy female for the following experiment). 20 minutes of continuous and stressful testing was determined to be the maximum to ensure no negative effects to the guppy's quality of life. 5 minutes was then seen to be a reliable amount of time to use to gain an insight into their behaviour. It was long enough for females to explore the whole maze and sample both males and then show favour to one of those two males. Results will be taken from the entire 10 minutes of demonstrator time for tests where the female just as asocial, and when she has social and asocial information.

2.9 Statistical Analysis

The data from this phase will be statisically analysed to look for any significant interactions of fixed effects such as colour, training, copying and treatment on the relative time a female spends in any zone.

All data will be used in this statistical analysis from both explorations of the maze by a single female. There will, however, be discrepancies in data for each female. This is because on the second run of the experiment the female is in possession of social information. This is taken into account when choosing a powerful method of statistical analysis.

The main form of statistical analysis is a generalised linear mixed model (GLMM). This model takes into consideration all aspects of the female individual and assesses

them against what the female did within the testing phase. The model will take the following aspects into consideration. The colour used (blue, green, yellow, pink), the treatment (0-hours, 24-hours and 48 hours of starvation), the conditioning of the female (was the zone marked with conditioned cues? 0=no, 1=yes), was the zone favoured by the demonstrator (0=no, 1=yes) and random effects (the arbitrary identification number given to each individual from 1-93) . These effects will be analysed against the amount of time a female spends in any zone (the independent variable of these tests).

Each zone has these particular effects attached to it. For example, a zone could be marked with a blue colour, the female could not have been conditioned to this blue colour, this zone could have been favoured by the demonstrator, the female entering this zone may have been starved for 48-hours before testing, and this female could have had an ID number of “68”. Each of these effects are measured against the 150 seconds she spent in this zone and after reduction, some of these effects may have had a significant effect on the independent variable.

The GLMM does this through modular reduction. This means that it will look for the most significant factors and slowly “knock off” the factors that have no significant effect on the time spent in a zone. Over many tests and reductions the only factors that are left are those that have most significant of an effect on the independent variable.

The GLMM also allows investigation of joint effects of two or more fixed effects on the independent variable. Imagine the previous example above, but two of the effects, for example the colour the zone was marked with and the treatment of the female, could have an interaction between them that could significantly impact the

amount of time spent in a zone. In this case the blue colour, not the other 3 colour possiblities, and the fact that the female was starved for 48-hours may both have made the female spend 150 seconds in this zone. The interaction that this study investigates is the two-way effect of the treatment of the female, how long she was starved for, and if the zone was favoured by the demonstrator.

The order of the results will follow in line with these questions. Firstly is there any singular interaction of any the fixed effects on the independent variable (time spent)? Secondly, is there a joint, two-way, interaction between the effects of demonstrator and treatment on the independent variable? Lastly the final question asks, “what do these interactions look like?”

2.10 Predictions

The first question’s predictions are that there will be a significant effect of conditioning on the amount of time a female spends in a zone because of the conditioning phase prior. There is not expected to be a significant effect between the colour and the independent variable of time because of the preliminary bias tests showing no significant bias of the females to any colour. The treatment is expected to have a significant impact on time spent because of the limitations on her activity due to the energy deficit inflicted. The effect of demonstrator is also predicted to show a significant impact on the time spent in zone, as this would show a female copying a male.

The second question’s prediction is that there will be an interaction between what the demonstrator did and the treatment of the female. The female is predicted to spend more time in the zone if that zone was preferred by a demonstrator if the female is in the 24-hour or 48-hour treatment groups. The reverse of this is that the females who

were not starved, the 0-hour treatment, are predicted to not show a propensity to copy and instead spend less time in the zone preferred by the demonstrator.

3 Results

3.1 Conditioning phase

The first question to be answered is “do fish show a preference for their zone of conditioning?”. As set out in the methods, this is done through two tests comparing data taken on day 1 and data taken on day 8. Results are shown in table 4. Paired analyses are taken from the same female. Because no food deprivation was inflicted upon the females, the “ALL” treatment is used. This is because at this conditioning stage there is no difference between any of the females, aside from the colour they are learning. Sample sizes vary between these treatments due to high mortality of females.

Overall females on day 1 first enter the zone of training 57% of the time, tested on 63 females, this is not seen to be significant (Binomial t= 0.314, successes= 36). On day 8, 71% of 56 females first enter the zone of training which is significant (Binomial t= 0.0018, successes = 40; table 4).

On day 1 there more time was spent in trained zones by an average of 23.275 seconds, this is not significant, however (Paired t-test = 1.944, df= 62, p= 0.176). On day 8 there is a similar positive time of 23.392 seconds in favour of trained, also non-significant (Paired t-test = 1.737, df= 55, p= 0.176).

First entry into trained zone					Time spent in trained zone				
Treatment	Day	Number of successes	Probability of first entry	Binomial test result	Relative time spent	t	DF	P	P values with FDR
ALL	1	36/63	0.57	0.313	23.274	1.944	62	0.056	0.176
ALL	8	40/56	0.71	0.0018*	23.398	1.736	55	0.088	0.176
0	1	10/20	0.50	1	9.75	0.414	19	0.68	0.68
0	8	16/19	0.84	0.004*	50.815	3.033	18	0.007	0.071
24	1	10/16	0.63	0.45	15.35	0.578	15	0.571	0.635
24	8	8/15	0.53	1	41.27	2.510	14	0.024*	0.124
48	1	8/13	0.62	0.581	1.950	48.661	12	0.074	0.176
48	8	8/10	0.80	0.109	1.051	32.34	9	0.320	0.400

Table 4: Do females first enter and spend more relative time in trained zones compared to non-trained zones?

This table shows the results from the first set of statistical analyses for the conditioning phase. Treatment, which of the 3 treatments was this female from, note the "ALL" treatment which lumps all data into one; Day, which day these results were taken; Number of successes, out of how many entries did the female enter the zone marked with cues matching her conditioning?; probability of first entry, number of successes divided by total observations; binomial test result, is the probability significantly different from random?; Relative time spent in trained, this is the time spent in the trained zone minus the time spent in the opposing zone; t, result from a paired t-test; DF, degrees of freedom for paired t-test; P, probability of t-test being significant; P with FDR, the probability that the t-test was significant when considering false discovery rate of significant results; *=$p<0.05$

There are some significant results worth mentioning, with 16 of 19 (84%), 0-hour fasted females, on day 8 of Trial 3, opting to first enter zones of training which is significantly different from what was predicted by the null hypothesis (Binomial t= 0.0044, successes = 16; table 4). On day 8, females from treatment groups 0-hour and 24-hour spent a considerable amount of time in zones marked with coloured cues (table 4). These were found to not be significant after accounting for false discovery rate (FDR; Benjamini and Hochberg, 1995). Aside from these indications of learning, however insignificant, there is no evidence of a significantly greater amount of time being spend in conditioned zones, relative to non-conditioned zones (table 4).

