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Can you see me, or can you smell me? An investigation into information transfer in *Betta splendens*

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**UNIVERSITY OF
PLYMOUTH**
Doctoral College

**Can you see me, or can you smell me? An investigation into information
transfer in *Betta splendens***

by

Alex Daniel Lynch

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RESEARCH MASTERS

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Author's Signed Declaration

At no time during the registration for the degree of Research Masters has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

This thesis has been proofread by a third party; no factual changes or additions or amendments to the argument were made as a result of this process. A copy of the thesis prior to proofreading will be made available to the examiners upon request.

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Abstract

Visual communication in *Betta splendens* has been studied in great detail. Chemical information transfer in this species has been studied less, and the interaction between chemical and visual information transfer less still. *Betta* may have a need for chemical information transfer; a need which is often neglected when housing this species in isolation from conspecifics. There is evidence that many fish species communicate using visual signals and spy on one another using chemical cues. Which type of information transfer is important and how they interact in which social context is less clear. Eavesdropping and mate choice has been studied extensively in *Betta*, most often with visual signals being monitored. This study both aimed to find whether male *Betta* 'wanted' to be in receipt of conspecific chemical information, and to investigate the relationship between chemical and visual information transfer in social interactions between *Betta* of both sexes. Experiments using T-mazes concluded that male *Betta* did choose to be in receipt of conspecific chemical information when given the option. Experiments utilising mismatching multimodal information transfer concluded that female mating behaviour was significantly affected when given mismatching chemical and visual signals from males. Male agnostic and mating behaviour was not significantly affected. Historically females having witnessed an interaction between males have been shown to consistently choose the winner of the interaction over the loser. These findings suggest that chemical information transfer is important to female *Betta* in regard to sexual selection. This study as a whole informs us as to the importance of chemical information transfer in *Betta*. In males in terms of preference and choice, and in relation to dominant-subordinate relationships. In females in terms of eavesdropping and mate choice. It informs welfare and husbandry practices by suggesting that male *Betta* would prefer to be in receipt of

conspecific chemical information, where they traditionally are kept in visual and chemical isolation from conspecifics. It also adds to our understanding of the role alternate modalities of information transfer play in the formation of dominance hierarchies and sexual selection.

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Introduction

What is Communication?

Communication is an essential feature of social organisms, providing individuals with the means to make decisions which will influence their behaviour based on information received from other individuals (Peake 2005). Communication in a dyad of individuals had classically been studied countless times (Dore *et al.* 1978) before more complex communication between networks of individuals was considered (see Communication Networks).

Historically there has been some contention regarding the actual definition of communication (Dawkins & Krebs 1978, McGregor & Peake 2000, Wisenden & Stacey 2005). How broad the definition is having been one of the main discussions. Dawkins & Krebs (1978) postulated that communication only took place when a signaller or receiver benefitted from the interaction. Bradbury & Vehrencamp (1998) argued that communication occurred only when both the signaller and receiver were to benefit. The discussion of how communication should be defined is too broad for this body of work. For ease of understanding, communication in this thesis is described as when a signal has been sent by a signaller and received by a receiver (Bradbury & Vehrencamp 1998, Wisenden & Stacey 2005). In this definition, a signal has been shaped by natural selection to carry information. This simpler definition ignores whether the interaction is beneficial for one or both individuals involved. There are costs associated with engaging in communication, it may take energy, time and special organ modifications in order to do so. There may therefore be evolved compensatory benefits to communication. The benefit may be direct, such as discouraging an enemy or gaining a mate. The benefit could also be indirect, as would be the case if the receiver's choice benefitted close kin of the signaller. A signaller will only give information to a receiver if the receiver's decision improves the fitness of the signaller more than the signal production reduces it. A receiver will pay attention to any signal source which is reliable and will improve their decision making, on average. There is a minimum amount of reliable information which will be provided before it becomes beneficial for signaller or receiver. The benefits minus costs must be positive before each party will engage in communication (Peake 2005).

There must be a minimum of two organisms involved for communication to occur, one signaller and one receiver (Oliveira *et al.* 1998). These individuals exchange signals which have been specialised to carry information, meaning that they have evolved a means of signal transmission specifically meant to alter the behaviour and/or physiology of the receiver (Peake & McGregor 2004, Wisenden & Stacey 2005). This likely evolved as a signaller will have sent a signal which may prove to be more beneficial to them than the receiver, deliberately exploiting the receiver (Dawkins & Krebs 1978). The receivers may in turn exert a selection pressure on signallers to the opposite effect, potentially increasing the reliability of the signal (Guilford & Dawkins 1991). Dawkins (1993) wrote that sensory capability and perception of receivers must match the signals sent to them, and the information within must be designed to increase how predictable a certain behaviour is or increase probability of detecting identity or spatial detection. Both receivers and signallers exert a selection pressure on signals to be clear and to help receivers make a physiological/behavioural decision (McGregor & Peake 2000).

Signals are purposefully designed and directed to change the behaviour and/or physiology of receivers, but receivers can also glean information from individuals by means of detecting 'cues' (Oliveira *et al.* 2001, Wisenden & Stacey 2005). This is an important part of information transfer in animals but is not communication by our definition as it involves no signal production (see Spying Networks). An example of cues would be female *Betta* choosing mates based on male size, colouration and fin length (Simpson 1968).

Communication is ubiquitous within the animal kingdom, having been shown in all Phyla (McGregor & Dabelsteen 1996, Earley & Dugatkin 2002, Wong & Candolin 2005). Discussions around bacteria and plants using signals to communicate have gained traction recently, however this is still an area of contention. The scientific consensus is that they do use and respond to cues (Wisenden & Stacey 2005, Forsatkar *et al.* 2014)

Why is Communication Necessary?

Social activities often involve interactions between conspecifics. Communication plays a central role in societies of animals and influences practically all these interactions (McGregor *et al.* 2001). Communication allows animals to co-ordinate

their behaviours, for example when mating, building nests or foraging in groups (Peake 2005). Courtship of females by territorial male animals can be a signal carrying information on sex, species, social status, reproductive motivation, general fitness and aggressiveness, among other things (Wong & Candolin 2005, Rutte *et al.* 2006). Group cohesion is also important in numerous social species, and it is governed by communication between conspecifics (Doutrelant & McGregor 2000).

Competing for mates, territory, food and other resources can be incredibly costly to animals (Peake 2005). It can lead to fighting, which can be detrimental to the survival of individuals involved due to either death or injury. Since the costs of this type of competition are so high, it can diminish the value of the resources being fought over. Therefore, there would be a selection pressure for individuals which can mediate or mitigate these costs. Animals which can gather more information on their potential opponent will have a greater ability to assess whether an agonistic encounter would be beneficial in relation to the resources at stake. Using this information they could choose to avoid combat and injury entirely (Matos & McGregor 2002, Dzieweczynski *et al.* 2005).

Communication itself can also be costly. A signaller risks making itself known to predators or conspecific rivals by exhibiting conspicuous behaviour or morphology (Peake & McGregor 2004). To mediate these threats signallers have been known to evolve specialised adaptations to make signal detection by predators more difficult. For example, great tits have evolved a way of communicating above the frequency hearing threshold of Sparrowhawks, one of their main predators (Klump *et al.* 1986). Signalling can be costly in other ways, for example they may need to be produced for an extended period, limiting time which could be used for other activities essential for survival (i.e., foraging, hunting). Signals carry an energetic cost, for example when animals display in an agonistic display or courtship ritual (Herb *et al.* 2003). Signals in the form of morphological traits can be costly in that they may interfere with the original evolutionary intention of the morphology, for example male fiddler crabs developing a signalling claw which is less effective at feeding than their smaller counterpart. Resources must also be devoted to the growth of said claw, and then the heavier claw must be carried around for the remainder of the crab's lifetime (Carvello & Cameron 1987). All these costs to the signaller must be counterbalanced

by the average benefit incurred from signalling, this inherent benefit could explain why communication has seemingly evolved ubiquitously (Peake 2005).

