Friendly neighbour or fiendish invader: individual recognition in the upland bully (*Gobiomorphus breviceps*).

Dominique Grace Harrison

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Department of Zoology

University of Otago

New Zealand

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In Loving Memory of Michael Peel Ethelston.

A wonderful grandfather with endless support for my endeavours.

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Abstract

Individual recognition behaviour is where an organism can identify another based on its individual characteristics. This behaviour is widespread throughout a variety of taxa. To be used successfully, an organism must be able to both send and receive signals. There are plenty of studies identifying recognition both intra- and inter-specifically, particularly in social animals. Territorial fish are a commonly used study species for recognition behaviour studies as these animals need to be able to differentiate threats and rivals from potential mates and familiar neighbours. This study aimed to investigate individual recognition through visual cues in a native New Zealand territorial fish.

In chapter two, a literature review was conducted on individual recognition, with a particular focus on visual cues. This review demonstrates that although individual recognition is widely studied, there are gaps in our understanding of the cues used for individual recognition. In particular, I focus on identifying pattern location on an animal's body and how this influences communication between individuals. There is a lack of research exploring how and why the visual cues need to be placed in a specific location on the body to allow for more direct communication. Another area which was covered in the review was how morphological constraints impact an organism's ability to use extravagant ornaments as visual cues. Animals' lifestyles vary among species, but for those with restricted body shapes, like fish, the use of a visual cue other than morphology is crucial for communication. This review provides a comprehensive review on individual recognition and what areas need further investigation.

In chapter 3, 30 upland bullies (*Gobiomorphus breviceps*), a native New Zealand territorial freshwater species, were collected from two locations in north Otago. Fish were paired together for a week to create a familiar pairing before being exposed to their familiar pair and then to an unfamiliar pair. Behaviours of the fish were filmed for 30 minutes when exposed to familiar and unfamiliar fish. The behavioural response of the upland bullies when exposed to familiar and unfamiliar fish were measured using video recordings analysed in BORIS (Behavioural Observation Research Interactive Software). Five behaviours were observed for analysis, aggression, movement, resting, duration inside the territory, and duration spent at the connective wall area. Neither males nor females showed any evidence for individual recognition, as none of the behaviours differed between familiar and non-familiar neighbours, which was likely due to a small sample size and seasonal restrictions due to the COVID-19 pandemic. The repeatability of the behaviours were also estimated. Overall, all behaviours, for

both males and females, were highly repeatable, suggesting that the upland bully exhibits consistent individual differences in behaviour (i.e., personality).

While the results did not support the hypothesis that upland bullies would differentiate familiar from unfamiliar fish based on visual cues, it poses new ideas around visual recognition cues and shows that the upland bully is displays consistent individual differences in behaviour study species, and thus could be a promising study species for more personality related studies. Future research should focus more on the specific cues used by territorial fish for identification.

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Chapter One: General Introduction

This chapter provides a general introduction to individual recognition with a focus on fishes, introducing some of the key concepts and ideas relevant to this thesis, followed by an outline of the thesis aims and structure.

1.1: Individual Recognition

Individual recognition is an important behavioural communication tool used by many taxa with substantial research behind it (Bass, 2001; Cheney & Seyfarth, 1986; Curé et al., 2016; Jouventin & Aubin, 2002; Snowdon & Cleveland, 1980; Tibbetts & Dale, 2007). Communication is crucial for many aspects of social interactions, and the methods of communication between individuals will vary depending on the species, environmental pressures, and the morphology of the animal (Bass, 2001; Costanzo & Monteiro, 2007; Mirza & Chivers, 2003; Tibbetts, 2002). Recognition can be used for identifying individuals, maintaining a social hierarchy, attracting and returning to a mate, or for defending territories or nest sites (Booksmythe et al., 2010; Chase, 1982; Cheney & Seyfarth, 1986; Frommolt et al., 2003; Issa et al., 1999; Jouventin et al., 1999; Jouventin & Aubin, 2002; Rosell et al., 2008; Tibbetts & Dale, 2007; Zenuto & Fanjul, 2002). Identifying a unique signal to recognise another individual requires the ability to send and receive a cue that is recognisable and unique to that individual (Tibbetts & Dale, 2007).

Common communication cues include visual (e.g., spot patterns), audial (e.g., song), and chemical cues (e.g., pheromones). Visual cues are observed in a variety of species, such as wasps (Tibbetts, 2002), sea lions (Wierucka et al., 2017) and chimpanzees (Parr & de Waal, 1999). Audial cues, such a songs or calls, are often observed in birds (Brunton et al., 2008; de Kort et al., 2009; Jouventin et al., 1999), marmosets (Snowdon & Cleveland, 1980), racoons (Sieber, 1986), bats (Balcombe & McCracken, 1992) and fish (Bass, 2001). Chemical cues (e.g., pheromones left from scent glands) are used by a wide variety of taxa, such as hyenas (Burgener et al., 2008), lizards (Carazo et al., 2008) and beetles (Steiger et al., 2008). Fish are known to exhibit variations of all three of these common cues (Cullis-Suzuki, 2016; Feng & Bass, 2016; Gherardi et al., 2011; Kohda et al., 2015; Mirza & Chivers, 2003; White & Gowan, 2013), but little is known about the specific cues used for individual recognition. Many studies investigating intraspecific communication make assumptions regarding the communication cues used by the studied organism. Often, the type of cue (e.g., visual) is already known due to the study set up, but few studies examine the specific cues used for individual recognition

(e.g., face shape, body patterns, colour). One study on the daffodil cichlid (*Neolamprologus pulcher*) investigated if the spot pattern on the face of these fish was the visual cue used to recognise a familiar fish (Kohda et al., 2015). By changing the spot pattern, researchers found fish were able to discern familiar fish from unfamiliar fish (Kohda et al., 2015). Further investigation into specific identifying cues would be beneficial to the scientific community for further behavioural studies.

1.2: Dear Enemy Phenomenon

The "dear enemy" phenomenon proposes that aggressive behaviours towards conspecific neighbours (i.e., familiar neighbours) is lower than aggression towards unknown individuals (Booksmythe et al., 2010; Brunton et al., 2008; Itzkowitz & Leiser, 1999; Thomson, 2016). From birds, to mammals, fish and reptiles, the "dear enemy" phenomenon has been a well-studied section of individual recognition (Langen et al., 2000; Moser-Purdy et al., 2017; Rosell & Bjørkøyli, 2002; Sogawa et al., 2016). Knowing that so many taxa can differentiate individuals from one another suggests a high level of cognitive ability in these animals (Brown, 2015; Delfour, 2006; Wascher et al., 2018).

An alternative hypothesis to the "dear enemy" phenomenon is the "nasty neighbours" hypothesis (Müller & Manser, 2007; Schradin et al., 2010). The "nasty neighbour" hypothesis proposes that individuals display higher levels of aggression toward a familiar neighbour than to a stranger. When there is a high threat level, an individual's response is influenced by the threat level presented by strangers and neighbours (Goll et al., 2017). Often, animals which live in social groups are more likely to display this "nasty neighbour" tactic than non-social animals (Müller & Manser, 2007). In the social banded mongoose (*Mungos mungo*), differentiation between neighbours and strangers were identified, and more alarm calls were elicited when investigating the neighbouring groups (Müller & Manser, 2007). The threat posed by the familiar neighbour was higher than the stranger within a social setting, as the threat of territory take over was higher with the familiar neighbours. When familiar neighbours already have an established territory, the biggest threat they pose is to an animals paternity (Moser-Purdy et al., 2017). In male song sparrows (*Melospiza melodia*), the "dear enemy" phenomenon was only displayed when their social mate was not fertile, and neighbours were not a threat to paternity. It is clear there are many threat levels which influence when the "dear enemy" phenomenon is used, and when the "nasty neighbour" tactic is more beneficial.

1.3: Territoriality

Territory defence is crucial but costly for territorial animals (Candolin & Voigt, 2001). The cost of energy for aggressive behaviours and fights are high (Grant & Noakes, 1987; Haller et al., 1996; Low, 2006; Neat, Taylor, et al., 1998; Taborsky, 1984) and can divert time which could be used for other essential activities, e.g., foraging for food or caring for young (Jaeger et al., 1983; Marzlufi & Heinrich, 1991; Viera et al., 2011; Wolf & Hainsworth, 1971). Being territorial can expose an animal to a greater risk of predation and injury (Candolin & Voigt, 2001; Jakobsson et al., 1995). For example, common bullies (*Gobiomorphus cotidianus*) were more likely to be eaten by rainbow trout (*Salmo gairdnerii*) during the bullies breeding season, probably due to the increase of territorial and aggressive behaviour which bullies display during their breeding season (Rowe, 1984). Defending a territory is crucial to maintain a mate and reduce any chance of another male establishing in that territory (Catchpole, 1983; Hamilton, 2004).

In fish, body size is important for female mate choice (Barber, 2002; Noonan, 1983), but the quality and the size of the nest can also determine the breeding success of a male in nesting species (Côte & Hunte, 1989). Within a population, individuals can experience different selective pressures which may cause contrasting results within a study. For example, a study on female mate choice in the upland bully (*Gobiomorphus breviceps*) showed there was no preference for males based on the fish size or the nest size (Hamilton et al., 1997). However, when a secondary study was conducted, there was the consensus that generalising a population based on one study was not prudent (Hamilton & Poulin, 1999). Variation within a population can cause generalisations to be inaccurate and cause false results, and further investigations into species which are effected individually by pressures would be ideal.

Territory location is key to successfully attracting a mate but can also come with its own challenges, such as increased defence of a resource (Grant, 1993; Gray et al., 2002). A territory rich with resources can attract rivals and increase attacks on the nest. Maintaining these territories often means the male has to be strong enough to defend and maintain these quality nests (Hamilton, 2004). Having conspecific neighbours with quality resources can increase attacks on neighbouring fish, typically due to individuals aggregating close to food resources (Hamilton, 2004).

1.4: Study Species

A popular study species for parasitism, mating behaviour and nesting choice, the upland bully (*Gobiomorphus breviceps*) is well studied, with information on its habitat and some behavioural traits established (Hamilton et al., 1997; Hamilton & Poulin, 1995, 1999; Hammond-Tooke et al., 2012; Poulin, 2000). However, limited research has been conducted on the social behaviours of upland bullies and their territorial behaviour. Male bullies defend a territory to attract females to lay eggs in his nest and will care for the eggs until they hatch. Upland bullies are commonly found in rocky, fast flowing streams both in the North and South Island of New Zealand at relatively shallow depths (less than 1m) (Jowett & Richardson, 1995) and are non-diadromous (do not migrate between salt water and freshwater) (Smith et al., 2005). The face and body of the upland bully exhibit a mottled spot pattern (Figure 1.1) and, when aggressive, male upland bullies will often turn jet black (based on personal observations). These physical and behavioural characteristics of the upland bully make it a useful study species for investigating individual recognition.

Figure 1.1: Mottled pattern of Upland Bully (*Gobiomorphus breviceps*).

1.5: Thesis Aims

This thesis investigates whether fish use visual cues for individual recognition. Due to the COVID-19 pandemic, I was unable to carry out the full extent of my intended MSc thesis as originally planned. My original intention was to conduct two behavioural experiments, but this became impossible due to COVID lockdowns. Instead, one chapter comprises a substantive literature review examining relationships between body patterns and individual recognition (Chapter 2). This chapter was in lieu of a second data chapter, and covers individual recognition, the communicative cues used by different taxa, why the location of visual cues is

important, and how morphological constraints can impact an animals communication cues. As this chapter contains significant overlap with my general introduction, I have kept my introduction brief to avoid repetition where possible.

Chapter Three outlines the experimental study of this thesis, the behavioural response of *G. breviceps* when encountering familiar and unfamiliar individuals. The hypothesis of this study was that upland bullies would show more aggression towards unfamiliar individuals compared to familiar. I predicted that the males would show more aggression than the female fish. I also predicted that the male fish would respond more to unfamiliar fish than the females. Five behaviours were recorded as they were related to how an individual would likely respond to a novel threat: aggression, movement, resting, duration spent inside the territory, and the duration spent at the contact zone (where both tanks met). Repeatability for each behaviour was calculated, to infer whether bullies display consistent individual differences in behaviour.

Chapter Two: A Review of Individual Recognition, Identifiable Cues in Fish, and the Impact of Morphological Constraints.

2.1: Introduction

Individual recognition, where one individual is able to recognise and distinguish between individuals, is one of the most remarkable skills displayed in the animal kingdom. This ability is seen in humans, where we recognise individuals through facial cues and other unique identifying features, but this ability has also been expressed in many other species, such as wasps, crayfish, trout, hyenas, and many more (Burgener et al., 2008; Gherardi et al., 2011; Tibbetts, 2002; White & Gowan, 2013). Individual recognition is commonly seen in animals living within a social group, or within a hierarchy (Brennan & Kendrick, 2006; Dunlop, 2018; Ewbank et al., 1974; White & Gowan, 2013). Conspecific consistent social relationships only work if individuals are able identify who they are interacting with. Differentiating between a neighbour and a stranger is crucial for social structures to be maintained (Goll et al., 2017; Saeki et al., 2018; Ydenberg et al., 1988).

Individual recognition is widespread among a variety of animals and is commonly seen in animals living within a social group, or a social hierarchy. However, attracting and nesting with suitable mates, offspring recognition, territorial defence, predator recognition and hierarchy rankings also involve individual recognition (Jordan et al., 2010a; Jouventin et al., 1999; Saeki et al., 2018; Wierucka et al., 2017). Individual recognition requires cues which are unique to each individual and vary among taxa. Visual cues are identifiable through colours, body markings and even facial recognition, and can be seen in wasps, (Tibbetts, 2002), sheep, (Kendrick et al., 2001), chimpanzees (Parr & de Waal, 1999) and fish (Kohda et al., 2015). Vocal or audial cues are used by birds (Weary et al., 1990), fish (Cullis-Suzuki, 2016) and in monkeys (Cheney & Seyfarth, 1980) for communication. Chemical cues are commonly seen in insects (Jackson & Morgan, 1993) as well as fish (Giaquinto & Volpato, 1997) and lizards (López & Martín, 2002). For this review, the main focus will be on visual communication and what cues can be utilised for visual communication. Visual cues can be through facial recognition, identifying unique patterns, markings or colours on an individual (Parr & de Waal, 1999; Tibbetts, 2002; Wierucka et al., 2017).