The next question for the conditioning phase is “is there any change in preference between day 1 and day 8 of the trial?”. This is measured in two ways. Firstly, what is the difference in probability of a female entering a zone of conditioning first, before another zone? This is calculated by conducting a paired t-test. Overall there is an increase in the probability of the female first entering the zone of conditioning, however this is not significant (figure 11; table 5; Paired t-test= -1.632, df= 116.9, p= 0.21). For the overall there is also a positive difference in mean time spent between day 1 and 8, of 5.695 seconds. This is not a significant difference between the two days (Paired t-test = 0.456, df= 116.47, p=0.9274). All data is shown below in table 5.

The 0-hour treatment group showed the greatest increase in first entry probability, however this 34% increase was seen to be non-significant when accounting for FDR. Figure 11, below, shows the difference in the probability of a female first entering a zone of training between day 1 and day 8. When lumped together, all fish show a strong improvement in learning where to enter first to find the high-quality male.

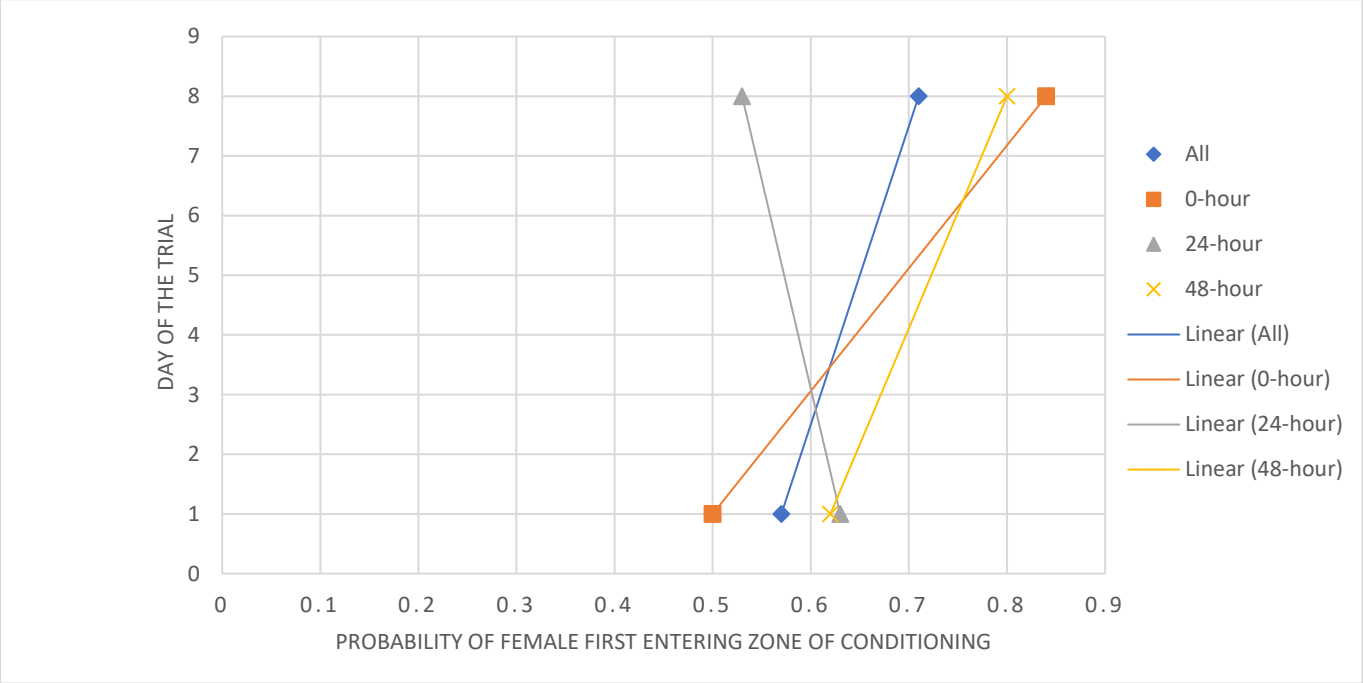


Figure 11: Graph to show the differences in probability of a female first entering a zone of training on day 1 compared with day 8.

This graph's X-axis represents the probability, from 0 to 1, of a female entering a zone that was marked with her conditioned cues. The Y-axis represents the day of the conditioning phase. Conditioning started on day 1 and finished on day 8. Lines on the graph represent change in propensity to first-enter trained zone over time. Blue Square, All data; Orange square, 0-hour treatment group; triangle, 24-hour treatment group; cross, 48-hour treatment group.

Difference in first entry into zones marked with conditioned cues between day 1 and day 8 of the conditioning phase					Difference in time spent in zones marked with conditioned cues between day 1 and day 8 of conditioning phase			
Treatment	Difference in probability observation	T1	DF1	P with FDR	Mean time	T2	DF2	P with FDR
ALL	+0.14	-1.6322	116.9	0.21	5.6949	0.45609	116.47	0.9274
0	+0.34	-2.386	34.758	0.09032	-1.83	-0.0916	36.475	0.9274
24	-0.01	0.05589	54.316	0.955	15.203	0.90758	53.952	0.9066
48	+0.18	-0.953	20.82	0.4666667	-5.33	-0.1437	20.191	0.9274

Table 5: Is there a difference between the female's propensity to enter and spend more time in trained zones compared to non-trained zones after conditioning?

This table shows the results from the second question asked of the conditioning phase. Is there any change to first entry and time spent in conditioned zones after 8 days of conditioning? Treatment, which of the 3 treatments was this female from, note the "ALL" treatment which lumps all data into one; Difference in probability of observation, probability of first entry into conditioned zone on day 8 minus the probability for same zone on day 1; t1, paired t-test result 1; DF1, degrees of

freedom 1; P with FDR, probability that the t-test was significant after taking false discovery rate into consideration; mean time in trained, time in trained zone for day 8 minus time in trained zone for day 1; t2, second paired t-test result for mean time spent; DF2, Degrees of freedom for second paired t-test; P with FDR, probability that second paired t-test shows a significant result after taking false discovery rate into consideration.

Overall, did females learn the association of a colour cue and the presence of a high-quality male? There is not enough evidence to reject the null hypothesis that there is not a successful interaction, and a significant change in first entry into conditioned zones nor significantly more time being spent in these zones relative to other zones. Females were not forming associations between a colour cue and the location of a high-quality male. How this failure impacts the testing phase will be mentioned in the discussion section with an explanation for why the experiment continued into the testing phase.

3.2. The Testing Phase

The first step in analysing the results of the testing phase is to revisit the questions set out in the methods section, and answer them with statistics. Argument of these results will continue in the following discussion section. The first question asks if there is any singular effect that impacts the independent variable (time spent in a zone)? This question is answered using results taken from a generalised linear mixed model after reduction of non-significant effects until a point that each individual effect is left on its own, showing its solo impact on the independent variable. Secondly is there a joint influence of demonstrator and treatment, acting in

a two-way interaction, on the independent variable. Lastly the final question asks, “what do these interactions look like?”