Communications in Aquatic Media

Within animal communication information can be transferred in myriad ways. Modalities of information transfer include tactile, electric, acoustic, visual and (semio)chemical (Oliveira *et al.* 2001, Wisenden & Stacey 2005). The reason for this often being the ecology of the habitat different species have. Information transfer would evolve differently in different organisms depending on selection pressures imposed in their specific habitat. It would for example be much harder for an animal inhabiting a dense forest to send a visual signal, but an acoustic signal would bypass most visual obstacles (Krebs & Davies 1993).

Tactile signals are often only effective at short range, as the individual must usually be touching another for it to be transferred. These types of signal are often energetically quite cheap to produce. Electrical signals are usually energetically costly to produce, but they are affected by obstacles and can travel only a short distance. Acoustic signals are commonplace in animal communication. Sound is advantageous in that it can travel relatively far and can be easy to locate. Sound also can be costly in terms of alerting predators or conspecific rivals of an individual's location. Visual signals can also be easily locatable but have much smaller range than an acoustic signal and can be blocked by any opaque obstacles. Invariably visual signals can be costly energetically, for example in the case of mating displays, or alerting predators of an individual's location (Wong & Candolin 2005). Chemical signals can be produced at relatively small cost and can be transmitted over large distances. Due to dispersal, they can sometimes not be as precise as visual or acoustic signals (Peake 2005, Wisenden & Stacey 2005).

Signalling can be performed in one or a combination of modalities. Animals will often use many types of signal alongside one another. The signals can mean something different individually or their combination could transfer new information entirely. It is also possible that multiple signals can be used to convey the same message, this is known as redundant signalling (Otto *et al.* 2013) These multimodal signals have been shown to increase the effectiveness of a signal in a variable or noisy environment (McGregor & Peake 2000).

Signalling in aquatic media has its own unique advantages and disadvantages. Acoustic signals travel faster in water as the molecules are packed much more closely together than in air, however the distance they travel is dependent upon ocean pressure and temperature. Whales can send signals which travel thousands of miles, the way pressure and temperature change in the ocean allow these sounds to refract up and down over huge distances (Miller 2006). Visual signals are widely used in underwater species, but face challenges such as low-light levels in turbid areas, deep water areas or areas dense in visually obstructive biodiversity. To combat this, bioluminescence has been widely employed by underwater creatures (Wilson & Hastings 1998).

Chemical communication has been recognised and studied broadly in aquatic species (Ingersoll *et al.* 1976, Oliveira *et al.* 2001, Dzieweczynski *et al.* 2006). Chemicals are not as limited by environmental barriers and are generally effective over distance. These signals however may disperse relatively quickly if in a turbid environment, or an environment with many competing chemical signals. Chemical signals are effective both during the day and the night. The signals can become redundant relatively quickly, as they may stay in the water after the signaller's internal state has changed (Bradbury & Vehrencamp 1998).

Communication Networks (Dyads Vs Networks)

Communication usually occurs within a network of individuals (McGregor & Dabelsteen 1996). Communication between organisms has often been studied in a dyad of receiver and signaller, however communication rarely happens solely in a dyad. McGregor (2005) explains that the “active space” (spatial area in which a signal can be detected reliably) of signals is large relatively, when compared with the average distance between individuals in a communication network. It is therefore expected that signals can be received by multiple receivers within this network, and these signals can potentially be interfered with by signals produced from different individuals. The core idea behind communication networks is that if the transmission range of a signal is greater than the average spacing between individuals, then we can say that communication happens within a network (McGregor & Peake 2000).

Previously we have discussed the costs and benefits associated with communication in a dyadic context. Communication in a network can incur additional costs due to

unintended receivers and limited signalling space due to signal competition. More than one signaller within the active space of a receiver can make it harder to gain information from a specific signaller. Some animals use a psychoacoustic process to discriminate a particular acoustic signal when mixed with other noise, for example when birds must locate their young amongst a crowd of many (Jouventin *et al.* 1999).

Adopting a network approach to communication is beneficial in that it allows explanation of processes which could otherwise not be explained, such as 'eavesdropping'. A signal may be directed at a target receiver, but there will also often be bystanders intercepting information from said signal for their own use (Peake 2005).

Eavesdropping

Eavesdropping has been found and studied in numerous animal species, spanning the entire animal kingdom (Peake 2005). In social situations, eavesdroppers can extract information from conspecifics which may benefit them in future interactions (Doutrelant & McGregor 2000). Eavesdropping provides a clear evolutionary benefit in that individuals can receive relevant information at little cost or risk to themselves (Oliveira *et al.* 1998). Eavesdropping can be divided into distinct categories; social and interceptive. Social eavesdropping describes a network in which information is extracted from a social interaction for use in future interactions. Intercptive eavesdropping describes when an eavesdropper is in receipt of a broadcast signal. A broadcast signal can often have a large active space, broadcast in the 'hope' of signalling the wanted receiver (McGregor & Dabelsteen 1996, Peake 2005).

Eavesdropping has been defined multiple times; It has been described as when signals are eavesdropped upon at a cost to the signaller (Wiley 1983) or at zero or negative benefit to signallers (Bradbury & Vehrencamp 1998). Eavesdropping was defined by Peake (2005) as "the use of information in signals by individuals other than the primary target." The present study prescribes to this definition as it is more useful, because in social eavesdropping signallers may benefit from interactions with eavesdroppers.

Eavesdropping has been shown to be present in numerous animal populations (Peake 2005). There have been many studies indicating that signallers change their

signalling behaviour when in the presence of an 'audience' of one or more potential eavesdroppers. Signallers will alter their signalling behaviour differently depending on the audience eavesdropping, for example *Betta* will increase the frequency and duration of aggressive signalling when there is a male audience and decrease aggression when there is a female audience. (Doutrelant *et al.* 2001, Bertucci *et al.* 2014).

Audience Effects

A communication network will typically consist of a signaller, a target receiver and any number of eavesdroppers. Since eavesdroppers may benefit from the information they intercept, often to the detriment of the signallers, there is a logical selection pressure on the evolution of signal production (Oliveira *et al.* 2001). Species often disguise their signals from detection (Wisenden & Stacey 2005) or alter their behaviour and/or physiology during signalling, depending on the characteristics or behaviour of the bystander(s) eavesdropping on them. This phenomenon is known as the audience effect (Dzieweczynski *et al.* 2012). Matos & Schlupp (2005) defined audience effect as, "changes in the signalling behaviour during an interaction between individuals caused by the mere presence of an audience." Audience effect is therefore an evolutionary manifestation of selection pressure from eavesdroppers. It has also been found that signallers may express their signal behaviours in response to an evolutionary audience (Matos & Schlupp 2005), which may or may not actually be present during signalling. An example of this is birds simultaneously warding off competition and attracting mates whilst singing (Searcy & Nowicki 2000).

Audience effect can influence different behaviours from signallers depending on the identity of the eavesdropper. For example, Doutrelant *et al.* (2001) showed that male *Betta splendens* (hereafter known as *Betta*) alter their signalling behaviour during an agonistic interaction with another male depending on the sex of the audience. The subjects showed decreased aggressive signalling and increased conspicuous signalling when in the presence of a female audience. Males are more aggressive when signalling in the presence of a male audience (Matos & McGregor 2002).