This review aims to give an overview on individual recognition in animal taxa, the mechanisms used, how morphology plays a key role, and key communication cues observed in research. To gather papers for this research, I searched using Google Scholar, Scopus and Web of Science and filtered through papers that focused on the topics I was after. Some examples of the key words are: "individual", "recognition", "fish", "dear enemy", "territorial", "pattern", "spot", "morphology". They were used all together, or sometimes paired up e.g., "fish" and "dear enemy" only.

2.2: Individual recognition

Communication is crucial for any social interaction, and conspecific individual recognition is needed in these social settings. Identifying mate, from kin, from nest mates, is essential, especially for social animals (Dugatkin & Earley, 2004; Parr & de Waal, 1999; Sharp et al., 2005; Tibbetts & Dale, 2007). Interspecific recognition is also key to many predatory/prey relationships (Gherardi et al., 2011; Griffin et al., 2001; Kelley & Magurran, 2003; Maloney & McLean, 1995; Mathis et al., 1996). Social animals need to have signalling and recognition to be able to identify and communicate within their social group (Tibbetts & Dale, 2007). Hierarchical animals also need to be able to identify who they are dominant and subordinate to (Barnard & Burk, 1979; Chase, 1982; Dugatkin & Earley, 2004; Issa et al., 1999).

Unique cues are needed to identify each individual from one another. As mentioned in the introduction, there are a variety of cues which are commonly seen throughout animals. These unique cues are learned and allow each individual to be identified clearly (Tibbetts & Dale, 2007). This precise form of communication can become extremely complicated, and as such has sparked an increase in research on this phenomenon. Research is often broken down into two parts: the receivers perspective, and the signallers perspective (Tibbetts & Dale, 2007). Receivers interpret whatever signal is being presented to them and have to make a choice how to behave or respond in response to this signal (Seddon & Tobias, 2010). Signallers have to send out the right cue to clearly communicate their intentions. For example, if they are trying to attract a mate, that signal may be different from alerting offspring that there is food. The evidence for unique cues is diverse and spreads across a huge range of taxa (Barnard & Burk, 1979; Brennan & Kendrick, 2006; Ewbank et al., 1974; Frommolt et al., 2003; Karavanich & Atema, 1998; Thom & Hurst, 2004; Tibbetts, 2002; Weary et al., 1990). These unique signals can often be what we see as individual variation within morphology, behaviours, colouration

and song. These patterns may seem similar, or sometimes even the same as another individual, but there is enough variation within these cues to remain unique to the individual.

Cue mechanisms are unique to different taxa, and often relate to the habitat and lifestyle the animal lives in (Tibbetts & Dale, 2007). For example, birds commonly communicate through vocal cues, as there is little variation in bird plumage (Brunton et al., 2008; Carlier, 1995; de Kort et al., 2009; Jouventin et al., 1999; Sharp et al., 2005), while fish will often use visual or chemical cues to identify a signal (Gherardi et al., 2011; Giaquinto & Volpato, 1997; Kelley & Magurran, 2003; Kohda et al., 2015). Often, multiple cues are used to communicate with conspecifics as well as amongst inter-specific individuals (Díaz-Gil et al., 2020; Kohda et al., 2015; Vilhunen & Hirvonen, 2003; Walther & Clayton, 2005; White & Gowan, 2013). Table 2.1 lists examples of studies which investigate recognition and whether or not the cues used by the species is specified.

Table 2.1. A sample of studies across a range of taxa which look at individual recognition (IR) since 2001. The IR type of cue is indicated in the table, along with what the specific cue is.

Animal	Cue type	Specific cue	Author(s)
Banded mongoose (*Mungos mungo*)	Chemical	Scent marks	Müller and Manser (2007)
Barking fox (*Alopex lagopus*)	Audial	Barking	Frommolt et al. (2003)
Eurasian beaver (*Castor fiber*)	Chemical	Scent marks	Rosell and Bjørkøyli (2002)
Rock hyrax (*Procavia capensis*)	Audial	Vocal calls	Goll et al. (2017)
Spotted hyena (*Crocuta crocuta*)	Chemical	Scent marks	Burgener et al. (2008)
Burying beetle (*Nicrophorus vespilloides*)	Chemical	Scent odour	Steiger et al. (2008)
Wasp (*Polistes dominula*)	Visual and chemical	Visual cue: facial pattern; chemical cue: scent from cuticular hydrocarbons	Cini et al. (2019)
Wasp (*Polistes fuscatus*)	Visual	Face pattern	Tibbetts (2002)
Adélie penguin (*Pygoscelis adeliae*) & Gentoo penguin (*Pygoscelis papua*)	Audial	The pitch of vocal calls	Jouventin and Aubin (2002)

Antbird (*Hypocnemis Peruviana* & *H. subflava*)	Audial	Vocal calls	Seddon and Tobias (2010)
Bellbird (*Anthornis melanura*)	Audial	Song	Brunton et al. (2008)
Kittiwake (*Rissa tridactyla*)	Audial	Vocal calls	Mulard et al. (2008)
Shearwater (*Calonectris diomedea*)	Audial	Vocal calls	Curé et al. (2016)
Skylark (*Alauda arvensis*)	Audial	Song	Briefer et al. (2008)
Brook Trout (*Salvelinus fontinalis*)	Visual	Unspecified	White and Gowan (2013)
Daffodil cichlid (*Neolamprologus pulcher*)	Visual	Facial spot pattern	Kohda et al. (2015)
Fiddler crab (*Uca mjoebergi*)	Unspecified	Unspecified	Booksmythe et al. (2010)
Iberian wall lizard (*Podarcis hispanica*)	Chemical	Scent marks	Carazo et al. (2008)
Crayfish (*Cherax dispar*)	Unspecified	Unspecified: presumed visual or chemical	Seebacher and Wilson (2007)

2.3: Individual recognition in fish

Fish are known to use individual recognition extensively, from expressing rank in dominance hierarchies to recognising neighbouring individuals (Hert, 1985; Itzkowitz & Leiser, 1999; Saeki et al., 2018; Sogawa et al., 2016; White & Gowan, 2013). Many species of fish live in complex social structures (Aspillaga et al., 2021; Barley and Coleman, 2010; Brown and Brown, 1993; Johnsson, 1997; Keenleyside and Yamamoto, 1962; Kohda et al., 2015; Laskowski et al., 2016; Sogawa and Kohda, 2018; White and Gowan, 2013), and to maintain these social structures individual recognition must be utilised. Strangely, there is little information on the mechanism(s) fish use to display these unique signals. Studies clearly show communication and recognition occurring between individuals, but fail to identify the specific cues (Frostman & Sherman, 2004; Thomson, 2016; White & Gowan, 2013). However, there are some key studies where the study design indicates the only possible signal has to be visual, hence some aspects of fish morphology, colouration or behaviour are the mechanisms involved in communication (Frostman & Sherman, 2004; Saeki et al., 2018; Thomson, 2016; White & Gowan, 2013). Surprisingly, many of these papers have not considered the actual mechanisms involved in these recognition behaviours and are only testing for evidence of individual recognition (Booksmythe et al., 2010; Seebacher & Wilson, 2007). These studies note that individual recognition is occurring e.g., increased aggression towards unknown individuals, but there was never any identification of the specific cue being used, visual, audial, or chemical.

Patterns and colours that produce unique body markings have been used by researchers in various studies to identify individuals (Arzoumanian et al., 2005; Dalibard et al., 2021; Huffard et al., 2008; Kim et al., 2005). The use of patterns in research for identification of individuals has been used as a non-invasive "tagging" method (Arzoumanian et al., 2005; Dennis & Shah, 2012; Elangovan et al., 2021). In fish like the giant kōkopu (*Galaxias argenteus*) which has a distinctive striped body pattern, researchers found that each pattern was unique and unchanging for each individual (David et al., 2007; Hansen et al., 2004; Hansen & Closs, 2005). Despite the complex-looking pattern, each kōkopu has a unique banded pattern across its body, allowing it to stand out as an individual to researchers. This can be seen in many taxa, but for animals restricted to a specific habitat (e.g. aquatic habitats) using this technique can be a useful way of identifying individuals for a study (Arzoumanian et al., 2005; David et al., 2007; Hansen et al., 2004; Hansen & Closs, 2005; Hustler, 2014; Wilson et al., 2006). The use

of unique and readily identifiable body patterns by human researchers does pose the question as to whether fish are able to use the very same body patterns for inter-individual recognition?

Unique cues used by fish can vary across species, but many forms of communication amongst fish are likely limited due to the hydrodynamic constraints on the morphology of fish. Fish bodies follow a similar structure, with the need to be streamlined for swimming, darting, and turning quickly. While there are obvious variations to fish body shapes due to lifestyle and habitat, a hydrodynamic body shape is needed to swim through water to hunt, forage, and survive (Tytell et al., 2010). This constraint on body morphology restricts how elaborate a fish can be in terms of body ornaments (Tibbetts, 2014; Zuk, 1991). To communicate without elaborate ornaments that would otherwise disrupt the streamlined morphology of fish, other cues must be utilised instead. Commonly used cues that do not require elaborate body ornaments are vocal communications, chemical cues and visual cues and displays. Fish are able to use all of these to recognise predators, kin, and rivals (Bass, 2001; Cullis-Suzuki, 2016; Feng & Bass, 2016; Gherardi et al., 2011; Giaquinto & Volpato, 1997; Kohda et al., 2015; White & Gowan, 2013). For example, Reebs (1994) investigated mate recognition in convict cichlids (*Cichlasoma nigrofasciatum*). Convict cichlids are known to use visual cues for recognition, but this study showed during nocturnal periods females could differentiate mates from strangers. Due to the lack of visual communication and no signs of differentiation at a far distance, researchers narrowed the secondary recognition cue to a chemical cue allowing recognition within these fish.

Visual cues are an easily observed form of communication, and there are many examples of fish with body patterns which could be used as an identifiable cue (Arzoumanian et al., 2005, 2005; Dalibard et al., 2021; Dawkins & Guilford, 1993). Body markings are a common form of communication in many species, but research on fish body patterns and their use is limited (Arzoumanian et al., 2005; Dalibard et al., 2021; Halloran et al., 2015; Harihar et al., 2010; Huffard et al., 2008). There are many studies which indicate that visual cues are being used for recognition, but no research into what these cues may be, or whether they occurred evolutionarily for communication purposes, or are perhaps a secondary use of physical features (Brown & Brown, 1993; Johnsson, 1997a; O'Connor et al., 2000; Sogawa & Kohda, 2018; White & Gowan, 2013).

Many studies which identify visual cue use are done through testing if the species utilises the "dear enemy" phenomenon, where recognition of individuals is crucial. The "dear enemy"

phenomenon is where it is more cost effective to not attack a familiar neighbour, and to conserve that energy to fight off an unfamiliar invader (Booksmythe et al., 2010; Brunton et al., 2008; Itzkowitz & Leiser, 1999; Langen et al., 2000; Sogawa et al., 2016; Sogawa & Kohda, 2018; Thomson, 2016). There have been multitudes of studies of the "dear enemy" phenomenon on territorial fish species, which means many species can clearly distinguish between familiar and unfamiliar fish (Itzkowitz & Leiser, 1999; Sogawa et al., 2016; Sogawa & Kohda, 2018; Thomson, 2016). Again, there is a lack of research into

cue is that various individuals are using, whether they are chemical, visual or audial. Many of these studies did not allow physical contact with other fish to reduce harm to the individuals, hence this also means that they are only visual cues in these studies, as individuals are typically separated with clear plexiglass or something similar (Frostman & Sherman, 2004; Itzkowitz & Leiser, 1999; Matsumoto et al., 2020; Saeki et al., 2018; Sogawa & Kohda, 2018). Clearly, the recognition in these fish is visual, but the lack of specificity on what kind of visual cues is a trend seen through many studies. The most obvious choice of what this cue could be is the body pattern on each individual, but few studies have investigated whether this potential cue is used (Kohda et al., 2015).

2.4: Morphological constraints on recognisable cues

The body morphology of many animals has been adapted to fit the environment in which they live in and caters to the specific needs required. There are many animals that cannot afford to change their morphological shape as it would inhibit them from living successfully in that habitat. The greater the constraints on shape that is imposed on an animal is to fit its habitat, the less variation in body shape that will occur (Fish, 1994; Lauder & Tangorra, 2015). Many taxa have a body shape that is made for its habitat, but also exhibit other unique differences e.g., size, colour, wing length, tail length, fur length (Stettenheim, 2000; Tytell et al., 2010). An example of an animal that is restricted morphologically are flying birds, which are subject to strong aerodynamic constraints. All flying birds have wings, and must have an aerodynamically streamlined body shape to allow efficient movement through the air (Stettenheim, 2000). Being aerodynamic allows flight with little drag and efficiency in movement, something aerial birds need to maintain successful flight (Muijres et al., 2012; Thomas, 1993). An unusual bird which is not aerodynamic but instead hydrodynamic is the penguin (Hui, 1988; Lovvorn et al., 2001). Using its wings to propel itself underwater instead of in the air, penguins have evolved under strong hydrodynamic morphological pressures to glide and swim through water with

little drag, and survive in its habitat (Hui, 1988; Lovvorn et al., 2001; Maeda et al., 2021). This is crucial for quick movements, swift swimming and easy turning and darting in aquatic animals. Being dynamic within an environment is crucial for the survival of many species.

With such aerodynamic and hydrodynamic morphological constraints restricting the scope for ornament usage, other forms of communication must be used. Most birds are aerodynamically restricted, and as such cannot afford to have ornaments which would reduce drag, inhibit flight or weigh them down (Evans, 2004). Instead, communication is often audial (Carlier, 1995; Curé et al., 2016; Mulard et al., 2008). Vocal cues do not restrain or inhibit flight patterns, and are commonly used among many bird species, even those which are not songbirds. Vocal calls are an excellent way to communicate and identify conspecific birds (Barnes et al., 2012; Carlier, 1995; Herbert & Herbert, 1965; Hustler, 1983). Peregrine falcons (*Falco peregrinus*) are not known for their singing abilities, but there has been substantial research that shows peregrine falcons communicate using vocal cues, such as wailing and creaking (Carlier, 1995). Vocal cues are a key communication and recognition for conspecific birds which cannot use ornaments for displays or communication. Similarly to birds, the morphology of fish are hydrodynamically constrained. Being streamlined for quick moves in the water does not allow for added ornaments to communicate. Many fish, however, use colour or body patterns as a form of visual communication (Bradner & McRobert, 2001; Dawkins & Guilford, 1993; Kohda et al., 2015). While many fish are streamlined, not every species needs swift speed to survive in their aquatic habitat. The leafy seadragon (*Phycodurus eques*) is a benthic fish which does not have a hydrodynamically streamlined body. Instead, this fish has elaborate ornaments on its body which allow it to camouflage into its habitat among seagrass and kelp (Stiller et al., 2017). Seadragons are not fast swimming fish, and do not need a sleek, streamlined body for fast movement through open waters (Connolly et al., 2002; Stiller et al., 2017). Blending into the kelp and seagrass habitat and being able to hide in these grasses is more beneficial than swimming quickly for these fish, so having a bulkier exterior is key to a seadragons survival in its habitat (Stiller et al., 2017). With no need for a typically "fishy" body shape, these seadragons are able to have large ornaments. Figure 2.1 shows a comparison of the ornamental leafy seadragon against the streamlined rainbow trout (*Oncorhynchus mykiss*).