Firstly, how do the fixed effects impact the amount of time an individual spends in a zone? There is no individual impact of any of the fixed or of the random effects on the independent variable (table 6). The strongest solo-contributor is the demonstrator effect, yet this is not significant (ChiSq= 2.5325, df= 2, p = 0.2819; table 6). This effect shows a slightly negative effect on the amount of time spent in a zone (figure 12). The boxplot (figure 12) shows that females spend more time in the zone that is not favoured by the demonstrator, relative to the zone favoured by the demonstrator. This is not significant after model reduction and therefore the null hypothesis for this question cannot be rejected.

Parameter ***Fixed Effects***	**Estimate**	**Standard error**	**df**	**Chisq**	**P**
Intercept	34.683	72.331	1		
Trained upon	-30.474	31.761	1	1.0148	0.3138
Demonstrator favoured	-61.999	46.718	2	2.5325	0.2819
Colour: Blue			3	2.3772	0.4979
Green	4.763	56.252			
Yellow	32.036	57.320			
Pink	71.303	57.778			
Treatment: 0hr			2	1.1411	0.5652
24hr	-22.578	47.201			
48hr	-45.739	45.021			
Demonstrator x Treatment			6	12.938	0.04403 *
Intercept	213.15	78.05			
Demonstrator favoured	-251.99	80.71			
Demonstrator favoured, 24hr	231.34	101.78			
Demonstrator favoured, 48hr	297.01	173.06			
Random Effects			**Df**	**Variance**	**Std. Dev**
Female ID			92	21721	147.4

Table 6: Table showing the results of the Generalised Linear Mixed Model after modular reduction

Table 6 shows results of GLMM. Fixed Effects include the colour the zone was marked with, whether the zone was favoured by the demonstrator, the treatment of the female, was the zone marked with conditioned cues; the random effect for this model was the female's ID number, which was assigned randomly.

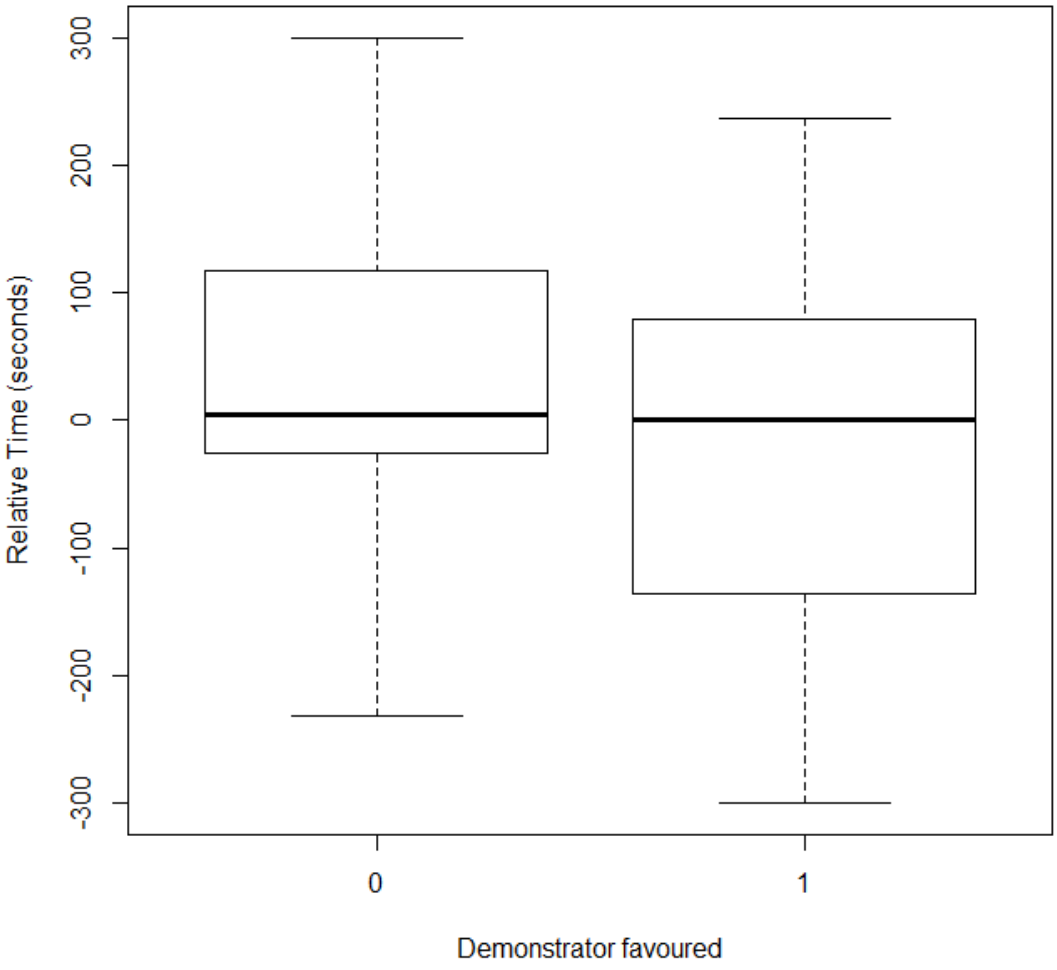


Figure 12: Graph to show the effect of demonstrator on the relative time spent in a zone by a female

Figure 12 is a box plot that shows the interaction between demonstrator and relative time spent in an arbitrary zone. On the Y-axis shows the relative time spend in any

zone; the X-axis shows whether this zone was favoured by the demonstrator; 1, demonstrator favoured zone; 0, demonstrator did not favour this zone.

Secondly, are there any joint interactions that have a significant impact on the independent variable? The GLMM shows a statistically significant effect for the two-way interaction of treatment and demonstrator on the relative time spent in a zone. This means that the treatment of the individual (how hungry she was) had an impact on the relationship between what the demonstrator did and where the observer spent her time. The hungrier the observer, the more likely she is to enter a zone previously favoured by the demonstrator (ChiSq= 12.938, df= 6, p = 0.044; figure 13). No other combination of effects was found to be significant in the GLMM.

What does this significant interaction look like? Figure 13 shows this graphically. This figure (13) shows the results of the GLMM for the interaction of demonstrator and treatment on the time spent in a zone. The graph is split into two grids, 0 (labelled at the top) represents a zone that was not favoured by the demonstrator; 1 represents a zone that was favoured by a demonstrator. Each of these two grids is split into the 3 treatments: 0-hour, 24-hour and 48-hour, shown on the X-axis. On the Y-axis, the numbers represent the relative amount of time a female spends in a zone compared to another zone. The lines show standard error. There is no standard error bar for the 48-hour group that's zone was favoured by the demonstrator because the sample size is too small. This suggests that the interaction found, albeit statistically significant, is based on a single result and is therefore extremely unreliable. This will be debated in the discussion section. There is however another interesting aspect of this graph (figure 13) and that is that the 0-hour treatment group seem to favour the zone not favoured by a demonstrator, suggesting that they are using asocial information and not copying.