Priming is the phenomenon of an individual behaving more aggressively in an agonistic interaction if they had been in the presence of a certain type of audience

prior to the agonistic interaction taking place or being more likely to enter into an agonistic interaction (Meliska & Meliska 1976, Bronstein 1989). Oliveira *et al.* (2001) demonstrated priming in an experiment where male cichlids (*Oreochromis mossambicus*) observed a contest between male conspecifics and consequently experienced an increase in androgen levels, which are linked directly to aggression; similarly, to the challenge hypothesis. Priming may use the same evolutionary processes as audience effects, altering the behaviour/physiology of individuals who have experienced an audience immediately prior to an interaction. Matos & McGregor (2002) demonstrated that exposing male *Betta* to an audience prior to an interaction with a male conspecific increased its aggressiveness in the subsequent interaction. This aggressiveness remained unchanged whether the audience was present during the interaction or not. This suggests that pre-exposure to an audience can override the actual presence of an audience during an interaction. Experiments involving communication networks must therefore take the priming effect into account to mitigate pre-exposure of subjects to an audience.

Spying Networks

Signal or cue?

Wisenden & Stacey (2005) define communication as explicitly dealing with signals, and not 'cues'. Signals are described as being specialised in information transfer. In order to produce a signal, a signaller must have evolved a specialised method of signal production. Bystanders however can gain information not just from eavesdropping on signals, but also by receiving cues from other organisms. While signallers have evolved specialised methods for producing and directing signals, cues are emitted by these 'originators' unintentionally. Receivers of both signals and cues have necessarily evolved a specialised means in receiving and processing this information. When a bystander receives information from a cue rather than a signal, this is referred to as spying (Wisenden & Stacey 2005).

Spying networks explain information transfer in a network without signal production. Signature-mixes are described by Wyatt (2010) as complex molecular chemical emissions produced by organisms. These emissions are processed (usually by conspecifics) through olfactory processes and can be used to inform on health, social status, familiarity and more. This information is not directed or broadcast at

any target receiver, but can still be received and interpreted by spies, who on average will benefit from it. While spying and communication networks are similar, they are functionally distinct in that spying networks may occur in dyads, where communication networks can only occur with three or more individuals. Audience effects also can only logically manifest in signallers, and not in originators of cues. These distinctions are important in the present study, as it deals with both spying and communication networks.

Model Species

Model Species (Betta splendens)

The domesticated strain of Siamese fighting fish (*Betta splendens*) (hereafter known as *Betta*) has been studied intensively regarding communication and spying networks. Both sexes of the species have been observed eavesdropping and/or spying, and both sexes engage in conspicuous display behaviour (Lucas 1968, Simpson 1968, Monvises *et al.* 2009, Peake 2005). These behaviours have been well-recorded and researched. Males will display to one another when they meet in an agonistic encounter; flaring their fins, extending their operculum and whipping their tails (Simpson 1968, Bronstein 1983). Once a male has become submissive, he will lower his fins, become a drabber colour and sink downwards. Females have been shown to eavesdrop on these encounters and use the information gathered to choose a prospective mate (Peake & McGregor 2004). When a female chooses a male to mate with, she begins a mating display and her usually duller flanks become bright stripes (Lucas 1968). It has been found repeatedly that females visually eavesdropping on male agonistic encounters will choose winning males most often (Herb *et al.* 2003, Dzieweczynski & Walsh 2011)

While it has been shown that females will consistently choose winning males when they have been visually observed winning, this does not necessarily mean that the visual modality is the only one used in deciding mate choice in this species. To the contrary, numerous species of fish have been shown to use chemical cues (which may be more honest and reliable as they are cues rather than signals) in this decision-making process (Earley & Dugatkin 2002, Peake 2005). Research has been done which allows water (chemical) flow between males and females while they are eavesdropping (simulating a more natural environment) (Clotfelter *et al.* 2006) and

research has been done which allows only visual eavesdropping, with no water flow between individuals (McGregor *et al.* 2001). Both methods are warranted depending on the question asked.

Hypotheses

This project is designed to investigate the importance of chemical information transfer in *Betta*. Information in this sense likely refers to cues in a spying network, rather than signals in a communication network. For the purposes of this study, 'chemical information' refers to both signals and cues.

The importance of chemical information in *Betta* has not been studied in detail. Some studies suggest that they are receptive to chemical cues from conspecifics (Ingersoll *et al.* 1976, Dore *et al.* 1978). Dzieweczynski *et al.* (2006) showed that the amount of steroids produced and excreted by *Betta* changed in different social environments. Since *Betta* are naturally predisposed to exhibit aggressiveness to conspecifics, they are often necessarily housed in isolation. They are housed not just in visual isolation, but often in chemical isolation. This study aims to inform us as to the 'need' *Betta* have for conspecific chemical information.

Need in this scientific context is more akin to 'want' in colloquial language. Dawkins (2004) suggested that we can simplify complicated welfare assessments by answering two key questions; "are the animals healthy?" and "do they have what they want?". We can offer an animal choice and determine if it chooses one option often enough to discount chance. This is a preference test (Dawkins 2004). This shows us which of multiple conditions an animal prefer at a given time. If an animal is willing to work harder to reach one option, then it can be said to want, or need that option (Dawkins 2004). Use of a T-maze is a well-established method for studying preference in animals and has been used previously with *Betta* (Craft *et al.* 2003, Shapiro & Jensen 2009).

We ask the question in this study; do male *Betta* express a need for conspecific chemical information? Using T-mazes, we will offer subjects various conditions, including conditions allowing access to said information. We hypothesise that the subjects will work harder to have access to this information and will therefore express a need for chemical information from conspecifics. There is a possibility that this need may translate to a response against a territorial intruder, however the

scope of this study only looked into whether subjects chose chemical cues over lack of. The results of these experiments will inform welfare and husbandry practices regarding male *Betta* being housed in social isolation, as well as improving our understanding of the importance of this type of information in terms of preference and choice.

In addition to this, we want to discover the importance of chemical information transfer in *Betta* in social contexts. In this thesis we pose the questions; do female *Betta* still choose winning males over losers after visually witnessing an agonistic display, if they receive mismatching chemical information from the losing male? And do male *Betta* in a dominant-subordinate relationship recognise a social hierarchy when receiving mismatching chemical and visual information during an agonistic interaction?

This will be investigated with experiments looking at mismatching multimodal information transfer. *Betta* are an aggressive species, as we have discussed previously. This however is moderated a great deal once a social dominance hierarchy has been established (Wallen & Wojciechowski-Metzlar 1985).

Subordinate individuals recognise dominant individuals and do not engage them for long, if at all. It is possible then to create a relative hierarchy among subjects by pairing them and letting them display to one another across a transparent barrier. Once a winner has been established, that pair can be allowed to display subsequent times. If each time the same subject remains dominant, then we can say that a dominant-subordinate relationship (DSR) is recognised between those subjects. This method is similar to that used by Wallen & Wojciechowski-Metzlar (1985). Once these DSR's are established, we will be able to predict the outcome of agonistic interactions.

We can then provide the subjects with mismatching information from different modalities and assess how it affects their behaviour. Providing a subject with a visual subordinate, but chemical information from a dominant opponent, we can attempt to understand the importance and interactions of each type of information transfer in this context. Using female eavesdroppers, we can employ similar techniques to better understand how chemical and visual information transfer affects mate choice in this species, building on our knowledge of sexual selection.