Figure 2.1. Comparison of ornamental fish and non-ornamental fish. The leafy seadragon *(Phycodurus eques)* to the left with ornamentation, and the rainbow trout *(Oncorhynchus mykiss)* on the right, with no ornamentation. Images from (Stiller et al., 2017) and (Colihueque et al., 2011) respectively.

Ornaments may be a successful way to attract a mate or communicate, but for animals which have more morphological restrictions, smaller, less intense forms of attraction and communication are utilised. Fish are excellent examples of species which have morphological restraints reducing elaborate ornamentation. The majority of fish and other aquatic based animals are constrained to this lifestyle, and must sacrifice bulky ornaments to stay streamlined, and instead focus on other features to attract a mate or communicate with other individuals (Nicoletto, 1991; Tytell et al., 2010; Woodward et al., 2006). Colouration is a common form of ornamentation in fish, where fish with the brightest colour are seen as more attractive to their prospective mates (Flamarique et al., 2013; Gomes-Silva et al., 2020; Ogita & Karino, 2019). Using colour as a tool for communication does not inhibit body morphology. These colours can indicate the health of a prospective mate, which is important when looking for a parent of offspring (Römer & Beisenherz, 2005). Chemical sensory systems are also used by fish for communication, allowing a fish to chemically identify predators or even potential mates (Gherardi et al., 2011; Giaquinto & Volpato, 1997; López & Martín, 2002; Zenuto & Fanjul, 2002). These communication cues are not as elaborate or costly to the individual, but still allow the fish to stand out without being weighed down as it were.

When looking into patterns for communication rather than mate attraction, morphological constraints can still influence what cues are able to be utilised. Colouration and patterns can be used to communicate without inhibiting the streamlined structure of a fish (Evans, 2004; Tytell et al., 2010). Patterns are cost effective, they do not restrict movement or create drag, and can still allow the animal to send out unique recognisable cues. Elaborate ornaments and bulky projections off the body are costly to fish which need to be streamlined and are not

ideal forms of communication. Many species both terrestrial and aquatic, use these patterns as forms of identification of individuals (Dalibard et al., 2021; Gore et al., 2016; Harihar et al., 2010; Speed et al., 2007). Visual communication has been noted in many fish behaviour studies, but there is no mention of what the specific cues could be (Gonçalves et al., 2000; Hert, 1985; Johnsson, 1997a; White & Gowan, 2013). Other cues which could be used to combat this morphological constraint would be audial cues, or chemical cues (Cullis-Suzuki, 2016; Feng & Bass, 2016; Gherardi et al., 2011; Giaquinto & Volpato, 1997; Mirza & Chivers, 2003). Body shape is not inhibited by chemical sensors or hearing as well as visual cues, and a combination of these three communication measures allows fish to still have elaborate colours or patterns without being restricted morphologically.

2.5: Physical pattern locations and their effect on communication

Species that use visual cues for communication often have clear and distinctively variable markings, patterns, or even colours, typically located in one area. For example, in some canines, pale patches along the chest and neck enhance the signals of submission to dominant individuals in the pack (Fox & Cohen, 1977; Ortolani, 1999). Patches of colour can be used to heighten other forms of communication, both within a group, or within a species, often for warnings. Mustelids are an excellent example of animals which use their colour patterns to communicate within their species. Having these light patterns on dark coloured fur stands out, especially in dusk when these animals are typically active (Pruitt & Burghardt, 1977). These lighter areas can draw attention to particular areas of the animals' body e.g., the dark circles on the paler fur on racoons brings the attention to expressive areas of the face (Pruitt & Burghardt, 1977). Enhancing facial expressions in these mammals allows for communication within species more effectively, and draws the attention to the correct area to focus on for those communications (Pruitt & Burghardt, 1977).

Pattern location is a crucial component for visual individual recognition, as individuals need to know where to look to identify one another. If communication occurs when two individuals are face to face, for example, then the visual cue must be located somewhere on the face (Kohda et al., 2015; Tibbetts, 2002). However, if communication for an animal is more easily done through side patterns, or through flashing a tail, then having the recognisable pattern on those areas is crucial for communication (Ortolani, 1999). These locations vary among species, but the concept of having the recognisable cues located on areas which will be seen most clearly for communication is carried through all species using visual recognition.

It is clear through the literature that pattern location is important when trying to communicate, and that it is used by many species for many different reasons. Communication is key when maintaining a social group, maintaining a hierarchy, or even as a defence mechanism when a predator attacks (Blest, 1957; Brennan & Kendrick, 2006; Cini et al., 2019; Ewbank et al., 1974; Murali & Kodandaramaiah, 2016; Ortolani, 1999; Stevens, 2005). There is a clear trend in the literature that patterns, both unique and general, can be used for enhanced expressions, defences and recognition. While there are few investigations into fish and what their spot or stripe patterns can be used for, there was a study by Kohda et al. (2015) which actually identified that the spots on the face were used for individual recognition. Further investigation into the use of these kinds of patterns would open up so many new ideas and explanations for the patterns on fish and many other species.

2.6: Conclusions

Identifiable cues that are unique to an individual are crucial for maintaining social relationships, attracting mates and even as a defence mechanism. Individual recognition is widespread throughout the animal kingdom, and some form of identification is needed for both intra-and inter-specific relationships (Bass, 2001; Cini et al., 2019; Gherardi et al., 2011; Itzkowitz & Leiser, 1999; Mirza & Chivers, 2003; Murali & Kodandaramaiah, 2016). Visual cues were the main point of focus for this review, and the focus on what these patterns and colours could be used for, why their location on the body was important and how morphology can restrict how elaborate these cues can be (Ortolani, 1999; Stettenheim, 2000; Stevens, 2005; Tytell et al., 2010; Woodward et al., 2006). There are clear areas within the literature that have been well studied, such as individual recognition within social taxa, but there is a lack of in-depth information on what actual cues used to communicate may be, why they are placed in a certain location, where they are found or how complex they may be. Going an extra step and investigating what the specific cue being used is would be useful for future research, allowing future studies to manipulate these cues and see how this affects behaviour. It is clear that while new studies are continuing to focus on individual recognition as a whole, further analysis into pattern location and how this can affect the relationship with the communicator and the receiver of those signals. Location is key to communicating signals with others and is crucial when used for predator deflection or attracting a mate. In turn, little focus has been directed to the morphological constraints many taxa have to obey. Some habitats restrict the ability for gaudy and expensive ornaments to be used when physical forms take precedence over these costly ornaments. The prime example I used for this study was fish, which need to

have a streamlined body that can be manoeuvred efficiently through water. Further research into how morphology can change how ornaments and communication cues are utilised by taxa would be an interesting and unique area to examine, as little focus has been placed on morphological constraints and its effect on the animal. Another area which could assist in identifying specific cues for individual recognition, particularly for visual cues, would be with the assistance of artificial intelligence or computers. Using computer programs to match photos of the same individual through the body markings and their distribution have been vital for many studies (Arzoumanian et al., 2005; Kim et al., 2005). Using these modern techniques to make alterations on specific parts of an animal e.g., altering face patterns and showing the image to a familiar conspecific, could provide further information on what specific cues are for visual communications. Using these technologies for recognition investigations could provide more answers about specific cues.

Chapter Three: Behavioural Responses of Upland Bullies to Familiar *vs.* Unfamiliar Individuals.

3.1: Introduction

The establishment and maintenance of territories is vital for many species, as it allows them to attract/keep mates and retain other resources, especially when they live within a social group (Barnard & Burk, 1979; Cheney & Seyfarth, 1986; Hoare & Krause, 2003). This social behaviour holds many benefits, including protection from predators, access to (and defence of) quality resources, and the ability to attract a mate and care for a brood (Goncalves & Radford, 2019). Territorial defence is often required to maintain access to these resources via the maintenance of territories, where both territorial animals and hierarchical groups will attempt to defend and maintain their territories (de Kort et al., 2009; Issa et al., 1999; Siracusa et al., 2019; Sogawa & Kohda, 2018; York et al., 2019). Hierarchies are formed through intragroup fights to gain dominance over the others in the group, as the dominant individual typically gains first access to quality resources, and the opportunity to reproduce (Hall & Fedigan, 1997; Issa et al., 1999). Territorial species may live within a hierarchy, but it is more common to see them living alongside "familiar" individuals (López & Martín, 2002; Temeles, 1994). These familiar individuals are usually of the same species and they often compete with one another for food and mate attraction (Jordan et al., 2010b; Saeki et al., 2018). There is also competition for the territories each individual inhabits although once territories have been established there is very little fighting between the neighbouring individuals (Neat, Huntingford, et al., 1998; Oliveira et al., 2011). Resources (e.g. food or water) may be shared between territories, and the ability to distinguish between familiar and unfamiliar individuals is important, as unfamiliar individuals may pose a greater threat to said resources (Booksmythe et al., 2010). Familiar neighbours could also become a potential threat, especially where shared resources are concerned, or when attempts are made to attract a mate (Sogawa & Kohda, 2018). However, studies of the "dear enemy" phenomenon show that reducing aggression to familiar individuals is more energy efficient. Conserving that energy to fight off invaders who may steal a territory is more important than continuously fighting neighbours which have established territories (discussed in Chapter One). This does not mean that territories are not invaded by familiar neighbours, but that it is less likely to occur; when it does, mistrust of that neighbour is commonly observed (Itzkowitz & Leiser, 1999; Leiser, 2003; Sogawa & Kohda, 2018).

Recognition of a familiar neighbour relies on the identification of unique cues which signals that they are a familiar neighbour, as knowing who to fight and who to ignore is key to territorial defence (Leiser, 2003; Temeles, 1994; Tibbetts & Dale, 2007). Expending energy on non-threatening individuals is not ideal, so reserving this expense for intruding individuals is often a better choice (Booksmythe et al., 2010; Brunton et al., 2008; Itzkowitz & Leiser, 1999; Rosell & Bjørkøyli, 2002; Sogawa et al., 2016).

3.1.1: Individual Recognition in Fish

While terrestrial animals (wasps, chimpanzees, birds etc,) are more commonly studied for individual recognition, there have been many studies on fish, both marine and freshwater (Höjesjö et al., 1998; Itzkowitz & Leiser, 1999; Myrberg & Riggio, 1985; Sogawa et al., 2016). White & Gowan (2013) demonstrated that brook trout (*Salvelinus fontinalis*) could find their place within a hierarchy using only visual cues. By observing other fish, the focal individual was able to discern where it stood within the ranking, likewise, a number of studies have investigated recognition in different species of cichlid fish (Chen & Fernald, 2011; Frostman & Sherman, 2004; Kohda et al., 2015; Reebs, 1994; Sogawa et al., 2016; Sogawa & Kohda, 2018). Many of these studies investigated the dear enemy phenomenon and found that this phenomenon was practiced. The "dear enemy" phenomenon is where it is more cost effective to not attack familiar neighbours, but to instead attack unfamiliar invaders. For example, the daffodil cichlid (*Neolamprologus pulcher*) was found to display the "dear enemy" phenomenon by Frostman & Sherman (2004). Likewise, Sogawa et al. (2016) used the same model and found that aggression decreased as the familiarity of the neighbouring fish increased, but when exposed to an unfamiliar fish, the aggression levels increased relative to aggression displayed to the familiar fish. Similarly, Reebs (1994) looked at mate recognition in female convict cichlid fish (*Cichlasoma nigrofusciaturn*) – females did not have a reaction when presented to their mates, but when non-mates were presented, the females had a change in behaviour, including head shakes, tail beats, and bites, suggesting the females could recognize the different males. In contrast, Thomson (2016) found no support that female upland bullies (*Gobiomorphus breviceps)* can differentiate between familiar and unfamiliar individuals. Male upland bullies should, however, be able to recognize individuals and display the dear enemy phenomenon, as males are highly territorial (Hamilton & Poulin, 1995).

Here I examine the behaviours of the upland bully following exposure to both familiar and unfamiliar individuals. Males bullies establish and defend territories to attract mates

(Hamilton et al., 1997; Jowett & Boustead, 2001). Territories consist of rocks, and the underside is used to hold the eggs laid by females (Hamilton et al., 1997). These territories are maintained until the eggs are hatched, around 3-5 weeks, during spring-summer (Hamilton et al., 1997). Studies have shown that male upland bullies will compete aggressively to gain and maintain optimal territories, but what happens after this territory is established? Do neighbouring fish continue to try and fight for the territory, or do they settle for suboptimal locations? Will they become dear neighbours, and behave less aggressively towards their familiar neighbours? Or will aggression levels remain high no matter if they are exposed to familiar or unfamiliar fish? My primary aim was to determine whether focal male individuals behave differently toward unfamiliar individuals than those that are familiar to them. I predicted an increase in aggression, movement and an increase in time spent in the contact zone, the wall where the fish could see each other when a focal fish was presented with an unfamiliar neighbour compared to a familiar neighbour. I also predicted that resting and duration in territory to decrease when the focal fish was exposed to the unfamiliar fish, due to the predicted increase in aggression and time spent at the wall contact zone. Unfortunately, many of the males turned out to be females upon dissection, so a secondary aim was to investigate difference in behaviours between focal male and female fish. Few territorial studies focus on the behavioural difference of the sex of the study subjects, but here I have the opportunity to investigate this further following on from the study Thomson (2016). From the Thomson (2016) study, I predicted that there will be lower levels of aggression in female fish, compared to focal males, and possibly no significant differences in behaviour to familiar or unfamiliar neighbours.