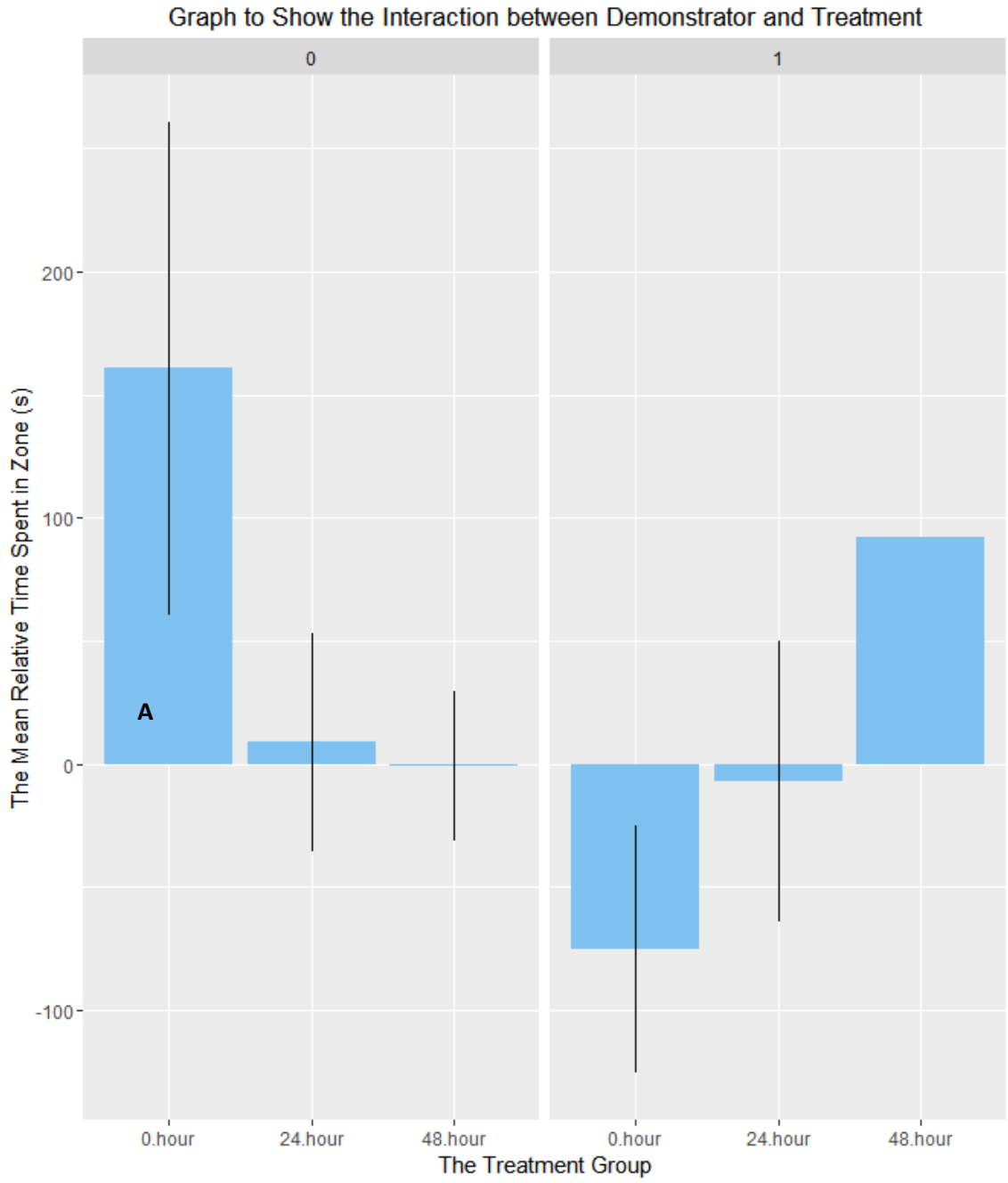


Figure 13: Graph to show the two-way interaction between demonstrator and treatment on the relative time spent in a zone by a female

Graph shows data from GLMM showing a two-way interaction that was found to be statistically significant. The left facet grid represents the zones that are favoured by the demonstrator (0); the right shows zones that were not favoured by the demonstrator (1); the Y-axis shows the relative amount of time a female spends in

that zone; the X-axis shows the treatment group of the female, which is how long the female was starved for (0,24 or 48 hours). For example, point "A" is the relative amount of time a female spends in a zone, compared to the opposing zone, when the demonstrator did not favour it, for a female from the 0-hour treatment group.

There is no evidence to suggest that any individual factor alone impacts the time spent in a zone after model reduction of the GLMM (table 6). The strongest individual factor is whether the zone was favoured by a demonstrator, however this is not significant alone and can only be made significant when paired with treatment.

4. Discussion

4.1 The Conditioning Phase

The conditioning phase was designed to give female guppies asocial information. By controlling this asocial information, and adding social information later, it allowed the study to investigate at what point the female changes from one source to another. This phase used sexual conditioning (Holloway and Domjan, 1993) as positive reinforcement to build this association. This phase had two questions that it attempted to answer; 1, do fish show a preference for their zone of conditioning? ; 2, is there any change in this preference between the beginning and the end of a trial? The prediction for question 1 is that females will spend significantly more time in zones of conditioning because these zones are also next to high-quality males. The prediction for the second question is that yes, females will positively change their preference for these zones as an association forms, making the decision easier for the female.

What was found, however, was a lack of significant results for either of these questions. Firstly, do females prefer their zone of conditioning? First entry data suggests this to be true (table 4) as females of every cohort show an equal or greater than first-entry probability into a zone of conditioning. The greatest indication of this is the average 71% chance of first-entry shown by all individuals on day 8 of trials. The better calculator of preference is how long females spend in those zones, for which there are no significant results. The greatest indication that there is preference is shown by the 0-hour treatment group on day 8, averaging 50.82 seconds more in a zone of training than in a zone not marked with conditioned cues.

However, most groups show no real difference between zone preference in terms of time spend.

Therefore, the conditioning phase was unsuccessful in forming associations between coloured cues and location of a high-quality male for female Trinidadian guppies. There are a few hypotheses that could explain the failure of this phase and careful remodelling of the experiment could lead to a more successful outcome in the future.