Methods

In order to test whether a domesticated strain of *Betta splendens* (hereafter known as *Betta*) are in 'need' of social (chemical) information, a set of experiments were done using choice mazes.

Male (n=8) and female (n=4) *Betta* were used in this study, they were a domesticated strain of the species commonly known as 'crowntails'. Subjects were sourced from one online commercial supplier; 'www.aquaticstoyourdoor.co.uk'. Through online communication it was made clear that males had no prior social contact with any other subjects, to control for familiarity. Females did not encounter one another during experimentation; therefore, familiarity was not an issue. There was no information on the genetic relatedness of subjects, however the operation of the pet trade and the physical and size similarities of subjects used suggests it likely they had been bred from the same stock, thereby limiting genetic variables. The males were all blue in colour while females were a duller brown colour, with flashes of blue and green. The age of subjects is unknown; however, they had all reached sexual maturity. The size of subjects varied little, with males ($\bar{x} \pm SE = 5.63 \pm 0.14$ cm) and females ($\bar{x} \pm SE = 5.35 \pm 0.21$ cm) being similar lengths, measured using standard length, from the tip of the nose to the base of the tail.

Housing

Subjects were housed individually in glass aquaria (10.5 x 10.5 x 13cm), in visual and chemical isolation. The aquaria were black on all sides except the front. They were kept in aged tap water from Newquay, Cornwall, which had been treated with standard water conditioner for tropical fish. Subjects were kept between 25-27°C and were fed on Tetramin® tropical flake food once a day. The light/dark cycle was controlled at 8.5/15.5 h. Water changes (20%) were made every 2 days in line with hygienic fishkeeping practices (Evans 1985, Oliveria *et al.* 1998). Subjects were left in the housing aquaria for 1 week prior to experimentation, to allow them time to acclimatise. The experiments took place during July and August 2017 at Cornwall College Newquay, Cornwall, UK.

Experiment 1 – Do male *Betta* ‘need’ to be in receipt of chemical information from conspecifics?

Experiment 1 was a preference test (Dawkins 2004), using an aquatic T-maze, similar to that used by Shapiro & Jensen (2009), which allowed subjects to choose one arm or the other. The maze was constructed from matt-black Perspex® sheets and aquarium-safe silicone sealant. Only male subjects were used in this experiment.

Using trapdoors (Perspex® sheets which could be removed) the T-maze was separated into sections (Fig. 1). These sections comprised of a start box, a choice box and a left and right arm. Trapdoors 1 & 2 allowed exit from / entry to the start and choice boxes. The first trapdoor allowed the subject to be contained in the start box at the beginning of the experiment, and the second prevented the subject from returning to the start box when choosing between the arm of the maze. ‘Decision lines’ were marked 3 cm into each arm, a further 3 cm beyond which was another trapdoor (3 or 4). The entire T-maze was 15cm high, and each compartment was 15cm long (compartments would be the left arm, choice box, right arm, box between choice and start box and the start box).

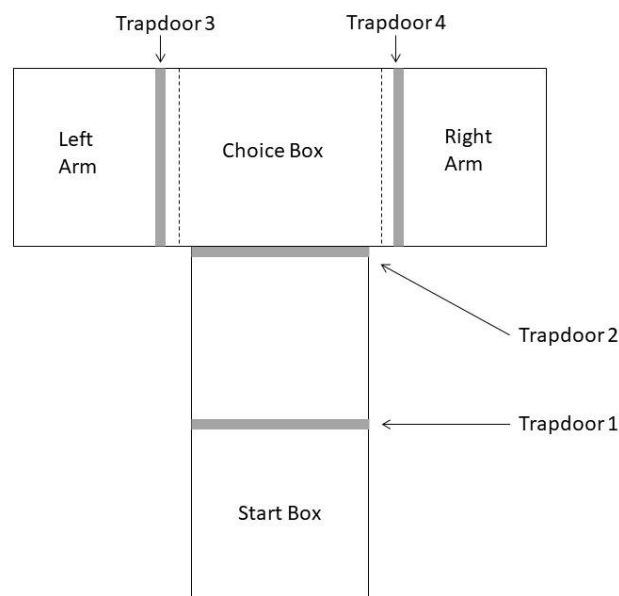


Figure 1. Aerial view of T-maze separated into sections, removable trapdoors (thick light-grey lines) and decision lines (dotted black lines).

The T-maze was filled with tap water from a large aquarium, which contained treated and aged water, used only to refill the T-maze. The T-maze was emptied, cleaned thoroughly and refilled after each run to ensure that no chemical information from conspecifics remained before the next run. The treated and aged water contained no known stimulus for *Betta*. To provide chemical cues during experiments, additional water could be pumped into either arm of the maze using a small peristaltic pump. This water was either the same as in the rest of the T-maze ('*no stimulus*') or contained chemical information from conspecifics ('*potential stimulus*'). The water containing chemical information from conspecifics was collected as a mixture from both male and female housing aquaria and put into an empty container, which then was connected to the peristaltic pump. Preliminary testing using dye indicated that the chemical cue was present throughout the arm within 20 seconds of the pump starting, but that no cue entered the choice box until the trapdoor was raised.

Phase 1: Experiencing the different stimuli

This method is similar to that used by Craft *et al.* (2003), allowing subjects to acclimatise to the different options available. A subject was transferred into the T-maze by placing their housing aquarium into the start box and allowing them to swim freely into this section of the maze, eliminating the need for using a net which is prone to cause stress (Evans 1985, Monvises *et al.* 2009). The subject was left there for 20 seconds.

The experiment began when trapdoors 1 & 2 were raised (allowing subjects to enter the choice box) and peristaltic pumps began filling the arms with water. Once the subject was in the choice box, trapdoor 2 was closed and trapdoor 3 with 'potential stimuli' was raised and the subject could experience the left arm and stimulus for at least 20 seconds. Once the subject moved back to the choice box of its own accord Trapdoor 3 was then lowered and 2 raised, allowing the subject to return to the start box. When they did so, trapdoor 1 was lowered and the subject was removed from the maze, using its housing aquarium in the same way that it was lowered into the maze. The T-maze was then flushed and refilled. The exact same process was then repeated ,except the subject experienced the right arm with no stimulus. The

stimulus arm did not change between replicates as the point was to allow the subject to become acclimatised to the stimulus coming from one particular direction. Water containing no stimuli was still pumped into the arm, so that any water-flow experienced coming from the arm to the choice box once the trapdoor was opened was the same. This was repeated so that the subject experienced each arm twice.

Phase 2: choosing arms

The same process as phase 1 was applied, however this time subjects were allowed to choose an arm of their own accord. An arm was labelled as chosen when a subject crossed (with the tip of its nose) a decision line (lines marked on T-maze by researcher; 3cm from the choice box on either side). Latency (the time elapsed between the fish entering the choice box until it chose an arm by crossing a decision line) was recorded in seconds using a digital stopwatch. Once an arm had been chosen, that trapdoor was then opened for 20 seconds and the subject could experience that arm. The subject was then ushered gently back the start box (and into its housing aquaria), at which point the housing along with fish was removed, the maze flushed thoroughly with tap/clean/aged/treated water to remove any remaining chemical cue, and then refilled with treated/aged water. After a 5-minute rest period, the process was repeated, until five iterations had been completed. The next fish was then introduced to the maze.

Phase 3: controlling for side-bias

Subjects had a resting period of 7-11 days before engaging in phase 3. Here the entire process of phases 1 and 2 were repeated, with the important exception that the potential stimulus was switched from the right to left arm, to control for side bias. After this phase, each subject had completed the maze 10 times, which was a number decided upon due to the number of replicates necessary for analysis, and time constraints of working within a college classroom.