3.2: Materials and Methods

3.2.1: Subjects and fish husbandry

For this study, 30 upland bullies (*G. breviceps*) were captured via electrofishing - 15 from the Waitaki river in the north of Otago, and another 15 from the Kauru river, a tributary of the Kakanui river. Two locations were used so the unfamiliar fish would be from a completely different river and would never have come into contact with the focal fish. The fish were transported back to the Department of Zoology, University of Otago in individual opaque one litre containers to reduce exposure to other individuals and were checked every hour to ensure temperatures were kept below 15 degrees Celsius.

Due to COVID-19 movement restrictions, I was only able to collect bullies in June/July of 2020. Originally, I planned to only collect males for this study during February, as the male bullies hold and maintain territories, but the aquaria tank delivery was delayed twice, which pushed my start date back by almost three weeks, into the start of lockdown. As I was unable to collect during breeding season (e.g., October to February), it was very difficult to visually identify the sex of the fish.

Prior to the experiment, fish were kept in individual 14 litre aquaria (31cm long, 18cm wide, 23.5cm high). Aquaria were not temperature controlled; ambient air temperature determined water temperature, which ranged from 16 to 22°C, and the light regime was set to 12:12 hours. Fish were fed daily with frozen bloodworms. For territories and shelters, a PVC tube with a diameter of 4cm and length of 10cm was attached to the base of the tank. Each tank had a single filter to aerate the water. The base of each tank was white, which was necessary for the filming technique. Each side of the tank was covered in black plastic to eliminate visual contact from other fish. When needed, the black plastic adjacent to the paired fish was removed to allow for visual contact. All animals were collected, maintained, and euthanised according to the permissions issued by the Animal Ethics Committee for the University of Otago (AUP-19-178).

3.2.2: Experiment

3.2.2.1: Experimental set up

The experiment was conducted at the University of Otago between August and September 2020. Two lateral tanks were set up with an adjacent side to allow direct visual contact between individuals in each tank. The remaining three sides of the tanks were covered in black plastic to prevent any other visual stimuli. A PVC tube was attached to the base of each tank in the same position within each tank. The fish were acclimated to conditions for two weeks before experiment began. For the first week, each fish did not have visual contact with their neighbouring fish (Figure 3.1). In the second week, the fish were visually exposed to one neighbour by removing the black plastic between tanks. All tanks were labelled to allow identification, including river of origin.

For each trial, fish were starved 24 hours prior to the trials and all tanks were labelled to allow identification, including river of origin. At the start of a trial, a focal fish was first exposed to a familiar fish, then an unfamiliar fish, and finally the familiar fish again. Exposing to the familiar fish a second time allowed me to test for repeatability of behaviours. Each fish was used as a focal fish, and each fish was also a familiar and unfamiliar fish, but not all in the same day. Footage was taken over the space of two months.

Figure 3.1: Experimental set up of tanks with fish in holding/acclimation. Paired fish have no black plastic between the shared wall.

3.2.2.2: First Familiar Trial

For each trial, the focal and familiar test fish originated from the same rivers. I also size matched the non-focal fish to within 10% of the body length of the focal fish to minimise any size bias that might influence the study. On the day of the trial, both tanks were moved to the filming station and an opaque barrier used to separate them. To control for manipulation,

neither fish were removed from their respective tanks, allowing them to maintain their familiar territory while the tanks were moved to the filming location. After a 5-minute re-acclimation period, the barrier was removed, and visual contact reinstated. Using a GoPro Hero3+, the behaviour of the fish was recorded for 40 minutes (Figure 3.2). Following the trial, the opaque barrier was returned, and the familiar fish was removed from the filming area and surrounded by opaque barriers, so it had no visual stimulus.

3.2.2.3: Unfamiliar Trial

After the first familiar trial, the familiar fish tank was removed to its original holding place and was replaced by an otherwise identical tank containing an unfamiliar fish. Criteria for the unfamiliar test trials were size matching to roughly 10%, originating from different rivers, and the unfamiliar fish had not lived in adjacent holding aquaria or been used previously in the same trial. The focal fish also had the tank removed and then immediately replaced into the same position to control for manipulation. A 5-minute acclimation period was given before the barrier was removed and the fish behaviour was filmed for 40 minutes. After the trial, the unfamiliar fish was placed back to its original set up with opaque barriers on all sides.

3.2.2.4: Second Familiar Trial

The second familiar trial took place after the unfamiliar trial. This trial took place to estimate repeatability of behaviours, to test whether focal fish altered their baseline behaviour after encountering an unfamiliar neighbour. This test followed the same protocol as the other two trials, where both tanks were moved to the filming station with barriers covering the shared wall. After a 5-minute acclimation period the fish were then visually exposed to each other again for 40 minutes. This was filmed using the GoPro Hero3+. Once this second trial was complete, the fish were removed from the filming station, and replaced adjacent to each other with visual contact.

In total, there were 30 trials, each consisting of a familiar, unfamiliar and second round familiar exposure trials, with each fish used as the focal fish once. Between each exposure, there was a five-minute rest period before the focal fish was introduced to the non-focal group (either the familiar or unfamiliar fish). Figure 3.3 shows an image of how the trials were filmed with both the focal and non-focal (either familiar or unfamiliar) fish could be observed. After all trials were completed, the fish were euthanized.

Figure 3.2: Filming set up in Zoology Department tank room. Black plastic was removed from the tanks for filming.
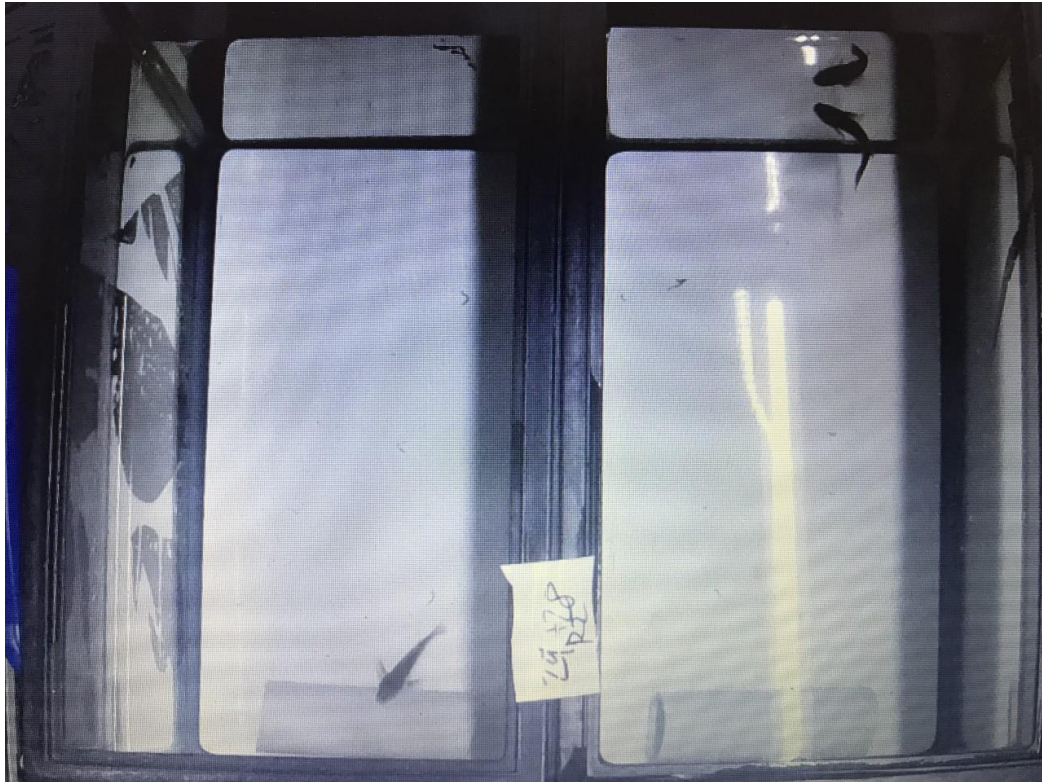
Figure 3.3: Close up image of the camera view. Both focal fish and non-focal fish were in view of the camera.

3.2.2.5: Dissections

At the conclusion of the behavioural trials, fish were euthanized by drug overdose of 2-phenoxy-ethanol. The fish were submerged in a mix of water and 2-phenoxy-ethanol until deceased. Fish were then stored in labelled containers of ethanol until dissection. Dissections were performed to identify the sex of the fish. I found that 14 of the fish were males, and 16 were females.

3.2.2.6: Quantifying behaviours from video footage

BORIS (Behavioural Observation Research Interactive Software) (Friard & Gamba, 2016) allowed for an ethogram input and I was able to monitor movement, resting, aggression and certain locations of the fish (Table 3.1), scoring the behaviour of both the focal and non-focal fish in each trial. Figure 3.4 shows an example of what the programme looked like in use.

Table 3.1. Behavioural ethogram used for Upland bully (*G. breviceps).*

Behaviour	Key	Description
Movement	M	Fish moves evenly around the tank.
Resting	R	Fish is motionless.
Aggression	A	Fish moves erratically or up and down the side of the tank looking into the neighbouring tank.
Wall	W	Fish is next to the shared wall with visual contact.
Territory	T	Fish is hidden in the territory, out of sight.
Peeking	P	Fish head peeks out from one end of the tank. Head only.
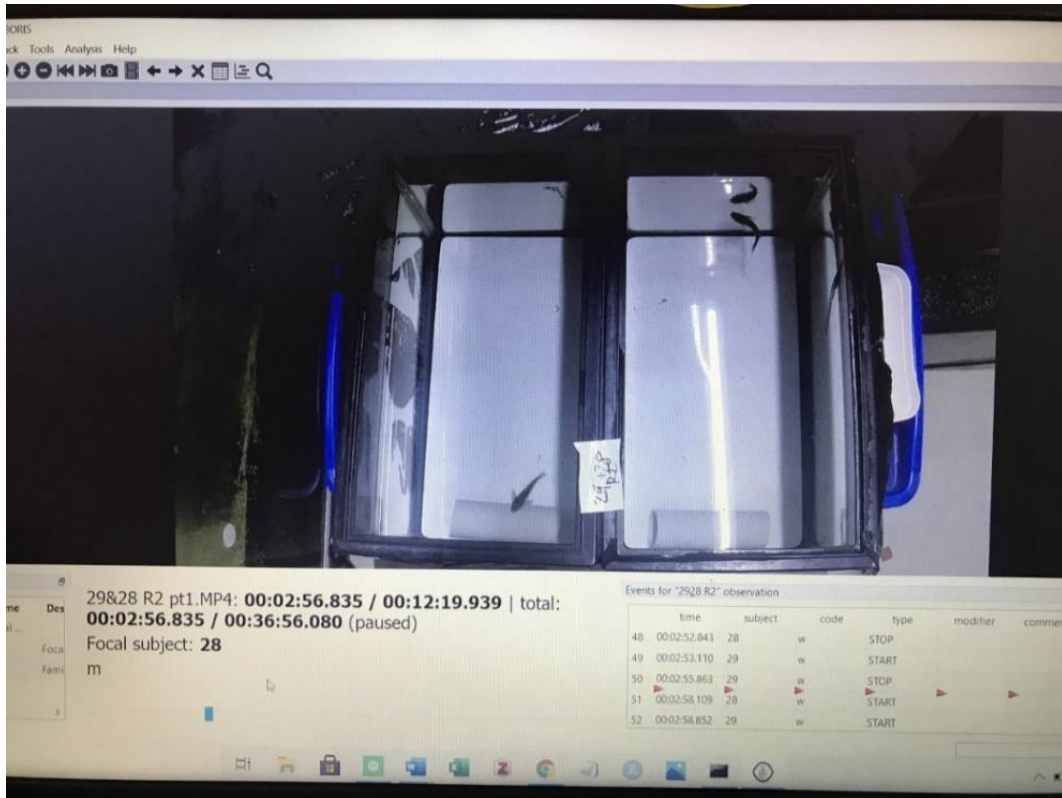
Figure 3.4: Image of BORIS Program with footage of fish 28 (left) and 29's (right) second familiar trial.

The videos included footage of myself turning the camera on and removing the black plastic, so the videos were edited so that the trial started as soon as I was out of vision of the camera. Trials were on average, 20 minutes. Unfortunately, due to shared tank room facilities and equipment failure, not all the videos were able to be used. In some cases, the camera stopped filming for no apparent reason leaving only 10 minutes of footage, and in others, people entered the filming room and disturbed the fish. This footage could not be used as the fish clearly reacted to the disturbance instead of the tank adjacent to them. Because of this, only 26 of the 30 trials were used in the final analysis.

3.3.3: Data Analysis

All statistical analysis were conducted in R version 4.0.4 (R Core Development Team, 2021). Once behaviour durations (aggression, movement, resting, territory and time in the contact zone) had been collected from BORIS, the behaviours were analysed using linear mixed models (LMM) in the lme4 package (Bates et al., 2014). The subject (identification number of each individual fish) was used as a random effect, and the effect of treatment (familiar vs.

unfamiliar neighbour), sex (male or female), and the non-focal sex (male or female) were included as fixed effects.

Across the 26 trials, there were four pairing types – MM (male and male, 22 trials), MF (male and female, 14 trials), FF (12 trials), and FM (16 trials). The first sex represents the focal fish, and the second sex represents the familiar or unfamiliar fish (non-focal); however, the focal/familiar and focal/unfamiliar pairings did not always match in terms of sex pairing type, as I was unable to identify the sex of the fish prior to the trials. Thus, I ended up with a small sample size for each complete sex pairing (e.g., male focal and all male non-focal, male focal, all female non-focal etc). Overall, I had 4 MM trials, 2 MF trials, 1 FF trial and 4 FM trials. These sample sizes were too small to conduct any meaningful analyses, so instead I conducted the analysis using mixed pairings, e.g., focal male, familiar male, unfamiliar female.

To test the repeatability of fish behaviour, repeatability estimates were obtained using the package "rptR" in R Studio, all with a Gaussian distribution using 1000 bootstrapping runs and 1000 permutations. The behaviour of the fish was used as the response variable and the identification was used as the random effect for the repeatability model. The repeatability of the behaviours was investigated for all individuals (both focal and non-focal), and males/females separately (Table 3.2).