Firstly, the sample size of females used (table 1) and the re-use of some females acts as a confounding variable on this phase. Having so few females, and having such a large proportion of those females die during testing means the effect size of statistical testing is much greater. This means that anomalies have a stronger impact on the statistical testing, exposing the test to high risk of type 1 and 2 errors. If this study is to be reconducted then the first place to start in its remodelling would be the acquisition and more careful use of a considerably larger sample of females and male Trinidadian guppies.

Because of high-mortality on small sample sizes, some treatment groups had differently sized groups. This makes the test unfair and unreliable as it exposes some treatment groups to a greater effect size, and therefore greater risk of type 1 and 2 errors, but not other groups. Having such a small sample size also brings the ethics of this experiment into question, as exposing these guppies to possible risks of research is only justifiable when there is a realistic chance to yield useful information. This phase did not yield useful information, and the mortality of lab subjects was far too high.

Why was this mortality rate so high? A recent hypothesis is that by splitting females into smaller and smaller groups their ability to shoal decreases. Shoaling works as

any grouping of social animals does in reducing predation risk and increasing quality of life. Taking a shoaling species and separating them over and over may be very stressful to the guppies. It also may make stressful situations, i.e. handling between tanks by laboratory technicians, more traumatic. Shoaling is also known to facilitate social learning of foraging behaviours in the guppy (Swaney et al, 2001), and reducing the ability to shoal may reduce an individual female's ability to find food. This will directly confound this study as this study specifically investigates the effect of hunger on behaviour. If hunger is already being impacted, and sample sizes show high variance between treatments, then this should be considered.

Mortality was so high that new females were brought in to make up the numbers of females lost to try and retain some methodological integrity. This, of course, does confound the study somewhat. The fact that some individuals could be used in multiple experiments and some would not have meant that some would get twice, if not triple, the amount of time in the conditioning phase. Some females are therefore being compared for propensity to form associations against those that had much more time to form those decisions, which is unfair. However, to rectify this imbalance, each female used in a trial was given a minimum of 2 weeks rest between trials. During this time, the female would be given shelter, unlimited food, and have zero human interaction. This rest was determined to be long enough for associations to be forgotten or reduced, and for all the stresses of the testing to be forgotten. New females entered tanks during this resting phase. Pseudo-replication is a confounding element to this study, but reasonable steps were taken, where possible, to reduce its impact.

Secondly, associative learning requires reinforcement. In this case it was thought that the positive reinforcement of seeing a high-quality male near a colour would be

enough to form an association. This was because it was thought that a female who had been in isolation from males for so long would be very interested in seeing a male, and this would stir enough interest to act as reinforcement. In future studies, perhaps allowing the females to interact with the males would act more as a biologically relevant reward or punishment.

Thirdly, the statistical analysis of this stage could be improved by using mixed models rather than multiple binomial tests and t-tests. More testing exposes results to the risk of false discovery rate, where there more tests you run, the chance of one of them turning up significant just by chance increases (Benjamini and Hochberg, 1995). FDR has been accounted for in all of the multiple testing of this study.

Fourth, there is no data for the behaviour of the males. The software used in this study did not allow for viewing multiple individuals. Time-in-zone may not be an accurate measurement or proxy for female attention if the males are not showing any courtship behaviours. Males were isolated for an extended amount of time, it was therefore assumed they would show excited, courtship behaviours when they viewed a female. Likewise, for the female's extensive isolation. The zones, (figure 2) were also measured to ensure that the female was close to the males, close enough that she wouldn't have just been swimming past, but showing an interest to that wall of the aquarium which does improve the accuracy of data measured.

Lastly, the female's age was not accurately calculated prior to testing. Body size was measured before using the females. Body size was determined to be a good indicator of female age (Laland and Williams, 1998; Swaney et al, 2001). It is important that females used are all relatively the same body size/ age because age is known to affect the propensity to use social information. The exact data for these

calculations has been lost, so no mean SE lengths and comparisons of body lengths among treatments using an ANOVA are available. If this study was redone it would be important to take this into consideration. Body size data was therefore not available during statistical testing, allowing it to potentially confound the analysis.

What does this mean for the testing phase? As this conditioning phase was unsuccessful females could not be seen to be using asocial cues in their decision making. They were, however, still making decisions. The colours do compromise the integrity of this study, but this experiment is still salvageable. As the colours were having no significant impact on the decision making of the female, it was deemed that they could continue to be used if they were accounted for in the testing phase's statistical analysis. This would mean they would be removed during reduction of the generalised linear model to determine independent fixed and random effects. Also, the fact that the zone was one that was trained upon by the female would also be removed in this phase.

In conclusion, there are numerous methodological shortcomings that may explain the failure of the conditioning phase to have female Trinidadian guppies form associations between a non-biological coloured cue and the location of a high-quality male. These include the poor sample size and pseudo-replication due to the high-mortality of lab subjects, the lack of biologically-meaningful interaction between the female and the male to solidify this association, the weakness of the statistical analysis and finally the lack of data concerning the age of females. These variables should all be taken into consideration if replication of this study is considered. However, this study continued onto the testing phase and tried to salvage what it could from the conditioning phase by accounting all variables possible in the testing

phase's statistical analysis. Discussion will continue the validity of this analysis in the following section.

4.2. The Testing Phase

The testing phase set out to test the central question of this experiment. That is, does hunger state impact the propensity of female guppies to mate-choice copy? This central question was broken down in the methodology into 3 questions. These were; 1, does any individual fixed effect have a significant impact on the time a female guppy spends in a zone; 2, is there a joint influence of demonstrator and treatment, acting in a two-way interaction, on the time a female guppy spends in a zone; 3, what do these interactions look like?

There is no significant impact of any singular fixed or random effect on the amount of time a female spends in a zone. The zone could be marked with any of the four colours, the demonstrator could have favoured or not favoured the zone, the female could have been trained or not trained upon that zone's colours and the female could have been from any treatment group, and still there would be no causative effect on the amount of time she spends in that zone. Therefore, we cannot say that females significantly show preference for any zone. This follows from the conditioning phase, where the colours and conditioning failed to show a significant association being formed.

There is a two-way interaction between two fixed effects that significantly impacts the amount of time a female spends in a zone, however (Table 6). This is an interaction between the hunger state of the female (treatment) and the behaviour of the demonstrator. When the demonstrator does or doesn't favour a zone, the result impacts the amount of time a female spends in there, depending on her treatment.

The central question asks does hunger state impact a female's use of social information. This result suggests that the central question can be with a "yes". There are, unfortunately, a few methodological shortcomings of this experiment that should be discussed in relation to the significance of this interaction. It is also important to answer the third question of this section, determining what this interaction may look like.

Figure 13 shows this interaction. The graph shows that the 0-hour treatment group spend a large amount of time in a zone that is not favoured by the demonstrator, relative to the zone that is favoured by a demonstrator. There are a few possible explanations for this behaviour. 0-hour treatment females were put under no hunger stress, they therefore have the energy to rely on their own asocial information. Their asocial information is data that they have acquired themselves, probably not through the failed conditioning phase of this experiment. When given social information, i.e. where the demonstrator goes, the female uses this and acts in a way that opposes the decision of the demonstrator. The female is not copying the demonstrator.