Phase 4: effect of additional cost

Here the entirety of phases 1-3 were repeated. However, the arm of the T-maze containing the potential stimulus was in each case extended by a further 15cm using Perspex and aquarium safe silicone sealant. This increased swimming distance made this option now costlier to reach.

Phase 5: decisions without stimulus

Again, phase 4 was repeated except that now the potential stimulus was removed and neither arm contained chemical information from conspecifics for the remainder of the experiment. This component of the experiment was to investigate whether captive *Betta* chose the longer arm purely on the merit of being able to swim further, as suggested by Shapiro & Jensen (2009).

Experiment 2 - Mismatching multimodal information transfer in *Betta*. How do subjects use visual communication and chemical cues in a social context?

All subjects were housed and cared for in the same way as in experiment 1. *Betta* are an aggressive species, but this is moderated a great deal once a social dominance hierarchy has been established (Wallen & Wojciechowski-Metzlar 1985). Subordinate individuals recognise dominant ones and do not engage them for long, if at all. It is possible then to create a relative hierarchy among subjects by pairing them and letting them display to one another across a transparent barrier (Wallen & Wojciechowski-Metzlar 1985). Once a winner has been established, the pair can be allowed to display subsequent times. If each time the same subject remains dominant, then we can say that a dominant-subordinate relationship (DSR) is recognised between those two subjects. This method is similar to that used by Wallen & Wojciechowski-Metzlar (1985).

Preliminary Dominant-Subordinate Relationship Establishment

Male subjects were paired and allowed to form DSR's using the above-mentioned method. The housing aquaria of males were placed in front of each other to initiate displaying. This usually began within seconds of visual acknowledgement (which was established as soon as a subject began displaying either fin flaring or gill cover erection behaviour). Subjects were labelled as losers when they became submissive, by lowering their fins, becoming a drabber colour and sinking to the bottom of the water. This is a well-established method of selecting winners/losers of agonistic displays in this species (Simpson 1968, McGregor & Peake 2000). If pairs followed the same pattern of winning and losing four times in a row, they were labelled as being in a DSR. If during DSR establishment subjects did not follow the same pattern of being either submissive or dominant, they were matched with another subject after a rest period of 24 hours and the process was repeated until one had been established. This type of established hierarchy is not the same as a more complex hierarchy found in the wild, as there were 4 DSR pairings, rather than a complete dominance hierarchy within a network of individuals. To establish a truly natural hierarchy of this type is possible only with specialised housing. There were now however had 4 pairs of individuals who visually recognised their paired partners as either dominant or subordinate. Water flow (using peristaltic pumps between

housing) was allowed between pairs as they displayed to one another, meaning that they were also receiving chemical cues from one another during this time. Once DSR's were established, subjects were allowed to rest 24 hours before the experiment began.

Phase 1

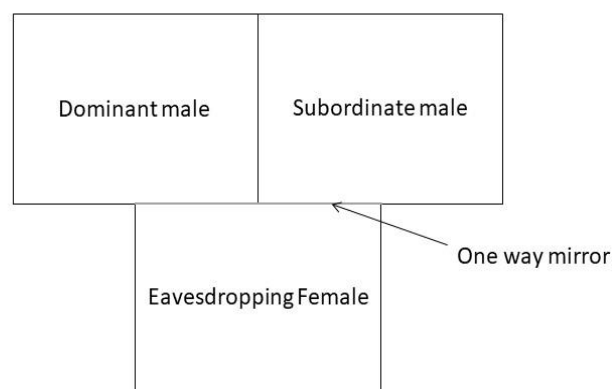


Figure 2. Eavesdropping female viewing agonistic display between a dominant and a submissive male via a one-way mirror which concealed her from being viewed by both male compartments.

DSR pairs were allowed to display to one another through a transparent barrier (shown in Figure 2), with a two-way water pump allowing chemical information transfer between dominant and subordinate males. Gill cover erection and fin flaring (agonistic display behaviours) were recorded as total time spent performing them (2 separate behaviours recorded separately) until the subordinate male became submissive. Female subjects were allowed to eavesdrop (see Eavesdropping) on these interactions (one female per pair of males) through a one-way mirror, so they could not exert an audience effect on the males. Following the display, the female was introduced to the winner and the loser, one at a time. This was achieved by simply moving the transparent containers holding the male and female to face one

another. In the first trial the female was introduced first to the winner and then to the loser, and this order of presentation was reversed for the next female. A peristaltic pump allowed chemical information transfer from male to female. Latency to developing mating stripes is a conspicuous behaviour in females (Lucas 1968) which is widely recognised as being positively correlated with female mate choice. This was recorded in seconds using a digital stopwatch. Latency to developing mating stripes has been shown to be significantly quicker when females are introduced to seen winners over losers (Doutrelant & McGregor 2000, Clotfelter *et al.* 2006).

Phase 2

The same male pairs were allowed to repeat their dominance interaction (after a break period of at least 1 hour), but on this occasion there was no water flow between subjects displaying to one another, meaning they would now only be receiving visual information from the subject opposite them. Two male-male interactions took place simultaneously, in separate aquaria. Water flow between dominants took place using two-way water pumps, as did water flow between subordinates. Now displaying subjects were receiving correct visual information, but incorrect chemical information. In both interactions, dominants displayed visually to subordinates, but receive chemical information from other dominant subjects. The opposite was true for subordinates.

Eavesdropping females were again introduced to winners and losers, one at a time. However, the female received chemical information from the loser when interacting with the winner and vice versa. This was done to study whether latency to developing mating stripes was affected by the female subjects receiving mismatching visual and chemical information from winners and losers. It is already well known that they will develop mating stripes significantly more quickly in the presence of seen winners over seen losers, but this study intended to establish whether the addition of mismatching chemical information would alter this response.

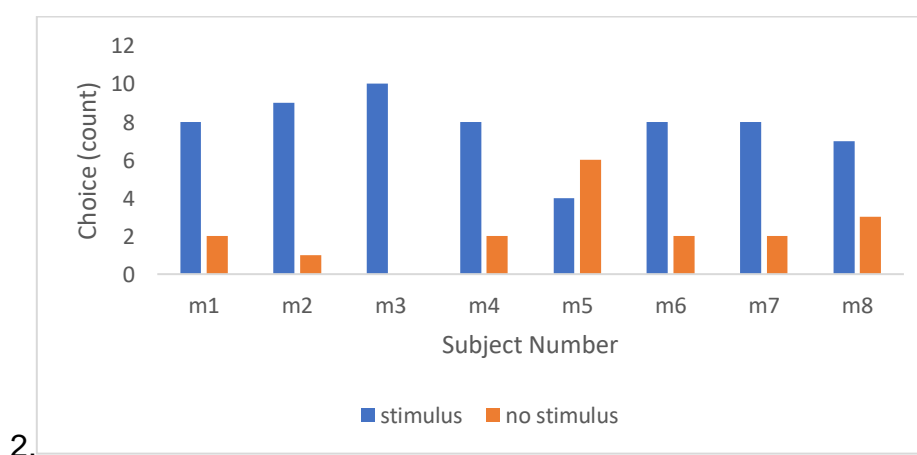
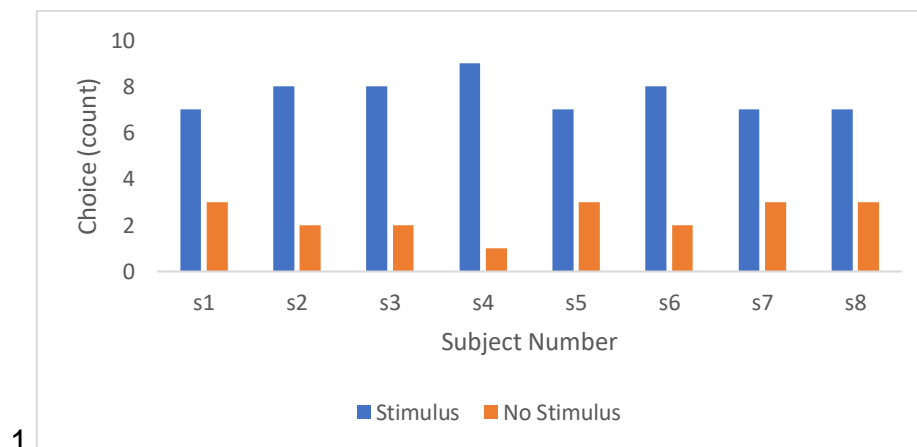
Results