Table 3.2. Repeatability values for all fish and for each sex estimated using the *rptR* package. Each behaviour was analysed for repeatability. *N=* 14 males and 16 females.

	k	Aggression	Movement	Rest	Territory	Wall Zone
Focal Fish	3	R=0.81 [0.6, 0.92]	R=0.87 [0.71, 0.95]	R=0.83 [0.63, 0.93]	R=0.57 [0.19, 0.8]	R=0.81 [0.59, 0.92]
Non-Focal Fish	3	R=0.8 [0.59, 0.91]	R=0.87 [0.72, 0.95]	R=0.83 [0.64, 0.93]	R=0.62 [0.28, 0.84]	R=0.81 [0.61, 0.92]
Male	3	R=0.69 [0.19, 0.9]	R=0.78 [0.39, 0.93]	R=0.67 [0.22, 0.89]	R=0.65 [0.11, 0.87]	R=0.65 [0.15, 0.87]
Female	3	R=0.94 [0.76, 0.99]	R=0.94 [0.78 0.99]	R=0.96 [0.86,0.99]	R=0.92 [0.66, 0.98]	R=0.97[0.88, 0.99]

3.3: Results

3.3.1: Aggression

Aggression was measured as the duration spent moving erratically, lunging, biting, and swimming up and down the side of the tank erratically. The aggression of the focal fish when exposed to a familiar fish ranged from 0 to 369.77 seconds, compared to the unfamiliar fish, which had a range of 0 to 484.79 seconds. Aggression was highly repeatable (Table 3.2) in the focal fish (R=0.81) and in the non-focal fish (R=0.8). Aggression was also highly repeatable for both male fish (R= 0.69) and female fish (R=0.94).

There was no difference in focal fish aggressive behaviour when presented with familiar or unfamiliar fish (t = -1.223, *P*=0.235; Figure 3.5A). The sex of the focal fish did not affect aggression (t= 1.08, *P*=0.29), nor did the sex of the non-focal individuals (t= 0.47, *P*=0.64). The mean time spent behaving aggressively for focal males was 99.02 sec ± 29.02 SE (standard error) and ranged from 0 to 369.77 seconds. The focal females spent a mean of 96.86 ± 34.9 SE seconds acting aggressive, ranging from 0 to 484.79 seconds. There was also no interaction between treatment, or sex of focal or non-focal fish on the aggression of the fish (t= 0.64, *P*=0.53). Focal males did tend to be more aggressive when presented with an unfamiliar neighbour compared to the familiar neighbour, whereas focal females showed the opposite pattern (Figure 3.5B), though there were no significant sex x treatment effects in my model (Table 3.3). When removing the three-way interaction (treatment, sex of focal fish, sex of non-focal fish), there were still no significant results (see appendix).

Figure 3.5: Duration of aggression measured for A) the focal fish when exposed to familiar and unfamiliar fish, and B) female familiar and unfamiliar fish, and male familiar and unfamiliar fish. Data was collected from 26 fish over a two-month period. Focal n= 42, familiar n= 21, unfamiliar n= 21. Female n= 16, Male n= 14. The dots represent outliers, and the vertical line represents the standard error. The horizontal black line represents the median of each group.

Table 3.3. Linear Mixed Model of aggressive behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the fixed variables, and the fish ID was used as the random effect. Df = degrees of freedom.

	Estimate	Std Error	df	t-value	Pr(>|t|)
Intercept	2.1	0.89	40.45	2.37	
Treatment	-1.02	0.84	23.65	-1.22	0.23
Focal Sex	1.33	1.23	41.39	1.08	0.29
Non-Focal Sex	0.43	0.92	26.29	0.47	0.64
Treatment x Focal Sex	-0.25	1.14	23.54	-0.22	0.83
Treatment x Non-focal Sex	1.44	1.14	23.66	1.26	0.22
Focal x Non-Focal Sex	-1.75	1.23	25.57	-1.42	0.17
Treatment x Focal Sex x Non-Focal Sex	1.03	1.6	24.15	0.64	0.53

3.3.2: Movement

Movement was measured as swimming around the tank, and not showing any aggressive behaviour. There was no difference in focal fish movement when presented to a familiar or unfamiliar fish (t = 0.22, *P*=0.82; Figure 3.6A; Table 3.4). The sex of the fish had no effect on movement (t= 0.89. *P*=0.38), nor did the sex of the non-focal fish (t=0.24, *P*=0.81). The three-way interaction between treatment, focal sex, and non-focal sex was also non-significant (t=0.38, *P*=0.70). While there is no statistical support, there is a visual difference between the focal males and the females, showing that the focal males moved more than the focal females (Figure 3.6B). When removing the three-way interaction in the LMM, there were still no significant findings (see appendix). The mean time spent moving for focal males was 549.86 ±91.15 SE and ranged from 0 to 1205.93 seconds. The focal females spent a mean of 376.03±75.5 SE seconds moving, ranging from 0 to 1087.48 seconds.

The overall movement of the focal fish was highly repeatable (R= 0.87), as was the repeatability for the non-focal fish (R=0.87). Time spent moving was also highly repeatable for both male (R=0.78) and female fish (R=0.94, Table 3.2).
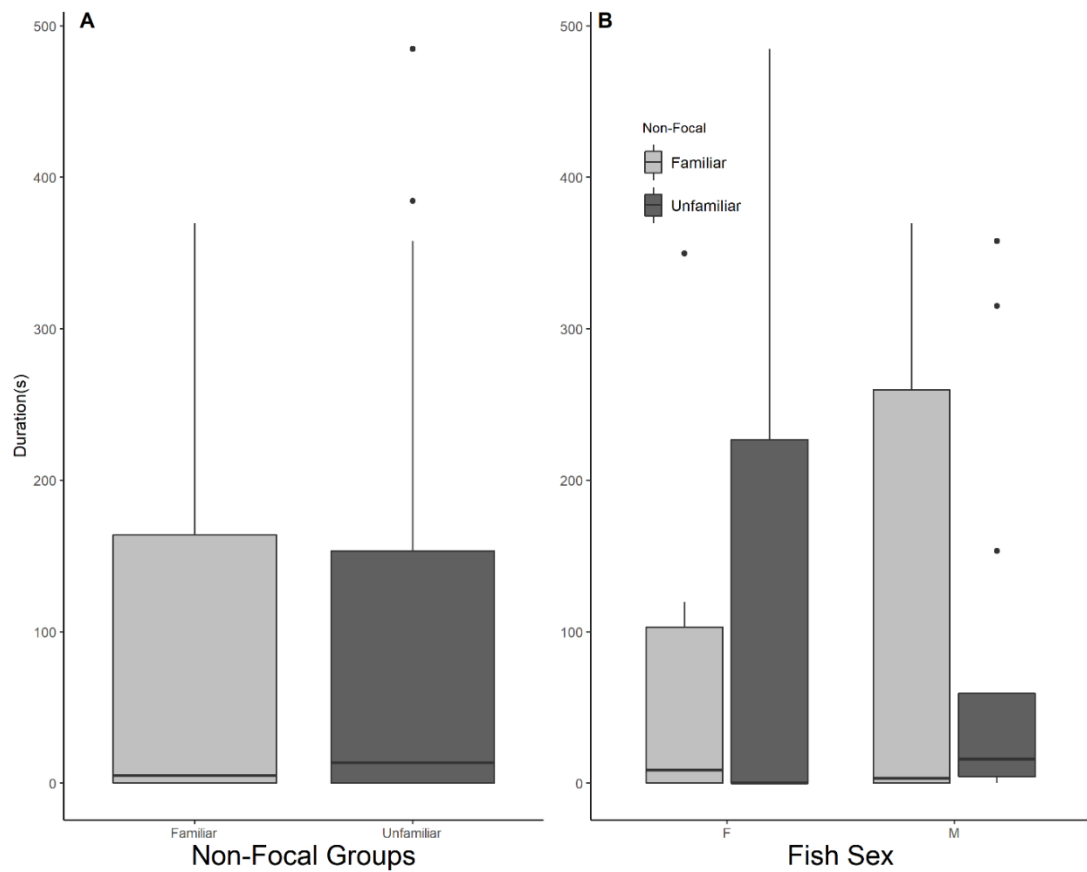
Figure 3.6: Duration of movement measured for A) the focal fish when exposed to a familiar and unfamiliar fish, and B) female familiar and unfamiliar fish, and male familiar and unfamiliar fish. Data was collected from 26 fish over a two-month period. Focal n= 42, familiar n= 21, unfamiliar n= 21. Female n= 16, Male n= 14. The dots represent outliers, and the vertical line represents the standard error. The horizontal black line represents the median of each group.

Table 3.4. Linear Mixed Model of movement behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. Df= degrees of freedom.

	Estimate	Std Error	df	t-value	Pr(>|t|)
Intercept	383.77	169.86	42.91	2.26	
Treatment	42.40	189.02	25.52	0.22	0.82
Focal Sex	210.35	237.56	43.00	0.89	0.38
Non-Focal Sex	48.80	203.35	30.66	0.24	0.81
Treatment x Focal Sex	-136.99	258.32	25.59	-0.53	0.60
Treatment x Non-focal Sex	-112.62	257.70	26.00	-0.44	0.67
Focal x Non-Focal Sex	-54.48	272.93	29.67	-0.20	0.84
Treatment x Focal Sex x Non-Focal Sex	138.02	359.18	27.18	0.38	0.70

3.3.3: Rest

Rest was measured as the duration of time the fish did not move at all. There was no difference in resting time for the focal fish when presented with a familiar or unfamiliar fish (t=-0.88, *P*=0.39; Figure 3.7A, Table 3.5). The sex of the focal fish also had no effect on the resting time (t=0.54, *P*=0.59), nor did the sex of the non-focal fish (t= -0.64, *P*= 0.53). The three-way interaction, treatment, focal fish sex, and non-focal fish sex, had no effect on the resting behaviour either (t= -0.92, *P*=0.37). However, when the two-way and three-way interactions were removed, there was a significant treatment effect, such that the focal fish spent less time at rest when in the presence of the unfamiliar neighbour (t =-2.83, *P*= 0.009, Table 3.6; Figure 3.7B). The mean time spent resting for focal males was 630.79 ±84.92 SE and ranged from 0 to 1205.93 seconds. The focal females spent a mean of 472.07±94.44 SE seconds moving, ranging from 0 to 1130.2 seconds.

Resting was also highly repeatable for the focal fish in this study (R=0.83), and for the non-focal fish (R=0.83). Resting time in both the males (R=0.67) and the females (R=0.96) was also highly repeatable (Table 3.2).
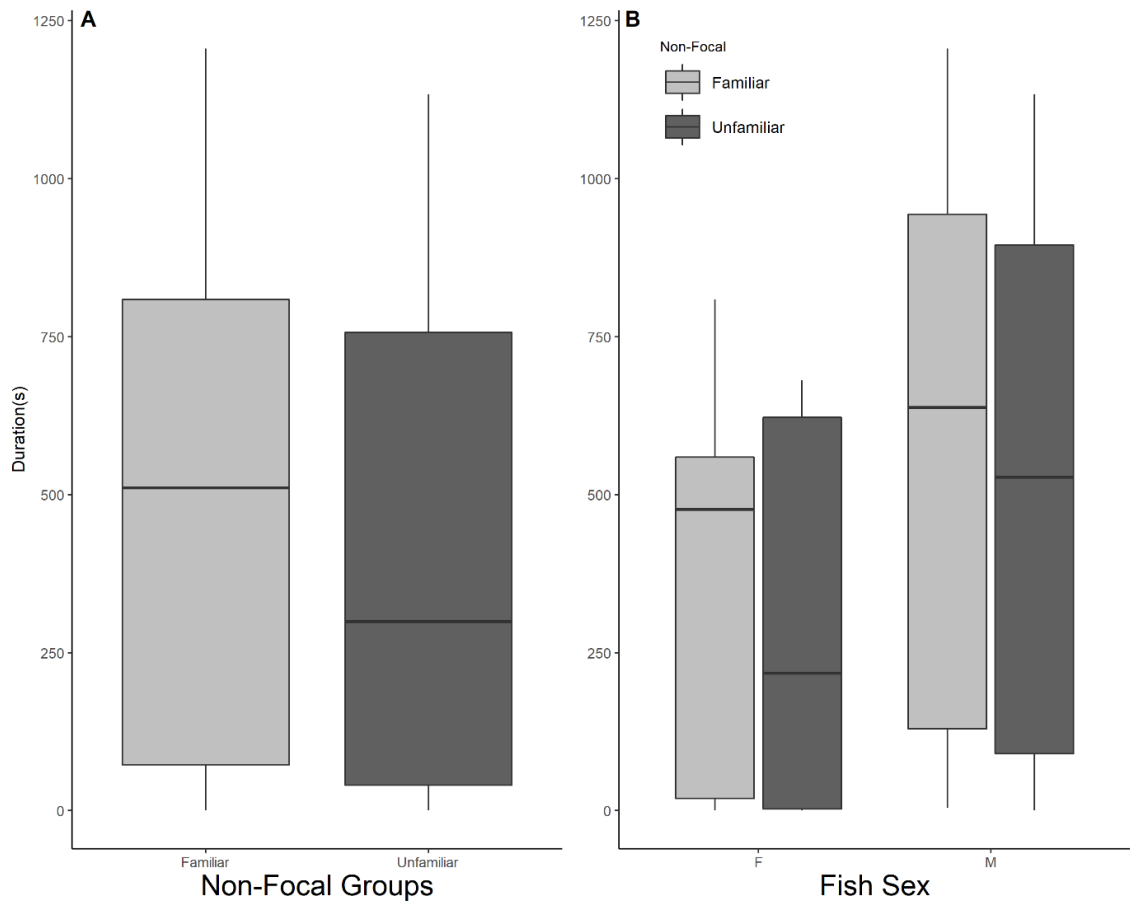
Figure 3.7: Duration of rest measured for A) the focal fish when exposed to a familiar and unfamiliar fish, and B) female familiar and unfamiliar fish, and male familiar and unfamiliar fish. Data was collected from 26 fish over a two-month period. Focal n= 42, familiar n= 21, unfamiliar n= 21. Female n= 16, Male n= 14. The dots represent outliers, and the vertical line represents the standard error. The horizontal black line represents the median of each group.

Table 3.5. Linear Mixed Model of resting behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. Df = Degrees of freedom.