This figure (13) also suggests that the hungriest cohort of females, the 48-hour treatment group, copied the demonstrator and spent more time in that zone, relative to the zone the demonstrator did not favour. This is what was predicted by this experiment, and could answer this study's central question. Hungrier females are not relying on their own, asocial information, but instead are copying conspecifics.

There are some issues with this two-way interaction, and there are many important points to be addressed before taking this significant result and using it to debate the null hypothesis and central question of this paper.

The first is the failure of the conditioning phase which then turns this experiment from a mate-choice copying investigation into a copying study. The link between asocial and social information being about mate-choice was broken when the females did not form associations between colours and mates. Colours in the testing phase had no significant effect, so females were just picking an arm of the maze to explore, ignoring the coloured indicators (figure 6, G1/G2). Females are following, or not following the demonstrators down an arm of the maze. When they are at their hungriest females are following the demonstrator, an explanation could be a food-finding strategy or searching for shelter. Females may not follow demonstrators when they are fully satiated, this could be because they do not want the competition of conspecifics when searching for food or shelter. The maze was not built in a way that the observer can view a demonstrator picking a mate, this part of the classic mate-choice copying reversal experiment (Dugatkin, 1992; Dugatkin and Godin, 1998) was removed.

Secondly, there is evidence to suggest that the significance of the two-way interaction is based on a singular data point for the 48-hour treatment group. No standard error bar on the graph for the 48-hour treatment group of females spending time in zones preferred by the demonstrator suggests that this is single result. A single result is not enough to base a statistically significant result on, there is a great chance that this result is anomalous and therefore interpretation is open to type 1 and 2 errors. The lack of data for the second run of the experiment that lead to this issue is because all data was used, yet some independent samples had more data than the other as that sample had data for a demonstrator's behaviour. This would be rectified by using a much larger sample size.

There are some methodological shortcomings in this phase, just like in the conditioning phase. Small sample size, pseudo-replication and high-mortality leading to unbalanced samples per treatment group makes this experiment very difficult to use to answer the central question. Re-using some females introduces a confounding variable, as discussed in the conditioning phase's conclusion. To try and rectify this issue, the resting phase between trials was introduced. 2 weeks of rest was assumed to return all females to an equal level in terms of association for colours. A possible explanation for the failure of the conditioning phase, and the controversial results of the testing phase, could be this pseudo-replication.

A shortcoming of the testing phase was the use of a male pair, like that used in the conditioning phase. Varying qualities of males was required in the conditioning phase to teach the association, however in the testing phase it would have made more sense to use equal quality males. This is because, theoretically, the use of social information in the mate choice context is more likely to occur when the female is uncertain about the two males she encounters. She is unsure about their qualities of being prospective mates. This experiment should therefore have used equal males, where either could be the higher-quality, and the female would have had to use social or asocial information to determine which.

4.3. Closing Statements

This experiment set out to investigate if mate-choice copying of the female Trinidadian guppy was affected by its hunger state. It predicted that the hungrier the female, the greater her propensity to copy the mate choice of a conspecific female demonstrator. The theory behind this prediction is that the hungrier the female, the less she can invest in sampling of males for their quality, when copying is a much

cheaper option. This experiment failed at providing enough evidence to reject the null hypothesis, and it cannot be said with significance that the hunger state of a female guppy impacts her propensity to mate choice copy.

The study was largely motivated by the previous studies of Dugatkin and Godin (1998) on the effect of hunger state on mate-choice copying and their use of a reversal experiment to test for an effect. This study used an interesting a novel approach (associative learning) to produce a source of asocial information in the form of a colour cue indicating the location of a high-quality mate. This was then pitted against social information to investigate the relative use of available asocial and social information by individual females in choosing between paired males in a binary-choice apparatus. Unfortunately, the technique used to provide the females with their asocial information was unsuccessful as the guppies did not learn to associate a particular cue with a particular male phenotype. Because of this the testing phase also suffered, and even a significant two-way interaction is so questionable it cannot be taken at face value.

Without being overly negative in the discussion of this experiment, there are numerous methodological shortcomings that may have had significant effect on the failure of this study. As the conditioning phase did not work, the testing phase couldn't as the investigation was no longer linked to mate-choice, but instead just following or not following for other reasons such as habitat choice or food-finding.

The study took an interesting and novel idea and produced a new, innovative methodology to test for a behaviour still shrouded in uncertainty through lack of information. This study attempted to investigate state-dependent mate-choice copying and provide insight into an area of behavioural ecology that exhibits both

evidence for and against the existence of mate-choice copying phenomena in the guppy (*Poecilia reticulata*).

If this study is to be reproduced there are a minimum of 3 alterations that should be made, and they are as follows:

1. Increased sample size with more limitations and measurements on the females to ensure equal age and size. This study did account for size, but did not include this data in its statistical analysis, and its sample size was far too small.

2. Improve the conditioning phase by allowing biological interaction between the male and female. This will improve the likelihood the female will make meaningful associations as the interaction with a male will directly act as positive reinforcement, a requirement for associative learning

3. In the testing phase, allow the observer to see the demonstrator interact with the male. This ensures that all behaviours exhibited relate to mate-choice, and any copying that occurs can be directly linked to mate-choice.

5. References

- Alkins-Koo, M., 2000. Reproductive timing of fishes in a tropical intermittent stream. *Environmental Biology of Fishes*, *57*(1), pp.49-66.
- Andersson, M. and Simmons, L. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, 21(6), pp.296-302.
- Applebaum, S. and Cruz, A. (2000). The Role of Mate-Choice Copying and Disruption Effects in Mate Preference Determination of Limia perugiae (Cyprinodontiformes, Poeciliidae). *Ethology*, 106(10), pp.933-944.
- Baerends, G., Brouwer, R. and Waterbolk, H. (1955). Ethological Studies On Lebistes Reticulatus (Peters). *Behaviour*, 8(1), pp.249-332.
- Bandura, A. (1977). *Social learning theory*. Englewood Cliffs: Prentice-Hall.
- Benjamini, Y. and Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the royal statistical society. Series B (Methodological)*, pp.289-300.
- Bikhchandani, S., Hirshleifer, D. and Welch, I. (1992). A Theory of Fads, Fashion, Custom, and Cultural Change as Informational Cascades. *Journal of Political Economy*, 100(5), pp.992-1026.
- Bikhchandani, S., Hirshleifer, D. and Welch, I. (1998). Learning from the Behavior of Others: Conformity, Fads, and Informational Cascades. *Journal of Economic Perspectives*, 12(3), pp.151-170.
- Blows, M., Chenoweth, S. and Hine, E. (2004). Orientation of the Genetic Variance-Covariance Matrix and the Fitness Surface for Multiple Male Sexually Selected Traits. *The American Naturalist*, 163(3), pp.329-340.
- Boyd, R. and Richerson, P. (1988). *Culture and the evolutionary process*. Chicago: University of Chicago Press.