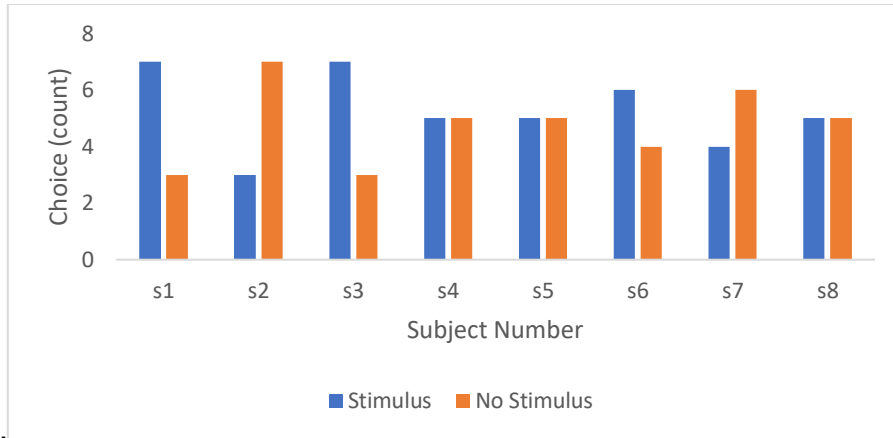
Experiment 1

Did subjects choose which decision line to cross in a similar way?

Following the choice tests, subjects chose the arm of the T-maze containing the stimulus more often than the arm containing no stimulus in phases 3 and 4 of experiment 1. They did not prefer either arm in phase 5 where no chemical cue was presented (Figure 3).

To test for statistical significance a chi squared test was run for each individual subject (n=8) across each individual phase of experiment 1. To account for the large number of degrees of freedom (24) the results were processed using Fisher's combinatorial method on Excel. A significant result was found in phase 1 ($p=0.0012$) and phase 2 ($p=0.0002$) but not in phase 3 ($p=0.9399$). There is a relationship between the way subjects cross a decision line when presented with chemical cues from conspecifics.





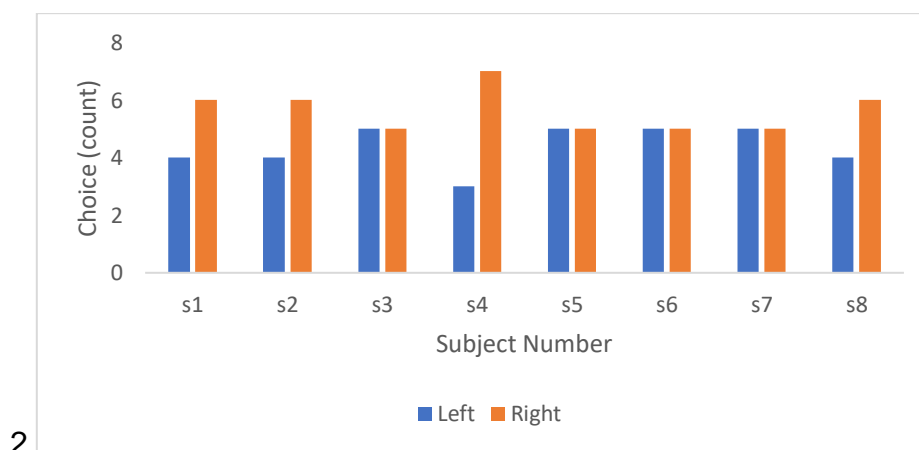
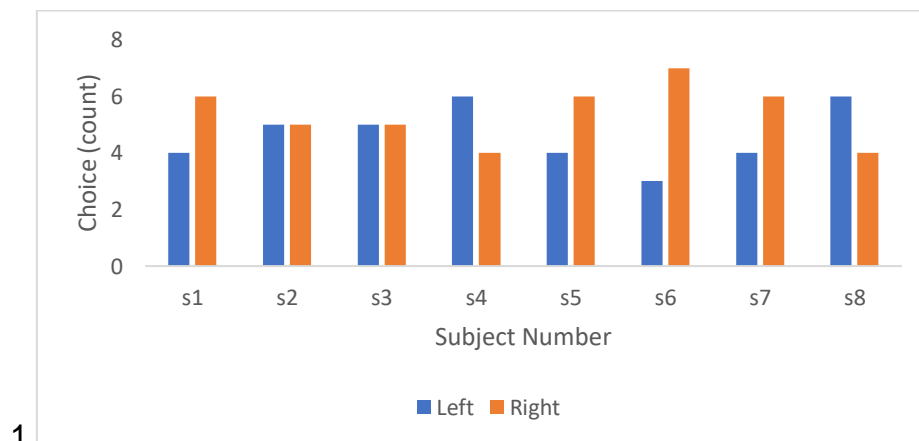
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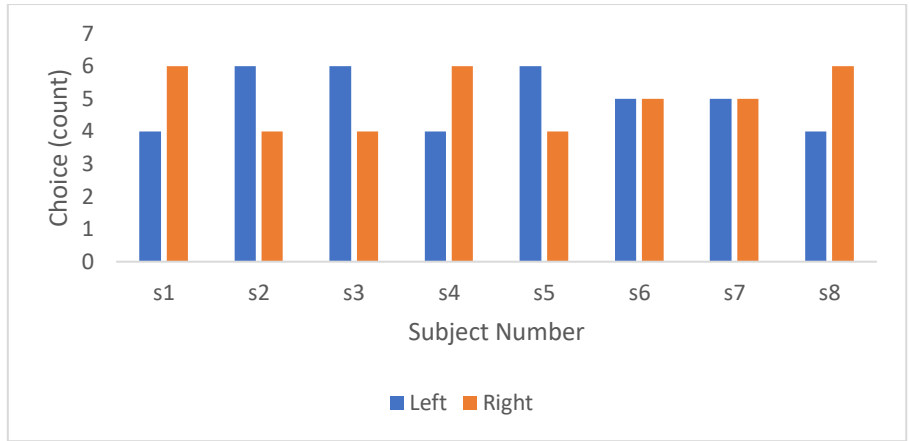
Figure 3. Counts of how often each subject (s1-s8) crossed the decision line of the arm of the T-maze containing chemical information (stimulus) or not (no stimulus). Graph 1 corresponds to phases 2 and 3 of experiment 1, graph 2 to phase 4 of experiment 1 and graph 3 to phase 5 of experiment 1.

Did subjects show a left/right arm bias when the variable of stimulus/no stimulus was not accounted for?

Subjects did not significantly favour either left or right in any of the phases of experiment 1 (Figure 2).