	Estimate	Std Error	df	t-value	Pr(>|t|)
Intercept	564.88	151.37	39.61	3.732	
Treatment	-120.89	137.35	23.56	-0.88	0.39
Focal Sex	113.52	209.44	40.65	0.54	0.59
Non-Focal Sex	-97.27	151.87	25.88	-0.64	0.53

	Estimate	Std Error	df	t value	Pr(>|t|)
Treatment x Focal Sex	-29.32	187.62	23.45	-0.16	0.88
Treatment x Non-focal Sex	111.46	187.49	23.54	0.59	0.56
Focal x Non-Focal Sex	200.16	202.56	25.23	0.99	0.33
Treatment x Focal Sex x Non-Focal Sex	-241.58	262.85	23.96	-0.92	0.37

Table 3.6. Linear Mixed Model of resting behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. Non-significant interactions (Table 3.5) were removed from this model. Significant results highlighted in black.

	Estimate	Std Error	df	t value	Pr(>|t|)
Intercept	546.15	130.93	30.29	4.17	
Treatment	-155.16	54.80	21.27	-2.83	**0.009**
Focal Sex	144.54	160.87	22.15	0.9	0.38
Non-Focal Sex	30.30	78.06	27.46	0.39	0.70

3.3.4: Territorial

Territorial behaviour was measured as the duration of time spent hidden in the opaque territory tube provided in each tank. There was no significant difference in the time the focal fish spent in the territory between familiar and non-familiar trials (t= 0.68, *P*=0.5; Figure 3.8A, Table 3.7). Likewise, neither the sex of the focal fish (t= -0.705, *P*= 0.49) nor the sex of the non-focal fish (t= 0.86, *P*=0.4) affected time spent in the territory. There was no significant three-way interaction (t=0.7, *P*= 0.49) and when removing the three-way interaction, there were still no significant findings (see appendix). However, there is a clear trend for the focal females to spend more time in the territory (Figure 3.8B). The mean time spent displaying territorial behaviour for focal males was 636.47±152.24 SE and ranged from 0 to 1833.72 seconds. The focal females spent a mean of 1024.48±165.09 SE seconds moving, ranging from 0 to 1877.54 seconds.

Repeatability measures for territorial behaviours were repeatable for the focal fish (R=0.57) and the non-focal fish (R=0.62). They were also repeatable for both the males (R=0.67) and the females (R=0.92).
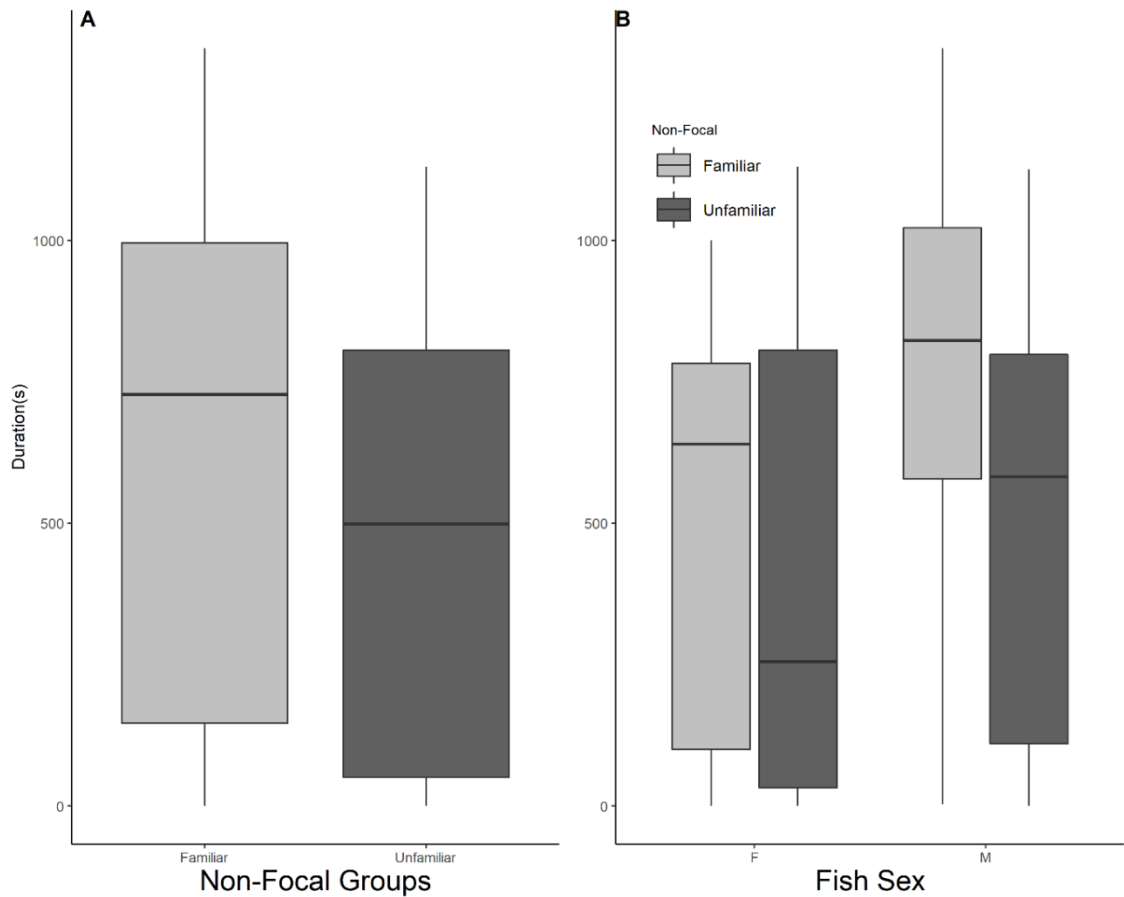

Figure 3.8: Duration of time spent in the territory for A) the focal fish when exposed to a familiar and unfamiliar fish, and B) female familiar and unfamiliar fish, and male familiar and unfamiliar fish. Data was collected from 26 fish over a two-month period. Focal n= 42, familiar n= 21, unfamiliar n= 21. Female n= 16, Male n= 14. The dots represent outliers, and the vertical line represents the standard error. The horizontal black line represents the median of each group.

Table 3.7. Linear Mixed Model of territory hiding behaviour for focal fish, with *n=* 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. Df = Degrees of freedom.

	Estimate	Std Error	df	t-value	Pr(>|t|)
Intercept	848.19	275.32	39.20	3.081	

Treatment	168.29	246.43	23.26	0.68	0.50
Focal Sex	-268.21	380.71	40.30	-0.71	0.49
Non-Focal Sex	234.67	272.77	25.49	0.86	0.4
Treatment x Focal Sex	106.79	336.61	23.15	0.32	0.75
Treatment x Non-focal Sex	-282.49	336.40	23.23	-0.84	0.41
Focal x Non-Focal Sex	-393.87	363.71	24.85	-1.08	0.9
Treatment x Focal Sex x Non-Focal Sex	329.77	471.69	23.63	0.7	0.49

3.3.5: Contact Zone

The wall zone was defined as activity at the contact zone between the adjacent wall to the non-focal tank. The focal fish showed no difference in activity at the contact zone when presented with familiar and unfamiliar fish (t= -0.45, *P*= 0.66; Figure 3.9A, Table 3.8). The sex of the focal fish also had no effect on the duration spent in the contact zone (t= 1.49, *P*= 0.14), and the sex of the non-focal also had no effect on time spent in this contact zone (t= -0.62, *P*= 0.53). There were no significant interactions (Table 3.8; Figure 3.9B). When removing the three-way interaction in the LMM, there were still no significant findings (see appendix). The mean time spent at the contact wall zone for focal males was 685.28±120.12 SE and ranged from 0 to 1810.09 seconds. The focal females spent a mean of 448.38±96.17 SE seconds moving, ranging from 0 and 1243.67 seconds.

Activity in the zone of contact was shown to be highly repeatable for focal fish (R=0.81) and non-focal fish (R=0.81). The males also showed repeatability levels (R=0.65), and the females showed high repeatability levels (R=0.97, Table 3.2).
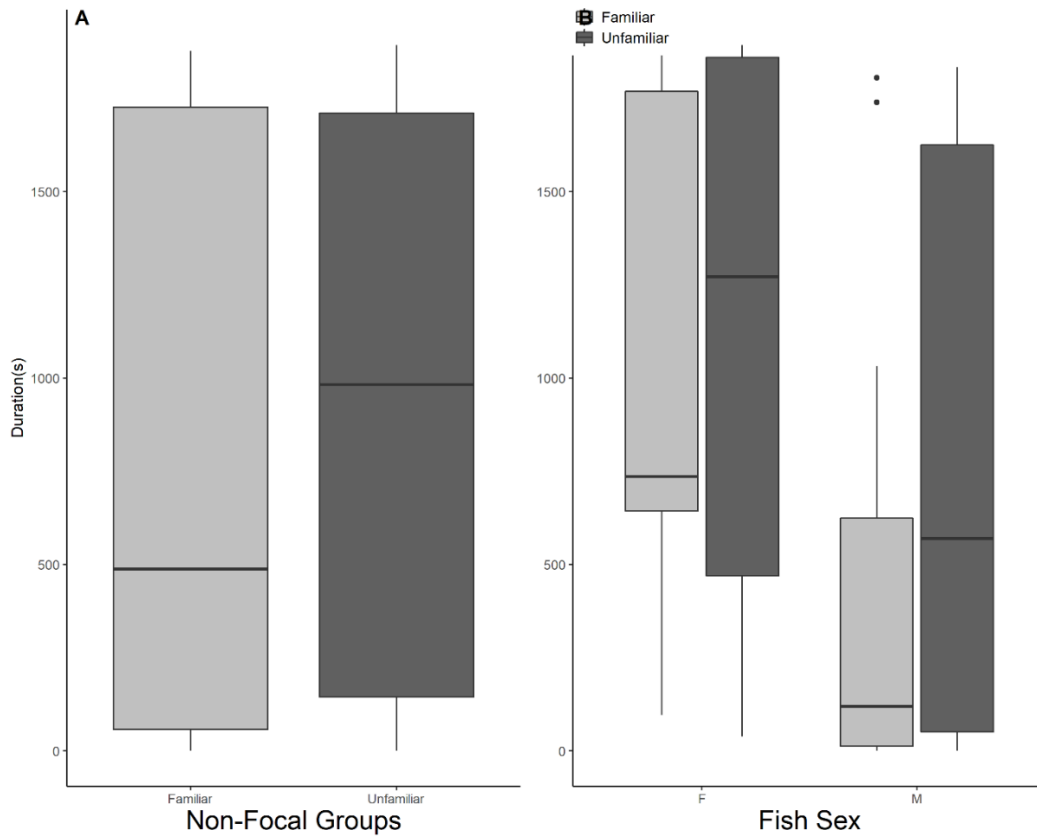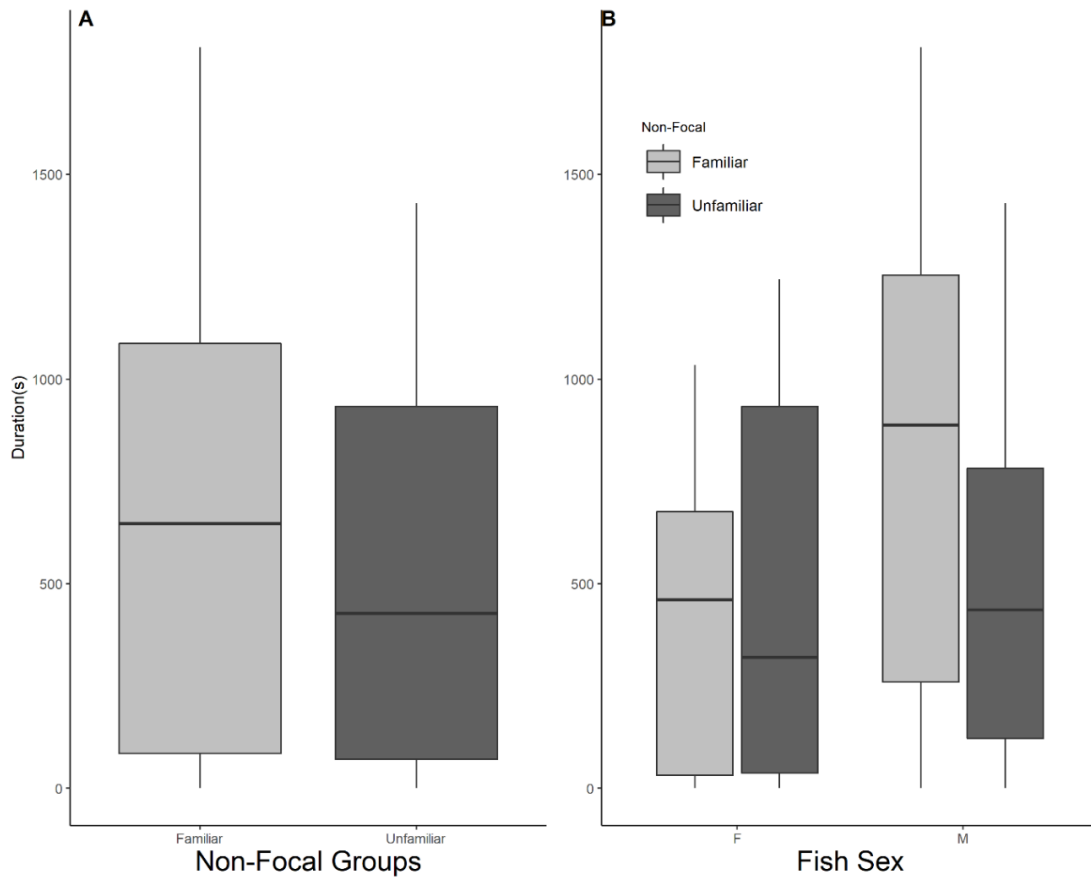
Figure 3.9: Duration of time spent in the contact zone for A) the focal fish when exposed to familiar and unfamiliar fish, and B) female familiar and unfamiliar fish, and male familiar and unfamiliar fish. Data was collected from 26 fish over a two-month period. Focal n= 42, familiar n= 21, unfamiliar n= 21. Female n= 16, Male n= 14. The dots represent outliers, and the vertical line represents the standard error. The horizontal black line represents the median of each group.

Table 3.8. Linear Mixed Model of activity in the contact zone for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. Df = Degrees of freedom.