- Boyd, R. and Richerson, P.J., 1988. An evolutionary model of social learning: the effects of spatial and temporal variation. *Social learning: psychological and biological perspectives*, pp.29-48.
- Briggs, S., Godin, J. and Dugatkin, L. (1996). Mate-choice copying under predation risk in the Trinigadian guppy (Poecilia reticulata). *Behavioral Ecology*, 7(2), pp.151-157.
- Bro-Jørgensen, J. (2010). Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends in Ecology & Evolution*, 25(5), pp.292-300.
- Brooks, R. (1996). Copying and the repeatability of mate choice. *Behavioral Ecology and Sociobiology*, 39(5), pp.323-329.
- Clutton-Brock, T. and McComb, K. (1993). Experimental tests of copying and mate choice in fallow deer (Dama dama). *Behavioral Ecology*, 4(3), pp.191-193.
- Coolen, I., Bergen, Y., Day, R. and Laland, K. (2003). Species difference in adaptive use of public information in sticklebacks. *Proceedings of the Royal Society B: Biological Sciences*, 270(1531), pp.2413-2419.
- Dall, S., Giraldeu, L., Olsson, O., Mcnamara, J. and Stephens, D. (2005). Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, 20(4), pp.187-193.
- Danchin, E. (2004). Public Information: From Nosy Neighbors to Cultural Evolution. *Science*, 305(5683), pp.487-491.
- Doligez, B., Cadet, C., Danchin, E. and Boulinier, T. (2003). When to use public information for breeding habitat selection? The role of environmental predictability and density dependence. *Animal Behaviour*, 66(5), pp.973-988.

- Dugatkin, L. and Godin, J. (1992). Reversal of Female Mate Choice by Copying in the Guppy (Poecilia reticulata). *Proceedings of the Royal Society B: Biological Sciences*, 249(1325), pp.179-184.
- Dugatkin, L. and Godin, J. (1998). Effects of Hunger on Mate-choice Copying in the Guppy. *Ethology*, 104(3), pp.194-202.
- Dugatkin, L. and Alfieri, M. (2003). Boldness, behavioral inhibition and learning. *Ethology Ecology & Evolution*, 15(1), pp.43-49.
- Dugatkin, L., Druen, M. and Godin, J. (2003). The Disruption Hypothesis Does Not Explain Mate-Choice Copying in the Guppy (Poecilia reticulata). *Ethology*, 109(1), pp.67-76.
- Dugatkin, L. (1992). Sexual Selection and Imitation: Females Copy the Mate Choice of Others. *The American Naturalist*, 139(6), pp.1384-1389.
- Dugatkin, L. (1992). Tendency to inspect predators predicts mortality risk in the guppy (Poecilia reticulata). *Behavioral Ecology*, 3(2), pp.124-127.
- de Chaumont, F. (2017). *Icy. an open bioimage informatics platform for extended reproducible research*. [online] Available at: http://http.//icy.bioimageanalysis.org [Accessed 25 Jul. 2017].
- Endler, J.A., Basolo, A., Glowacki, S. and Zerr, J., 2001. Variation in response to artificial selection for light sensitivity in guppies (Poecilia reticulata). *The American Naturalist*, *158*(1), pp.36-48.
- Feldman, M., Aoki, K. and Kumm, J. (1996). Individual Versus Social Learning: Evolutionary Analysis in a Fluctuating Environment. *Anthropological Science*, 104(3), pp.209-231.
- Fisher, R. and Bennett, J. (2009). *The genetical theory of natural selection*. Oxford [u.a.]: Oxford Univ. Press.

- Galef Jr, B.G., 1990. An adaptationist perspective on social learning, social feeding, and social foraging in Norway rats.
- Galef, Jr, B. and WHITE, D. (1998). Mate-choice copying in Japanese quail,Coturnix coturnix japonica. *Animal Behaviour*, 55(3), pp.545-552.
- Galef,Jr, B. (1995). Why behaviour patterns that animals learn socially are locally adaptive. *Animal Behaviour*, 49(5), pp.1325-1334.
- Gibson, R. and Bradbury, J. (1985). Sexual selection in lekking sage grouse: phenotypic correlates of male mating success. *Behavioral Ecology and Sociobiology*, 18(2), pp.117-123.
- Gibson, R., Bradbury, J. and Vehrencamp, S. (1991). Mate choice in lekking sage grouse revisited: the roles of vocal display, female site fidelity, and copying. *Behavioral Ecology*, 2(2), pp.165-180.
- Giraldeau, L., Valone, T. and Templeton, J. (2002). Potential disadvantages of using socially acquired information. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 357(1427), pp.1559-1566.
- Godin, J., Herdman, E. and Dugatkin, L. (2005). Social influences on female mate choice in the guppy, Poecilia reticulata: generalized and repeatable trait-copying behaviour. *Animal Behaviour*, 69(4), pp.999-1005.
- Godin, J. and Dugatkin, L. (1996). Female mating preference for bold males in the guppy, Poecilia reticulata. *Proceedings of the National Academy of Sciences*, 93(19), pp.10262-10267.
- Goodale, E., Beauchamp, G., Magrath, R., Nieh, J. and Ruxton, G. (2010). Interspecific information transfer influences animal community structure. *Trends in Ecology & Evolution*, 25(6), pp.354-361.

- Grether, G.F., Kolluru, G.R., Rodd, F.H., De la Cerda, J. and Shimazaki, K., 2005. Carotenoid availability affects the development of a colour-based mate preference and the sensory bias to which it is genetically linked. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1577), pp.2181-2188.
- Grüter, C., Leadbeater, E. and Ratnieks, F. (2010). Social Learning: The Importance of Copying Others. *Current Biology*, 20(16), pp.R683-R685.
- Head, M., Wong, B. and Brooks, R. (2010). Sexual Display and Mate Choice in an Energetically Costly Environment. *PLoS ONE*, 5(12), p.e15279.
- Höglund, J., Lundberg, A. and Alatalo, R. (1990). Copying the Mate Choice of Others? Observations on Female Black Grouse. *Behaviour*, 114(1), pp.221-231..
- Höglund, J., Alatalo, R., Gibson, R. and Lundberg, A. (1995). Mate-choice copying in black grouse. *Animal Behaviour*, 49(6), pp.1627-1633.
- Holloway, K. and Domjan, M. (1993). Sexual approach conditioning: Unconditioned stimulus factors. *Journal of Experimental Psychology: Animal Behavior Processes*, 19(1), pp.38-46.
- Hoppitt, W. and Laland, K.N., 2013. *Social learning: an introduction to mechanisms, methods, and models*. Princeton University Press.
- Houde, A.E., 1987. Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, *41*(1), pp.1-10.
- Houde, A.E., 1997. *Sex, color, and mate choice in guppies*. Princeton University Press.
- Houde, A.E. and Torio, A.J., 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioral Ecology*, *3*(4), pp.346-351.