To test for statistical significance a chi squared test was run for each individual subject (n=8) across each individual phase of experiment 1. To account for the large number of degrees of freedom (24) the results were processed using Fisher's combinatorial method on Excel. No significant result was found in phase 1 (p=0.2969), phase 2 (p=0.5363) or phase 3 (p=0.2969). The lack of significant results leads me to accept the null hypothesis; there is no difference in the directional (left or right) aspect of subject's choices in the T-maze.





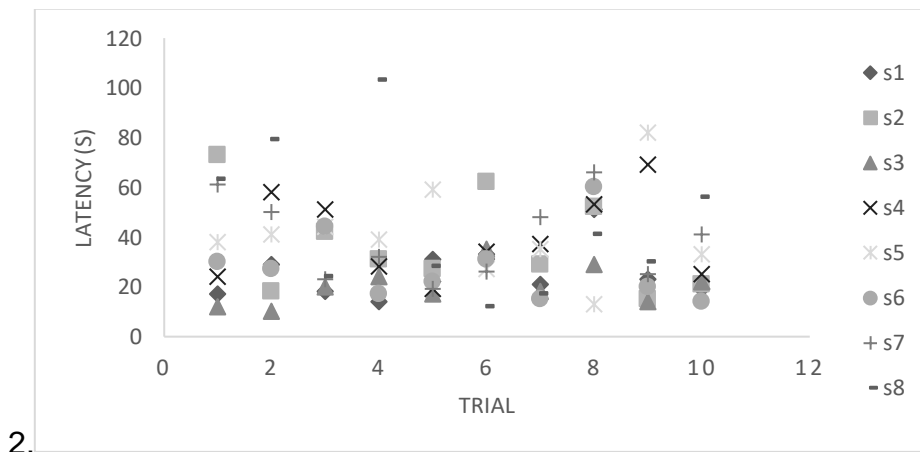
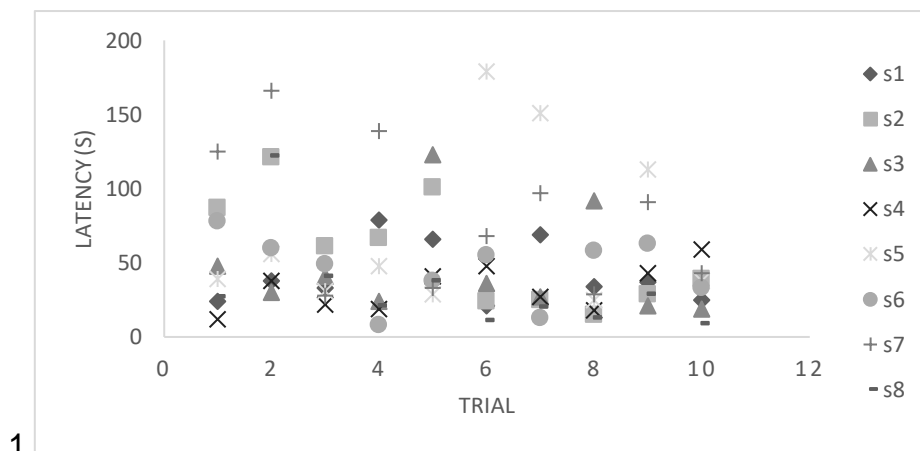
3.

Figure 5. Counts of how often each subject (s1-s8) crossed a decision line in a specific direction (left or right) irrespective of any potential stimulus. Graphs 1, 2 and 3 correspond to the 3 phases of experiment 1.

Did subjects cross a decision line more quickly as experiment 1 progressed?

Subjects did not choose decision lines significantly more quickly as the experiment progressed in any of the phases 1-3 (Figure 3).

Data was non normal and so was analysed using Wilcoxon signed ranks test in AQB. Subjects did not choose a decision line significantly more quickly between the first and last trials of phase 1 ($V=28$, $p=0.161$), phase 2 ($V=28$, $p=0.195$) or phase 3 ($V=21$, $p=0.742$) of experiment 1. The lack of significant results leads me to accept the null hypothesis; there is no difference in how quickly subjects crossed a decision line between the first and last trials in any phase of experiment 1.



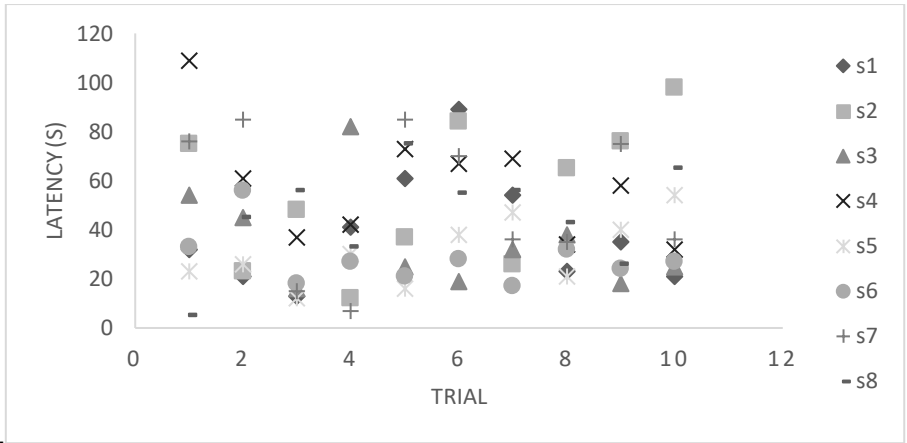


Figure 6. The relationship between subjects (s1-s8) latency to crossing a decision line across trials 1-10. Graphs 1,2 and 3 correspond to the 3 phases of experiment 1.

Experiment 2

Interactions affecting latency to developing mating stripes in female subjects.

The difference in latency to developing mating stripes in eavesdropping female subjects was recorded when introduced to male winners and losers of agonistic interactions across two conditions: being in receipt of correct visual and chemical information and being in receipt of correct visual information and mismatching chemical information. A non-parametric analysis of variance with replication was applied using AQB.

The two factors were factor A (winning/losing males) and factor B (correct/mismatching information). There was no significant main effect of factor A ($F=0.35$, $p=0.5651$, $df=3$), there was no significant main effect of factor B ($F=0.64$, $p=0.4393$), however there was a significant interaction between these factors ($F=15.93$, $p=0.0018$).