	Estimate	Std Error	df	t-value	Pr(>|t|)
Intercept	497.553	221.304	42.998	2.248	
Treatment	-155.564	259.016	25.779	-0.45	0.66
Focal Sex	463.154	310.465	42.859	1.49	0.14
Non-Focal Sex	-172.696	275.027	32.398	-0.63	0.53
Treatment x Focal sex	-508.653	353.856	26.011	-1.44	0.16
Treatment x Non-focal Sex	356.472	352.573	26.681	1.01	0.32
Focal x Non-Focal sex	6.003	369.944	31.365	0.02	0.99
Treatment x Focal sex x Non-Focal sex	179.187	489.839	28.300	0.37	0.72

3.4: Discussion

In this study, I aimed to investigate whether upland bullies (*Gobiomorphus breviceps*) displayed different behavioural patterns when exposed to familiar and unfamiliar individuals. Overall, there were no significant differences in focal fish when presented with familiar vs. non-familiar neighbours, though some sex-specific patterns were evident and there was an effect with the resting behaviour. Interestingly, bully behaviour was highly repeatable over time, suggesting that individuals exhibit consistent individual differences in behaviour (i.e., personality).

My study aim was based on comparing the behaviour of male bullies, as male upland bullies are highly territorial - they establish territories to attract females for spawning and guard the nest post-spawning, until offspring hatch (Hamilton, 1998). Unfortunately, many of my 'males' were female, and my sample size of true male-male comparisons (i.e., where both the familiar and non-familiar fish presented to the focal fish were also male) was not large enough for a meaningful analysis (n = 4 replicates). Hence, the study compares the behaviour of both males and females when presented with familiar vs. unfamiliar neighbours. However, there is limited data to suggest that females show territorial/aggressive behaviours. Thompson (2015) explored female upland bullies and the "dear enemy" phenomenon, but aside from her research there are very few investigations on the behaviour of female territorial fish. Her results showed no significant difference in the behaviour of the female fish when exposed to familiar and unfamiliar fish. There is no evidence or studies supporting the idea that female upland bullies are territorial or maintain a territory. However, there are freshwater fish species that show females are territorial, so it is possible that female upland bullies could display these territorial behaviours. Sogawa & Kohda (2018) investigated territorial behaviour in female cichlids (*Neolamprologus pulcher*) and found them to have increased aggression when defending their nest from a rival fish. Other dear enemy studies on territorial fish species have found significant differences in behaviour when the focal fish was exposed to a familiar and unfamiliar, with Kohda et al (2015) even investigating what the specific identification cues might be (Itzkowitz & Leiser, 1999; Sogawa et al., 2016; Sogawa & Kohda, 2018). These studies have been done on both male and female fish, which indicates that female freshwater fish can be territorial.

3.4.1: Behaviours associated with aggressive response (Aggression, Movement and Territory)

I predicted an increase in aggression when the focal fish was exposed to an unfamiliar fish, compared to when exposed to a familiar fish. The data does not support this prediction, as there was no significant difference in the focal fish behaviour for male or female fish. This is in contrast to the findings of other studies. For example, Frostman & Sherman (2004) found that male cichlids (*Neolamprologus pulcher*) spent a significantly longer time at the territory boundary when exposed to unfamiliar fish. They also found an increase of aggressive behaviours towards these unfamiliar fish in comparison to the familiar fish. My results may be confounded by the presence of females in my data. Many studies focus on male-male interactions in territorial fish, but in some cases the female fish may also display aggression (Barley & Coleman, 2010). The assumption is that females are less likely to be aggressive as they do not territory guard, but this study shows there is a trend towards females being more aggressive to unfamiliar fish, so it is possible that female fish also use the dear enemy complex (Thomson, 2016). This territorial behaviour could be useful for trying to find a mate, knowing where they rank relative to known females compared to the unknown quantity of non-familiar females. Sogawa & Kohda (2018) found that under laboratory conditions, female daffodil cichlids (*Neolamprologus pulcher*) displayed tit for tat behaviour and were able to recognise familiar from unfamiliar fish. They found that the female fish was displaying territorial behaviours within the harem. The females attacked familiar and unfamiliar individuals when they were in their territory but stopped attacking the familiar fish when it returned to its territory. However, when the unfamiliar fish returned to its territory, the female focal fish carried on its aggressive behaviour. It is possible that female upland bullies display similar behaviours. While they do not display any territorial guarding behaviours, they could have territories which are separate from the male territories. Investigating female territorial behaviour would be an interesting step to extend upland bully studies. There have also been studies on the convict cichlid (*Archocentrus nigrofasciatus*) which show that females had varying levels of aggression depending on the habitat structure (Barley & Coleman, 2010). Aggressive behaviours for territory defence was needed in certain habitats compared to others for female convict cichlids.

The assay for territorial behaviour is difficult to quantify. What was seen in the videos could have been territory defence, or a refuge-seeking behaviour. The literature seems to show that territory defence comes from outside the nest, as the nest is often the underside of a rock, so it is possible that the hiding in the territory is refuge-seeking rather than territorial defence. Even though I was unable to differentiate between territory defence and refuge-seeking, I decided to still monitor the behaviour and see if there was a difference in time spent inside the territory between familiar and unfamiliar exposures. I predicted that I would see limited time spent in the territory when exposed to the familiar fish, but for time spent inside the territory to increase when exposed to the unfamiliar fish. I also expected to see a higher duration of time spent in the territory for the females overall, as I expected the females to hide from aggressive behaviours of the unfamiliar fish. The results did not show any significant difference between focal fish presented with familiar vs unfamiliar fish. However, there appears to be a slight trend between the familiar and unfamiliar males (Figure 3.8). When investigated further, there was no significant difference between familiar and unfamiliar males. Aggressive behaviours are often classed as "territory defence". Because I was looking at aggression as a separate behaviour, territory hiding was less likely to be a defence mechanism, but rather a way to hide from an assailant. Hamilton (2004) found that when one neighbouring fish was inside its refuge, there was an increase of theft by the other fish on the refuged fish's resources. Larger fish also seemed to use the territory less often. It is possible that something similar was happening with the bullies in this study. It could also be related to a fear response. As there was no physical contact between the two fish, and no resource to be fighting over, hiding in the territory could have been a fear response to attacks from the other fish. Visual contact meant each fish could see the behaviour of the other fish, and it may have been safer to hide in the territory instead of attack. The presence of a wall barrier may have also aided in the time spent in the territory. Knowing that the other fish is unable to enter the same territory could provide a sense of "comfort" where the fish does not feel as threatened. There is no clear idea as to how upland bullies behave when becoming territorial, i.e., whether they sit in the entrance, rest on top, swim around the entrance etc. Sheltering is energy efficient for fish which live in fast moving rivers, so it is likely that sheltering behaviour of upland bullies could be spending more time inside the territory (Chrétien et al., 2021). Territorial behaviour is often identified as aggressive encounters, and there is little mention of fish behaviour around the territory in studies on fish territoriality. This complicates the classification of territory defence for upland bullies, and further observation on bully territorial behaviour would help to identify the behavioural traits of territoriality.

Duration spent in the contact wall zone was predicted to increase in the focal fish when exposed to the unfamiliar fish. The contact zone was the best location for one fish to see the other fish in the adjacent tank. Due to the territoriality of upland bullies, I expected to see a high duration spent observing or attacking the opposing fish in the opposite tank. The results (Figure 3.9) do not support these predictions, which is in contrast to other research that has demonstrated that unfamiliar fish are more likely to be subjected to higher levels of aggression (Frostman & Sherman, 2004; Saeki et al., 2018; Sogawa et al., 2016). The point of contact in my experiment was at the contact wall zone - it would be the appropriate place to fight an unfamiliar fish who may have been perceived as invading the territory, as seen in many other studies (O'Connor et al., 2000; Saeki et al., 2018; Sogawa et al., 2016). Perhaps if there had of been physical contact between the two fish, then there would have been instances where one fish invaded the other's tank and if that would have sparked more aggressive, territorial behaviours between the fish. Studies on trout have used both contact and no contact studies (Elliott, 1990; Johnsson, 1997b; Newman, 1956; White & Gowan, 2013) depending on what the research was investigating. The majority of individual recognition studies separate the fish, but studies focused on dominance hierarchies will often leave two fish in a tank together to establish this relationship (Abbott et al., 1985; Johnsson, 1997b; Newman, 1956; White & Gowan, 2013).

3.4.2: Behaviours not associated with aggressive response (Movement, Resting)

I predicted the duration of movement would decrease when the focal fish was exposed to the unfamiliar fish. This decrease was expected as I expected to see an increase in aggression. Aggressive responses did involve some movement, but because there were aggressive acts occurring with the movement, e.g., biting while erratically swimming up and down the lateral side of the tank, I did not count that behaviour as movement, just as aggression. I found that where there was no significant difference between the duration of movement when presented with familiar and unfamiliar fish, for either sex. Movement is not measured in many other dear enemy studies. Thomson's (2016) study on female upland bullies found no difference in the mobility of the bullies when they were exposed to familiar and unfamiliar fish either. It could be that movement is not a significant behaviour to focus on and may have no correlation to fish aggression. However, distance moved or swimming speed are standard

measures in most behaviour studies (Dzieweczynski & Crovo, 2011; Laskowski et al., 2015; Thomson et al., 2020; Waller & Cairns, 1972) particularly environmental stressor studies (Kelley & Magurran, 2003; Lamb et al., 2020). The most common take away from these studies is that fish often pick the "freeze" option from the "fight", "flight" or "freeze" response options when exposed to a stressor (Galhardo et al., 2011; Vilhunen & Hirvonen, 2003; Yue et al., 2004). Galhardo et al (2011) found that fish increased freezing responses when they were exposed to an unpredictable stressor. It is possible that the unfamiliar fish was not as much of a stressor as I believed it to be, and therefore the movement had no significant difference between the familiar and unfamiliar fish. "Freezing" behaviour was not clearly observed in this study and was not a behaviour included in the ethogram. It is possible that it did not occur at all, but it could help to explain the resting behaviours displayed by the focal fish.

I predicted that the duration of time spent resting would be higher when exposed to a familiar fish compared to an unfamiliar fish. This was because I predicted that aggression levels would be high when the focal fish was faced with an unfamiliar fish. Once I excluded the non-significant interaction from my model, the effect of treatment on resting behaviour became significant, with focal fish resting more in the presence of familiar neighbours. However, this result only appears to be a trend if you examine the figure (Figure 3.7). As mentioned above, there is often the case of fish displaying the "freeze" response when faced with a stressor (Galhardo et al., 2011; Vilhunen & Hirvonen, 2003; Yue et al., 2004). Many of these studies use a chemical stressor, and then measure the levels of stress hormones in the blood. Few studies focus purely on the behaviour of the fish. However, these studies can still give us an insight as to how a fish may behave during an interaction with an unfamiliar fish. Resting or "freezing" responses are likely to have increased if the focal fish was exposed to an unpredictable stressor. As the focal fish had previously been exposed to a familiar fish, I do not think that the unfamiliar fish would have been an unpredictable stressor. While the fish was different, the procedure of moving the tanks to the filming station was the same, and the reveal of the adjacent tank was the same. The unfamiliar fish was not suddenly presented to the focal fish. Alongside this, I predicted that aggression levels would have increased when presented to an unfamiliar fish, so the resting behaviour would have decreased.

I estimated repeatability to be low based on the study by Thomson (2016). The repeatability estimates for all the behaviours measured were very high, for both male and female fish, which is in contrast to that reported by Thomson (2016). This study also used live tracking to gather data on the fish, while my study was not analysed live. It is possible that this data

collection method had an effect on the repeatability levels between Thomson's (2016) study and my own. Females appeared to be more repeatable than males, which was an unexpected result. A study by Thomson et al. (2020) investigated the individual behaviours and personality of zebrafish (*Danio rerio*). Behaviours including distance travelled, time at the bottom of the tank, time stationary and overall exploration were analysed over a 5-month period. Males were found to have much higher repeatability than females. In my study, I observed the opposite. It is unlikely that the repeatability of male and female behaviour would be significantly different, as the confidence intervals overlapped for most behaviours. Other studies report mixed findings when it comes to repeatability in male and female fish. There have been studies where both males and females have repeatable behaviours, or just one sex is repeatable (Kodric-brown & Nicoletto, 1997; O'Neill et al., 2018; Piyapong et al., 2010; Rushbrook et al., 2008; Thomson et al., 2020). This suggests that further research into female upland bullies and their personalities would be repeatable, despite the results found in the study by Thomson (2016), who found very low levels of repeatability from female upland bullies. These high repeatability scores indicate that there are some consistent individual differences in behaviour (i.e., personality) in individual upland bullies, and this could have had an influence over the behaviours of the fish when presented to familiar and unfamiliar individuals. Consistent individual differences in behaviour is observed in many fish species (Kerr & Ingram, 2021; O'Neill et al., 2018; Stamps & Groothuis, 2010). These highly consistent behaviours could mean that bullies would be unlikely to respond differently to familiar and unfamiliar neighbours. It is possible that they treat everything as a threat, which is why there was no differentiation between the non-focal groups. The opposite might even be occurring, where the bullies perceive the non-focal groups to be all familiar, or non-threatening. It is possible also that the bullies are not territorial "enough" to respond to these stimuli.

Investigation into individual recognition was inconclusive due to the inability to take this study further and investigate the unique patterns on the opercular side of the fish. As my results were not conclusive, I cannot confidently say that upland bullies have the ability to identify one individual from another. However, I do not think that this experiment gave an accurate result due to the unknown sex pairings, females in the study, and the fish not being collected during breeding season. I believe that upland bullies are likely to be able to recognise familiar from unfamiliar individuals, but nothing can be said with certainty until further research investigates this.

3.4.3: Limitations of the study

There were several limitations within this study which may have impacted the findings. As mentioned earlier, I aimed to only have male upland bullies within this study, as they are known to be territorial. However, due to COVID-19 I was only able to collect the bullies outside of breeding season. At the height of breeding season, the males can become sexually dimorphic and turn jet black when exposed to a stressor, e.g., a human and when defending a territory. In my preliminary study in 2019, I collected 10 male bullies in the breeding season, and all 10 of the bullies turned black when I was feeding them. In contrast, there was no colour change in any of the fish when I collected them in 2020, and I believe this is because they were caught outside of breeding season. Further, as we were unable to correctly identify males from females, we were unable to get true pairings (e.g., male focal fish paired with male familiar fish, and exposed to male unfamiliar fish). This resulted in the focal fish often being displayed to different sexes, and this could contribute to a lack of power to detect any significant findings.