- Johnstone, R.A., 1995. Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biological Reviews*, *70*(1), pp.1-65.
- Von Frisch, K., 1967. The dance language and orientation of bees.
- Kameda, T. and Nakanishi, D., 2002. Cost–benefit analysis of social/cultural learning in a nonstationary uncertain environment: An evolutionary simulation and an experiment with human subjects. *Evolution and Human Behavior*, *23*(5), pp.373-393.
- Kendal, R.L., Coolen, I., van Bergen, Y. and Laland, K.N., 2005. Trade-offs in the adaptive use of social and asocial learning. *Advances in the Study of Behavior*, *35*, pp.333-379.
- Kodric-Brown, A., 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology and Sociobiology*, *25*(6), pp.393-401.
- Krebs, J.R., 1973. Social learning and the significance of mixed-species flocks of chickadees (Parus spp.). *Canadian Journal of Zoology*, *51*(12), pp.1275-1288.
- Lafleur, D.L., Lozano, G.A. and Sclafani, M., 1997. Female mate-choice copying in guppies, Poecilia reticulata: a re-evaluation. *Animal Behaviour*, *54*(3), pp.579-586.
- Laland, K.N., 2004. Social learning strategies. *Learning & behavior*, *32*(1), pp.4-14.
- Laland, K.N. and Williams, K., 1998. Social transmission of maladaptive information in the guppy. *Behavioral Ecology*, *9*(5), pp.493-499.

- Liley, N.R., 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour. Supplement*, pp.III-197.
- Long, K.D. and Houde, A.E., 1989. Orange spots as a visual cue for female mate choice in the guppy (Poecilia reticulata). *Ethology*, *82*(4), pp.316-324.
- Magurran, A.E., 2005. *Evolutionary ecology: the Trinidadian guppy*. Oxford University Press on Demand.
- Moscarini, G., Ottaviani, M. and Smith, L., 1998. Social learning in a changing world. *Economic Theory*, *11*(3), pp.657-665.
- Nordell, S.E. and Valone, T.J., 1998. Mate choice copying as public information. *Ecology Letters*, *1*(2), pp.74-76.
- Pomiankowski, A. and Iwasa, Y., 1993. Evolution of multiple sexual preferences by Fisher's runaway process of sexual selection. *Proceedings of the Royal Society of London B: Biological Sciences*, *253*(1337), pp.173-181.
- Powell, G.V., 1985. Sociobiology and adaptive significance of interspecific foraging flocks in the Neotropics. *Ornithological Monographs*, pp.713-732.
- R Core Team (2017). R. *A language and environment for statistical computing. R Foundation for Statistical Computing*. [online] Available at: http://http.//www.R-project.org/ [Accessed 25 Jul. 2017].
- Riebel, K., Holveck, M.J., Verhulst, S. and Fawcett, T., 2010. Are high-quality mates always attractive? State-dependent mate preferences in birds and humans: State-dependent mate preferences in birds and humans. *Communicative & integrative biology*, *3*(3), pp.271-273.
- Riley, J.R., Greggers, U., Smith, A.D., Reynolds, D.R. and Menzel, R., 2005. The flight paths of honeybees recruited by the waggle dance. *Nature*, *435*(7039), p.205.

- Rodd, F.H., Hughes, K.A., Grether, G.F. and Baril, C.T., 2002. A possible non-sexual origin of mate preference: are male guppies mimicking fruit?. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*(1490), pp.475-481.
- Seppänen, J.T., Forsman, J.T., Mönkkönen, M. and Thomson, R.L., 2007. Social information use is a process across time, space, and ecology, reaching heterospecifics. *Ecology*, *88*(7), pp.1622-1633.
- Smith, J.W., Benkman, C.W. and Coffey, K., 1999. The use and misuse of public information by foraging red crossbills. *Behavioral Ecology*, *10*(1), pp.54-62.
- Spottiswoode, C.N., Begg, K.S. and Begg, C.M., 2016. Reciprocal signaling in honeyguide-human mutualism. *Science*, *353*(6297), pp.387-389.
- Stöhr, S., 1998. Evolution of mate-choice copying: a dynamic model. *Animal Behaviour*, *55*(4), pp.893-903.
- Stoner, G. and Breden, F., 1988. Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy (Poecilia reticulata). *Behavioral Ecology and Sociobiology*, *22*(4), pp.285-291.
- Swaney, W., Kendal, J., Capon, H., Brown, C. and Laland, K.N., 2001. Familiarity facilitates social learning of foraging behaviour in the guppy. *Animal Behaviour*, *62*(3), pp.591-598.
- Templeton, J.J. and Giraldeau, L.A., 1996. Vicarious sampling: the use of personal and public information by starlings foraging in a simple patchy environment. *Behavioral Ecology and Sociobiology*, *38*(2), pp.105-114.

- Valone, T.J., 2007. From eavesdropping on performance to copying the behavior of others: a review of public information use. *Behavioral ecology and sociobiology*, *62*(1), pp.1-14.
- Valone, T.J., 1989. Group foraging, public information, and patch estimation. *Oikos*, pp.357-363.
- Valone, T.J. and Templeton, J.J., 2002. Public information for the assessment of quality: a widespread social phenomenon. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *357*(1427), pp.1549-1557.
- van Bergen, Y., Coolen, I. and 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*(1542), p.957.
- Waynforth, D., 2007. Mate choice copying in humans. *Human Nature*, *18*(3), pp.264-271.
- Widemo, F. and Owensi, I.P., 1995. Lek size, male mating skew and the evolution of lekking. *Nature*, *373*(6510), pp.148-151..
- Winge, O., 1937. Succession of broods in Lebistes. *Nature*, *140*, p.467.
- Witte, K. and Ryan, M.J., 1998. Male body length influences mate-choice copying in the sailfin molly Poecilia latipinna. *Behavioral Ecology*, *9*(5), pp.534-539.
- Witte, K. and Ryan, M.J., 2002. Mate choice copying in the sailfin molly, Poecilia latipinna, in the wild. *Animal Behaviour*, *63*(5), pp.943-949.
- Wourms, J.P., 1981. Viviparity: the maternal-fetal relationship in fishes. *American Zoologist*, *21*(2), pp.473-515.

- Zahavi, A. and Zahavi, A., 1999. *The handicap principle: a missing piece of Darwin's puzzle*. Oxford University Press.
- Zahavi, A., 1975. Mate selection—a selection for a handicap. *Journal of theoretical Biology*, *53*(1), pp.205-214.