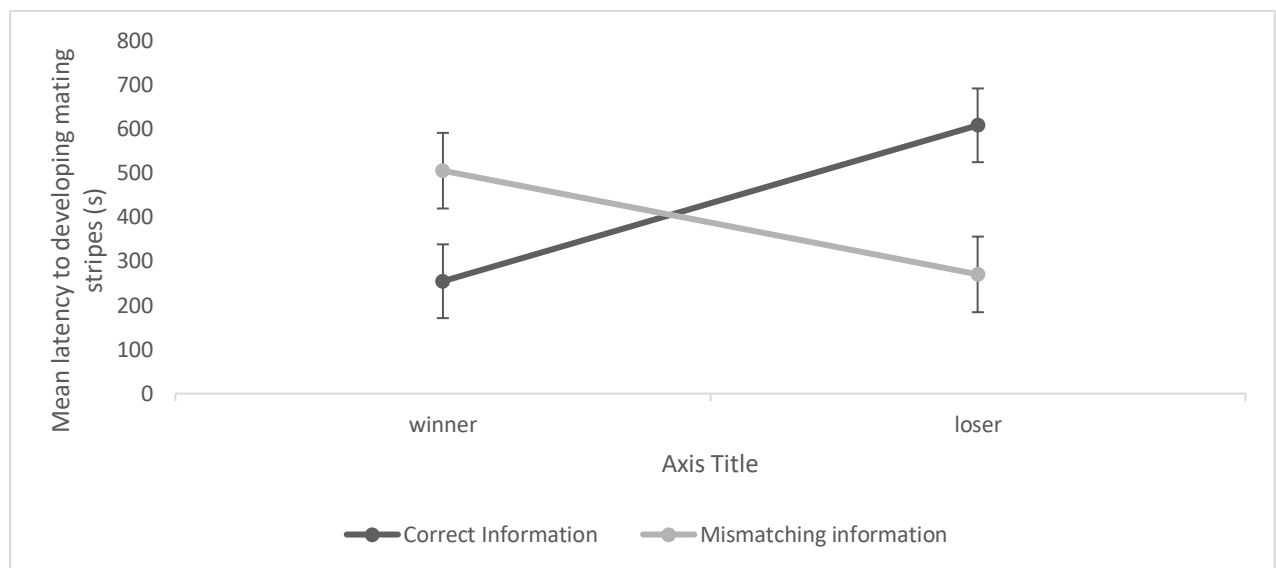


Figure 7. Interaction plot of mean latency to developing mating stripes in female *Betta* once introduced to seen winners/losers of agonistic interactions, both under the conditions of receiving correct visual/chemical information and receiving correct visual/incorrect chemical information.

Difference between time spent performing courtship displays in males across trials.

The difference in time spent performing operculum extension and fin flaring in males (dominant and subordinate) was compared across trials (correct and mismatching). T-tests were applied in AQB.

No significant difference was found in dominant males in either trial in operculum extension ($t=0.11$, $p=0.9157$, $df=14$) or fin flaring ($t=0.36$, $p=0.7294$, $df=14$). No significant difference was found in subordinate males in either trial in operculum extension ($t=0.76$, $p=0.4737$, $df=14$) or fin flaring ($t=1.70$, $p=0.1408$, $df=14$). The lack of significant results leads me to accept the null hypothesis; there is no difference in how male subjects visually displayed in agonistic interactions in either phase of experiment 2.

Difference in latency to submission in males across trials.

Under both conditions of subjects receiving correct and incorrect chemical information whilst performing agonistic displays, only subordinate males became submissive. A T-test in AQB was used to establish the difference in latency to submission in subordinate males between phases 1 and 2 of experiment 2.

No significant difference was found ($t=1.14$, $p=0.2966$). The lack of significant results leads me to accept the null hypothesis; there is no difference in how male subjects became submissive following agonistic interactions in either phase of experiment 2.

Discussion

The aim of this study was to research the importance of chemical information transfer in *Betta splendens* in regard to the species need to be in receipt of it (Dawkins 2004), and its effect on social (agonistic and mating) interactions. The results add to the body of knowledge surrounding communication and spying networks (Peake 2005, Wisenden & Stacey 2005), in addition to providing possible new insights into the mechanisms behind social communication in this model species used in laboratory experimentation.

This study found in experiment 1 that male *Betta* exhibited a preference for conspecific chemical cues in a T-maze choice test, when presented with said cues or an alternative of no cue. This suggests that this species may have a need (Dawkins 2004) for chemical information from conspecifics. This was the case in all phases of experiment 1 with the exception of phase 3, where no cue was present in either arm of the maze. This adds confidence to the findings and suggests further that when given the straight choice between chemical cue or no chemical cue, *Betta* will choose the cue. This is the case even when the energetic cost is increased, suggesting that they will pursue the cue over no cue even when it is more costly to them. *Betta* did not choose an arm purely on the merit of being able to swim for longer in an enclosed environment (experiment 2, phase 3), as suggested by Shapiro & Jensen (2009). They also showed no bias for left or right, adding further confidence to the results found.

This information together suggests that *Betta* may be in need of conspecific chemical information, which could be important from a welfare perspective, as they are often housed in complete isolation. This may prove to be detrimental to their welfare as they are being deprived of a social aspect of their lives which they would rather be in receipt of. Future studies could research how the welfare of *Betta* is affected by being housed in not just visual but chemical isolation for extended periods of time, or indeed their entire lives.

Experiment 1 could benefit from being researched again using a larger T-maze, as the arms were fairly small, and the extended arms in phase 2 and 3 could also be larger to add to the energetic cost aspect of the experiment. Given the limited resources and space that had to be accounted for in this study, it was not possible. If

more time were available, more replicates could be used in each trial also to improve confidence in the statistical results. This experiment used only male subjects, again due to time and resource constraints. Future studies could perform the same experimental method using females to expand on these findings.

In experiment 2 it was found that male *Betta* did not alter their display behaviour (fin flaring or gill cover erection) or their latency to submission significantly when receiving mismatching chemical information during agonistic interactions. Dominant males always remained dominant and vice versa for subordinates. This suggests that while male *Betta* have been shown to use chemical cues in these circumstances (Dzieweczynski & Walsh 2011) they may rely more on visual communication to determine their agonistic behaviour during a display interaction with rival males. Females on the other hand were shown to significantly change their behaviour when in receipt of mismatching chemical and visual information. Females developed mating stripes more quickly in the presence of seen winners when receiving correct chemical information, and more quickly in the presence of seen losers when receiving mismatching chemical information from the winners. This suggests that females in this species rely more heavily on chemical cues than visual communication when involved in a mating interaction with males. This could be because wild *Betta* live mainly in heavily vegetated or muddy habitats, and chemical signals are easier to detect than obstructed visual signals. It could also be that the chemical is a more reliable signal as it is more honest, and less prone to deception by the signaller. Herb *et al.* (2003) showed that male *Betta* display more to females which did not witness them lose an agonistic interaction, essentially tricking non-eavesdropping females into thinking they were winners. They however will produce less testosterone following a loss than the winners (Oliveira *et al.* 2001, Dzieweczynski *et al.* 2006). Therefore, it is possible that females may have evolved to use chemical information over visual in the context of sexual selection as it is a more honest appraisal of a potential mate's fitness.

Experiment 2 could have benefitted from many more replicates to improve confidence in the statistical results. In this case it was not possible due to limited resources (number of subjects of each sex available). The repeated measures aspect of the experiment meant that female subjects could not be introduced to multiple pairs of males in dominant-subordinate relationships, severely limiting the

number of trials possible. Future studies could replicate this experiment with a large number of subjects. It is also possible in both experiments that the limited sample size was not enough for a valid representation of the population.

During experimentation it was noted that males occasionally noticed their own reflections within the display aquaria before they were introduced to rival males. Displays were directed only towards rival males, not reflections; however, the possibility of a priming effect (Bronstein 1989) cannot be discounted. If a priming effect were experienced, all subjects were exposed to it equally. In future work specific lighting techniques or aquaria wall material could be employed in an attempt to minimise reflections within an aquarium.

The key points are that male *Betta* did choose to be in receipt of chemical information from conspecifics in a T-maze choice test, and that they did not appear to change their agonistic display or submission behaviour when in receipt of mismatching visual and chemical information from other displaying males. Female *Betta* did however develop mating stripes more quickly in mating interactions with males when in receipt of chemical information from winning males, whether visually seeing a winner or a loser of an interaction they had eavesdropped upon. This suggests that male *Betta* do have a need to be in receipt of chemical conspecific information transfer, although in an agonistic interaction they may rely more on visual communication. Females will use a combination of visual communication and chemical cues in mate choice but may rely more on chemical cues in this particular social interaction. To date there have been no studies asking the question of the importance of each type of information transfer in these contexts, and the novel results add to the body of knowledge surrounding communication/spying in this species.

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