3.4.4: Conclusions

Overall, my results suggest the upland bullies are unable to differentiate between familiar and unfamiliar fish. While I expected to see more significant results in behaviours such as aggression, duration in the contact zone and territory, this research has shown that the individuality of the fish may have more of an influence on this study than expected. None of the results matched my predictions. It is possible that this is due to females being included in this research, reducing power to detect an effect. I had hoped to investigate the "dear enemy" phenomenon in upland bullies further by altering the face patterns on the familiar fish to test if this altered behaviour, but I ran out of time. Further research into the visual patterns, and if there are any visual differences between individuals, would help to further understand whether upland bullies exhibit individual recognition or not. Investigating the differences in sex and behaviour more clearly would be an excellent next step to take as well. While efforts were made to reduce presentation bias, it is possible that it may have still occurred. By presenting the familiar fish to the focal fish twice, I expected the behaviour of the focal fish to remain the same if it were able to identify the familiar fish from the unfamiliar fish. This was done instead of randomisation to reduce any bias from myself as the researcher, but next time it may be worthwhile trying to randomise the order in which the focal fish observes the familiar and unfamiliar fish. Finally, as mentioned above, collecting fish during breeding

season would be a more reliable way to collect males or females for future research into upland bully behaviours and focusing on whether upland bullies can identify familiar from unfamiliar individuals. Individual recognition is seen in many fish species, and further investigation into upland bullies and their ability to distinguish other individuals would be an asset to the research on fish behaviour. Future research on upland bully behaviour should focus on recognition cues and if these cues are unique spot patterns on the individual.

Chapter Four: General Discussion

4.1: Introduction

The primary aim of this thesis was to investigate individual recognition in fish. Chapter 2 was a literature review of individual recognition studies, while chapter 3 investigated whether individual recognition was observed in the upland bully (*Gobiomorphus breviceps*), specifically, whether upland bullies could distinguish between familiar and unfamiliar individuals.

Overall, I found little support for the idea that upland bullies could differentiate between familiar and unfamiliar individuals, as there were no significant differences in their behaviours, except for resting behaviour, where the focal fish spent less time resting than the non-focal fish. These findings suggest that the focal fish displayed alternative behaviours when presented to non-focal fish and shows potential for individual recognition in bullies. While the results of my study did not support my hypothesis, I did find that the repeatability measures for each behaviour and for the sex of the fish was repeatable. This is a good indicator for future studies on both male and female upland bullies.

This thesis highlights the need to investigate the behaviours of understudied freshwater fish. While the upland bully is not in decline, the congeneric Tarndale bully (*Gobiomorphus alpinus*) which is only found in Marlborough, New Zealand, is an at-risk species. Bullies are not a popular study species for behavioural studies; thus, little is known about their behaviours especially when exposed to familiar and unfamiliar individuals. Studying and learning more about the behaviours of freshwater fish with similar lifestyles to those in decline may provide novel insights into ways to prevent their numbers from declining while providing more information of the lifestyle of these fish. Using a fish that is not in decline but lives in a similar habitat could provide a useful alternative to direct studies of a declining species, as such study may induce unnecessary stress or mortality. Investigating aggression behaviours against both intra-and inter-specific species could give an indication on how these fish behave when faced with a potential threat or predator. Knowing these behavioural responses to aggressive attacks could help researchers assess how threatened species respond to predation and the risks taken to defend territories or protect eggs and nests.

4.2: Chapter 2: Literature Review

My literature review on individual recognition and identifiable cues was conducted in lieu of a second data-based chapter. This literature review investigated the literature surrounding individual recognition across taxa, how morphological constraints may restrict the forms of communication available to an animal, and how the location of patterns or unique visual cues are crucial for communication. This review demonstrated that while there was ample knowledge of individual recognition, how it works and what it is used for, there is a lack of knowledge regarding the unique visual cues used by many organisms. For example, many studies simply stated that visual recognition was occurring, but did not specify or identify the specific cue(s) used (e.g., face shape, colouration, patterning, etc.). Future research on individual recognition should investigate the specific cues used by different species for the recognition of familiar/unfamiliar individuals.

Morphological constraints can also impact the visual cues available to an individual. Restrictions on body shapes and ornaments which could be used for signalling means that other forms of cues must be used. Patterns, colouration, chemical scents or audial cues can all combat morphological constraints which do not allow for such elaborate ornamentations. Large and extravagant ornaments increase the risk of predation, disrupt hydrodynamic and aerodynamic streamlining, and cost energy to develop and maintain (Evans, 2004; Nicoletto, 1991; Weiss & Kirchner, 2010; Zuk, 1991). Fish and volant birds are two clear examples of animals that have been morphologically constrained, and as such do not have the elaborate ornaments that ground-dwelling birds or mammals may have (Evans, 2004; Thomas, 1993; Tytell et al., 2010; Zuk, 1991). Overall, there is a clear understanding of individual recognition and how it can be used for both inter-and intra-specific communications. However, further investigations into pattern locations and morphological constraints on visual cues could beneficial our knowledge of individual recognition.

The location of patterns on the body is important for clear visual communication (Ortolani, 1999; Parchem et al., 2007). Being able to identify the cue itself is crucial, and the positioning of these visual cues needs to be in a clear, corresponding location. This location is also important for the signal receiver, as they have to know where to look to find and process this cue. Often, colours or patterns are used to enhance expressions which are cues themselves for how an animal is feeling (Hamilton & Poulin, 1999; McGraw, 2005; Ortolani, 1999). The use

of patterning or colouration in a specific location is often crucial for successful communication.

4.3: Chapter 3: Behavioural study

Chapter 3 consisted of an investigation chapter focused on the behaviour of the upland bully (*G. breviceps*) where a focal fish was exposed to both familiar and unfamiliar fish. I found no significant behavioural differences, although there were some trends in certain behaviours. Originally, the study aimed to focus solely on male upland bullies as they display territorial behaviours, nest guard, and defend these nests to attract a mate. Unfortunately, I was unable to collect only males, and females were also sampled in this study. This was a limitation that was unable to be resolved as the sex of the fish was only determined post-mortem. While there was no effect of sex on focal fish behaviour, having females in the study did reduce the sample size of male-male comparisons significantly, which may have obscured my ability to detect a differences in the behaviour of male bullies to familiar vs. unfamiliar neighbours. Indeed, Thomson (2016) found that there was no significant difference in behaviour between female upland bullies when testing for the dear enemy phenomenon. While there were males in the study, having females decreased my male vs male pairing sample size. Sex did not show to have any effect on the treatment interactions, but with over half of my sample being females plus only being able to determine sex accurately post-mortem left me with a very small sample size of males. There has been no indication that female upland bullies display any aggressive tendencies (Thomson, 2016), so it is possible that being exposed to females could have influenced a decrease of aggression. It is also possible that upland bullies are not as aggressively territorial as I had anticipated, and it may require more than an unfamiliar fish to prompt an aggressive response.

4.4: Limitations of the study

Unfortunately, there were many limitations to this study which were out of my control. The first was the COVID-19 pandemic and lockdown. There were approximately three months where I was unable to continue with this study due to the pandemic restrictions and delays for necessary equipment due to arrive. I was due to start sampling in February, but these delays pushed me into March which was when New Zealand went into a lockdown. This prevented me from collecting male bullies during their breeding season, when their territorial behaviour is most prominent. Studying bullies during their breeding season would have made it easier to

determine the sex of the fish and ensured that they would display nest guarding behaviours. When male bullies become territorial, a common response is for them to turn completely black except for the stripe on their dorsal fin. Unfortunately, I sampled a mixture of males and females, and their sex was only determined post-mortem. There was not enough time to repeat the study using only males, so the decision was made to continue. Additionally, I also struggled to collect fish of a mature size. There were very few fish in the rivers when we looked for them, and most of the ones we caught were very small. I did not want to sample small fish, as they may not be sexually mature and would therefore not display the territorial tendencies of mature males. Furthermore, I did not want to collect female bullies, which are typically smaller than their male counterparts.

Unfortunately, due to these setbacks, female individuals were sampled. Female bullies have not been well studied, and there is no literature on their territoriality or aggressive behaviours. It is assumed that they are not territorial as the males are the ones which nest guard and look after the eggs (Hamilton, 1998; Hamilton et al., 1997). Because I could not differentiate the males from the females, I was unable to pair females with females, and males with males. This complicated the statistical analysis, and when I tried to analyse only the "true pairs" (i.e., male vs male familiar and unfamiliar, female vs female familiar and unfamiliar, etc.), this resulted in insufficient sample sizes, with only 4 "true pairs" for the male vs male fish. Thus, the data was particularly difficult to analyse. When graphs displayed significant results, the analysis was not powerful enough to detect patterns due to the complexity of including the sex trial combinations. A larger sample size of "true pairs" would drastically improve the statistical power of this research.

4.5: Suggestions for future research

This study indicates many avenues for future research. For example, upland bullies exhibit spot patterns along the sides of their bodies and upon their face. Photo analysis of these spot patterns could be used to determine whether these patterns are individually unique and used to identify/recognise specific individuals. Using methods similar to Arzoumanian et al. (2005) and Gore et al. (2016), further research into the body patterns could be undertaken. These studies used computer software and images to identify individuals based on spot patterns on the body of the animal. By uploading multiple images of individuals, the programs can filter through these images and match up photos of the same individual with a high level of

accuracy. As seen in Figure 4.1, upland bullies have unique markings that look superficially similar but are markedly different from one another.

Figure 4.1. Fish 5 (on the left) and Fish 1 (on the right). Lateral spot patterns are similar, but different in shape, size and colour

An additional avenue of research would be to monitor the response of a focal fish to a familiar fish with an altered body pattern. Kohda et al (2015) conducted such an experiment on cichlid fish, manipulating images so that the faces of familiar individuals were displayed upon the bodies of unfamiliar individuals (and vice versa). These authors found that the focal fish was able to recognise familiar faces on unfamiliar bodies (Kohda et al., 2015). This methodology could be applied to the upland bully to help identify the visual cues potentially used to identify individuals.

Finally, little research has been undertaken on the behaviours of female upland bullies and it may be worthwhile to conduct a long-term study and investigate their behaviour. Thomson (2016) studied female upland bullies and the dear enemy hypothesis but did not obtain any conclusive results regarding their behaviours towards familiar and unfamiliar fish. Additionally, my repeatability results differed significantly from those of Thomson (2016), where my results suggested high repeatability for females whereas Thomson (2016) reported low repeatability's. Despite females not being territorial, there could be mate competition between females and seasonal differences in behaviour which could explain the trends seen in this study, such as in many female cichlid species (Reebs, 1994; Sogawa & Kohda, 2018).

4.6: Conclusions

This study aimed to give a review on individual recognition and how morphology and body locations are important for communication, as well as to investigate the behavioural differences of the upland bully (*G. breviceps*) when exposed to familiar and unfamiliar

individuals. The literature review explored how animals communicate and identify individuals, while also unpacking how morphology has shaped specific animals to communicate in specific ways. Fish and birds are constrained to their environment, and as such much adapt their forms of communication to fit into their niche. Song, patterns and colours are non-adverse forms of presenting information to other individuals without creating costly or expensive ornaments. The location of these patterns are also crucial for communication, as the receiver of the signal must be able to identify and see these cues. Investigation into studies looking at individual recognition showed that many identified individual recognition occurring but failed to specify what cues were being used. Including what cue was, or might have been, used would allow further investigations into these animal's forms of communication. Obtaining this sort of information could improve a study, as well as the knowledge on that animal.

In my data chapter, I investigated five behaviours: aggression, movement, resting, time in territory, and time at the contact wall. Overall, I found no statistically significant behavioural differences when a focal fish was exposed to a familiar or unfamiliar individual, except for resting behaviour, suggesting that the focal fish spent less time at rest than the non-focal fish. Not resting could mean that the focal fish was moving, showing aggression, or hiding in the territory more often than resting compared to the non-focal fish. Repeatability of all the behaviours was high, both across and between sexes. This high repeatability indicates that upland bullies are a good study species for behavioural studies, as their behaviours are consistently repeatable. Further investigation into the "dear enemy" phenomenon on male upland bullies would show if they do display this territorial behaviour consistently, or if there is consistency in no behaviour change. Having both males and females in this study caused difficultly in comparisons for sex combinations (e.g., focal males vs non-focal males, focal males vs non-focal females) due to the small sample size for these true combinations.

Obtaining a broader knowledge of upland bully behaviour will provide a better understanding of how they behave in the wild. This knowledge can be applied to assist with conservation of the Tarndale bully (*G. alpinus*), which is in decline. It could also provide more insight into the behaviour of freshwater fish and their use of individual recognition. New Zealand contains a wide range of endemic freshwater fish, many of which are threatened (Bowie et al., 2018), and investigating their lifestyles and behaviours could help us conserve them.

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Appendix

Linear Mixed Model of behaviours with no interaction

Table A1.1. Linear Mixed Model of aggressive behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. There was no three-way interaction used in this LMM.

	Estimate	Std. Error	df	t value	Pr(>|t|)
Intercept	1.96	0.81	30.3	2.42	
Treatment	0.11	0.34	21.21	0.32	0.76
Focal Sex	0.42	0.99	22.08	0.42	0.68
Non-Focal Sex	0.36	0.48	27.45	0.75	0.46

Table A1.2. Linear Mixed Model of moving behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. There was no three-way interaction used in this LMM.

	Estimate	Std. Error	df	t value	Pr(>|t|)
Intercept	425.88	133.64	33.71	3.19	
Treatment	-56.66	71.48	20.48	-0.79	0.44
Focal Sex	147.54	154.49	21.24	0.96	0.35
Non-Focal Sex	8.17	96.63	31.53	0.09	0.93

Table A1.3. Linear Mixed Model of territorial behaviour for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. There was no three-way interaction used in this LMM

	Estimate	Std. Error	df	t value	Pr(>|t|)
Intercept	936.28	238.42	29.89	3.927	
Treatment	181.62	99.10	20.91	1.833	**0.08**
Focal Sex	-339.35	293.32	21.79	-1.157	0.26
Non-Focal Sex	-87.89	141.31	27.02	-0.622	0.54

Table A1.4. Linear Mixed Model of duration spent at contact wall zone for focal fish, with *n*= 14 males and 16 females. Treatment, focal fish sex and non-focal fish sex were the variables, and the fish ID was used as the random effect. There was no three-way interaction used in this LMM.

	Estimate	Std. Error	df	t value	Pr(>|t|)
Intercept	467.56	173.41	38.24	2.7	
Treatment	-137.47	118.41	21.60	-1.16	0.26
Focal Sex	231.05	182.60	22.14	1.27	0.22
Non-Focal Sex	94.98	145.65	39.29	0.65	0.52